Inflammatory cerebral amyloid angiopathy: the overlap of perivascular (PAN-like) with vasculitic (Aβ-related angiitis) form: an autopsy case

Grażyna M. Szpak, Eliza Lewandowska, Anna Śliwińska, Tomasz Śypek, Sylwia Tarka, Tadeusz Mendel, Janina Rafalowska

1Department of Neuropathology, Institute of Psychiatry and Neurology, 2II Department of Neurology, Institute of Psychiatry and Neurology, 3Department of Forensic Medicine, Warsaw Medical University, 4Department of Experimental and Clinical Neuropathology, Mossakowski Research Medical Institute, Warsaw, Poland

Abstract

Beside advanced age, cerebral amyloid angiopathy (CAA) and hypertension (HTA) are the two most important risk factors for haemorrhagic stroke. Inflammatory changes of amyloid-laden vessels have been reported only in rare sporadic CAA cases. We present the case of a 78-year-old woman with a history of hypertension, dementia and haemorrhagic stroke of the right frontal lobe 2 years before admission. She was admitted with recurrence of symptoms of transient aphasia and central, right-side facial paresis that occurred an hour before her arrival at the hospital. In the admission unit, she was only slightly confused, with no other neurological deficits. An urgent CT scan revealed a recent haemorrhagic stroke in the left frontal lobe. In an hour her condition suddenly deteriorated. After a generalized seizure she presented with right-side hemiparesis with signs of uncal herniation and remained unconscious. A control CT scan showed a large haemorrhagic expansion that comprised the whole left brain hemisphere with 2 cm midline shift. She died about 10 hours after the onset of symptoms. At autopsy chronic inflammation of the thyroid gland, bronchopneumonia, fibrous and fatty heart degeneration and kidney haemorrhagic infarcts were documented. Amyloid deposition and systemic immune disorders in the inner organs were not demonstrated. In neuropathological examination we diagnosed inflammatory form of CAA with coexistence (the overlap) of two, perivascular and vascular, subtypes of CAA-related inflammation.

Key words: inflammatory cerebral amyloid angiopathy, CAA-related inflammation, Aβ-related angiitis (ABRA), overlapping syndrome, immunohistochemistry, electron microscopy.

Communicating author:
Grażyna Maria Szpak, Department of Neuropathology, Institute of Psychiatry and Neurology, Sobieskiego 9, 02-957 Warsaw, Poland, e-mail: szpak@ipin.edu.pl
Introduction

Sporadic CAA is a common disease of small and medium-sized CNS blood vessels of the elderly. Its incidence and severity as well as association with other CNS diseases increase with age [9]. Beside advanced age, CAA and hypertension are the most important risk factors for haemorrhagic stroke, but CAA may also occur as a separate syndrome and cause lobar CAA-related intracerebral haemorrhage (CAA-ICH), or dementia alone [8,21]. The most common and a strong overlap is between CAA and Alzheimer’s disease pathological changes (CAA/AD) [11].

Primary angiitis of CNS (PACNS), is an uncommon syndrome in which vascular inflammatory changes are limited to the brain and spinal cord. In a comparative study of PACNS with and without CAA, performed biopsies revealed that both forms show two histological patterns: lymphocytic and acute necrotizing or granulomatous [18].

Inflammatory changes of amyloid-laden vessels morphologically similar to PACNS have been reported only in rare sporadic cases of CAA [1,3,10,20]. Usually CAA and PACNS as well as hypertensive angiopathy (HTN) are distinct vascular diseases devastating small vessel walls of the CNS.

Currently there is no evidence-based treatment or preventive strategy for CAA-ICH or CAA-I-ICH (CAA-inflammation related intracerebral haemorrhage). However, contrary to CAA alone and CAA/AD, inflammatory CAA is a rare syndrome whose symptoms may respond to immunosuppressive treatment; hence biopsy studies revealed that both forms show two histological patterns: lymphocytic and acute necrotizing or granulomatous [18].

Inflammatory changes of amyloid-laden vessels morphologically similar to PACNS have been reported only in rare sporadic cases of CAA [1,3,10,20]. Usually CAA and PACNS as well as hypertensive angiopathy (HTN) are distinct vascular diseases devastating small vessel walls of the CNS.

