

Cerebral Hemorrhage With Biopsy-Proved Amyloid Angiopathy

William H. Yong, MD; Marie E. Robert, MD; Diana Lenard Secor, MS;
Theodore J. Kleikamp, MD; Harry V. Vinters, MD

• **Clinical, radiological, and immunohistochemical findings in brain biopsy specimens from six patients with cerebral amyloid angiopathy-associated intracerebral hemorrhage were reviewed. Acute clinical presentations included headache, nausea and vomiting, loss of consciousness, and focal neurological deficits such as hemiplegia and blindness. Transient ischemic attacks experienced by one patient and referable to one hemisphere did not indicate impending hemorrhage in that region. Computed tomographic scans revealed acute, irregular, superficial, lobar hemorrhage with occasional ring enhancement. Immunohistochemical studies were performed on biopsy specimens using primary antibodies against portions of the Alzheimer A4 (β -) peptide or γ -trace peptide (the vascular amyloid protein in**

patients with hereditary cerebral hemorrhage with amyloidosis-Icelandic type). In all patients, anti-A4 and anti- γ -trace labeled cerebral microvessels. Immunoreactive senile plaques were few compared with the numbers of stained microvessels. Reactive astrocytes in some patients were labeled by both antiserum samples, suggesting uptake or production of these proteins by the astrocytes. This study demonstrates the heterogeneous clinical and radiological features of cerebral amyloid angiopathy-related brain hemorrhage and the value of anti-A4 and anti- γ -trace immunohistochemical study of biopsy material from patients with suspected cerebral amyloid angiopathy-related intraparenchymal bleeding.

(*Arch Neurol.* 1992;49:51-58)

munohistochemical staining with primary monoclonal or polyclonal antibodies to the Alzheimer A4 peptide and to a peptide related to γ -trace or cystatin C (a protease inhibitor that composes the microvascular amyloid in hereditary Icelandic cerebral hemorrhage [HCHWA-I]) has been shown to localize amyloid in microvessel walls in brain tissue from appropriate patients.^{17,18} In this study, we examine the efficacy of immunohistochemical methods in detecting CAA in brain biopsy specimens from six patients with peripheral lobar cerebral hemorrhages. Clinical and radiological features of the patients are emphasized.

SUBJECTS AND METHODS

The charts of six patients who ranged in age from 54 to 86 years were reviewed with respect to clinical presentation, age, sex, history of hypertension and smoking or other risk factors for vascular disease, dementia, neurological and psychiatric symptoms, imaging studies, surgical procedures, treatment, and outcome. Two of the patients (cases 1 and 4) were seen and treated at St Vincent Hospital and Medical Center, Portland, Ore, and four (cases 2, 3, 5, and 6) were followed up at the UCLA Medical Center. Initial diagnosis of CAA in biopsy material (available from all patients) was by Congo red staining of paraffin sections and examination with polarization microscopy. Immunohistochemical (immunoperoxidase) staining of tissues was carried out utilizing primary polyclonal antibodies raised against synthetic peptides representing a 28-amino-acid fragment from the Alzheimer A4 (β -) peptide^{19,20} or an 18-amino-acid fragment from a γ -trace-like protein initially isolated from cerebral microvessels of patients with HCHWA-I.^{17,21,22} The immunocytochemical method involved formic acid pretreatment of brain tissue sections to improve immunola-

Cerebral amyloid angiopathy (CAA) is characterized by the accumulation of amyloid in the media and adventitia of medium and small arteries, arterioles, and occasionally veins.^{1,2} It is responsible for up to 10% of primary, nontraumatic brain hemorrhages or 1% of all strokes.³ Cerebral amyloid angiopathy commonly occurs in otherwise healthy and nondemented persons without cerebral hemorrhage or infarcts.² The amyloid in most patients with CAA

is virtually identical biochemically to that found in patients with Alzheimer's disease (AD) senile or neuritic plaques, both lesions containing the Alzheimer A4 or β -peptide.^{4,6} Cerebral amyloid angiopathy occurs in over 90% of brains from patients with AD or senile dementia of the Alzheimer type (SDAT).⁶ Thus, understanding the pathophysiological nature of CAA may provide insights into AD and SDAT.⁷ Cerebral amyloid angiopathy appears to increase in extent and severity with age.² There are associations between CAA and syndromes of familial cerebral hemorrhage,^{8,9} Down's syndrome,¹⁰ isolated cerebral vasculitis,^{11,12} leukoencephalopathy,^{13,14} and cerebrovascular malformations.^{15,16} Traditionally, CAA has been diagnosed by staining tissue sections with Congo red and viewing them under polarized light. Recently, im-

Accepted for publication August 1, 1991.

From the Department of Pathology (Drs Yong, Robert, and Vinters and Ms Secor) and Brain Research Institute (Dr Vinters), UCLA School of Medicine; and Department of Neurology, St Vincent Hospital and Medical Center, Portland, Ore (Dr Kleikamp).

Reprint requests to Department of Pathology (Neuropathology), CHS 18-170, UCLA School of Medicine, Los Angeles, CA 90024-1732 (Dr Vinters).

being²¹ and a standard avidin-biotin-peroxidase technique for visualization of immunoreactivity. Control sections from all patients were treated with serum samples preabsorbed with the immunogen to which the antibody was raised as a measure of antibody labeling specificity. Sections of AD brain with widespread parenchymal and microvascular A4 amyloid were immunolabeled with anti-A4, while paraffin-embedded brain tissue from patients with HCHWA-I was stained with anti- γ -trace antibody to serve as positive controls and ensure consistent levels of staining in each run.

REPORT OF CASES

Table 1 summarizes the clinical features of all patients, and Table 2 summarizes the histological features of the cerebral biopsy specimens; relevant details of both are presented below.

CASE 1.—A 54-year-old white man with a questionable history of mild hypertension and a two- to three-pack per day smoking history presented with loss of consciousness in 1984. In the previous year, he had complaints of numbness in the right hand and occasional episodes of dropping objects from that hand. At that time, a computed tomographic (CT) scan of the head was negative, but electroencephalography (EEG) showed excessive synchronous 2- to 3-Hz slow-wave activity. He was started on a regimen of phenytoin sodium, 400 mg/d. He remained asymptomatic for 4 months and then had an episode of aphasia followed by a tonic seizure. Magnetic resonance imaging (MRI) immediately before this episode demonstrated multiple regions in the periventricular and centrum semiovale regions bilaterally, consistent with plaques of multiple sclerosis. Neuropsychological testing was consistent with organic dementia and depression. In 1987, his symptoms worsened, and MRI revealed additional small foci of probable demyelination involving the brain stem. The patient then suffered acute confusion and blindness. Computed tomography revealed a large hemorrhage in the right occipital and posterior parietal regions with mass effect. Cerebral arteriography failed to demonstrate an arteriovenous malformation (AVM) but showed mild atheromatous change of the cerebral vessels. He showed mild clinical improvement and in mid-1987 underwent a right temporal lobe biopsy that revealed CAA.

