Universitat Autònoma de Barcelona

# Sporadic cerebral amyloid angiopathy, beyond lobar intracerebral hemorrhage: multimodal biomarker studies of atypical presentations

**María Carmona Iragui**

DOCTORAL THESIS

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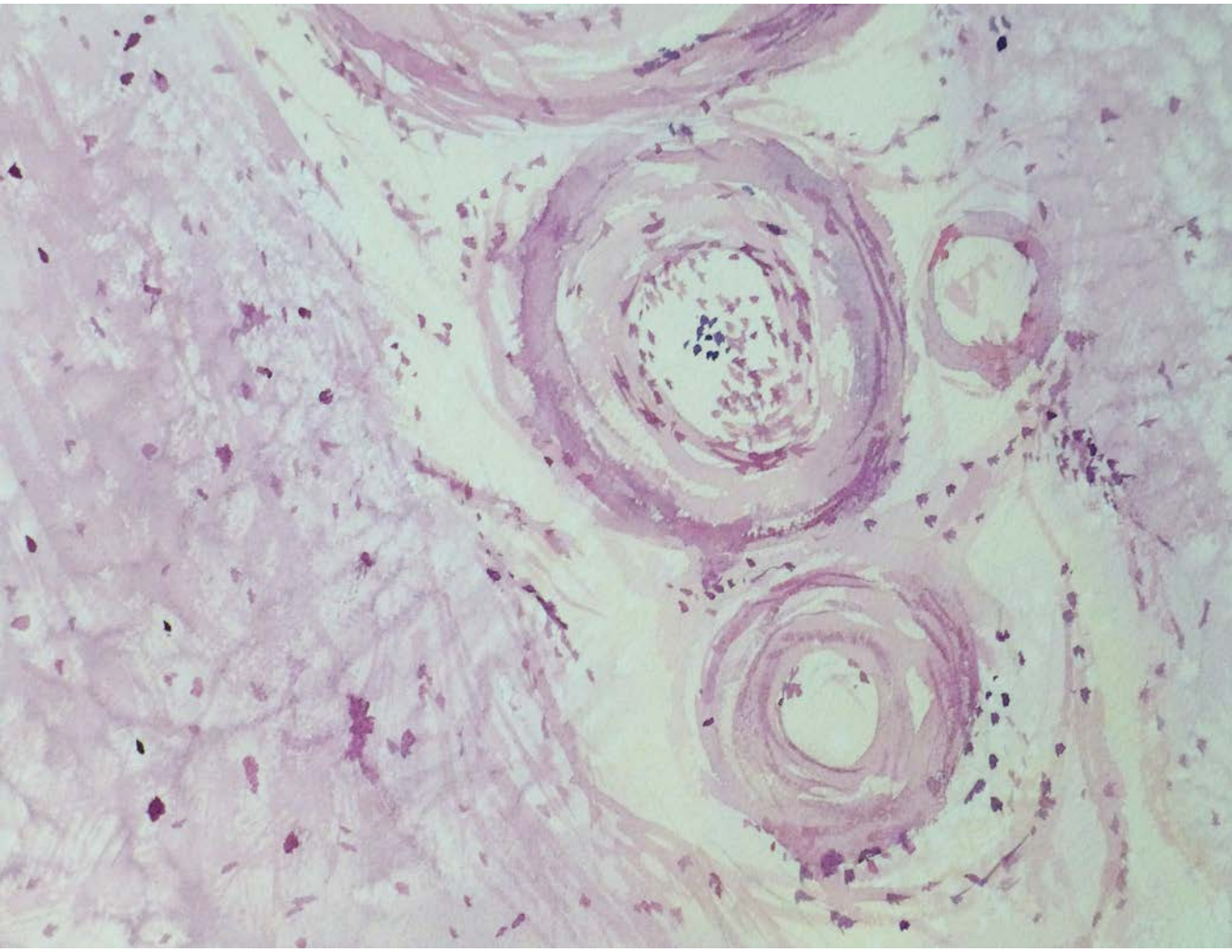

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## Doctoral thesis

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Cover figure: watercolour made by César Viteri based on an image courtesy of Ellen Gelpi representing structural changes due to cerebral amyloid angiopathy in leptomeningeal arterioles, in hematoxylin-eosin staining.

