Stereologic Analysis of Microvascular Morphology in the Elderly: Alzheimer Disease Pathology and Cognitive Status

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Abstract

The presence of microvascular changes has been documented both in brain aging and Alzheimer disease (AD), although the relationship between the morphometry of brain capillaries and cognitive impairment is still unknown. We performed an analysis of capillary morphometric parameters and AD-related pathology in 19 elderly individuals with variable degrees of cognitive decline. Cognitive status was assessed prospectively using the Clinical Dementia Rating (CDR) scale. Total capillary length and numbers as well as mean length-weighted diameter, total neurofibrillary tangle (NFT) and neuron numbers, and amyloid volume were estimated in entorhinal cortex and the CA1 field. Total capillary numbers and mean diameters explained almost 40% of the neuron number variability in both the CA1 and entorhinal cortex. Total capillary length and numbers in the CA1 and entorhinal cortex did not predict cognitive status. Mean capillary diameters in the CA1 and entorhinal cortex were significantly related to CDR scores, explaining 18.5% and 31.1% of the cognitive variability, respectively. This relationship persisted after controlling for NFT and neuron numbers in multivariate regression models. Consistent with the growing interest about microvascular pathology in brain aging, the present data indicate that changes in capillary morphometric parameters may represent independent predictors of AD-related neuronal depletion and cognitive decline.

Key Words: Alzheimer, Cognition, Aging, Dementia, Microvasculature.

INTRODUCTION

Despite much research, the morphologic substrates of cognitive decline in brain aging and Alzheimer disease (AD) remain a matter of debate. The consistent development of neurofibrillary tangles (NFTs) and amyloid deposits in cognitively intact elderly individuals initially led to the hypothesis that their density and regional distribution are more important determinants of cognitive status than their mere presence within the cerebral cortex (1). Unlike amyloid deposits, which are known to be poor correlates of cognitive status in the elderly (2–11), several studies pointed to the importance of NFT and neuronal loss in terms of clinicopathologic correlations (6, 12). After the neuropathologic work of Braak and Braak, who first identified the role of AD neuronal pathology within the entorhinal cortex and CA1 field in age-related neurodegeneration (13, 14), several clinicopathologic studies showed that NFT formation and progressive neuronal depletion in these areas significantly contributes to cognitive decline in the course of aging (4, 8, 9, 15–17). However, these neuropathologic hallmarks are not sufficient to explain the cognitive variability in the elderly. In fact, our recent work showed that stereologic estimates of all AD-related neuropathologic variables (i.e., total NFT and neuron numbers) in the CA1 field and entorhinal cortex may explain less than 50% of the variability in Clinical Dementia Rating (CDR) scores in both brain aging and AD (18, 19).

Besides AD-related pathology, structural parameters of the cerebral vasculature may also determine cognitive performances in the elderly. For instance, a substantial decrease of smooth muscle actin immunoreactivity was described in the arachnoid, grey and white matter in AD cases when compared to age-matched control subjects (20). Several age-related alterations of the microvascular ultrastructure (e.g., perivascular collagen deposits, atrophy of endothelium, basement membrane thickening, and pericyte degeneration) as well as qualitative changes in microvascular structure (such as glomerular loops and twisted capillaries) have been also described both in the aging brain and in AD (for review, see [21, 22]). In contrast, quantitative analyses of structural parameters in brain capillaries led to controversial data. Increased age-related capillary density attributed to tissue shrinkage in the human neocortex and hippocampus has been reported earlier (23), but more recent animal and human studies challenged these findings (24–28).
Similarly, an age-related increase of capillary diameters and decrease of capillary length has been found in the aging human hippocampus (29) but not in neocortical areas (27, 30). Recently, the development of modern design-based stereologic techniques allowed for an accurate assessment of age-related changes in the capillary network (31, 32). To date, there is no study addressing the relationship between the morphometric characteristics of human capillaries and cognitive impairment in the elderly. We report here an analysis of capillary morphometric parameters and AD-related pathology, including stereologic estimates of total capillary length and number as well as mean length-weighted diameters, total NFT and neuron numbers, and amyloid volume in the entorhinal cortex and CA1 field, in 19 elderly individuals with variable degrees of cognitive decline.