Postoperatively, the patient was obtunded, and a CT scan demonstrated edema but no new hemorrhage. Dexamethasone sodium phosphate was administered, with clinical improvement. In late 1987, a CT scan revealed an additional left-sided occipital lucency. Magnetic resonance imaging in April 1988 revealed multiple serpiginous channels with high signal intensity in the temporal region, suggestive of an AVM. In retrospect, and since an angiogram 1 year earlier had not shown AVM, this area may have represented acute hemorrhage in a gyral pattern. His condition improved slightly until October 1988, when his vision and ability to carry out activities of daily living deteriorated. Computed tomographic and MRI scans in November 1988 demonstrated a right-sided parieto-

Patient No./Age, y/Sex	HTN/Smoker	Dementia at Onset	Clinical Presentation	Subsequent Symptoms	Location of Hemorrhages (Onset/Subsequent)	Surgical Procedures
1/54/M	?/+	No	Loss of consciousness (h/o TIAs, R hemiparesis 1 y?)	Seizures, dementia, depression, blindness, obtundation	L, R periventricular/R occipitoparietal (X2), R temporal, L occipital, R hemisphere	R temporal biopsy
2/58/F	-/+	Yes	Confusion, difficulty walking, urinary incontinence, aphasia, HA, N/V (h/o depression, 38 y)	Memory loss, irritability, depression, paranoia	L, R frontal/none	L frontal biopsy
3/60/F	-/+	No	L hemiparesis, HA, N/V, progressive lethargy (h/o TIAs with R hemiparesis 7 y, L hemiparesis 6 mo)	Seizures, aphasia, L hemiparesis, L paresthesias	R frontal/none	R frontal evacuation of hematoma
4/71/M	+/+	Yes	HA, confusion, ataxia, L homonymous hemianopsia, L hemiplegia (h/o carotid and peripheral vascular disease)	Confusion, L hemiparesis, L paresthesia, memory loss, irritability	R frontal, L cerebellar/L occipital, R cerebellar	R frontal biopsy
5/82/F	-/-	No	HA, slurred speech, R hemiparesis, obtundation	No change	L occipital/none	L occipital evacuation of hematoma
6/86/M	?/?	No	Malaise, N/V, ataxia, obtundation, L hemiparesis	L hemiparesis, L homonymous hemianopsia	R parieto-occipitotemporal/none	R parieto-occipitotemporal evacuation of hematoma

*CAA indicates cerebral amyloid angiopathy; HTN, hypertension; TIA, transient ischemic attack; h/o, history of; HA, headache; and N/V, nausea and vomiting. Ages are age at onset of first hemorrhage. The periventricular lucencies in patient 1 probably represented infarcted and demyelinated areas.

Case No.	Staining of Cerebral Microvessels			Staining of Senile Plaques		Staining of Astrocytes	
	Congo Red	Anti-A4	Anti- γ -Trace	Anti-A4	Anti- γ -Trace	Anti-A4	Anti- γ -Trace
1	+	+	+	+	-	++	+
2	+	+	+	+	-	++	++
3	+	+	+	CNA	CNA	CNA	CNA
4	+	+	+	+	-	+	++
5	+	+	+	CNA	CNA	CNA	CNA
6	+	+	+	+	-	+	-

*CAA indicates cerebral amyloid angiopathy; CNA, cannot assess. With the minimal amount of brain tissue sometimes available, immunolabeled sections did not always contain reactive astrocytes or senile plaques, and it was therefore not possible to comment on their immunolabeling properties.

occipital hemorrhage (Fig 1, top and center). Magnetic resonance imaging in April 1989 showed maturation of the parieto-occipital hemorrhage. Following 1 month of increasing dementia in May 1989, the patient became acutely unresponsive, and a CT scan demonstrated an extensive, multilobar right

hemispheric hemorrhage with substantial subarachnoid spread (Fig 1, bottom). An EEG obtained during obtundation revealed marked abnormalities in the right hemisphere consisting of focal slowing over the right frontotemporal regions superimposed on generalized bilateral slowing. He suffered

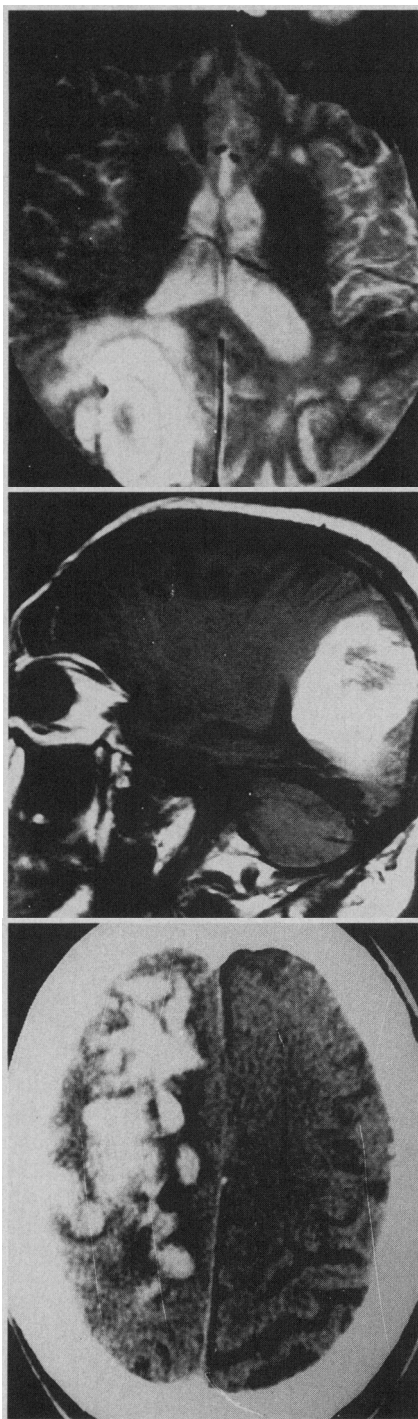


Fig 1.—Patient 1. Top, This T₂-weighted magnetic resonance imaging (MRI) image (repetition/echo times, 2800/80 ms) demonstrates a serpiginous lobar lesion of high signal intensity, representing hemorrhage in the right occipital region (November 9, 1988). Center, A sagittal T₁-weighted MRI image (600/20 ms) of the right occipital lesion (same date) demonstrates an area of high signal intensity (indicating a chronic or subacute hemorrhage) surrounding a central region of lesser intensity. Bottom, Computed tomographic scan without contrast of a multilobar right cerebral hemorrhage (May 17, 1989) demonstrating delineation of superior gyri probably secondary to subarachnoid involvement.

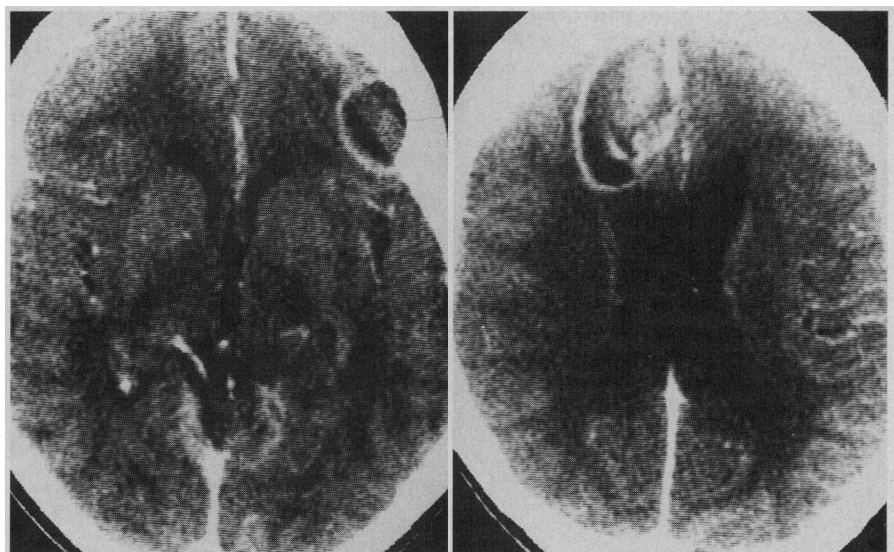


Fig 2.—Patient 2. Left, Enhanced computed tomographic scan of a low-density, ring-enhancing, superficial left frontal lobe lesion. Right, Computed tomographic scan with contrast of a simultaneous hemorrhage in the right frontal lobe. An area of ring enhancement is visible with a central region of mixed density.

recurrent grand mal seizures refractory to phenytoin and died 2 months later.