A Santi y a Mikel

# LIST OF CONTENT

## LIST OF ARTICLES INCLUDED IN THIS THESIS

The main body of this thesis consists of a compilation of the following articles:

1. Martínez-Lizana E*, **Carmona-Iragui M***, Alcolea D, Gómez-Choco M, Vilaplana E, Sánchez-Saudinós MB, Clarimón J, Hernández-Guillamon M, Munuera J, Gelpi E, Gómez-Anson B, de Juan-Delago M, Delgado-Mederos R, Montaner J, Ois A, Amaro S, Blesa R, Martí-Fàbregas J, Lleó A, Fortea J. Cerebral amyloid angiopathy-related atraumatic convexal subarachnoid hemorrhage: an ARIA before the tsunami. *J Cereb Blood Flow Metab. 2015;35(5):710-7.* doi: 10.1038/jcbfm.2015.25. *Both authors equally contributed to this work.

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IF 2015: 3.92. IF 5 year: 4.079. JCR Category: Neurociencies. Quartile in category: Q2.

3. **Carmona-Iragui M***, Balasa M*, Benejam B, Alcolea D, Fernández S, Videla L, Sala I, Sánchez-Saudinos MB, Morenas-Rodriguez E, Ribosa R, Illán-Gala I, Gonzalez-Ortiz S, Clarimón J, Schmitt F, Powell D, Bosch B, Lladó A, Rafii M, Head E, Molinuevo JL, Blesa R, Videla S, Lleó A, Sánchez-Valle R, Fortea J. Cerebral amyloid angiopathy in Down syndrome and sporadic and autosomal dominant Alzheimer's disease. *Alzheimers Dement*. 2017 Apr 29 [Epub ahead of print]. *Both authors equally contributed to this work.

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Chapter 1

# Outline and introduction

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# 1. OUTLINE

This thesis widens our knowledge of key aspects of cerebral amyloid angiopathy (CAA). To achieve this, we have studied clinical, neuroimaging, genetic, and cerebrospinal fluid biomarkers in subjects with infrequent presentations of CAA. We aimed to strengthen the links between clinical manifestations and imaging and biochemical biomarkers to allow greater reliance on efficient surrogate markers for CAA.

Chapter 1 sets the framework and general context for this thesis by giving an overview of the current knowledge in the field of CAA and CAA-related biomarkers. In particular, atypical presentations of CAA, different from lobar intracerebral hemorrhage, and features of CAA associated with Alzheimer's disease are detailed in this section.

In chapter 2 the hypotheses and objectives of this doctoral thesis are described.

In chapter 3, we provide a detailed study of a multicenter cohort of subjects with an atraumatic convexal subarachnoid hemorrhage (cSAH). This kind of hemorrhage is a rare cerebrovascular disease in which the bleeding is located in one or several adjacent sulci in the convexities of the brain. Clinical data, magnetic resonance imaging studies, Apolipoprotein E genotyping, and cerebrospinal fluid biomarkers were evaluated and results were compared with data from healthy controls, non-cSAH CAA patients, and Alzheimer's disease patients.

Chapter 4 addresses CAA-related inflammation and includes a biomarker study of four patients with this rare form of CAA. This is another infrequent presentation of CAA; however it has recently gained attention due to its similarities to amyloid-related imaging abnormalities as observed in clinical trials of anti-amyloid therapies in patients with Alzheimer's disease.

In chapter 5, we aimed to study CAA in the context of Alzheimer's disease. We compared neuroimaging, cerebrospinal fluid biomarkers, and genetic features of CAA in genetically determined forms of Alzheimer's disease to those in sporadic Alzheimer's disease. We recruited a multicenter international cohort of subjects

with autosomal dominant Alzheimer's disease, Down syndrome, or sporadic early onset Alzheimer's disease.