Case report

We present the case of a 78-year-old woman with a history of hypertension, dementia and haemorrhagic stroke of the right frontal lobe two years before admission. She was admitted with symptoms of transient aphasia and central, right-side facial paresis. An urgent CT scan revealed a recent haemorrhagic stroke in the left frontal lobe (Fig. 1A). In an hour her condition suddenly deteriorated. After a generalized seizure she presented right-side hemiparesis with signs of uncal herniation and remained unconscious. A control CT scan showed a large haemorrhagic expansion that comprised the whole left brain hemisphere with 2 cm midline shift. She died about 10 hours after the onset of symptoms.

Material and methods

Gross examination of the brain was performed on coronal section after fixation in 4% formaldeyde buffered to pH 7.4. Blocks obtained from the brain autopsy material were embedded in paraffin. For histological examination, samples were stained in H&E, Congo red, PAS, azocarmine, Mallory-trichrome, PTAH, Klüver-Barrera as well as Gallyash and Gomori impregnation. Immunohistochemical (IHC) reactions were performed according to the labelled streptavidin-biotin complex methods in 5 µm selected sections using antibodies against amyloid-β (Aβ total 8-17), tau, ubiquitin, α-actin (SMA), HLA-DR, CD68, and GFAP (all DAKO), and CD45Ro (T lymphocytes) and CD20 (B lymphocytes) (Novocastra). Double IHC/histological reactions were performed in selected sections.

Vonsattel’s grading scale of CAA severity, modified according to Greenberg and Vonsattel, was used [9]. The scale comprises four grades: (1) indicates the presence of some congophilic staining in an otherwise normal-appearing vessel; (2) shows complete replacement of the media by congophilic material; (3) refers to cracking of the amyloid-laden vessel wall.
creating a vessel-within-vessel (double barrel appearance); and (4) denotes the presence in an amyloid-laden vessel of fibrinoid necrosis or lipohyalinosis and/or miliary aneurysms.

For electron microscopic examination (EM) of material after formalin fixation, brain tissue fragments were taken from the paraffin blocks from the region of the highest incidence of vascular amyloid changes. After deparaffinization, the material was fixed in 2.5% glutaraldehyde and postfixed in 2% OsO₄ and processed routinely to Spurr resin. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined under an Option DPS 109 electron microscope.

Results

Pathological findings

At autopsy chronic inflammation of the thyroid gland, bronchopneumonia, fibrous and fatty heart degeneration and kidney haemorrhagic infarcts were documented. Amyloid deposition and systemic immune disorders in the inner organs were not demonstrated. Neither fibrinoid necrosis of glomerular capillaries nor endarteritis of arterioles and small arteries, regarded as characteristic changes in malignant hypertension, were found.

CT and neuropathological findings

Computed tomographic brain scan (CT) and neuropathological examination showed two lobar intracerebral haemorrhages of different age, recent and past. The recent intracerebral haemorrhage was localized in the left frontal and parietal lobes associated with subarachnoid haemorrhage and extensive vasogenic oedema resulting in significant midline shift (Figs. 1A-B). The post-haemorrhagic cortical and subcortical cicatrices in the right frontal lobe were seen along with dispersed small ischaemic cerebral and cerebellar changes. Some amyloid-laden small vessels and their miliary aneurysms and splitting walls arterioles in microbleeds surrounding the haemorrhage and in the subarachnoid space were observed (Fig. 1C).

The coexistence of a few vascular changes of different pattern in the walls of small cerebral vessels was revealed. In cerebral and cerebellar, as well as in leptomeningeal and cortical vessels, Congo red- and β-amyloid (Aβ)-positive depositions were found.