Autopsy (restricted to the brain) revealed mild atherosclerosis except for a large basilar artery plaque. The frontal lobes bilaterally demonstrated recent hemorrhage. A recent 3-cm encapsulated hematoma was located at the left frontotemporal junction. There was bilateral cortical loss in the temporal regions, with a resorbed hemorrhagic lesion on the left. The right parieto-occipital region revealed a resorbed hemorrhagic cortical lesion that extended to the ventricles. A resorbed 2-cm hemorrhagic lesion was noted to the left of the splenium of the corpus callosum. The ventricular system was dilated, especially the temporal horns of the lateral ventricles. A “degenerative vasculopathy” with extensive amyloid deposition in cerebral cortical leptomeningeal microvessels was noted. Bilateral infarcts were noted, as were multiple old and acute microinfarcts of the cerebellar cortex. Senile plaques were not as prominent as amyloidotic microvessels in the neocortex.

CASE 2.—A 58-year-old normotensive black woman with a 44-pack per year history of smoking presented in 1984 with a 2- to 3-week history of progressive confusion, difficulty walking, and incontinence. Beginning at the age of 20 years, she had experienced episodes of depression requiring prolonged hospitalization. Treatments had included electroconvulsive therapy and antipsychotic medications. She subsequently received the diagnosis of bipolar disorder with psychotic features and was also thought to have a mixed personality disorder. In the 2 years before the most recent admission, she had undergone increasing numbers of hospitalizations for major depressive episodes. Two weeks before admission, gait and speech difficulties developed. Two days before admission, the patient had nausea, vomiting, and urinary incontinence. On the day before hos-

pitalization, a headache developed. A CT scan of the brain with contrast revealed ring-enhancing lesions, one in each frontal lobe, that were thought to be consistent with malignant neoplasia (Fig 2). Results of a left-sided frontal craniectomy with ultrasound-guided needle biopsy revealed CAA, gliosis, and hemorrhage, but no evidence of malignant neoplasia. A follow-up CT scan revealed resolving lesions consistent with prior hematomas. She continued to have episodes of depression and experienced increased memory loss and irritability. Three years postoperatively, an EEG demonstrated intermittent, generalized theta wave and polymorphic delta wave activity consistent with encephalopathy (toxic, metabolic, or degenerative) in the left anterior region. The EEG changes were not thought to be attributable to resolving hematomas.

CASE 3.—A 60-year-old normotensive white woman with a 25-pack per year smoking history presented with a 12-hour history of left-sided hemiparesis in 1988. She had a 9-year history of transient ischemic attacks (TIAs). During the first 7 years, she had had three TIAs characterized by right-sided facial and extremity paresthesias and weakness. The first two TIAs had also included an expressive aphasia. Computed tomographic scanning of the brain was repeatedly normal, as was a carotid artery duplex scan. Six months before admission, she suffered a TIA characterized by left-sided facial and extremity paresthesias and weakness, and possibly a seizure. Both CT and MRI scans were normal. On the day before admission, a headache and episodes of vomiting developed. In the 12 hours before admission, left-sided weakness developed as before, but she also demonstrated a rapidly progressing lethargy that culminated in a somnolent though pain-arousable state. A CT scan of the brain revealed a right frontal lobe hematoma (Fig 3), which was evacuated. The biopsy material

revealed CAA. Follow-up CT scans revealed no further hemorrhage. She suffered seizures postoperatively and was treated with phenytoin and acetazolamide sodium. Six months postoperatively, she had continuing complaints of slurred speech and left-sided numbness and weakness.

CASE 4.—A 69-year-old white male smoker (two packs per day of unknown duration) with a history of controlled hypertension and carotid and peripheral artery disease was admitted for mental status changes and difficulties with activities of daily living in 1989. Four days before admission, headaches developed. On the day before admission, he drove into a parked car. No clear history of head trauma was obtained. Subsequently, slight confusion and difficulty with ambulation and dressing developed. On examination, he had a left-sided homonymous hemianopsia, a left-sided hemiplegia, and ataxia.

A right frontal lobe biopsy specimen was believed initially to be consistent with a sterile abscess or cerebritis. Subsequent Congo red staining demonstrated CAA. Postoperatively, he experienced fluctuating mental status changes with periods of confusion and disorientation and periodic episodes of left upper extremity weakness and numbness. Three months postoperatively, he suffered a severe episode of acute confusion. He was given colchicine (0.5 to 1 mg/d), which appeared helpful in reducing his neurological deficits for several months. By 1 year postoperatively, he had increasing memory loss and irritability marked by rage attacks.

CASE 5.—An 82-year-old white woman was admitted for severe headache, slurred speech, and right-sided hemiparesis. Her medical history included intraductal carcinoma in 1987, a history of myocardial infarction, and congestive heart failure. Her family reported that she had had frequent headaches for several years. She did not smoke and was not hypertensive. She was living independently at home, where she cared for her husband. Three months before admission, she fell at home and reported minor aches and pains, which resolved. In December 1990, she suffered a severe headache, slurred speech, and a right-sided hemiparesis. A head CT scan demonstrated a large left-sided occipital subdural and intraparenchymal hematoma with a 1.0-cm shift of midline structures to the right. A subdural and intraparenchymal hematoma measuring 6.0 × 4.0 × 2.0 cm was evacuated, and CAA was demonstrated in the biopsy material. Postoperatively, neurological status improved only minimally. A repeated head CT scan demonstrated some increased cerebral edema and continued right shift. Recovery of neurological function seemed a remote possibility at the time of manuscript preparation of this article.

CASE 6.—In January 1991, an 86-year-old Japanese man with a vague history of cardiac disease had an episode of malaise, nausea, and vomiting. He became ataxic and confused. On examination, he had left-sided hemiparesis and was obtunded. A CT scan without contrast revealed a hemorrhage in the right parietal and temporal lobes with extension into the ventricles and a 1-cm midline shift to the left. He underwent a right-

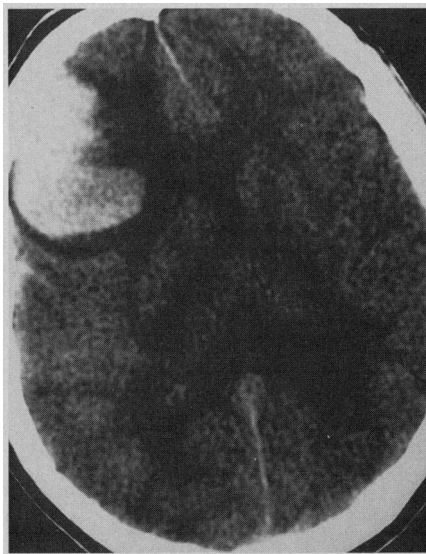


Fig 3.—Patient 3. Computed tomographic scan without contrast of a superficial frontal lobe hemorrhage. There is acute hemorrhage with surrounding edema (low density) and midline shift.