Lastly, in chapter 6 we provide a general discussion, the concluding remarks, and future perspectives of this thesis.

In summary, in this thesis we use neuroimaging, cerebrospinal fluid biomarkers, and genetics to study CAA from a translational perspective in subjects with infrequent presentations of the disease and in rare genetically determined Alzheimer's disease populations. A multimodal approach such as this is essential to provide new insights to disease processes, to establish new accurate diagnostic tools, and, potentially, to discover new therapeutic targets.





# 2. INTRODUCTION

## 2.1. AN OVERVIEW OF CEREBRAL AMYLOID ANGIOPATHY

### Definition and risk factors

Cerebral amyloid angiopathy (CAA) is a small vessel disease characterized by the progressive deposition of the ß-amyloid protein (Aß) in the walls of leptomeningeal and cortical arteries, arterioles, capillaries, and, in rare cases, in veins. It is the main cause of spontaneous intracerebral hemorrhage (ICH) and contributes to cognitive impairment in the elderly. It can occur as a genetic disorder due to mutations or duplications of some specific genes; however the most common presentation is sporadic, sometimes associated with Alzheimer's disease (AD) [1].

The need for a neuropathological examination for a definite diagnosis means that the prevalence of CAA in the general population are likely underestimated. However, it is clear that the prevalence of CAA increases with age [2,3]. Population-based autopsy studies have revealed a prevalence of CAA of 20-40% in subjects without dementia and 50-60% in elderly populations with dementia [4–6]. CAA is even more prevalent and severe in patients who meet neuropathological criteria for AD, where it is present (to any degree) in more than 85% of the cases, and moderate to severe categories are present in about 25% of the cases [7–9].

In life the prevalence of CAA is also difficult to estimate. The diagnosis of possible or probable CAA is made based on clinicoradiological criteria. The Boston criteria for CAA-related hemorrhage were clinico-pathologically validated to attribute an ICH *in vivo* to CAA based on age, an appropriate clinical history, magnetic resonance imaging (MRI) findings and pathological data [10]. The radiological findings of the classic Boston criteria include single or multiple lobar hemorrhages restricted to lobar, cortical or cortico-subcortical regions. Sensitivity is improved by including cerebral lobar microbleeds as a CAA-related feature in the criteria. It is considered that both cerebral lobar microbleeds and lobar ICH represent independent vascular rupture events which are assumed to offer equal evidence for the presence of CAA [11,12]. In 2010, cortical superfi-

cial siderosis (cSS) and convexal subarachnoid atraumatic hemorrhage (cSAH) were included in the modified Boston criteria for CAA, providing an enhancement in sensitivity without a loss in specificity [13]. Table 1 shows the modified Boston criteria for CAA-related hemorrhage. Although the value of detecting lobar microbleeds, cSS, and cSAH has been validated in cohorts of patients who presented with symptomatic lobar ICH, these radiological features might also have a role in the diagnosis of patients presenting without major ICH, but with other syndromes associated with CAA.

**TABLE 1: MODIFIED BOSTON CRITERIA FOR CEREBRAL AMYLOID ANGIOPATHY-RELATED INTRACEREBRAL HEMORRHAGE.** Adapted from [13].