Fig. 1. A) CT scan of the brain. The lobar brain haemorrhage localized in the left frontal lobe. B) Gross autopsy findings. The brain and subarachnoid haemorrhage localized in the left frontal and parietal lobes associated with oedema and midline shift. C) Small vessels in the cortical microbleeds. Amyloid-laden walls of miliary aneurysm. Congo red 200× (Left). Microscopic appearance of cortical arteriole at sites of fibrinoid necrosis and rupture (Right). Mallory 200×.
There were striking perivascular inflammatory cell infiltrates, dense focally or encircling Congo red-positive and Aβ-reactive leptomeningeal vessels and in their aneurysms (Figs. 2A-D). Inflammatory infiltrates surrounding amyloid-laden vessels in the subarachnoid space were mostly composed of immunoreactive CD45Ro T-lymphocytes and CD68-positive macrophages of varied density (Figs. 2B-C). Intramural infiltration mainly composed of T lymphocytes without multinucleated cells was seen in some leptomeningeal vessels and miliary aneurysms. Lymphocytes usually infiltrated adventitia of some vessels. In a few arteries the inflammation penetrated through the media, usually without involving the full thickness of their walls (Fig. 2D). Inflammatory changes differed in time. Chronic inflammatory changes with fibrosis leptomeninges, histiocytic cells and occasionally perivascular giant-like macrophages (arrow) were seen in the subarachnoid space (Figs. 3A-C). Moreover, large CD-68 positive macrophages were found in amyloid-laden vessel walls (Fig. 3B).

The CAA-affected cortical vessels were mostly smaller arteries, arterioles and capillaries. CAA cortical vessels had markedly thickened walls with concentrically narrowed lumina (CAA grade 2). Not all vessels with CAA exhibited inflammation (Fig. 4A) and not all vessels exhibited CAA.

Inflammatory infiltrates were mainly confined to the perivascular space amyloid-laden or necrotic walls of cortical arterioles (Fig. 4B). Panarteritis-like (PAN-like) type inflammatory infiltrates (Fig. 4B) were mainly composed of T-lymphocytes. Transmural infiltrations of capillaries were mainly composed of mononuclear cells, lymphocytes and macrophages.
Inflammatory cerebral amyloid angiopathy: the overlap of perivascular (PAN-like) with vasculitic (A-related angiitis) form: an autopsy case

(Fig. 4C). The microglial activation of the dispersed and perivascular pattern in the form of the reactivity in the pericyte position, concomitant with CAA-cortical arterioles, were found (Fig. 4D).

Both leptomeningeal and cortical vessels with severe CAA manifested numerous angiodestructive changes. There were partial or complete thromboses, recent and organized with recanalisation narrowing the lumen of small parenchymal and leptomeningeal CAA vessels (Figs. 5A-C). Advanced CAA changes, including double-barrel appearance, “vessel in the vessel” (CAA grade 3), were found mainly in leptomeningeal medium-sized and small vessels (Figs. 5B-C).

The most severe segmental angiodestructive changes, comprising lipohyalinosis and/or fibrinoid necrosis (CAA grade-4), were prominent in multiple leptomeningeal and cortical vessels (Figs. 6A-D), as well as in walls of their miliary aneurysms (Figs. 7A-C). The fibrinoid necrosis differed in changes of the medial vessel layer, which were usually more homogeneous, fibrinogen immunoreactive and selectively azocarmine-positive stained, whereas lipohyalinosis contained some macrophages and histiocytic cells between homogeneous and fibrous bunches (Figs. 6B-D).

The necrotic or healed walls of several leptomeningeal miliary aneurysm formations contained homogeneous deposits of fibrinoid necrosis or multiple foamy cells. Large macrophages were visible between fibrous changes of CAA-related lipohyalinosis (Figs. 7A-B). In some of them, intimal and medial cell proliferation with prominent narrowing of the lumen was found (Fig. 7C). All vascular and cerebral changes, lobar haemorrhage, advanced vascular amyloid deposition, angiodestructive lesions and inflammatory vascular infiltrations were asymmetric and mainly on the left side.

The ultrastructural examination showed arterioles with different degrees of thickened basement membrane and degenerated vascular smooth muscle cells (VSMC). In early stages of degenerative changes, VSMC exhibited degeneration of cellular structures and thickened basement membrane (Fig. 8). Advanc-
ed changes of arteriole walls comprised degeneration and loss of VSMC and strong thickening of basement membrane (Figs. 9, 10A). In some of them, thickened vessel walls were loaded with numerous amyloid fibres and the remainder showed strong degeneration of VSMC. Collagen fibres were also visible (Fig. 10A). Arterioles not exhibiting amyloid fibres had a very thickened basement membrane and less destroyed VSMC. The arterioles both with and without amyloid fibres showed deposits of fibrin below the intima as well as collagen fibres (Figs. 9, 10A). In high magnification, fibrin consisted of deposits of dense granular material (Fig. 10B).