**MATERIALS AND METHODS**

The series included 19 very old individuals (mean age, 90.8 ± 4.1 years; age range, 82–101 years; Table 1) who died and were autopsied at the Department of Geriatrics and Psychiatry of the University of Geneva School of Medicine. Only cases with a CDR scale score established within the last month before death were included in the present series. In addition, all our cases underwent neuropsychologic assessment within the last 6 months before their death. To guarantee the quality of clinicopathologic correlations, CDR scores were used for further statistical analysis. Clinical data were obtained from the medical records of the patients and from the neuropathologic examination records of the Department of Geriatrics and Psychiatry, University of Geneva School of Medicine, Geneva, Switzerland. Cases with a history of stroke or other central nervous system disorders (i.e., tumors, inflammation, Parkinson disease, Lewy body disease, frontotemporal dementia, and argyrophilic grain disease) and premortem hypoxia related to agonal states were excluded from the present study. Cases with substantial microvascular pathology (i.e., cortical microinfarcts, deep white matter, and periventricular demyelination) as well as silent lacunar infarcts in the routine neuropathologic examination were also excluded. To assess the cognitive impact of capillary morphometric parameters in the absence of major changes in capillary integrity, cases with cerebral amyloid angiopathy were also excluded from the present series. In all of the cognitively impaired cases, AD severity was further confirmed neuropathologically by the presence of numerous NFT and neuritic alterations in the hippocampal formation and neocortex using Braak and Braak staging (14), and by the presence of amyloid plaques (33). All cases were also neuropathologically assessed using the National Institute on Aging (NIA)-Reagan Institute neuropathologic criteria (34). All procedures involving the use of postmortem human brain were conducted after written consent of the patients and their families was obtained, and were approved by the relevant ethics committees at the University of Geneva School of Medicine.

All brains were hemisected at autopsy (postmortem delay 6–60 hours and fixed in 15% formaldehyde for 4 weeks. After hemisection, the left hemisphere was used for diagnostic purposes. The entire right hippocampal formation,

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**TABLE 1. Stereologic Data for Mean Diameters, Total Capillary Lengths, Numbers and Capillary Length/Neuron Number in the Present Series**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Braak NFT</th>
<th>Braak Alß Staging</th>
<th>CDR</th>
<th>D (m) (cap)</th>
<th>L (m) (cap)</th>
<th>W (m) (cap)</th>
<th>L (cap)/N (neur)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91</td>
<td>F</td>
<td>II</td>
<td>2</td>
<td>0</td>
<td>8.72</td>
<td>7.74</td>
<td>30.06</td>
<td>10.35</td>
</tr>
<tr>
<td>2</td>
<td>96</td>
<td>M</td>
<td>III</td>
<td>2</td>
<td>0</td>
<td>6.45</td>
<td>6.43</td>
<td>30.06</td>
<td>10.35</td>
</tr>
<tr>
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<td>96</td>
<td>F</td>
<td>III</td>
<td>4</td>
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<td>8.87</td>
<td>7.76</td>
<td>30.06</td>
<td>10.35</td>
</tr>
<tr>
<td>4</td>
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<td>I</td>
<td>3</td>
<td>0</td>
<td>7.55</td>
<td>7.64</td>
<td>30.06</td>
<td>10.35</td>
</tr>
<tr>
<td>5</td>
<td>82</td>
<td>M</td>
<td>I</td>
<td>1</td>
<td>0.5</td>
<td>6.12</td>
<td>6.58</td>
<td>30.06</td>
<td>10.35</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
<td>M</td>
<td>III</td>
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<td>0.5</td>
<td>6.80</td>
<td>6.95</td>
<td>30.06</td>
<td>10.35</td>
</tr>
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<td>7</td>
<td>91</td>
<td>M</td>
<td>I</td>
<td>3</td>
<td>0.5</td>
<td>6.56</td>
<td>5.99</td>
<td>30.06</td>
<td>10.35</td>
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<tr>
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<td>F</td>
<td>II</td>
<td>3</td>
<td>0.5</td>
<td>7.82</td>
<td>8.00</td>
<td>30.06</td>
<td>10.35</td>
</tr>
<tr>
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<td>1</td>
<td>6.20</td>
<td>6.20</td>
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<td>10</td>
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<td>5.99</td>
<td>30.06</td>
<td>10.35</td>
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<td>11</td>
<td>92</td>
<td>M</td>
<td>V</td>
<td>4</td>
<td>1</td>
<td>7.58</td>
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<td>83</td>
<td>F</td>
<td>V</td>
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<td>2</td>
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<td>5.45</td>
<td>30.06</td>
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<td>VI</td>
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<td>30.06</td>
<td>10.35</td>
</tr>
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<td>97</td>
<td>M</td>
<td>V</td>
<td>3</td>
<td>2</td>
<td>5.48</td>
<td>5.02</td>
<td>30.06</td>
<td>10.35</td>
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<td>17</td>
<td>97</td>
<td>F</td>
<td>V</td>
<td>3</td>
<td>2</td>
<td>5.79</td>
<td>5.33</td>
<td>30.06</td>
<td>10.35</td>
</tr>
<tr>
<td>18</td>
<td>97</td>
<td>F</td>
<td>III</td>
<td>2</td>
<td>2</td>
<td>6.78</td>
<td>5.85</td>
<td>30.06</td>
<td>10.35</td>
</tr>
<tr>
<td>19</td>
<td>97</td>
<td>F</td>
<td>V</td>
<td>3</td>
<td>2</td>
<td>6.02</td>
<td>5.37</td>
<td>30.06</td>
<td>10.35</td>
</tr>
</tbody>
</table>