| | MODIFIED BOSTON CRITERIA |
|---|---|
| **DEFINITE CAA** | Full postmortem examination demonstrating:<br>• Lobar, cortical, or corticosubcortical hemorrhage<br>• Severe CAA with vasculopathy<br>• Absence of other diagnostic lesion |
| **PROBABLE CAA WITH SUPPORTING PATHOLOGY** | Clinical data and pathologic tissue (evacuated hematoma or cortical biopsy) demonstrating:<br>• Lobar, cortical, or corticosubcortical hemorrhage<br>• Some degree of CAA in specimen<br>• Absence of other diagnostic lesion |
| **PROBABLE CAA** | Clinical data and MRI or computerized tomography demonstrating:<br>• Multiple hemorrhages restricted to lobar, cortical, or corticosubcortical regions (cerebellar hemorrhage allowed) or<br>• Single lobar, cortical, or corticosubcortical hemorrhage and focal[a] or disseminated[b] superficial siderosis<br>• Age ≥55 y<br>• Absence of other cause of hemorrhage or superficial siderosis |
| **POSSIBLE CAA** | Clinical data and MRI or computerized tomography demonstrating:<br>• Single lobar, cortical, or corticosubcortical hemorrhage or<br>• Focal[a] or disseminated[b] superficial siderosis<br>• Age ≥55 y<br>• Absence of other cause of hemorrhage or superficial siderosis |

CAA: cerebral amyloid angiopathy. [a]: siderosis restricted to ≤ 3 sulci. [b]: siderosis affecting at least 4 sulci.





The population-based Rotterdam study found that 13.3% of subjects over 60 years of age met criteria for possible or probable CAA based on the presence of strictly lobar cerebral microbleeds [14]. Although sporadic, CAA-related symptoms are uncommon at ages younger than 60-65, in rare cases, they can also affect individuals in their 50s.

Aging is the strongest known clinical risk factor for developing CAA [12,15]. There are no differences associated with sex. The risk for CAA is not accounted for by conventional vascular risk factors (other than age), albeit that they do increase the risk of CAA-related ICH and other CAA-related manifestations [16]. Arterial hypertension, thrombolytic, antiplatelet, and anticoagulant therapy are also risk factors for CAA-related ICH [17–20].

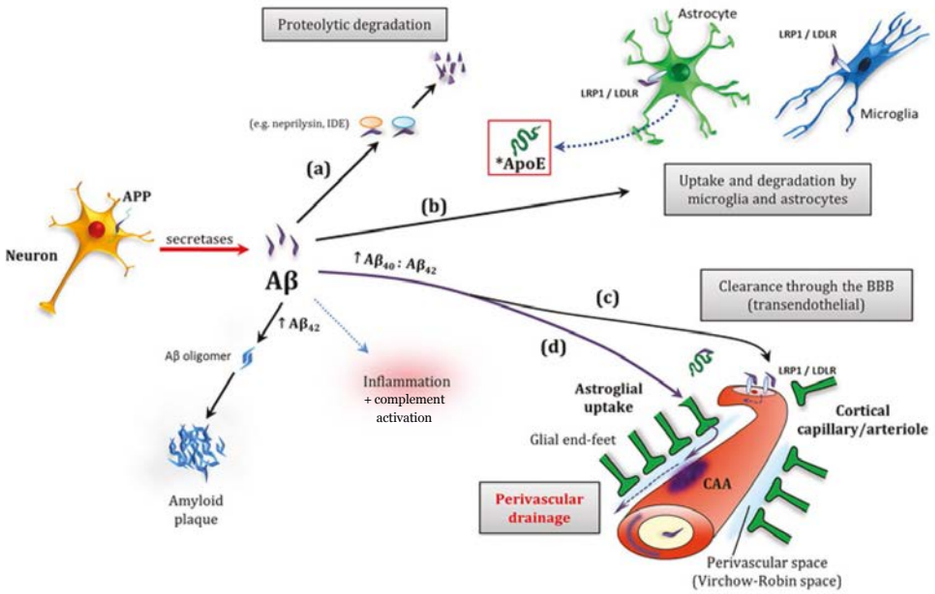
Variation in the Apolipoprotein E (*APOE*) gene is the best established genetic risk factor for sporadic CAA [21]. *APOE*-ε2 and *APOE*-ε4 alleles are both associated with more severe CAA [22] and increase the risk of sporadic CAA related -ICH [23]. Moreover, an epistatic interaction between the *APOE*-ε2 and *APOE*-ε4 alleles means that patients with both alleles present the earliest disease onset and the highest risk of early CAA related-ICH recurrence [12]. It has been proposed that the two alleles might promote CAA-related ICH through separate mechanisms: *APOE*-ε2 would favor amyloid-laden vessels to undergo the structural vasculopathic changes that lead to rupture while *APOE*-ε4 would enhance amyloid deposition [24]. Several genetic polymorphisms and rare coding variants in other genes, such as transforming growth factor (TGF)-ß1, low-density lipoprotein-receptor related protein (LRP-1), angiotensin-converting enzyme (ACE) or the complement component receptor 1 gene (CR1) have been proposed as potential risk factors for CAA. However their exact role has yet to be determined. A CR1 polymorphism which has been reported to increase AD risk has also been associated with increased risk of CAA-related ICH and CAA [25].

