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Small Vessel Disease Pathological Changes in Neurodegenerative and Vascular Dementias concomitant with Autonomic Dysfunction

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Abstract

We performed a clinicopathological study to assess the burden of small vessel disease (SVD) type of pathological changes in elderly demented subjects, who had clinical evidence of autonomic dysfunction, either carotid sinus hypersensitivity or orthostatic hypotension or both or had exhibited unexpected repeated falls. Clinical and neuropathological diagnoses in 112 demented subjects comprised dementia with Lewy bodies (DLB), Parkinson's disease with dementia (PDD), Alzheimer's disease (AD), Mixed dementia (mostly AD-DLB) and vascular dementia (VaD). Of these, 12 DLB subjects had no recorded unexpected falls in life and therefore no evidence of concomitant autonomic dysfunction. A further 17 subjects were assessed as ageing controls without significant pathology or signs of autonomic dysfunction. We quantified brain vascular pathological changes and determined severities of neurodegenerative lesions including α-synuclein pathology. We found moderate-severe vascular changes and high vascular pathology scores ($P < 0.01$) in all neurodegenerative dementias and as expected in VaD compared to similar age controls. Arteriolosclerosis, perivascular spacing and microinfarcts were frequent in the basal ganglia and frontal white matter (WM) across all dementias whereas small infarcts (<5 mm) were restricted to VaD. In a sub-set of demented subjects, we found that vascular pathology scores were correlated with WM hyperintensity volumes determined by MRI in life ($P < 0.02$). Sclerotic index values were increased by ~50% in both the WM and neocortex in all dementias compared to similar age controls. We found no evidence for increased α-synuclein deposition in subjects with autonomic dysfunction. Our findings suggest greater SVD pathological changes occur in the elderly diagnosed with neurodegenerative dementias including DLB and who acquire autonomic dysfunction. SVD changes may not necessarily manifest in clinically overt symptoms but they likely confound motor or cognitive dysfunction. We propose autonomic dysfunction promotes chronic cerebral hypoperfusion to impact upon ageing-related neurodegenerative processes and characterise their end-stage clinical syndromes.

Keywords

Alzheimer's disease, Autonomic Dysfunction, Dementia, Dementia with Lewy Bodies, Microvascular Pathology, Mixed Dementia, Parkinson's Disease with Dementia, Small Vessel Disease, Vascular Dementia

Abbreviations

AD, Alzheimer's disease; CSH, carotid sinus hypersensitivity; CSM, carotid sinus massage; DLB, dementia with Lewy bodies; H&E, Haematoxylin and Eosin; OH, orthostatic hypotension; PD, Parkinson's disease; PDD, Parkinson's disease with dementia; SI, sclerotic index; SVD, small vessel disease; WM, white matter; WMH, white matter hyperintensity.

Introduction

Autonomic dysfunction occurs commonly after 50 years of age. At least 40% of the elderly with Dementia with Lewy bodies (DLB), Parkinson's disease with dementia (PDD), vascular dementia (VaD) and Alzheimer disease (AD) will exhibit some form of autonomic dysfunction. They will invariably develop carotid sinus hypersensitivity (CSH), orthostatic hypotension (OH) or neurocardiovascular instability, associated with hypotension and bradycardia (2, 7, 23). CSH involving an excessive fall in arterial pressure (53) is reported to occur in 35-58% of elderly with AD and DLB (24). OH develops in nearly 50% of Parkinsonians (16, 18, 43, 49, 50) and may be present in over 90% of PDD patients (2). OH is an independent risk for incident dementia (9, 55) and often accompanied by disabling symptoms, impaired quality of life, dizziness, nausea, sweating, and loss of consciousness (1, 4, 32).

The vascular hypothesis of autonomic dysfunction proposes that recurrent episodic hypotension results in chronic cerebral hypoperfusion, especially in the frontal lobe. This in turn causes anoxic damage and leads to impaired cognitive function (45). While the true burden of asymptomatic autonomic dysfunction is unknown (31), it is crucial to protect the ageing brain from fluctuations between hyper- and hypotension, which are known to be associated with white matter (WM) changes (58). We previously showed increased WM hyperintensities (WMHs) (24) in DLB patients with autonomic dysfunction. WMHs are also associated with a postural drop in blood pressure in PD (39). Here, we tested the hypothesis

that there is increased small vessel disease (SVD) or microvascular pathology in patients with clinical evidence of autonomic dysfunction or in those with repeated unexplained falls or syncope and progress to dementia. We performed clinicopathological studies to explore the extent of SVD pathological changes (51) in patients exhibiting signs of autonomic dysfunction and had developed dementia.

Materials and Methods

Study design and subjects

This study involved the assessment of a total of 129 subjects. Demographic, clinical and pathological details of all the subjects are given in Tables 1 and 2. Initially, we screened clinical records and post-mortem reports filed between 1992 and 2017. Of the 350 records screened, we identified 77 demented subjects, who were assessed for autonomic function in the Falls and Syncope Service (FASS) at the Royal Victoria Infirmary, Newcastle upon Tyne and had had a post-mortem (Table 1). We then found 23 additional dementia cases with clinical records reporting repeated unexplained falls prior to death. This information was used as a proxy for the presence of autonomic dysfunction (1, 2). In total, we identified 100 demented subjects with direct clinical or reported evidence for autonomic dysfunction (Table 2). The unexplained falls in these individuals were deemed to be for reasons other than arthritis, osteoporosis or joint disease. We also identified an additional 12 DLB cases with no reported unexplained falls prior to death (Table 2). This group with likely no or minimal autonomic dysfunction was compared with those DLB subjects, who had clear evidence of autonomic dysfunction or repeated unexplained falls. We could not find any cases diagnosed with PDD, AD, VaD or mixed dementia and without evidence of autonomic dysfunction and had come to post-mortem. Furthermore, we identified 17 similar age controls with no evidence of reported falls, syncope or autonomic dysfunction (Table 2). We could not find any control subjects, who were clinically assessed in the FASS for autonomic function or had falls or syncope and had had a post-mortem. There were also no dementia cases tested in the FASS without autonomic dysfunction and had had an autopsy.