NFT, Neurofibrillary tangle; CDR, Clinical Dementia Rating scale; D, diameter; L, length; W, total capillary numbers; N, total neuron numbers; EC, entorhinal cortex.
including the entorhinal cortex, was then dissected out and cut at regular intervals using a specially designed multiblade knife (35). This knife is composed of 15 disposable microtome blades secured with brass machine screws and separated by washers of variable size depending on which slab thickness is appropriate. This results usually in a total up to 12 blocks in the human hippocampus, comprising its entire rostrocaudal extent and therefore representing an exhaustive sample (35). These slabs were numbered in order, taking note of the rostral surface of each block. Blocks were embedded in paraffin and cut on a microtome at 50 μm. Sections were kept in strict anatomic order and any gap in the series was noted. Special care was taken to lose a minimal amount of tissue at the interface between blocks. One of every 40 sections was processed alternatively for histochemistry and immunohistochemistry, and depending on the case, five to eight sections were analyzed to sample exhaustively the region considered using a systematic random sampling scheme (35–37). In particular, capillary morphology was assessed using modified Gallyas silver impregnation. Briefly, sections were placed in 2-propanol at room temperature during 4 hours and then transferred to acetic acid 1% for 5 minutes. After treatment with periodic acid 5% for 30 minutes, they were rinsed in acetic acid 1% for 5 minutes followed by incubation in a solution of NaOH 4% for 30 minutes. Finally, they were rinsed in acetic acid 1% for 5 minutes and the classic Gallyas silver staining was used (38). This method allowed for optimal visualization of capillary structure in all cases studied (Fig. 1). To assess the relationship between total amyloid volume and mean capillary diameters in multimodal association areas, the entire superior frontal gyrus was dissected out in an independent series of 11 nonagenarians and centenarians (Table 2). Tissue processing was identical to that described in the previous paragraph.