## Pathophysiology of cerebral amyloid angiopathy

Aß is generated by sequential cleavage of amyloid precursor protein (*APP*) by ß- and ɤ- secretases. Under normal conditions, Aß peptides can either be degraded

by proteolytic enzymes, remain in solution, or be cleared, via perivascular drainage pathways into the blood or lymph, or recirculated in the cerebrospinal fluid (CSF) across the blood-brain barrier (BBB) [26]. An imbalance in the production and clearance of Aß is one of the main mechanisms for CAA development, although the factors that promote Aß deposition in sporadic CAA are not clear. Recently, a hypothesis has emerged that the integrity of cerebral microvessels is critical for solute clearance from the interstitial fluid of the brain as these molecules pass from the brain into the perivascular drainage system and the systemic lymphatic circulation [27]. Cerebrovascular accumulation of Aß can thus be viewed as an indicator of perivascular drainage failure. Figure 1 represents the production, elimination and deposition of Aß in CAA.

**FIGURE 1. AB PRODUCTION, ELIMINATION, AND DEPOSITION IN CEREBRAL AMYLOID ANGIOPATHY (CAA).** ADAPTED FROM [12].




Aß is eliminated from the brain by four major pathways: (a) proteolytic degradation by endopeptidases; (b) receptor mediated clearance by cells in the brain parenchyma, like microglia, astrocytes and neurons; (c) active transport into the blood through the blood-brain barrier; (d) elimination along the perivascular pathways by which interstitial fluid drains into the periarterial space and towards the subarachnoid space in an opposite direction to blood flow.





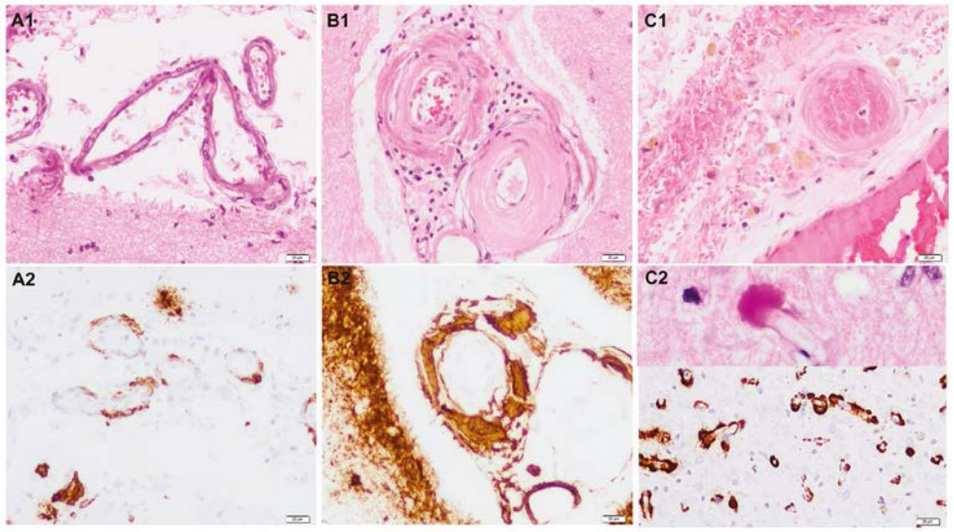
## Neuropathology of cerebral amyloid angiopathy