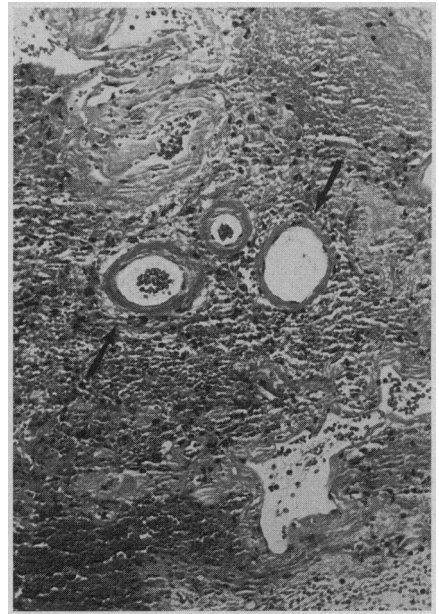


Fig 4.—Patient 3. Cerebral biopsy specimen shows abundant acute hemorrhage and thickened ectatic amyloid-laden microvessels (arrows) (hematoxylin-eosin, ×200).

sided parieto-occipital craniectomy, and the hematoma was evacuated. Microscopic examination showed CAA. Postoperatively, he was alert and oriented but had a left-sided homonymous hemianopsia and left-sided hemiparesis. A CT scan revealed a largely evacuated hematoma and decreased midline shift. On postoperative day 5, pneumonia developed. Treatment with antibiotics was followed by return to a fully oriented state after 1 day. He was subsequently transferred to a rehabilitation facility.

RESULTS

Summary of Biopsy Findings

Brain tissue from all patients demonstrated the characteristic light microscopic features^{2,6} of CAA, with thickened, hyaline, eosinophilic vessel walls (Fig 4). Congo red staining confirmed the finding in all cases (Table 2). Bielschowsky's (silver) staining in selected cases showed a relative lack of senile (neuritic) plaques compared with large numbers of amyloidotic microvessels. Quantitation of this finding was difficult in view of the relatively small size of some of the biopsy specimens.

All specimens revealed spongiosis and inflammatory cells. A frontal lobe biopsy specimen of patient 3 was remarkable for markedly ectatic amyloidotic vessels (Fig 4). A right frontal lobe biopsy specimen from patient 4 revealed focally prominent histiocytes and clusters of mononuclear cells, primarily in a perivascular location. Mitoses (including granular forms) were seen among the reactive cells. Some larger arteries demonstrated fibrous intimal hyperplasia and others showed or-

ganizing thrombi. Excessive numbers of thick-walled blood vessels initially suggested the presence of a vascular malformation.

Both anti-A4 and anti- γ -trace consistently labeled leptomeningeal and cerebral microvessels in all patients (Fig 5). The anti-A4 labeled the few plaques observed, while γ -trace did not. The anti-A4 and anti- γ -trace inconsistently labeled reactive astrocytes (Table 2). In patient 2, gliosis was prominent, and strong immunoreactivity for both peptides was observed in numerous astrocytes. In patient 4, reactive astrocytes were only lightly stained by anti-A4, while anti- γ -trace labeling showed strong immunoreactivity. Conversely, in patient 1, anti- γ -trace revealed minimal staining of astrocytes, but anti-A4 reacted strongly. The astrocytes from patient 6 showed only light staining by anti-A4.

Rarely, strongly immunostained vessels (whether reactive for anti-A4 or γ -trace) were surrounded by acute hemorrhage (Fig 6, left). Less commonly, sites of apparent extravasation of blood from immunoreactive vessels were encountered (Fig 6, right). Large meningeal arteries, when present within the biopsy material, sometimes showed immunoreactive material largely confined to their adventitial component (Fig 7).

COMMENT Clinical Features

The age of the patients with CAA-associated hemorrhage is comparable

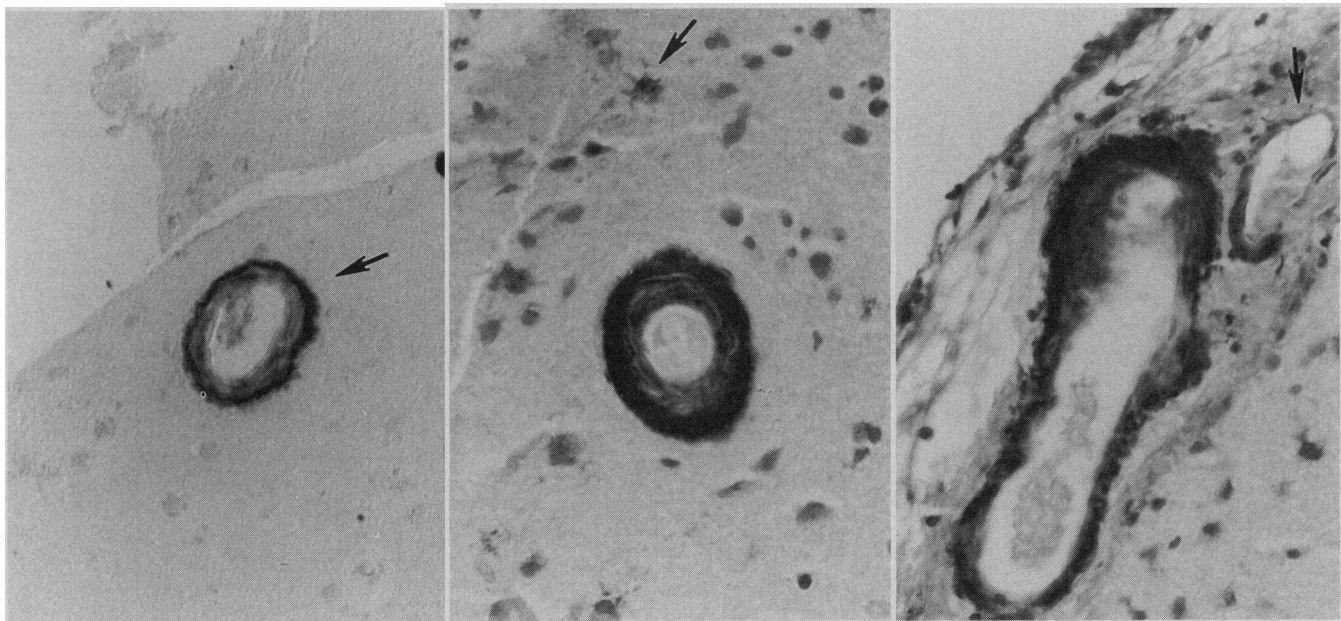


Fig 5.—Patient 1. Left, A section of brain biopsy specimen immunostained for Alzheimer A4 peptide demonstrates prominent staining of media and adventitia (arrow) of a small artery. Note the virtual absence of parenchymal (senile plaque) immunoreactivity. Center, Another section immunostained for γ -trace peptide shows intense labeling of the vessel wall. Note rare astrocytes in the surrounding neuropil that show light cytoplasmic immunostaining (arrow). Right, Leptomeningeal vessels, immunostained for γ -trace. Note the patchy nature of peptide deposition within the vessel wall. The smaller of the two labeled vessels shows segments of its wall that do not demonstrate immunolabeling (arrow) ($\times 550$).