For both routine neuropathologic evaluation and stereologic estimates of AD neuronal pathology, materials were stained using AT8 (Innogenetics, Gent, Belgium), a monoclonal antibody that recognizes tau proteins phosphorylated at residues Ser199/Ser202 to visualize NFT, neuropil threads, and neuritic plaques. After pretreatment with a mixture of 0.25% potassium permanganate and 1% PAL solution, antibody AT8 was used at a working dilution of 1:3000 overnight at 4°C. Specific labeling was revealed with a horseradish peroxidase–conjugated antimouse antibody (1:100; Dako, Glostrup, Denmark) and 3,3'-diaminobenzidine as a chromogen. The sections were then counterstained with cresyl violet for stereologic determination of unaffected neurons (cresyl violet only-stained neurons), neurons with intracellular NFTs, and extracellular NFTs. In all cases, the antibody AT8 penetrated through the full depth of the sections processed for stereologic analysis. Total amyloid volume assessment was made in 50-μm-thick freefloating sections that were treated with a methanol/H₂O₂ solution (3/1 v/v) for 30 minutes and then washed in phosphate-buffered saline for 30 minutes. Subsequently, sections were pretreated with 88% formic acid for 5 minutes to enhance amyloid detection. Incubation with the primary antibody was performed overnight at 4 °C using monoclonal antibody 4G8 (1:3000; Signet Laboratories, Dedham, MA [34]) diluted in PBS and 0.5% Triton X-100 and 3% BSA. Sections were then rinsed and incubated with biotinylated goat antirabbit or goat antimouse IgG (H+L) secondary
TABLE 2. Stereologic Data for Mean Capillary Diameters and Total Amyloid Volume in Area 9 in 11 Nonagenarians and Centenarians

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Gender</th>
<th>CDR</th>
<th>Braak NFT</th>
<th>D (μm)</th>
<th>Vol Amyloid (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>91</td>
<td>F</td>
<td>0</td>
<td>II</td>
<td>11.80</td>
<td>84.2</td>
</tr>
<tr>
<td>2</td>
<td>96</td>
<td>F</td>
<td>0</td>
<td>I</td>
<td>13.18</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>97</td>
<td>F</td>
<td>0.5</td>
<td>I</td>
<td>12.26</td>
<td>21.4</td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>F</td>
<td>0.5</td>
<td>I</td>
<td>11.57</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>91</td>
<td>F</td>
<td>2</td>
<td>III</td>
<td>12.58</td>
<td>6.9</td>
</tr>
<tr>
<td>6</td>
<td>92</td>
<td>M</td>
<td>2</td>
<td>V</td>
<td>11.50</td>
<td>748.8</td>
</tr>
<tr>
<td>7</td>
<td>97</td>
<td>F</td>
<td>2</td>
<td>V</td>
<td>11.08</td>
<td>252.4</td>
</tr>
<tr>
<td>8</td>
<td>101</td>
<td>F</td>
<td>2</td>
<td>V</td>
<td>10.65</td>
<td>453.0</td>
</tr>
<tr>
<td>9</td>
<td>101</td>
<td>F</td>
<td>2</td>
<td>V</td>
<td>10.50</td>
<td>540.9</td>
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<tr>
<td>10</td>
<td>93</td>
<td>F</td>
<td>3</td>
<td>VI</td>
<td>9.57</td>
<td>185.5</td>
</tr>
<tr>
<td>11</td>
<td>94</td>
<td>M</td>
<td>3</td>
<td></td>
<td>7.26</td>
<td>132.2</td>
</tr>
</tbody>
</table>

D, Diameter; CDR, Clinical Dementia Rating score; Braak NFT, Braak NFT staging score; Vol amyloid, total amyloid volume.

antibody (1:300; Vector Laboratories, Burlingame, CA) for 1 hour. Sections were processed for Nissl staining and coverslipped with DePex (Fluka, Milwaukee, WI).