Patients suspected of autonomic dysfunction with recurrent symptoms of syncope or falls had evidence of CSH or OH or both (Table 1). CSH was diagnosed by an exaggerated haemodynamic response to longitudinal carotid sinus massage (CSM). The exaggerated response was defined as >3 seconds of asystole (cardioinhibitory CSH) or a fall in systolic

blood pressure in excess of 30 mmHg whilst supine or 50 mmHg whilst in the upright position (vasodepressor CSH) during 5 seconds of carotid sinus stimulation by longitudinal massage over the carotid sinus (24, 28, 29, 37). OH was defined as >30 mmHg drop in systolic blood pressure after an active stand test (Head-up tilt test) (11, 54). Patients diagnosed with OH had undergone CSM and were confirmed not meet the clinical diagnostic criteria for CSH. The mean interval between the time of autonomic function assessment and death was 1.92 years with a range from 0 to 7 years. Almost 80% of the subjects died 3 years after they were assessed in the FASS. The neuropsychometric assessments included the revised Cambridge Cognition Examination (CAMCOG) battery and Mini-Mental State Examination (MMSE). Sub-scores for cognitive domains including memory, orientation and other domains of executive function were generated from the total scores (3). The suspected clinical diagnosis of dementia was confirmed at monthly clinicopathological consensus meetings where clinicians met with the pathologists to designate a final diagnosis in accordance with current criteria including the diagnostic and statistical manual of mental disorders, 4th Edition (DSM-IV).

Study subjects were participants of the Newcastle longitudinal prospective dementia series. They had a clinical diagnosis of either DLB, PDD, mixed dementia (Mixed), AD or VaD. VaD subjects were participants of the Newcastle Cognitive Function After Stroke study (3). Age-matched control subjects aged >70 years were either part of previous prospective studies or based on unrelated brain donations to the Newcastle Brain Tissue Resource (NBTR). They were included as controls if they had not been diagnosed with autonomic dysfunction, cognitive impairment or any neurological or psychiatric illness. Ethics approval was granted by local research ethics committees of the Newcastle upon Tyne NHS Foundation Hospitals Trust. Permission for use of brains for post-mortem research was also granted by consent from the participants themselves or next-of-kin or family member. Brain tissues were retained in and obtained from the NBTR.

Magnetic Resonance Imaging

A total of 42 subjects who had post-mortem had undergone brain MRI in life. T2-weighted MRI images (T2WI) were obtained in each case, and WMH volumes (vWMHs) estimated using previously validated in house software (12). The mean time from MRI scans to death (years $\pm$ SEM) for all the subjects was 6.4 $\pm$0.6 years. For each group this was as follows:

DLB 3.6 ±0.7 years; PDD 4.3 ±1.0 years; Mixed 4.6 ±0.6 years; AD 8.1 ±1.1 years and VaD 6.4 ±1.3 years. For controls, without autonomic dysfunction it was 12.2 ±1.4 years (n=5). The percentage of WMH volume per total brain volume (%vWMH/vTB) in disease cases was calculated. We then performed correlation analysis between WMH volume and vascular pathology scores from all the demented subjects with autonomic dysfunction (Figure 2).

Brain tissues and Neuropathological analysis

Neuropathological assessment was carried out as described previously (3). Briefly, haematoxylin and eosin (H&E) staining was used for assessment of structural integrity and infarcts, Nissl and Luxol Fast blue staining for cellular patterns and myelin loss, Bielschowsky's silver impregnation and amyloid-β for Consortium to Establish a Registry for Alzheimer's Disease (CERAD) rating of neuritic plaques, Gallays stain for neuritic pathology, and tau immunohistochemistry for Braak staging of neurofibrillary tangles. Large sections from the frontal, temporal (including hippocampal formation), parietal and occipital lobes, caudate-putamen, brain stem including midbrain, pons and medulla oblongata, and cerebellum were examined from sampled regional blocks as identified in the Newcastle brain map (20, 42). The clinical diagnosis of DLB and PDD were confirmed according to established criteria (30). The clinical diagnosis of AD was confirmed on evidence of significant Alzheimer's-type pathology incorporating Braak stages V–VI, moderate-severe CERAD (26) and high ABC scores per National Institute of Aging-Alzheimer's Association guidelines (36), in the general absence of significant vascular pathology (Table 2). The clinical diagnosis of vascular dementia (VaD) was made when there were multiple or cystic infarcts, lacunae, border-zone infarcts, microinfarcts and small vessel disease, and pathologically confirmed as Braak stage ≤ IV (20, 21). Mixed AD and VaD case was classified when there was sufficient degree of pathology to reach Braak V–VI and significant vascular pathology (6). We also included cases with Mixed dementia, who met two or more neuropathological diagnostic criteria for DLB, PDD and AD.