From a neuropathological point of view, CAA primarily involves neocortical and leptomeningeal arterioles, capillaries are less frequently affected and the involvement of venules is very rare. Two distinct pathological subtypes of CAA have been described: CAA type 1, characterized by Aß in cortical capillaries (with or without involvement of other vessels); and CAA type 2, where Aß deposits are restricted to leptomeningeal and cortical arteries, arterioles and, in rare cases, veins [28]. *APOE*-ε2 is typically associated with CAA type 2 while *APOE*-ε4 is associated with CAA type 1. CAA type 1 appears to be more closely associated with parenchymal amyloid deposition in AD [29].

The vascular amyloid in CAA is mostly composed of the 40 amino acid fragment (Aß40), in contrast with Aß plaques found in AD, which are predominantly composed of Aß42. Vascular deposition is facilitated by factors that increase the Aß40/Aß42 ratio (while increased Aß42 leads to oligomerisation and the amyloid plaques found in AD). Vascular deposition of amyloid interferes with the perivascular drainage, reducing the efficiency of efflux of Aß along the perivascular pathways [30]. Since clearance mechanisms fail with age, Aß becomes increasingly blocked from the perivascular drainage pathways, trapped in the basement membranes of capillaries and arterioles of the brain leading to CAA.

In early stages of CAA, vessels show Aß vascular deposits in the adventitia and media of the microvasculature (Figure 2: A2). As the disease progresses, vascular media degradation, loss of smooth muscle cells, and acellular thickening of the vessel wall take place (Figure 2: A1). These structural modifications may be able to trigger secondary events including the release of proinflammatory components, oxidative stress, changes in the BBB permeability and cell toxicity [31]. At advanced stages, the microvessel structure is severely altered and luminal narrowing, concentric splitting of the vessel walls, fibrinoid necrosis, hyaline degeneration, obliterative intimal changes, microaneurysm formation, and perivascular microhemorrhages are present (Figure 2: B1, B2). Deregulation of matrix metalloproteinases (MMP) has been suggested as an important contributor to CAA-related lobar ICH [32].

**FIGURE 2: HISTOPATHOLOGICAL FEATURES OF CEREBRAL AMYLOID ANGIOPATHY** (COURTESY OF DR. ELLEN GELPI, NEUROLOGICAL TISSUE BANK OF THE BIOBANC-HOSPITAL CLINIC-INSTITUT D'INVESTIGACIONS BIOMEDIQUES AUGUST PI I SUNYER (IDIBAPS), BARCELONA, SPAIN).




Morphological changes of the vessel walls of leptomeningeal arterioles, in hematoxylin-eosin staining (A1-B1-C1) and immunohistochemistry applying anti-betaA4 amyloid antibodies (A2-B2-C2). In A1 and A2, only minimal structural changes can be detected. B1 and B2, where a severe affectation is present, structural alterations and amyloid deposition in the vessel walls are found. C1 displays an intra-hemorrage located vessel with surrounding hemosiderin deposits, while C2 shows amyloid deposition in the wall of capillaries (capillary CAA).

Topographically, sporadic CAA frequently affects posterior cortical regions. The occipital lobe is the most severely affected, followed by the frontal, temporal, and parietal lobes. The cerebellum can also be affected however the white matter, brainstem, thalami and basal ganglia are typically spared. The distribution of CAA pathology can be described as patchy where affected and spared regions might be adjacent. The practical consequence of this is the possibility that cerebral biopsy could miss CAA pathology leading to a false negative diagnosis.