We used the criteria of Amal and Insauti for identifying the anatomic boundaries of the CA1 field and entorhinal cortex (37, 39). To perform stereologic analysis of total amyloid volume in area 9, it was first necessary to establish the anatomic boundaries of the considered region with as much precision as possible to ensure accuracy and reliability of the stereologic sampling and validity of the estimates. To address this methodologic problem, we relied on chemoarchitectonic patterns to recognize the location of Brodmann area 9 using monoclonal antibody SMI-32 raised against nonphosphorylated neurofilament proteins (Sterneberger Monoclonals, Lutherville, MD), which labels a subpopulation of pyramidal neurons in the human neocortex. This antibody has been shown to provide remarkable chemoarchitectural details about laminar and region-specific distribution of pyramidal neurons, permitting reliable establishment of anatomic boundaries among neocortical domains. Antibody SMI-32 was used at a working dilution of 1:1000 on a series of sections adjacent to those stained with antibody 4G8 and processed as described previously. Before immunoreaction, all sections for SMI-32 labeling were pretreated in a water bath in 10 mM sodium citrate buffer (pH 6.5) for 10 minutes at 95°C for optimal antigenicity retrieval. SMI-32 immunoreactivity was further enhanced using 0.005% OsO₄. These parallel series of sections were then used as a guide to distinguish the borders of area 9 from adjacent neocortical regions (i.e. Brodmann areas 8, 46, 10, and 32) and permitted an assessment of the variability of the extent of this region and its divergence from classic descriptions (3, 40, 41).

All quantitative analyses were performed using a computer-assisted morphometry system consisting of a Zeiss Axiosplan 2 photomicroscope equipped with an Applied Scientific Instrumentation MS-2000 XYZ computer-controlled motorized stage, a DAGE-MTI DC-330 video camera, a Gateway microcomputer, the StereoInvestigator morphometry and stereology software (MicroBrightField, Wiliston, VT), and Imaris 4.2.0 software (Bitplane, Switzerland). For stereologic analyses, the cycloid optical fractionator method was used to estimate total numbers of neurons and NFTs (37). For total capillary length and numbers, we applied in detail the protocol described by Lokkegaard et al. (32). The distinction between capillaries and other microvessels was based on their diameter and regional distribution. Vessels with diameters ≤12 μm were considered as part of the capillary network. The diameters of the capillary profiles with odd shapes were not included in the estimates of the mean diameters. On average, 200 to 500 profiles were counted in each case according to the rules for systematic sampling (42). In the entorhinal cortex, capillary parameters were assessed separately for layers II and V. In the absence of significant differences between these measures, capillary data from layers II and V were pooled for statistical analysis (Fig. 1). Sections were first viewed at low magnification (10×) for outlining the entorhinal cortex or CA1 onto a live computer image (35–37). Pyramidal layers could be recognized unequivocally on the Nissl-counterstained sections. The resulting contours were selected. The software placed dissector frames using a systematic random design within each contour outlining the cortical layers to account for a predetermined fraction of the outlined area. This fraction (approximately 2% in the present study) was established in pilot studies including the full range of CDR scores (0, 0.5, 1, 2, 3) and set to accommodate both sufficient sampling of neurons (200–600 profiles in each case). The neurons and NFTs that fell within these dissector frames were then counted at high magnification using a 1.4 n.a. Zeiss Plan-Apochromat 100× objective, and their total numbers were then estimated as previously described (37, 43, 44). A dissector height of 10 μm was consistently explored at each location. For capillaries, the area of the dissector frame varied from 15,625 to 67,600 μm² depending on the region analyzed (i.e. CA1 field, entorhinal cortex, area 9). The capillaries that fell within the dissector frames and that were cross-cut by a cycloid were then counted at higher magnification using a 0.6 n.a. Zeiss Plan-Apochromat 20× objective and Koehler illumination to achieve optimal optical resolution. For neurons and AD pathology, the area of the dissector frame was kept at 900 μm² throughout the study.
FIGURE 2. Relationship between clinical severity and raw stereologic data ($\times 10^3$) for neurofibrillary tangle (NFT) (A, B) and neuron (E, F) numbers as well as amyloid volume (in mm$^3$) (C, D) in the CA1 field (A, C, E) and entorhinal cortex (B, D, F). Despite the substantial interindividual variability, there was a significant positive relationship between total NFT numbers in the CA1 field and Clinical Dementia Rating (CDR) scores (A) and a negative relationship between total neuron numbers and CDR scores in the same area (E). The Alzheimer disease-type neuronal pathology in the entorhinal cortex was not related to the clinical severity (B, F). Note also the absence of relationship between total amyloid volume and CDR scores in both areas (C, D).
FIGURE 3. Relationship between clinical severity and total capillary numbers (A, B), lengths (C, D), and mean diameters (E, F) in the CA1 field (A, C, E) and entorhinal cortex (B, D, F). Only decreased mean capillary diameters (E, F) were related to the Clinical Dementia Rating scores.
Individual coefficients of error were all within 10% (45, 46). For total amyloid volume assessment, the volume of each region was estimated using a point-counting grid and the Cavalieri principle as previously described (36). The total amyloid volume for each region of interest was calculated as