with that of previous reports of CAA, all indicating that the incidence of this CAA-related complication increases in those over 50 years of age.^{2,3,23,24} Four of the patients in this study are near or below the mean ages (male, 71 years; female, 73 years) of those with CAA-associated hemorrhages.³ No sex predominance has been consistently documented,³ and there was none in this small sample. The presentation of CAA may include nonspecific symptoms such as headache, nausea, and vomiting in addition to focal neurological deficits.²⁵ Recurrent hemorrhages often occur.³ One report describes a 58-year-old woman who suffered a total of eight strokes.²⁶ In our study, patient 1 appears by imaging studies to have had seven hemorrhagic or ischemic events, though not all presented clinically as strokes. Of particular interest is patient 3, who experienced three TIAs referable to the left cerebral hemisphere over the course of 7 years and subsequently had a TIA referable to the right hemisphere. Ultimately, she experienced hemorrhage in the hemisphere most recently affected by TIAs. It is unclear why the hemisphere that first produced symptoms was not the one in which clinically significant bleeding first occurred. Possibilities include two distinct types of CAA lesion (one more likely to hemorrhage than the other), or different rates of evolution in CAA-re-

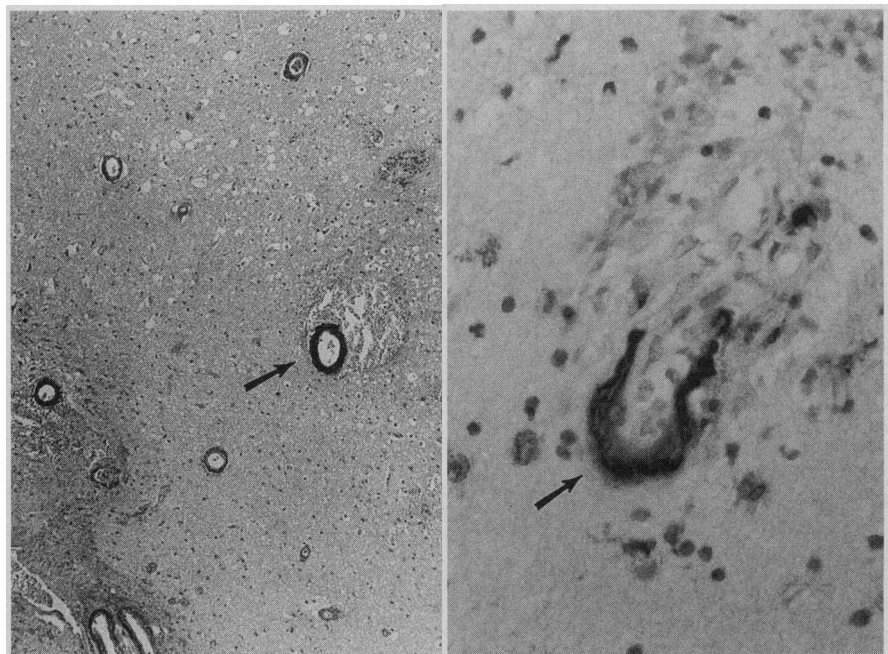


Fig 6.—Left, Anti-Alzheimer A4 peptide-immunostained section from patient 6 shows labeled parenchymal and meningeal vessels but virtually no senile plaques. An occasional parenchymal vessel (arrow) is surrounded by acute hemorrhage ($\times 95$). Right, A microvessel from patient 5 shows γ -trace immunoreactivity (arrow) along one portion of its wall, while there is acute extravasation of blood at its opposite end. Rare labeled astrocytes (arrowhead) are present (Anti-A4 and anti-gamma trace immunostaining performed as described in Vinters et al,¹⁷ $\times 625$).

lated lesions. Transient ischemic attacks referable to CAA have previously been described,²⁷ and this patient's history reinforces this rare origin of TIAs,

though the mechanism of such TIAs is not apparent. Microinfarcts or microhemorrhages have been suggested as the cause.³

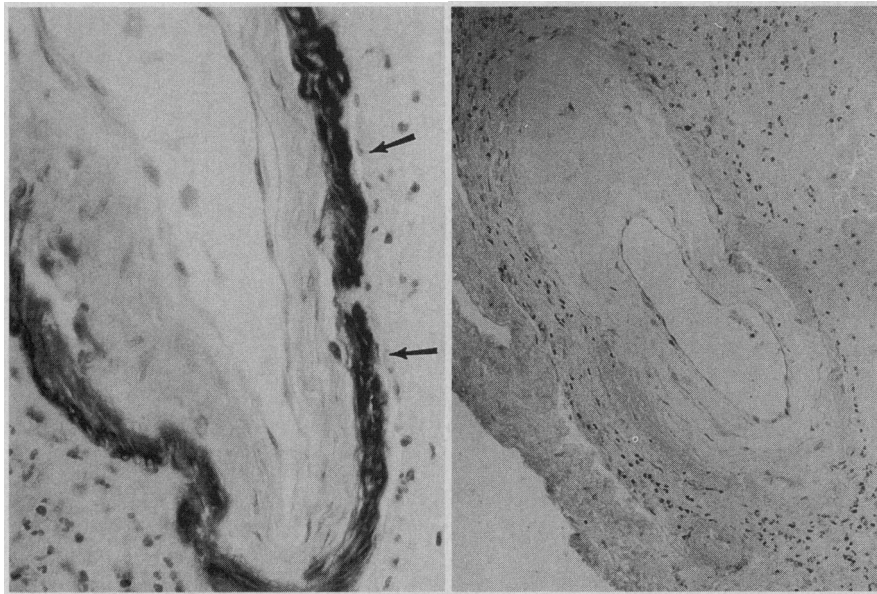


Fig 7.—Patient 5. A large meningeal artery (anti- γ -trace stain) shows prominent adventitial peptide (arrows, left) that is not visible in a parallel section (right) stained with adsorbed antibody (see "Subjects and Methods" section) (left, $\times 625$; right, $\times 245$).

While Torack²⁸ has reported cerebral bleeding after and others²⁹ have noted frequent hemorrhage during neurosurgical procedures, interventions ranging from biopsy to hematoma evacuation did not exacerbate intraoperative or postoperative hemorrhage in our patients. A study of 11 patients with CAA also suggested that neurosurgery may be safely performed.³⁰ Hypertension is not believed to be a major risk factor for CAA-associated hemorrhages, and only one of the six patients in this study (patient 4) had a clear hypertensive history. Four of our patients were normotensive, with diastolic systemic blood pressures consistently not exceeding 90 mm Hg. There is no statistically significant difference in the incidence of infarcts or hemorrhages in hypertensive and nonhypertensive patients who have CAA associated with AD/SDAT,³¹ nor is there a correlation between elevated blood pressure and the presence of CAA.³²

A history of heavy smoking, was present in at least four of six patients. Given the advanced age of patients 5 and 6 and their fulminant presentation, an absence of smoking history may not reflect their life experience. In patient 6, smoking history was not documented at all. Since smoke toxins are known to damage blood vessels,^{33,34} it is interesting to speculate on a possible association between smoking and symptomatic forms of this vasculopathy. In a study of patients with "probable AD" by National Institutes of Health criteria, 37% of fe-

males and 98% of males had used tobacco, but only 10% of those females and 14% of those males were still smoking at the time of AD onset.³⁵ The mean interval between smoking cessation and disease onset was 18.3 years for women and 16.6 years for men. Average daily consumption before cessation of smoking was 18.2 and 18.8 cigarettes per day, respectively, and mean use was 32 years for females and 44 years for males. Since almost 100% of patients with AD have CAA, smoking may play a role in this vasculopathy. Unlike the majority of those clinically diagnosed with AD, four of our patients had not stopped smoking. Caution must be taken in drawing parallels between the AD smoking study and our patients, since none of the patients in our study was given the diagnosis of AD by clinical criteria, and no independent assessment of CAA was made in the AD smoking study. A more extensive and rigorous study on a larger sample will be necessary to demonstrate conclusively an association between smoking and CAA.