Discussion

The presented 78-year-old patient with history of hypertension and dementia died suddenly a few hours after the onset of recurrent lobar brain haemorrhage associated with subarachnoid haemorrhage. Some amyloid-laden small vessels and their miliary aneurysms were observed in microbleeds surrounding the haemorrhage and in the subarachnoid space. On neuropathological examination the coexistence of a few vascular changes of different pattern in the walls of small cerebral vessels was revealed. Advanced cerebral amyloid angiopathy of leptomeningeal and cortical vessels was associated with differing in time perivascular and vascular inflammatory changes and leptomeningeal lymphocytosis. Inflammatory changes destroyed vascular walls and most of them were connected with perivascular subtype inflammation. However, mural inflammatory infiltrations of vascular subtype in some thickened vessel walls were also found.
Since the time when Jellinger [11] revealed that CAA is an integral part of Alzheimer’s disease, several reports on CNS diseases associated with CAA have been published. Currently, more than ten CNS diseases have been associated with various forms of CAA, including a common and strong pathological overlap between Alzheimer’s disease and CAA [8] and the recently reported overlap between AD/CAA and brain iron accumulation [2]. There were some diffuse forms of amyloid plaques in our patient; however, their number did not fulfill AD criteria.

The first case of amyloid angiopathy overlapping angitis of the nervous system was reported in 1974 [15]. Unlike CAA alone, CAA-related inflammation (CAA-I) is a rare syndrome whose symptoms may respond to immunosuppressive treatment [12,13].

Two subtypes of CAA-I have been described under several different names [1,3,8,10,20].

First, a non-vasculitic or a perivascular form is characterized by perivascular (PAN-like) pattern inflammation and extensive brain oedema. Eng et al. [3] and recently Chung et al. [1] proposed the commonly accepted term CAA-related inflammation or CAA-associated with inflammation (CAA-I), whereas Harkness et al. [10] divided cerebral angiopathies into inflammatory and non-inflammatory and proposed the term cerebral amyloid inflammatory vasculopathy or inflammatory cerebral amyloid angiopathy.

Second, a vasculitic form is characterized by transmural and intramural inflammation of the vessel wall with the occasional presence of granulomas, hence called transmural granulomatous or non-granulomatous angitis (TGA). Scolding et al. [20] proposed for this second type the term amyloid β-(or Aβ)-related angitis and the acronym “ABRA” or primary angitis of the central nervous system associated with CAA (PACNS associated with CAA).

The reduction of vascular smooth muscle cells (VSMC) and pericytes characteristic for CAA might predispose them to higher destruction and weakness. Recent results of studies on cultures of smooth muscle cells have revealed that steroidal anti-inflam-
matory agents, such as dexamethasone, reduced not only vasogenic oedema but also Aβ-related inflammation of the vessel wall and thus the loss of vessel wall integrity [18].

The beneficial effects of corticosteroid treatment could be dependent on the pathological subtype of CAA-I and were reported mainly in the perivascular form [12,13]. Hence cerebral biopsy studies are a gold standard for definite diagnosis and therapy with special attention to CAA-I pathological subtypes [1,3,8,9,10,20]. In our sporadic case, loss of smooth muscular actin (SMA) immunoreactivity of VSMC and pericytes was comparable with that previously found in several hereditary small vessel diseases: CAA with familial AD (CAA/FAD) and CADASIL [22]. It makes the brain biopsy a potentially invasive study in both CAA and CAA-I.

Recently, Chung et al. [1] proposed diagnostic criteria for CAA-I on the basis of clinical and radiological imaging findings without requiring biopsy.