Vascular pathology scores were derived from the presence of vascular lesions/pathologies in brain areas, including the frontal lobe at the level of the olfactory bulbs, temporal lobe at level of the anterior hippocampus, and basal ganglia at level of mamillary body. Lesions including arteriolosclerosis, cerebral amyloid angiopathy (CAA), perivascular haemosiderin deposition, perivascular space dilatation in the deep and juxtacortical WM,

myelin loss, and cortical micro (<0.5 mm), small (<5 mm) and large (>0.5 cm) infarcts were recorded with increasing severity resulting in greater scores (10). These measures were compatible with the recently established vascular cognitive impairment neuropathology consortium criteria (46). The vascular pathology scores were determined as detailed previously (10) with >95% agreement between scoring performed by VD and RNK. CAA was scored using the system described previously (25). Tissues from control subjects had occasional ageing-related pathology and were classified as 'no pathological diagnosis' (Table 2). Except for neuropathological examination (TP, RHP, PI and RNK), all subsequent morphological analyses were always undertaken under operator blinded conditions. Samples were identified with coded sequential numbers. In addition, at least two of both positive and negative controls were included to monitor the quality of staining.

Assessment of vascular sclerotic index (SI)

H&E stained coronal frontal lobe (Brodmann area 9) sections were analysed to assess SI in a total of ~4,000 brain vessels, essentially as described previously (8, 56). Twenty images from each entire depth of the cerebral cortex and the deep WM were randomly captured using a bright field microscope (Leitz DIALUX 20, Leica) with a 10x objective lens coupled to a lumenera infinity digital camera (Lumenera Corporation, Canada). Using VasCalc software (56), internal diameter and external diameter of each vessel was measured in three opposing axis. Sclerotic index (SI) was calculated using the following formula: SI = (External diameter - Internal diameter) / (External diameter). Three SI values were calculated from three measured axis in each vessel and averaged. The inter-rater reliability between the assessors was 0.9 either DV and RK (SVD pathology scores) or YH and RK (SI scores).

Statistical analysis

Statistical analysis was carried out using SPSS (IBM, version 23.0, IBM Corporation, Armonk, NY, USA) with the level of significance set at $P<0.05$. First, distribution of values was tested using the Shapiro-Wilk test followed by one-way analysis of variance (one-way ANOVA) with post-hoc Tukey's tests for normally distributed values or Kruskall-Wallis H tests for non-normally distributed values to compare data amongst autonomic dysfunction plus dementia and control group. Student's t-test or Mann-Whitney U test was used for

normally or non-normally distributed data respectively to assess the differences between cortex and WM data. Spearman's rho correlation was used to assess the relationship between WM hyperintensity volumes and vascular pathology. Pearson's correlation was used to assess the correlation between vascular pathology and microinfarcts/microbleeds. Anonymized data will be shared by request from any qualified investigator. All the data we have generated in this study have been included in this paper.

Results

Clinical features in the autonomic dysfunction plus dementia subjects

Tables 1 and 2 provide the mean age and gender distributions of all the dementia subjects with relevant clinical manifestations. We noted that the mean age of PDD subjects was marginally lower compared to controls and other autonomic dysfunction plus dementia subjects ($^{\dagger}P<0.01$) (Table 1 and 2). Of the 77 dementia subjects tested in the FASS, 29 (38%) had evidence of both OH and CSH (Table 1). The total CAMCOG and MMSE scores indicated all subjects had evidence of dementia at least 6 months prior to death. There were no differences in CAMCOG memory and executive sub-scores amongst the autonomic dysfunction plus dementia groups (Table 1). We further noted that on average 55% of the autonomic dysfunction plus dementia subjects exhibited hypertension and often had more than one other vascular disease risk factor including diabetes mellitus (DM), ischaemic heart disease (IHD) and smoking (Table 1). The frequency of hypertension in DLB without falls subjects was 33%, which was not different compared with that in dementia subjects who had autonomic dysfunction (Chi-square test).

Cerebrovascular Pathological changes in Dementia with Autonomic Dysfunction

Table 2 provides details of clinically tested autonomic dysfunction subjects and ageing controls assessed for SVD or microvascular pathology. None of the control subjects had reported unexplained falls in life or had been tested in the FASS. Subjects with dementia exhibited the expected pathology defining each type of disease. The Mixed and AD subjects had the highest amount of neurofibrillary tangle (Braak stage) and neuritic or amyloid β plaque burden (Table 2). The DLB, PDD and VaD groups had relatively lower burdens of AD-related hallmark pathologies and there was even less in ageing controls (*P<0.01;

**P<0.01) (Table 2). In addition, nigral Lewy body pathology and neuronal loss were characteristically most severe in DLB, PDD and Mixed dementia subjects (Table 2). Of the 13 VaD subjects clinically diagnosed with autonomic dysfunction, 3 had some degree of Lewy body pathology (Table 2).

VaD subjects expectedly exhibited the highest vascular and WM pathology scores (10). However, remarkably all neurodegenerative dementia groups including DLB, PDD, Mixed and AD had high vascular pathology scores compared to ageing controls (‡P<0.01) (Table 2). AD subjects had the highest ABC scores (36) and the greatest frequency of moderate to severe CAA (Table 2). The types of vascular changes seen in subcortical regions in the autonomic dysfunction plus dementia subjects predominantly constituted those akin to SVD or microangiopathy, which included arteriolosclerosis, intimal thickening, fibroid necrosis, hyalinisation, perivascular spacing, microinfarcts, WM rarefaction and cerebral amyloid angiopathy (10) (Figure 1A, a-f). WM scores incorporating demyelination (17) were also greater in the dementia cases compared to controls. Variable calcification was present in numerous vessels within the extrapyramidal regions particularly in and vicinity of the globus pallidus (Figure 1B, a-d) that we also observed, albeit to a lesser degree in normal ageing controls. The diameters of the calcified vessels ranged 40 to 160 μm. However, distribution of the vascular pathology scores in different brain regions indicated that higher scores were evident in the frontal lobe and the basal ganglia (Figure 1C). There was high predilection for microinfarcts in the frontal lobe WM as well as the basal ganglia across all the dementias.