Patient 2 demonstrated a long history of depression that appeared to be exacerbated in the period preceding her CAA-associated hemorrhage, though whether this represents a truly organic effect is unclear. It is extremely unlikely, however, that her initial depression was due to CAA, since it began at the age of 20 years, when nonfamilial CAA is not known to occur.³ Of note is the information that her daughter also suffers from depression.

Radiological Features

The radiological features of CAA may mimic other forms of brain abnormality that enter into the clinical differential diagnosis. While it has been suggested that CAA should be considered in the differential diagnosis when multiple subcortical lobar lesions appear in the normotensive elderly person, in practice it may be difficult to distinguish from other lesions without a biopsy.³⁶ When examined by CT scan without contrast, CAA hemorrhages tend to be superficial, lobar, dense when acute and hypodense when subacute or chronic, and somewhat irregular, and at times to show "fingers" or extensions, even into the subarachnoid space.³⁶⁻³⁹ Hemorrhages may be multiple, bilateral, and recurrent. Resolution can result in leukomalacia and localized ventricular dilatation.³⁹ Extension from the cortical and subcortical areas into the subarachnoid space appears common, though it does not usually delineate the gyri, as seen in Fig 1, bottom. However, pure subarachnoid hemorrhage secondary to CAA is rare.^{37,40} Hemorrhage favors the frontoparietal cortex and white matter, though CAA may predominate in the parieto-occipital regions.^{2,40} While most CT scan studies of CAA-related hemorrhages have been done without contrast, the few done with contrast indicate that ring enhancement may occur.^{36,38} Filloux and Townsend³⁸ noted that ring enhancement persisted up to 2 weeks after the first CT scan. Thus, waiting for a hemorrhagic CAA lesion to resolve as a means of distinguishing it from tumor may not be optimal. In particular, multiple, peripherally placed ring-enhancing lesions that would be consistent with metastatic tumor in an elderly patient may require biopsy to rule out the latter possibility. Magnetic resonance imaging may also be superior to CT scan in the detection of subacute or remote hemorrhage.

Patient 1 demonstrated multiple periventricular white-matter lesions (PWMLs) by MRI that were at first thought to represent demyelination secondary to multiple sclerosis. However, autopsy revealed old infarcts and hemorrhage related to CAA. Similar MRI findings of leukoencephalopathy in the periventricular regions have been noted in spontaneous and Dutch variants of CAA.^{41,42} However, patchy white matter lesions may be detected by MRI in 20% to 30% of asymptomatic subjects and are associated with increased age and vascular risk factors such as hypertension, diabetes mellitus, coronary artery disease, and TIAs.⁴³⁻⁴⁵ While PWMLs appear to be common in hereditary cerebral hemorrhage with amyloi-

dosis-Dutch type (HCHWA-D),⁴⁶ they appear less commonly in spontaneous CAA.^{14,46} The occurrence of PWMLs has been attributed to hypoperfusion of the deep white matter due to stenosis of long perforating arterioles.¹⁴ Periventricular white-matter lesions in HCHWA-D were not related to dementia according to Haan et al,⁴⁶ since dementia was approximately equally prevalent in patients with and without PWML.

Microscopic and Immunocytochemical Features in Relation to Pathogenesis of CAA

Senile plaque cores in AD and SDAT, and amyloid in CAA, are composed of a 4200-d molecular weight protein that is only minimally different.⁴⁷ This 4200-d Alzheimer A4 or β -peptide is cleaved from a 695-amino acid precursor encoded by a gene on chromosome 21.⁴⁸⁻⁵⁰

In our study, the immunohistochemical methods used involve polyclonal antibodies against the C-terminal end of the Alzheimer A4 precursor molecule. The site of origin of the A4 precursor molecule is controversial.⁶ Some patients demonstrate exclusive anti-A4 staining of cortical arteriolar or capillary walls, and others have strong perivascular and/or parenchymal immunolabeling.^{17,20}

In this study, arterioles, capillaries, and reactive astrocytes were labeled by both anti-A4 and anti- γ -trace. Colocalization of both types of antibodies has also been observed in microvessels of patients with AD.⁵¹ The finding of prominent colocalization within vessel walls of A4 and γ -trace peptides in this group of six patients with cerebral hemorrhage supports the view that γ -trace peptide may be a significant cofactor in the pathogenesis of bleeding in CAA.^{17,18,51} Our patients were also of interest insofar as they usually showed predominantly or exclusively microvascular deposition of brain amyloid, in this regard resembling patients with HCHWA-D, though examination of more extensive brain tissue may show that significant amounts of plaque amyloid were also present.

The role of astrocytes in cerebral amyloidogenesis is unclear. The reactivity of astroglial cells with anti-A4 and anti- γ -trace antibodies raises the possibility that these cells are phagocytosing peptides from the parenchyma, microvessels, or perivascular regions. Initial production of A4 or cystatin C may be by other cells. Anti-cystatin C serum has labeled neurons in neocortical biopsy specimens of patients with cerebral tumors⁵² and in postmortem hypothalami.⁵³ In vitro, human astrocytes have demonstrated phagocytic potential.⁵⁴ However, in animal models, adult astro-

cytes do not appear to phagocytose colloidal carbon, though there appears to be evidence that neonatal astrocytes have that capability.^{55,56} Alternatively, the astroglial cells themselves may be producing the amyloid peptides. Microglial and astroglial cells are capable of constitutive secretion of lysozyme and cystatin C in vitro.⁵⁷ Interleukin 1 produced by microglial cells stimulates astrogliosis.^{58,59} Possibly, the stimulus for astrocytes to produce glial fibers may also trigger the increased production of both A4 precursor protein and cystatin C. Since not all astrocytes were immunolabeled, there may be either local differences in the quantities of amyloid protein available for phagocytosis or varying levels of stimulus for the production of amyloid.