\[ V_{\text{AT}} = \left( \frac{V_{\text{t}}}{V_{\text{r}}} \right) \times V_{\text{ref}} \]

with \( V_{\text{t}} \) being the local amyloid volume determined for each section, \( V_{\text{r}} \) the local volume of the region determined for each section, and \( V_{\text{ref}} \) the volume of reference for an entire region (36). The validity of all estimates was assessed as both investigators (CB, PRH) counted the same regions in several cases with a high interrater reliability (κ = 0.90). The interindividual variability of capillary parameters was assessed using the coefficient of variation (calculated as CV = SD/mean; values close to 1 indicate substantial variability among cases).

After normalization of the neuropathologic variables, the relationship between capillary parameters (i.e. total length and number, mean diameter, mean length/neuron as the dependent variables) and age, total NFT numbers, total neuron numbers, and amyloid volume (the independent variables) in each area was studied using linear regression in both univariate and multivariate models. A one-way analysis of variance was used to explore possible gender-related differences in capillary parameters. For clinico-pathologic correlations, the association between CDR scores (as the dependent variable) and neuropathologic parameters (as the independent variables) was studied using maximum likelihood ordered logistic regression, which makes possible to measure the relationship between an ordinal outcome variable (CDR) and several independent variables. This method can also evaluate the amount of variability of the outcome variable (i.e. the CDR score) that can be explained by the independent variables (i.e. capillary parameters, neuron and NFT numbers, and amyloid volume) and thus provide an estimate of the strength of the relationship. Given the limited number of cases, only two neuropathologic variables were entered in each multivariate model. Statistical analyses were performed using the Stata software package, release 9 (College Station, TX).

RESULTS

Total NFT and amyloid volume as well as total neuron numbers as a function of CDR scores are illustrated in Figure 2. Total NFT numbers were inversely associated with neuron numbers in CA1 (\( R^2 = 0.45, p < 0.01 \)) but not entorhinal cortex. Total amyloid volume was not related to neuron numbers in the areas studied (data not shown). There was no significant relationship between AD-related markers and CDR scores in the entorhinal cortex. In contrast, both NFT (\( R^2 = 0.26, p < 0.01 \)) and neuron numbers (\( R^2 = 0.14, p < 0.05 \)) in the CA1 field predicted CDR scores (Fig. 2).

Mean capillary diameters as well as total capillary number and length and capillary length/neuron in CA1 and entorhinal cortex in all cases are summarized in Table 1. Among the capillary morphologic parameters, the highest interindividual variability was observed for total numbers (CV = 0.32) followed by capillary lengths (CV = 0.28). These values were substantially lower for capillary length/neuron (0.20) and mean diameters (0.11). The CV values were not related to CDR scores. Across CDR groups, the coefficient of error for mean diameters was low (0.02–0.08). In contrast, these values were higher for total capillary numbers (0.08–0.28) and lengths (0.05–0.26 for the entorhinal cortex). The entorhinal cortex had consistently higher capillary length per neuron compared with CA1 field of the hippocampus independently of the CDR score.