Further verification of our observations on clinically tested subjects was evident from retrospective assessment of DLB subjects, who had had no apparent autonomic dysfunction (Table 2) and of demented subjects who had had repeated unexplained falls in life (Table 2). The vascular pathology features and scores in most of the dementia subjects were similar or higher compared to those who were clinically tested in the FASS (Table 2). We also could not find differences in SVD features in demented individuals whether they were clinically tested in the FASS or experienced unexplained falls as proxy for autonomic dysfunction. However, the key observation was that DLB subjects without reported unexplained falls or autonomic dysfunction, had lower vascular pathology scores compared to dementia subjects who had autonomic dysfunction. The vascular pathology scores in DLB subjects without autonomic dysfunction were also not different from ageing controls (P=0.236) (Figure 1C). We also found a positive correlation between severity of CAA and haemosiderin deposition (proxy for microbleeds) (r=0.88, P=0.01) but not between CAA and microinfarcts (P>0.05).

We further noted that the degree of arteriolosclerosis was strongly correlated with numbers of microinfarcts ($r=0.87$, $P=0.012$) (Table 2).

Correlation between Vascular pathological changes and WMH volumes upon MRI

To strengthen the evidence that WMHs associated with the various dementias in life (Figure 2A) reflected greater SVD or microvascular pathology, we tested the relationship between vWMHs determined by T2WI MRI and vascular pathology scores evaluated after post-mortem in those subjects who exhibited autonomic dysfunction. We found there was a positive correlation ($\rho= 0.40$, $P=0.016$) between vWMH and vascular pathology scores across the dementias (Figure 2B). However, there was no clear relationship between vWMH and any of the other neurodegenerative disease markers such as tau or α-synuclein pathology ($P>0.05$).

Sclerotic Index and autonomic dysfunction plus dementia

We then quantified the degree of arteriolosclerosis using the SI (Figure 3A) in the deep WM and entire depth of the cortex of the frontal lobe. The SI was consistently higher by 40-50% in all of the autonomic dysfunction plus dementia subjects compared to controls in both the frontal cortex and the WM (**$P<0.01$ and $^{\dagger\dagger}P<0.01$ respectively) (Figure 3B). Dementia subjects exhibited higher SI values in the cortex compared to the WM, particularly in AD subjects ($^{\P}P<0.05$), whereas controls showed similar SI values in both the cortex and WM (Figure 3B). However, it was notable that DLB subjects without falls exhibited similar SI values as ageing controls and lower SI values, particularly in the WM compared to all dementia with evidence of autonomic dysfunction ($^{\ddagger}P<0.035$) (Figure 3B).

Discussion

We previously reported that deep WMHs correlated with autonomic dysfunction in DLB subjects (24). In this study, we provide robust evidence for the presence of significantly greater degree of SVD pathology including WM changes in dementia subjects with clinical evidence of concomitant autonomic dysfunction. The remarkable finding is that irrespective of common dementia type whether DLB, PDD, Mixed dementia, AD or VaD they all

exhibited greater SVD type of pathological changes in the presence of autonomic dysfunction. This was corroborated by low vascular pathology scores and SI values in DLB subjects without evidence of autonomic dysfunction. Whereas the highest burden of SVD pathology (51) consisting of microinfarcts, lacunar infarcts, severe arteriolosclerosis, WM rarefaction and perivascular spacing was present in VaD, it was rather surprising that subjects diagnosed with neurodegenerative dementias including PDD, DLB and AD exhibited these similar microvascular changes particularly severe arteriolosclerosis and perivascular spacing. SI values were higher by 45-50% signifying moderate-severe arteriolosclerosis in dementia cases with autonomic dysfunction. Arteriolosclerosis and microinfarcts are strongly associated with cognitive impairment (5, 19, 52), and were consistent features across all dementias. Vascular pathology scores, particularly in the WM were also increased and correlated with vWMHs in those demented subjects with autonomic dysfunction. These observations collectively suggest arterial wall changes and thickening may impact on blood rheology to impair cerebral blood flow and perfusion (20).

With reference to other features of SVD such as CAA, except in AD, dementia subjects with and without autonomic dysfunction exhibited similar CAA pathology to ageing controls. This further suggested that CAA was not necessarily a factor in the observed arteriosclerotic differences in demented subjects with and without autonomic dysfunction. While, we observed strong correlations between arteriolosclerosis and microinfarcts and between CAA and haemosiderin deposition or microbleeds, we did not observe correlations between CAA and microinfarcts (40, 47). The latter is probably because we had low numbers of cases in the analysis and had included VaD subjects, who showed relatively low CAA but high burden of microinfarction. The frequency of hypertension in 'DLB without falls' group was not different from other dementia groups. Moreover, DLB subjects without falls exhibited similar SI values as ageing controls but had lower SI values, particularly in the WM, compared to all dementias with concomitant of autonomic dysfunction. This suggests hypertension per se was not necessarily a factor in the observed differences in SVD pathology in demented subjects with and without autonomic dysfunction.

Large vessel disease may also contribute to SVD type of changes in the WM and to microinfarcts (20, 51, 57). In comparison to the neurodgenerative dementias, VaD subjects bear greater degrees of large vessel disease i.e. intracranial and extracranial atherosclerotic disease. In a recent study (14), we noted that regardless of severity of carotid artery stenosis, most of the infarcts were small (<5mm in size) and most were located in the cerebral cortex.

In a previous it was further noted that cerebral atherosclerosis was not only expectedly associated with cystic infarcts but importantly microinfarcts (57). It would appear microinfarcts arise from a combination of mechanisms including artery-to-artery embolism, cardiogenic embolism and haemodynamic impairment that likely involve microemboli.