In nonhereditary CAA, chronic inflammation may be one starting point for amyloid formation. The late age at onset of CAA is consistent with a prolonged exposure to some inflammatory agent. Since abnormalities of cerebral microvessels as seen in AVMs or angitis^{11,12,15,16} are associated with CAA, an inflammatory agent, in a similar manner, may change the properties of the microvessel walls over time to permit amyloidosis. Smoke may be such an agent, since it is known to cause damage to fetal and adult vessels in animal models and to adult vessels in humans.^{33,34,60,61}

The blood-borne toxin may stimulate ameboid microglial cells (which are believed to be the primary source of interleukin 1 in the brain⁶²), macrophages, and possibly other cells to secrete that factor. Interleukin 1 produced by macrophages in the brain or in extracranial sites may in turn stimulate production of A4 precursor protein in their respective locations. As suggested above, astrocytes may be one source of the peptides. Under the influence of interleukin 1 or perhaps additional factors, peripheral mononuclear or localized cell wall proteases may cleave A4 precursor protein to form A4. Localized proteolytic enzymes have been suggested to explain the selective distribution of CAA lesions.⁶³ By postulating a factor similar to amyloid enhancing factor, a glycopeptide that accelerates amyloid deposition in animal models of systemic amyloidosis,⁶⁴ and differing local levels of it, one could explain varying evolutionary rates of CAA and related lesions.

Finally, this study reemphasizes the importance of carefully examining evacuated nontraumatic cerebral hematomas, especially from elderly patients, in an attempt to establish a causative mechanism for the bleeding.⁶⁵ Such examination is enhanced by the use of immunohistochemical techniques (such as

we have described) in tissue from appropriately selected patients.

This research was supported in part by US Public Health Service grant R29 NS26312 and P30 AG10123 (Dr Vinters).

A portion of this work was submitted in fulfillment of senior thesis requirements of the UCLA School of Medicine (Dr Yong).

Illustrations were prepared by Carol Appleton and Stephen Kaufman, MS.

References

1. Robert ME, Yong WH, Secor DL, Vinters HV. Diagnosing cerebral amyloid angiopathy in material from cerebral hemorrhages: the value of immunocytochemistry. *Lab Invest.* 1991;64:102A. Abstract.
2. Vinters HV, Gilbert JJ. Cerebral amyloid angiopathy: incidence and complications in the aging brain, II: the distribution of amyloid vascular changes. *Stroke.* 1983;14:924-928.
3. Vinters HV. Cerebral amyloid angiopathy: a critical review. *Stroke.* 1987;18:311-324.
4. Masters CL, Multhaup G, Simms G, Pottgiesser J, Martins RN, Breyreuther K. Neuronal origin of a cerebral amyloid: neurofibrillary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels. *EMBO J.* 1985;4:2757-2763.
5. Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochem Biophys Res Commun.* 1984;120:885-890.
6. Vinters HV, Miller BL, Pardridge WM. Brain amyloid and Alzheimer disease. *Ann Intern Med.* 1988;109:41-54.
7. Glenner GG. On causative theories in Alzheimer's disease. *Hum Pathol.* 1985;16:433-435.
8. Gudmundsson G, Hallgrímsson J, Jónasson TA, Bjarnason O. Hereditary cerebral haemorrhage with amyloidosis. *Brain.* 1972;95:387-404.
9. Wattendorff AR, Bots GTAM, Went LN, Endtz LJ. Familial cerebral amyloid angiopathy presenting as recurrent cerebral haemorrhage. *J Neurol Sci.* 1982;55:121-135.
10. Glenner GG, Wong CW. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochem Biophys Res Commun.* 1984;122:1131-1135.
11. Reid AH, Maloney AFJ. Giant cell arteritis and arteriitis associated with amyloid angiopathy in an elderly Mongol. *Acta Neuropathol.* 1974;27:131-137.
12. Gray F, Vinters HV, Le Noan H, Salama J, Delaporte P, Poirier J. Cerebral amyloid angiopathy and granulomatous angitis: immunohistochemical study using antibodies to the Alzheimer A4 peptide. *Hum Pathol.* 1990;21:1290-1293.
13. Heffner RR Jr, Porro RS, Olson ME, Earle KM. A demyelinating disorder associated with cerebrovascular amyloid angiopathy. *Arch Neurol.* 1976;33:501-506.
14. Gray F, Dubas F, Roulet E, Escourrolle R. Leukoencephalopathy in diffuse hemorrhagic cerebral amyloid angiopathy. *Ann Neurol.* 1985;18:54-59.
15. Peterson EW, Schulz DM. Amyloid in vessels of a vascular malformation in brain. *Arch Pathol.* 1961;72:480-483.
16. Hart MN, Merz P, Bennett-Gray J, et al. β -Amyloid protein of Alzheimer's disease is found in cerebral and spinal cord vascular malformations. *Am J Pathol.* 1988;132:167-172.
17. Vinters HV, Secor DL, Pardridge WM, Gray F. Immunohistochemical study of cerebral amyloid angiopathy, III: widespread Alzheimer A4 peptide in cerebral microvessel walls colocalizes with gamma trace in patients with leukoencephalopathy. *Ann Neurol.* 1990;28:34-42.
18. Maruyama K, Ikeda SI, Ishihara T, Allsop D, Yanagisawa N. Immunohistochemical characterization of cerebrovascular amyloid in 46 autopsied cases using antibodies to beta protein and cyst-

tatin C. *Stroke*. 1990;21:397-403.

19. Vinters HV, Pardridge WM, Yang J. Immunohistochemical study of cerebral amyloid angiopathy: use of an antiserum to a synthetic 28-amino-acid peptide fragment of the Alzheimer's disease amyloid precursor. *Hum Pathol*. 1988;19:214-222.

20. Vinters HV, Pardridge WM, Secor DL, Ishii N. Immunohistochemical study of cerebral amyloid angiopathy, II: enhancement of immunostaining using formic acid pretreatment of tissue sections. *Am J Pathol*. 1988;133:150-162.

21. Ghiso J, Pons-Estel B, Frangione B. Hereditary cerebral amyloid angiopathy: the amyloid fibrils contain a protein which is a variant of cystatin C, an inhibitor of lysosomal cysteine proteases. *Biochem Biophys Res Commun*. 1986;136:548-554.

22. Ghiso J, Jenson O, Frangione B. Amyloid fibrils in hereditary cerebral hemorrhage with amyloidosis of Icelandic type is a variant of gamma-trace basic protein (cystatin C). *Proc Natl Acad Sci U S A*. 1986;83:2974-2978.

23. Tomonaga M. Cerebral amyloid angiopathy in the elderly. *J Am Geriatr Soc*. 1981;29:151-157.

24. Hauw JJ, Vignolo P, Duyckaerts C, et al. Étude neuropathologique de 12 centenaires: la fréquence de la démence sénile de type Alzheimer n'est pas particulièrement élevée dans ce groupe de personnes très âgées. *Rev Neurol*. 1986;142:107-115.

25. Ishii N, Nishihara Y, Horie A. Amyloid angiopathy and lobar cerebral hemorrhage. *J Neurol Neurosurg Psychiatry*. 1984;47:1203-1210.

26. Finelli PF, Kessimian N, Bernstein PW. Cerebral amyloid angiopathy manifesting as recurrent intracerebral hemorrhage. *Arch Neurol*. 1984;41:330-333.

27. Smith DB, Hitchcock M, Philpott PJ. Cerebral amyloid angiopathy presenting as transient ischemic attacks: case report. *J Neurosurg*. 1985;63:963-964.

28. Torack RM. Congophilic angiopathy complicated by surgery and massive hemorrhage: a light and electron microscopic study. *Am J Pathol*. 1975;81:349-365.

29. Roosen N, Martin JJ, De La Porte C, Van Vyve M. Intracerebral hemorrhage due to cerebral angiopathy: case report. *J Neurosurg*. 1985;63:965-969.