The diagnosis of probable CAA-I requires the following: typical symptoms (headache and mental or behavioural changes); focal neurological signs and seizures and the onset of symptoms at the age of 40 or more; MRI should show patchy or confluent T2-weighted or FLAIR lesions with or without mass effect and with or without leptomeningeal or parenchymal enhancement; evident pre-existing CAA (multiple lobar microhaemorrhages (micro-bleeds) and/or recent or past lobar intracerebral haemorrhages); and the absence of neoplastic, infectious or other causes. Definite diagnosis of CAA-I requires all the above plus histopathological confirmation with amyloid deposition within vessels.

Inflammatory cerebral amyloid angiopathy: the overlap of perivascular (PAN-like) with vasculitic (A-related angiitis) form: an autopsy case

in the brain, leptomeninges, perivascular, transmural and/or intramural vascular inflammation.

Microscopic findings with the use of immunohistochemical and ultrastructural studies turned our attention to specific features of the examined material:

First, predomination of remarkable severity of amyloid angiopathy of grade 3-4 in leptomeninges and restriction of inflammatory and angiodestructive changes exclusively to small and medium-size leptomeningeal and cortical blood vessels with advanced CAA. This predisposition of ABRA has already been noted in previous reports [3,18,20].

Second, the cortico-subcortical location of lobar haemorrhage and secondary rupture to the subarachnoid haemorrhage characteristic for CAA-related ICH [11,20] is an important feature of CAA-I related ICH. In our case, the predominance of more pronounced angiodestructive changes in leptomeningeal and cortical vessels was compatible with the characteristic meningeal and cortical distribution of CAA-related haemorrhage.

Third, the advanced vascular amyloid deposits can also obliterate the vessel lumen, leading to ischaemia, infarction or leukoaraiosis. Contrary to usually symmetric changes in CAA alone [9,11], asymmetric lesions characteristic for CAA-I [19] were found in our case.

Fourth, the advanced fibrinoid necrosis in the walls of arterioles and their miliary aneurysms of CAA-I blood vessels. A) Medial fibrinoid necrosis surrounded by Aβ-reactive adventitia. Aβ/PAS 400×. B) Lipohyalinosis. PAS 400×. C) Intimal and medial cell proliferation following obliteration of lumen miliary aneurysm. Aβ/PAS 400×.

In summary, the co-existence of different types of cerebral small vessel diseases, present in our patient, poses a few questions:

• Firstly: Is CAA-I overlapping HTN?
Hyperension frequently coexists with CAA and CAA-I with and without haemorrhage in the older population. The co-existence of HTN- and CAA-related vascular changes is sometimes termed “mixed microangiopathy”. This common overlap of high blood
pressure may exacerbate the tendency to CAA-related haemorrhage. However, recently the increased risk of haemorrhage is not supported by available data [8,9]. Like parenchymal brain cells [2], vessel wall cells also have a limited repertoire of response to injury. Angiodestructive changes, especially the fibrinoid necrosis of vessel walls and miliary aneurysms which were documented in multiple cerebral vessels, can be due to hypertension as well as advanced CAA-I; however, clinical and histological criteria for malignant hypertension, including fibrinoid necrosis in vessels outside the brain, were absent. This is in agreement with the results of the reported cases in which fibrinoid necrosis in the brain of patients with benign hypertension was found. Rosenblum [16,17] concluded that in the brain, arterioles may display fibrinoid necrosis in patients with clinical and histological criteria of benign hypertension, because the brain arterioles are more susceptible to high blood pressure of moderate severity. Therefore in the brain, the severe change in cerebral

**Fig. 8.** Arteriole with degenerating vascular smooth muscle cells (VSMC) and thickening of basement membrane (BM); EC – endothelial cell. Orig magn. 7000×.

**Fig. 9.** Arteriolar wall with markedly thickened basement membrane (BM) and dense granular deposits of fibrin (F); L – lumen, EC – endothelial cell. Orig magn. 1200×.
Inflammatory cerebral amyloid angiopathy: the overlap of perivascular (PAN-like) with vasculitic (Aβ-related angiitis) form: an autopsy case

Blood vessels in benign hypertension may be identical to that appearing elsewhere only in malignant hypertension.