In comparison to ageing controls with no recorded autonomic dysfunction and DLB subjects without reported falls, overall our findings indicate SVD pathological changes are common in elderly subjects with autonomic dysfunction irrespective of the type of dementia they acquire. This may compound effects of co-existing pathologies including neurofibrillary or α-synuclein pathology or amyloid deposits, which albeit may occur even in cases of pure autonomic failure such as idiopathic OH or Bradbury-Eggleston syndrome (pure autonomic failure causing idiopathic OH) (ROC and RNK, unpublished observations). It seems reasonable to conclude that cerebral SVD changes contribute to the clinical syndrome in elderly subjects who develop dementia and exhibit autonomic dysfunction. While these ageing-related SVD changes may not result in overt neurological signs or cerebrovascular events except in VaD, they likely collectively contribute to the declining brain health of the elderly with autonomic dysfunction.

Our findings underscore that vascular protection is important in patients who develop any type of dementia and are symptomatic or even asymptomatic for autonomic dysfunction. We enrolled symptomatic autonomic dysfunction plus obviously demented subjects. However, some patients with neurodegenerative diseases and dementia develop autonomic dysfunction prior to motor and cognitive symptoms. A previous study reported that up to 40% of PD patients with autonomic dysfunction were 'asymptomatic', and even 'asymptomatic' autonomic dysfunction caused higher prevalence of falls, worsened activities of daily life (ADL) and affected their quality of life (QOL) (31). In a follow up study (32), the severity of autonomic symptoms progressed by 20% over the course of a year and was independently associated with impairment in ADLs and QOL. Symptomatic and asymptomatic OH were both associated with increased falls and health care utilization. Thus, 'asymptomatic' autonomic dysfunction has almost similar detrimental effects to being symptomatic. Importantly, if 'asymptomatic' autonomic dysfunction could be established clinically at early stages of cognitive impairment, relevant treatment or intervention(s) has the potential to prevent further cognitive decline in patients with neurodegenerative dementias.

While our study provides good evidence to support our hypothesis in that autonomic dysfunction is linked to alterations in the cerebral vasculature of subjects with neurodegenerative diseases, it is unclear whether autonomic dysregulation is a consequence or an instigator of dementia. A recent study (38), provided novel evidence for autonomic dysfunction in mild cognitive impairment. This was associated with orthostatic blood pressure dysregulation and suggests that dysautonomia is an early feature in the development of cognitive dysfunction. CSH is an important risk factor for hypertension, IHD and systemic atherosclerosis (48). In our cohort, autonomic dysfunction plus dementia subjects displayed frequent vascular risk factors. These may contribute to atherosclerosis in the carotid arteries, affecting the baroreceptors (27) and possibly intracranial cerebral arteries to cause deep WM changes (15, 24) and worsen cognitive function. Alternatively, CSH may independently cause chronic or episodic hypotension promoting abnormal perfusion with resultant changes in cerebral small vessels and the WM leading to cognitive decline. While previous studies have confirmed the association between features of autonomic dysfunction e.g. OH and selective cognitive deficits, albeit in PD, they have rejected the hypothesis that this is underlined by the development of cerebrovascular disease (43). The underlying mechanism could be mediated by development of cerebrovascular disease induced by chronic or episodic hypoperfusion but until now the extent of brain vascular load in PD or DLB patients with OH was not elucidated.

Another hypothesis is that generalised brain α-synuclein pathology or focal α-synucleinopathy in the sympathetic ganglia relates to autonomic dysfunction and dementia. Clinical and post-mortem neuropathological findings in patients with PD and DLB, both clinically diagnosed as pure autonomic failure (PAF) revealed that neurodegeneration in PD and DLB may originate from outside the CNS in autonomic postganglionic neurons (22). Involvement of postganglionic sympathetic nerves including the cardiac sympathetic nerves were predominant in PD and PAF (41), suggesting that the cardiac but not the medullary sympathetic neurones are affected in people with PD and PAF. Cardioinhibitory CSH in patients with Lewy body pathology was also related to severity of the cardiac sympathetic neurons rather than in CNS (44). Furthermore, severity of autonomic dysfunction was associated with cognitive dysfunction in patients with PD (50). These reports strongly support our findings in that PDD and DLB, which exhibit extensive brain Lewy body pathology, showed similar SVD pathologies and cognitive dysfunction as those cases with autonomic dysfunction plus dementia without significant Lewy body pathology. Thus,

extensive Lewy body pathologies in the brain may not directly affect SVD pathological changes to lead to dementia but autonomic dysfunction related cerebral hypoperfusion likely contributes to microvascular pathology, WM changes and cognitive decline in patients with Lewy body pathology. It is plausible, however, that the observed SVD pathological changes result from neuronal damage in the brainstem nuclei or disruption of the monoaminergic neuronal system (27, 35). While further investigation is necessary, we observed alpha-synuclein pathology in sympathetic ganglia of some VaD subjects, suggesting autonomic dysfunction in VaD could partly result from alpha-synuclein pathology in the sympathetic nervous system.