30. Greene GM, Godersky JC, Biller J, Hart MN, Adams HP Jr. Surgical experience with cerebral amyloid angiopathy. *Stroke*. 1990;21:1545-1549.

31. Ferreira JA, Ansbacher LE, Vinters HV. Stroke related to cerebral amyloid angiopathy: the significance of systemic vascular disease. *J Neurol*. 1989;236:267-272.

32. Masuda J, Tanaka K, Ueda K, Omae T. Autopsy study of incidence and distribution of cerebral amyloid angiopathy in Hisayama, Japan. *Stroke*. 1988;19:205-210.

33. Kaufmann RC, Amankwah KS, Weberg AD. The effect of maternal smoke exposure on the ultrastructure of fetal peripheral blood vessels in the mouse. *J Perinat Med*. 1986;14:309-317.

34. Bylock A, Bondjers G, Jansson I, Hansson

HA. Surface ultrastructure of human arteries with special reference to the effects of smoking. *Acta Pathol Microbiol Scand Section A*. 1979;87:201-209.

35. Barclay L, Kheifets S. Tobacco use in Alzheimer's disease. *Prog Clin Biol Res*. 1989;317:189-194.

36. Wagle WA, Smith TW, Weiner M. Intracerebral hemorrhage caused by cerebral amyloid angiopathy: radiographic-pathologic correlation. *AJNR*. 1984;5:171-176.

37. Ohshima T, Endo T, Nukui H, Ikeda S, Allsop D, Onaya T. Cerebral amyloid angiopathy as a cause of subarachnoid hemorrhage. *Stroke*. 1990;21:480-483.

38. Filloux FM, Townsend JJ. Congophilic angiopathy with intracerebral hemorrhage. *West J Med*. 1985;143:498-502.

39. Brown RT, Coates RK, Gilbert JJ. Radiographic-pathologic correlation in cerebral amyloid angiopathy: a review of 12 patients. *J Can Assoc Radiol*. 1985;36:308-311.

40. Gilbert JJ, Vinters HV. Cerebral amyloid angiopathy: incidence and complications in the aging brain, I: cerebral hemorrhage. *Stroke*. 1983;14:915-923.

41. Loes DJ, Biller J, Yuh WTC, et al. Leukoencephalopathy in cerebral amyloid angiopathy: MR imaging in four cases. *AJNR*. 1990;11:485-488.

42. Haan J, Roos RAC, Algra PR, Lanser JBK, Bots GTAM, Vegter-van der Vlis M. Hereditary cerebral haemorrhage with amyloidosis-Dutch type: magnetic resonance imaging findings in 7 cases. *Brain*. 1990;113:1251-1267.

43. Bradley WG Jr, Waluch V, Brant-Zawadzki M, Yadley RA, Wycoff RR. Patchy periventricular white matter lesions in the elderly: a common observation during NMR imaging. *Noninvasive Med Imaging*. 1984;1:35-41.

44. Gerard G, Weisberg LA. MRI periventricular lesions in adults. *Neurology*. 1986;36:998-1001.

45. Awad IA, Spetzler RF, Hodak JA, Awad CA, Carey R. Incidental subcortical lesions identified on magnetic resonance imaging in the elderly, I: correlation with age and cerebrovascular risk factors. *Stroke*. 1986;17:1084-1089.

46. Haan J, Algra PR, Roos RAC. Hereditary cerebral hemorrhage with amyloidosis-Dutch type: clinical and computed tomographic analysis of 24 cases. *Arch Neurol*. 1990;47:649-653.

47. Prelli F, Castano E, Glenner GG, Frangione B. Differences between vascular and plaque core amyloid in Alzheimer's disease. *J Neurochem*. 1988;51:648-651.

48. Kang J, Lemaire HG, Unterbeck A, et al. The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature*. 1987;325:733-736.

49. Tanzi RE, Gusella JF, Watkins PC, et al. Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science*. 1987;225:880-884.

50. Tanzi RE, St George-Hyslop PH, Gusella JF. Molecular genetic approaches to Alzheimer's disease. *Trends Neurosci*. 1989;12:152-158.

51. Vinters HV, Nishimura GS, Secor DL, Par-

dridge WM. Immunoreactive A4 and gamma-trace peptide colocalization in amyloidotic arteriolar lesions in brains of patients with Alzheimer's disease. *Am J Pathol*. 1990;137:233-240.

52. Löfberg H, Grubb AO, Brun A. Human brain cortical neurons contain gamma-trace: rapid isolation, immunohistochemical and physicochemical characterization of human gamma-trace. *Biomed Res*. 1981;2:298-306.

53. Bernstein HG, Järvinen M, Pöllänen R, Schirpke H, Knöfel B, Rinne R. Cystatin C containing neurons in human postmortem hypothalamus. *Neurosci Lett*. 1988;88:131-134.

54. Watabe K, Osborne D, Kim SU. Phagocytic activity of human adult astrocytes and oligodendrocytes in culture. *J Neuropathol Exp Neurol*. 1989;48:499-506.

55. Al-Ali SY, Al-Zuhair AGH, Dawod B. Ultrastructural study of phagocytic activities of young astrocytes in injured neonatal rat brain following intracerebral injection of colloidal carbon. *Glia*. 1988;1:211-218.

56. Ling EA, Penney D, Leblond CP. Use of carbon labeling to demonstrate the role of blood monocytes as precursors of the 'ameboid cells' present in the corpus callosum of postnatal rats. *J Comp Neurol*. 1980;193:631-657.

57. Zucker-Franklin D, Warfel A, Grusky G, Frangione B, Teitel D. Novel monocyte-like properties of microglial/astroglial cells: constitutive secretion of lysozyme and cystatin-C. *Lab Invest*. 1987;57:176-185.

58. Giuliani D, Young DG, Woodward J, Brown DC, Lachman LB. Interleukin-1 is an astroglial growth factor in the developing brain. *J Neurosci*. 1988;8:709-714.

59. Giuliani D. Ameboid microglia as effectors of inflammation in the central nervous system. *J Neurosci Res*. 1987;18:155-171.

60. Boutet M, Bazin M, Turcotte H, Lagacé R. Effects of cigarette smoke on rat thoracic aorta. *Artery*. 1980;7:56-72.

61. Kjeldsen K, Astrup P, Wanstrup J. Ultrastructural intimal changes in the rabbit aorta after a moderate carbon monoxide exposure. *Atherosclerosis*. 1972;16:67-82.

62. Giuliani D, Baker TJ, Shih LCN, Lachman LB. Interleukin 1 of the central nervous system is produced by ameboid microglia. *J Exp Med*. 1986;164:594-604.

63. Glenner GG. Amyloid β protein and the basis for Alzheimer's disease. *Prog Clin Biol Res*. 1989;317:857-868.

64. Brandwein SR, Sipe JD, Skinner M, Cohen AS. Effect of colchicine on experimental amyloidosis in two CBA/J mouse models: chronic inflammatory stimulation and administration of amyloid-enhancing factor during acute inflammation. *Lab Invest*. 1985;52:319-325.

65. Hinton DR, Dolan E, Sima AAF. The value of histopathological examination of surgically removed blood clot in determining the etiology of spontaneous intracerebral hemorrhage. *Stroke*. 1984;15:517-520.