- Secondly: Is CAA overlapping PACNS?
  Sporadic CAA and PACNS are generally considered to be two distinct devastating diseases of cerebral vessels. Their overlapping cannot be ruled out; however, some authors have proposed that co-localization of amyloid and inflammation provides strong evidence against the possibility of affecting blood vessels by overlapping [1,3,21]. Vascular Aβ-deposition has emerged as a trigger of vascular inflammation, particularly in older patients with common CAA pathology [9].

- Thirdly: Is CAA-related perivascular inflammation (CAA-I/PAN-like subtype) overlapping CAA-related vascular inflammation (ABRA subtype) of the inflammatory CAA?
  Both pathological forms of CAA-related inflammation (clinical remarkably similar) can co-occur, suggesting at least their partial overlap [12,13]. We diagnosed in our patient this rare overlap of two subtypes of mixed form of inflammatory CAA: non-vasculitic (perivascular) [1,3] and vasculitic (vascular) [8,10,20].

Over the last decades, it has been discussed whether advanced vascular deposition of Aβ-amyloid may be due to inflammation associated with CAA, or whether the inflammation is an immune response to CAA triggered by the vascular Aβ deposition. It is possible that inflammation may result in the increase of vascular amyloid deposition due to the failure of perivascular drainage of Aβ by analogy to the vascular hypothesis of the development of amyloid plaques in AD. However, amyloid deposition may also trigger immune-mediated vascular inflammation [1,6,20,21]. The presence of morphological PAN-like changes, similar to those observed in pararteritis (periarteritis nodosa), may indicate a possible immunological origin of inflammation in our CAA-I patient [3,20].

Inflammatory perivascular and vascular changes were different in time in our case. On neuropathological examination, vascular infiltration by multiple giant-like macrophages and leptomeningeal infiltrations by histiocytes and perivascular and vascular lymphocytic acute inflammation were found. Histiocytes between lymphocytic infiltrations were associated with the fibrous changes of the leptomeninges and subarachnoid space, which may be the cause of failure of perivascular amyloid drainage and progression of disease. Similar increase of macrophage activation, including multinuclear cells in amyloid-laden vessel walls, and clusters of activated microglia surrounded Aβ-laden and inflammatory was...
interpreted as an immune activation of phagocyte cells [25]. A retrospective study carried out at the Mayo Clinic on PACNS material has suggested that vasculitic form inflammation (ABRA) associated with CAA can be triggered by vascular AP deposition [18], whereas chronic cellular infiltrates may be interpreted as an immune foreign body reaction evoked by amyloid [1,20]. It has recently been demonstrated that the presence of angitis relative to CAA may have implications for AD immunotherapy [24]. More recently, development of antibody-related vasogenic brain oedema (VE) induced by anti-amyloid immunotherapy was revealed and similarities between antibody-related VE and the syndrome of spontaneous CAA-related vascular inflammation were reported [7].

The patient rapidly developed brain oedema with secondary fulminant bleeding to the ventricle and mesencephalon. Extensive vasogenic brain oedema due to blood-brain barrier breakdown is frequently associated with perivascular form of CAA-I, hence the positive response of this CAA-I subtype to immunotherapy [14,24].

The coexistence of pathological changes of all three distinct small vessel diseases, including hypertension, cerebral amyloid angiopathy and inflammation, cannot be ruled out in our case. However, several observations suggest that inflammatory cerebral amyloid angiopathy is not simply an association and CAA overlapping PACNS [1].

In summary our patient died following the exacerbation of probably the chronic stage of mixed type CAA-related inflammation with overlap of two forms of CAA-I pathology, presenting fulminant progression of lobar cerebral haemorrhage and vasogenic oedema. The perivascular and vascular patterns of inflammatory infiltrations were consistent with perivascular and vascular lymphocytic and necrotizing PAN-like form of inflammatory CAA.

Striking co-localization of CAA AP deposition and inflammation as well as angiodegenerative changes, especially the fibrinoid necrosis, suggests their causative relationship. Thus the presented inflammatory changes of cerebral vessel walls appeared as \"AP-related\", not only as \"AP-associated\" CAA [1,18,20]. Time will show which of the cited authors is right.

References
Inflammatory cerebral amyloid angiopathy: the overlap of perivascular (PAN-like) with vasculitic (A-related angiitis) form: an autopsy case