We emphasise that one of the main limitations of our study is that we were not able to find and compare groups of dementia subjects who were tested in the FASS, did not have evidence of autonomic dysfunction and had come to post-mortem. As suggested by several clinical studies (cited above), high numbers of dementia patients may be 'asymptomatic' for autonomic dysfunction and it is likely that they bear considerable burden of SVD pathology. We previously demonstrated the spectrum of vascular pathology in different dementias including VaD, AD and DLB (10). In that previous post-mortem cohort, while we were not aware whether any of the patients had autonomic dysfunction or were asymptomatic for it, on the whole they tended to exhibit relatively low SVD pathology scores. Another limitation is that we did not quantify microvascular pathology in all the regions of the brain used to score vascular pathology. Such analysis is profoundly cumbersome but in accord with our previous hypothesis (13), we concentrated on the deep WM of the frontal lobe and incorporating the centrum semiovale. Finally, in terms of elucidating some mechanistic approaches we did not have enough frozen tissues from cases without clear evidence of autonomic dysfunction to substantiate study power and enable biochemical assays of myelin degrading proteins or gliovascular unit markers to demonstrate consequences of microvascular damage (13, 17).

Autonomic functions are regulated in the brainstem, e.g. medulla and its nuclei (34). In patients with CSH, tau pathology was evident in the medullary baroreflex nuclei (35) and medullary vessels were degenerated in patients with multiple system atrophy (MSA) (33). Microvascular changes in the brainstem also relate to or are caused by autonomic dysfunction. A future expanded analysis of SVD pathological changes involving other brain regions, particularly in the brainstem in this cohort may provide more evidence to elucidate the pathophysiological mechanisms and impact of autonomic dysfunction on microvascular changes in dementia patients.

Conclusions

In summary, autonomic dysfunction irrespective of final diagnosis of dementia type is associated with consistent higher burden of SVD pathological changes, particularly in the deep WM. These SVD changes do not necessarily manifest into overt clinical signs but likely add to the dementia syndrome. Our findings implicate that autonomic dysfunction is an important risk factor of chronic cerebral hypoperfusion, which may differentially affect neurodegenerative processes to produce the end-stage pathology. We propose the importance of assessing autonomic dysfunction in patients with dementia, particularly at an early stage. Treatment for detrimental autonomic dysfunction (even when it is asymptomatic) implementing stricter control of vascular risk factors, pacemakers, life-style intervention or pharmacological approaches might be effective therapeutic strategies to reduce SVD during ageing and prevent or slow down cognitive decline in patients with autonomic dysfunction plus dementia, in addition to reducing their immediate risk of falls.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Author Contributions

YH and RNK conceived the study and wrote the first drafts of the manuscript. YH, EH, CP, LJLC, WS, MJF, TP and RNK performed or contributed to different aspects of the quantitative analysis. CB, RAK, LMA were responsible for original set up and clinical evaluation of the cohorts. TMP, VD, RHP, PI and RNK provided the pathological diagnosis. YH, TMP, MJF, ROC, KH, LMA and RNK contributed to critically revising the manuscript for important intellectual content, and all approved the final version of the manuscript for submission.

Conflict of interests

The authors have no disclosures or conflicts of interest in relation to this manuscript.

Ethical Approval

Ethical approvals were granted by local research ethics committees of the Newcastle upon Tyne Foundation Hospitals Trust. Permission for use of brains for post-mortem research was also granted by consent from next-of-kin or family. All the brain tissues were retained in and obtained from the Newcastle Brain Tissue Resource.

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Table 1: Clinical features in the autonomic dysfunction plus dementia subjects assessed in the FASS

Variable	All dementias	DLB	PDD	Mixed	AD	VaD
Number of subjects	77	16	13	17	18	13
Age, years, mean (range)	82.3 (64-98)	79.6 (69-96)‡	72.8 (64-81)†	83.0 (72-93)	87.8 (76-96)	88.1 (75-98)
OH / CSH / OH and CSH, number	58, 42, 29	12, 10, 7	11, 5, 3	12, 11, 8	14, 11, 9	9, 5, 2
Total CAMCOG score (/100), mean (range)	54.2 (20-80)	53.8 (24-80)	61.1 (39-74)	51.0 (23-68)*	44.0 (20-73)	61.2 (25-80)
Memory sub-score (/27), mean (range)	13.7 (4-23)	14.9 (8-21)	16.2 (9-23)	13.5 (4-23)	10.0 (5-14)	14.1 (4-22)
Executive sub-score (/28), mean (range)	11.2 (2-19)	12.1 (4-23)	9.6 (5-18)	11.2 (7-15)	12.3 (10-17)	10.6 (2-19)
MMSE score (/30), mean (range)	13.1 (0-24)	13.8 (6-20)	14.2 (3-20)	11.9 (2-19)	8.5 (0-16)	17.2 (8-24)¶
Hypertension, number (%)	42 (55%)	11 (69%)	7 (54%)	7 (41%)	9 (50%)	9 (69%)
Systolic BP, mean (range)	146 (101-200)	149 (122-187)	152 (101-200)	142 (109-186)	141 (110-165)	146 (131-172)
Diastolic BP, mean (range)	76 (51-120)	74 (60-86)	82 (57-120)	74 (53-98)	72 (51-95)	76 (52-92)
Other VRF, number (smoking / DM / IHD)	32/5/15	7/0/4	6/1/3	6/1/3	5/3/2	8/0/3

All dementia subjects had clinical evidence of autonomic dysfunction. All dementias group represent all causes of dementia including DLB, PDD, Mixed, AD and VaD; Mixed dementia: 7 =AD+DLB, 6 =AD+VaD, 3=AD+DLB+VaD and 1= PD+DLB+VaD.