There were no gender-related differences in total capillary length, number, and mean diameters. Age was not related to any capillary morphologic parameters in the CA1 field. A significant negative relationship was observed between age and total length of capillaries in the entorhinal cortex (\( R^2 = 0.28, p < 0.05 \)). Both mean diameters and total capillary numbers were strongly related to total neuron numbers in the CA1 field (\( R^2 = 0.30 \) and 0.41, respectively, \( p < 0.05–0.01 \)) and entorhinal cortex (\( R^2 = 0.24 \) and 0.42, respectively, \( p < 0.05–0.01 \)). In a multivariate model, including total NFT and capillary numbers (or diameters) in CA1 field, both variables significantly explained neuron number variability (\( p < 0.01 \)). In contrast, there was no significant association between total capillary length and neuron number in the areas studied. No relationship was found between AD-related lesions (i.e. total NFT numbers and amyloid volume) and capillary morphologic parameters in the CA1 field and entorhinal cortex. This was also the case when Braak NFT and amyloid staging scores were considered. The study of an independent series of 11 nonagenarians and centenarians revealed no relationship between amyloid volume and mean capillary diameters in area 9.

Univariate regression analysis showed that total capillary length and numbers as well as mean capillary length/neuron in the CA1 field and entorhinal cortex did not predict cognitive status. This was also confirmed in multivariate models controlling for total neuron numbers, NFT numbers, or total amyloid volume. In contrast, mean capillary diameters in the CA1 field and entorhinal cortex were significantly related to CDR scores (Fig. 3). In a univariate model, they explained, respectively, 18.5% and 31.1% of the cognitive variability. Importantly, these associations persisted when total neuron numbers (\( R^2 = 0.22 \) in CA1 field and 0.36 in entorhinal cortex), NFT numbers (\( R^2 = 0.36 \) in CA1 field and entorhinal cortex), or amyloid volume (\( R^2 = 0.31 \) in CA1 field and 0.40 in entorhinal cortex) were considered. Despite the limited number of cases studied (Table 2), the inverse relationship between CDR scores and mean capillary diameters was also confirmed in area 9 (\( R^2 = 0.36 \)).

DISCUSSION

To our knowledge, this is the first study addressing the role of capillary morphometric parameters in cognitive deterioration. In terms of total capillary numbers and lengths in CA1, our results are consistent with the only stereologic study in this field (32), stressing the reliability of these estimates of human capillary parameters. Only total capillary length in the entorhinal cortex was negatively related to age.
after 85 years. In addition, our results challenge the vascular
theories of CA1 vulnerability in the oldest-old by showing
that although this area displays significantly lower capillary
length per neuron values compared with the entorhinal
cortex, this ratio as well as the other capillary parameters
remained quite stable in this particular age group (32).
However, given the narrow age range, no definite conclu-
sions about the age effect on capillary parameters can be
drawn from the present series.

The present data provide novel information about the
relationship between capillary morphologic parameters and
AD-related pathologic markers in the elderly. In a recent
stereologic analysis of microvascular parameters in double
amyloid precursor protein/presenilin-1 transgenic mice that
displayed severe cerebral amyloid angiopathy and amyloid
deposits, Lee et al reported decreased total capillary numbers
in white matter but no changes in total capillary length
compared with nontransgenic littermates (31). Based on these
observations, they postulated that amyloid deposition within
the capillary walls alter the formation of new capillaries
without affecting their length. The present study does not
support a direct relationship between amyloid parenchymal
deposits and capillary parameters in brain aging. Although
one could argue that this absence of relationship may be
partly the result of the selection of hippocampal areas known
to be relatively spared by amyloid deposits in cases with
mild cognitive impairment and mild AD, this is an unlikely
scenario because our additional data in 11 independent cases
showed no relationship between total amyloid volume and
mean capillary diameters in area 9. One limitation should,
however, be considered when interpreting these data. To
avoid the confounding effect of major qualitative changes in
capillary structure, cases with cerebral amyloid angiopathy
were not included in the present study. We cannot therefore
exclude that this particular form of amyloid pathology
influences quantitative capillary parameters in the human
cerebral cortex.

In contrast to AD-related lesions, the present results
reveal that total capillary numbers may explain more than
40% of the neuron number variability in CA1 field and
entorhinal cortex, supporting a strong relationship between
microvascular changes and AD-related neuronal depletion.
This correlation points to the necessity of multivariate
analyses including these variables when addressing the
cognitive impact of capillary pathology in brain aging. From
a biologic viewpoint, the temporal link between changes of
the cerebral microvasculature and neuronal loss is still
controversial. The decrease of total capillary numbers could
simply reflect the adaptive response of the capillary network
to neuronal depletion-related decrease of energetic demands.
However, one should keep in mind that microvascular
changes might also influence directly neuronal loss in these
areas. In fact, previous studies postulated that decreases in
capillary number and diameter could disrupt the balance
between energy requirements and cerebral blood supply,
rendering the brain more vulnerable to oxidative stress
damage and ultimately neuronal death (47–49). In particular,
an early animal study showed that chronic brain hypoperfu-
sion in rats induces ultrastructural capillary changes in the
CA1 field that were accompanied by a substantial compro-
mise of spatial memory (50). Supporting a primary role of
cerebral hypoperfusion in triggering AD-related pathology, a
single-photon emission computed tomography study
revealed the presence of regional cerebral perfusion abnor-
malities that preceded clinical symptoms in presenilin-1
mutation carriers (51). Future studies including detailed
assessment of capillary morphologic parameters, neuron
numbers, and oxidative stress markers at different time points
in aged rodents are warranted to elucidate the mechanisms
surrounding a possible deleterious effect of microvascular
changes on neuronal homeostasis in brain aging.

In terms of clinicopathologic correlations, our data
indicate that mean capillary diameters in entorhinal cortex
and, to a lesser degree, the CA1 are independent predictors
of cognitive status in very old individuals, further supporting
a direct effect of microvascular pathology on cognitive
decline. Consistent with previous stereologic work (8, 9,
17–19, 52), NFT and neuron numbers in the CA1 field were
reliable predictors of cognitive status. It is, however, note-
worthy that mean capillary diameters (but not AD-related
neuronal pathology) in entorhinal cortex predict more than
30% of the CDR variability. Moreover, the association
between capillary diameters and CDR scores persisted after
controlling for total NFT and neuron numbers even in the
CA1 field. Importantly, no other capillary parameters were
related to the cognitive outcome in the hippocampal
formation pointing to the specificity of these results (31).
In addition, the study of 11 independent oldest-old cases
showed a significant inverse association between mean
capillary diameters in area 9 and CDR scores, suggesting
that our observations may also be valid for multimodal
association areas. However, a detailed analysis of a larger
cohort of nonagenarians and centenarians is warranted to
confirm this observation.

The biologic significance of these findings is, however,
unclear. A critical assumption that has to be made in this
context is that the capillaries observed in postmortem
material correspond to the structure in its in vivo state. Such
a correlation has been reported (53, 54), and it seems likely
that instead of the recruitment of additional capillaries,
increased cognitive load induces differential distribution of
flow (55), heterogeneity in blood flow velocity (56), and
changes in diameters (57). Changes in the flow distribution
are usually regulated in the capillary network itself through
the local diffusion of nitric oxide (58), serotonin, and other
neurotransmitters (59, 60). Decreased capillary diameters
may lead to impaired microcirculation within the hippo-
campal formation and thus prevent adaptive responses to
local changes in metabolic demands.

Strengths of the present study include the prospective
assessment of dementia severity, quantitative analysis of total
capillary lengths, and numbers as well as mean diameters
using unbiased rigorous stereologic methods and use of
multivariate models to assess the predictive value of these
variables taking into account their possible interaction with
AD-related pathologic changes. However, several limitations
should be considered when interpreting our data. First, as is
frequently the case in very old cohorts, the limited number of
cases contributes to the relatively high interindividual variability observed for total capillary numbers and lengths. Second, the present analysis was confined to the hippocampus and entorhinal cortex and did not address in detail the cognitive impact of capillary structural changes in neocortical association areas. Third, as a global measure of dementia severity, CDR score may be influenced by noncognitive parameters related to functional impairments. Additional stereologic studies in larger series, including both younger and oldest-old cases combined with ultrastructural characterization of capillary abnormalities in the entire spectrum of brain aging, are needed to explore further the role of microvasculature in the development of cognitive decline.

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