Age, †P<0.01 vs Control, DLB, Mixed, AD and VaD; ‡P<0.01 vs AD and VaD; P=0.057, Mixed vs AD; P=0.053, Mixed vs VaD; OH, orthostatic hypotension; CSH, carotid sinus hypersensitivity; 'OH / CSH / OH and CSH, number' indicates number of cases who had OH / CSH / both OH and CSH in each group. For example, in DLB group, OH / CSH / OH and CSH are described as 12, 10, 7. This means 12 cases in DLB had OH, 10 subjects confirmed to have CSH and 7 had evidence to have both OH and CSH (OH and CSH overlapped in some cases); Total CAMCOG score, *P=0.034 vs PDD, *P=0.028 vs VaD; CAMCOG executive and memory subscore, n.s. between groups; MMSE score, ¶P=0.041 vs DLB, ¶P=0.021 vs Mixed, ¶P=0.003 vs AD; Hypertension, number (%), n.s. between groups (Chi-square test); Systolic and Diastolic BP, n.s. between groups. Clinical features other than age and gender in Controls and DLB without falls cases were not completely available as they were not assessed at the falls and syncope clinic (FASS).

Abbreviations: AD, Alzheimer's disease; BP, blood pressure; CAMCOG, revised Cambridge Cognition Examination; DLB, Dementia with Lewy Bodies; DM, diabetes mellitus; F, female; IHD, ischaemic heart disease; M, male; Mixed, Mixed dementia; MMSE, Mini-Mental State Examination; PDD, Parkinson's disease with dementia; VaD, Vascular dementia; VRF, vascular risk factor; WML, white matter lesion.

Table 2

Neuropathological features of all dementia cases and similar age controls in the study

Variable	Controls	DLB without Falls	All dementias	DLB	PDD	Mixed[‡]	AD	VaD
Number of subjects	17	12	100	24	18	22	18	18
Age, years, mean (range)	86.9 (72-99)	80.0 (74-90)[&]	82.0 (64-98)	79.6 (69-96)[‡]	74.6 (64-89)[†]	83.4 (72-93)	87.8 (76-96)	85.8 (71-98)
Gender, number (F/M)	13 / 4	1 / 11	52 / 48	11 / 13	9 / 9	16 / 6	9 / 9	7 / 11
Autonomic Dysfunction (n=)[§]	0	0	100	24	18	22	18	18
Braak Stage, mean (range)	1.9 (0-4)	3.0 (0-6)	3.3 (0-6)	2.2 (0-4)	2.0 (0-4)	4.8 (2-6)*	5.6 (5-6)**	1.6 (0-4)
CERAD, mean (range)	0.5 (0-2)	1.2 (0-2)	1.7 (0-3)	1.5 (0-3)[ϕ]	0.5 (0-2)	2.6 (0-3)*	2.9 (2-3)**	0.8 (0-3)
Alzheimer's Disease Neuropathologic Changes; A, B, C (mean)[♯]	A0.5, B1.2, C0.5	A1.2, B1.6, C1.2	A1.5, B1.8, C1.7	A0.9, B1.3, C1.5	A0.4, B1.1, C0.5	A2.5, B2.6, C2.6	A3, B3, C3	A0.6, B1.2, C0.8
Vascular pathology score, mean (range)[♯]	6.7 (0-10)[‡]	8.1 (5-10)[&]	11.4 (5-18)	9.9 (5-13)	10.8 (7-17)	11.9 (7-15)	11.2 (6-16)	13.6 (10-18)[¶]
White matter lesion score, mean (range)	0.5 (0-2)[§]	1.6 (1-3)	2.2 (0-3)	2.0 (1-3)	2.1 (1-3)	2.1 (0-3)	1.8 (0-3)	2.9 (2-3)[ψ]
White matter/Vascular lesions, moderate - severe (%)	17.6**	41.7*	94	83	94	91	72	100

Lewy body pathology, number (limbic/neocortical)	0 / 0	2 / 7	16 / 36	3 / 19	5 / 10	5 / 7	0 / 1	3 / 0
SN neuronal loss (n=), (none/mild/moderate/ severe)	N/A	3/1/3/5	19/25/28/24	0/4/11/8	0/1/4/12	3/5/9/4	7/8/2/0	9/7/2/0
CAA, total (%)	17.6	8.3	18.6	8.3	5.6	18.2	66.7**	27.8
CAA, moderate-severe (%)	5.8	8.3	10.1	0.0	5.6	9.1	38.9**	16.7
Arteriolosclerosis, moderate-severe (%)	5.9**	25.0	60.6	45.9	61.1	63.4	61.1	66.7
Microinfarct, total (%)	35.3**	25.0**	81.1	79.1	88.9	77.3	66.7	100*
Microinfarct, moderate-severe (%)	0.0*	0.0*	30.5	25.0	33.3	27.2	15.6	76.9**
Haemosiderin deposition (microbleeds), total (%)	0.0	0.0	15.0	8.3	11.1	9.1	33.3**	16.7
Brain only necropsy (%)	100	25	72.0	70.8	88.9	95.4	66.7	33.3
Intracranial MCA stenosis, moderate-severe (%)	N/A	16.7	14.0	4.2	5.6	0.0	11.1	90.9
Extracranial ICA stenosis, moderate-severe (%)	N/A	0.0	16.8	N/A	0.0	N/A	11.1	100
α-Synuclein pathology in the sympathetic ganglia, moderate-severe (n=)	N/A	N/A	5	N/A	1	N/A	N/A	4

[§]All dementia subjects had clinical symptoms or evidence of autonomic dysfunction. ǂMixed dementia group represents: 7=AD+DLB, 7=AD+VaD, 4=AD+DLB+VaD, 3=DLB+VaD and 1=PD+DLB+VaD.

Age, [†]P<0.01 vs Control, DLB, Mixed, AD and VaD; [‡]P<0.01 vs AD and VaD; [&]P<0.01 vs AD and VaD; Braak Stage, **P<0.01 vs Control, DLB without Falls, DLB, PDD and VaD; **P<0.05 vs Mixed; *P<0.01 vs Control, DLB and PDD; *P<0.01 vs DLB without Falls and VaD; CERAD, **P<0.01 vs Control, DLB, PDD and VaD; *P<0.01 vs Control, DLB, PDD and VaD; [ϕ]P<0.05 vs Control; [ϕ]P<0.01 vs PDD; ♯Alzheimer's Disease Neuropathologic Changes **(36)**; ♯Vascular Pathology Score (10), [‡]P<0.01 vs DLB, PDD, Mixed, AD and VaD; [&]P<0.01 vs PDD, Mixed, AD and VaD; [¶]P<0.05 vs DLB without Falls, DLB and PDD; White matter lesion score, [§]P<0.01 vs DLB without Falls, DLB, PDD, Mixed, AD and VaD; [Ψ]P<0.01 vs DLB without Falls, DLB, PDD, Mixed and AD; White matter/Vascular lesions, **P<0.01 vs Control, DLB, PDD, Mixed, AD and VaD; *P<0.05 vs Control, DLB, PDD, Mixed, AD and VaD (Chi-square test); Lewy body pathology, only the number of limbic/neocortical cases are shown. Fifteen Controls, 7 Mixed, 15 AD and 14 VaD cases had no Lewy body pathology. Two controls, 1 DLB, 2 Mixed and 1 AD showed Lewy body pathology in brain stem. Data were not available for 1 case in DLB and PDD groups due to limited autopsy; Neuronal loss in the substantia nigra, data was not available for 1 case in each group other than VaD due to limited autopsy. CAA total (%) and CAA moderate-severe (%), **P<0.01 (Chi-square test); Arteriolosclerosis moderate-severe (%), **P<0.01 (Chi-square test); Microinfarct total (%) and CAA moderate-severe (%), **P<0.01, *P<0.05 (Chi-square test); Microbleeds total (%), **P<0.01 (Chi-square test).

Abbreviations: AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy; CERAD, Consortium to Establish a Registry for Alzheimer's Disease; DLB, Dementia with Lewy Bodies; F, female; ICA, internal carotid artery; M, male; Mixed, MCA, middle cerebral artery; Mixed dementia; PDD, Parkinson's disease with dementia; SN, substantia nigra; VaD, Vascular dementia; WML, white matter lesion.

Figure Legends

Figure 1: Spectrum of SVD pathology (subcortical cerebrovascular lesions) in autonomic dysfunction plus dementia subjects. A (a-f), Representative images (H&E staining) of different vascular pathologies in frontal WM **(a-d)** and basal ganglia **(e and f)** observed in autonomic dysfunction plus DLB and PDD subjects. **A (a)**, vascular wall thickening with perivascular spacing; **A (b)**, perivascular spacing; **A (c)**, small infarct (arrows); **A (d)**, perivascular microinfarct; **A (e)**, microvascular hyalinosis with perivascular spacing; **A (f)**, rarefaction (arrows). **B (a-d)**, Representative images (H&E stain) of microvascular changes and variable calcification observed in DLB and PDD subjects. **B (a-d)**, microvascular hyalinosis; **B (b, c and d)**, calcification in the vessel wall. **C**, Bar chart showing the total vascular pathology scores and distribution of individual vascular pathology scores in different regions of the brain. All dementias with autonomic dysfunction groups showed consistently higher total ($^{¶}P<0.008$) and individual vascular pathology scores compared to controls, particularly in both the frontal lobe and the basal ganglia ($^{**}P<0.016$ and $^{††}P<0.000$ respectively). In the temporal lobe, DLB, Mixed, AD and VaD subjects showed higher vascular pathology scores compared to controls ($^{‡}P<0.019$). Vascular pathology scores in the hippocampus remained same across all dementias and control subjects. High scores were evident in VaD subjects who also exhibited lacunar infarcts. Subjects with dementia also exhibited microinfarcts particularly in the frontal WM and basal ganglia with scores of 5/6 and 3/4 respectively (10). Total and individual vascular pathology scores were not different between controls and DLB without Falls. Scale bar represents 100 µm (A, a-f) and 20 µm (B, a-d).

Figure 2: Relationship between WM hyperintensity volumes and vascular pathology in autonomic dysfunction. A (a-f), Axial views of T2-weighted brain MRI images at the level of basal ganglia and corona radiata to illustrate degrees of vWMHs in different dementias **(b-f)** in comparison to a control subject without autonomic dysfunction **(a)**. The mean percentage of vWMH per total brain volume and vascular pathology scores in the controls (n=5) was 10.4% and 8.8 respectively **(a)**. **B**, Correlational analysis between percentage of vWMH per total brain volume and vascular pathology scores of demented subjects with autonomic dysfunction. Spearman's rho correlation analysis revealed that vascular pathology

score exhibited a positive correlation with % vWMH per total brain volume (ρ=0.40, P=0.016).

Figure 3: Assessment of vascular sclerotic index in brain vessels. **A**, Representative profile of a mildly hyalinised microvessel indicating the dimensions used to determine the sclerotic index (SI). The dotted lines mark inner diameters of the vessel. The normal lines indicate external diameters. Scale bar represents 50 µm. **B**, Histogram showing SI values in the WM and the cortex in controls and autonomic dysfunction plus dementia groups. All dementias with autonomic dysfunction groups showed consistently higher mean SI values compared to controls in both the WM and the cortex (**P<0.005 and ††P<0.005 respectively). SI values were not different between controls and DLB without Falls in both the WM and the cortex. In the WM, DLB without Falls showed lower SI values compared with all dementia with autonomic dysfunction groups (‡P<0.035). In autonomic dysfunction plus dementia subjects, higher SI was evident in the cortex compared with WM, particularly in AD subjects (¶P=0.019).