This CAA-associated vasculopathy leads to the development of hemorrhagic lesions, cerebral ischaemic damage, encephalopathies, and cognitive impairment

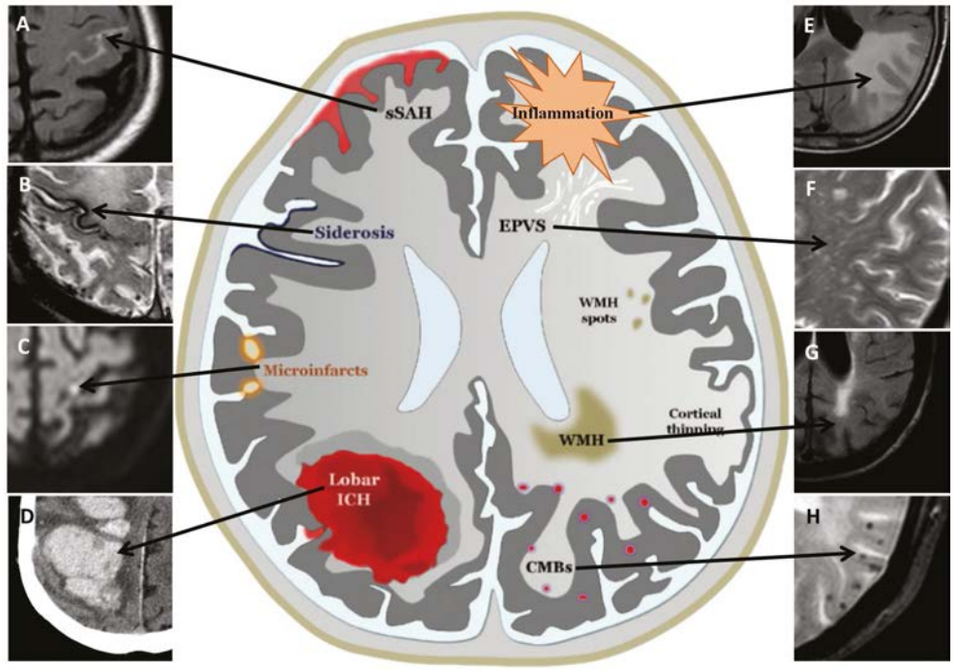




## 2.2. CLINICAL AND NEUROIMAGING CORRELATES OF CEREBRAL AMYLOID ANGIOPATHY

CAA is associated with several clinical syndromes and neuroimaging features as a consequence of both hemorrhagic and non-hemorragic small vessel-related brain damage (Figure 3). The relative frequency of the different clinical and neuroimaging CAA presentations is difficult to assess, as it is heavily dependent on making an accurate diagnosis of underlying CAA in different settings and using appropriate MRI sequences.

**FIGURE 3: SCHEMATIC REPRESENTATION OF THE NEUROIMAGING SPECTRUM OF HEMORRHAGIC AND NON-HEMORRHAGIC MANIFESTATIONS OF SPORADIC CEREBRAL AMYLOID ANGIOPATHY.** MODIFIED FROM [33].




A. Convexal subarachnoid atraumatic hemorrhage in a FLAIR sequence. B. Cortical superficial siderosis in T2*- gradient echo sequence. C. Microinfarcts in diffusion weighted imaging. D. Lobar intracerebral hemorrhage in a computerized tomography scan. E. Inflammatory reaction in FLAIR sequence. F. Dilated perivascular spaces in T2-weighted sequence. G. White matter hyperintensities in FLAIR sequence. H. Cortico-subcortical microbleeds in susceptibility weighted imaging.

## Hemorragic manifestations of cerebral amyloid angiopathy

### *Cerebral amyloid angiopathy-related intracerebral hemorrhage*

The most common clinical manifestation of CAA and the basis for the Boston criteria for CAA (Table 1) diagnosis is spontaneous lobar ICH [34]. The term lobar refers to the site of the hemorrhage in the cortex and subcortical white matter (Figure 3D), as opposed to the basal ganglia, brainstem, and cerebellum characteristic of the hypertensive hemorrhages (Figure 3D).

After hypertension, CAA is the second most common cause of spontaneous ICH in the elderly [35,36]. There are several classification systems to assess the etiology of ICH. A recently introduced classification scheme, the H-ATOMIC system (Hypertension, CAA, Tumour, Oral anticoagulants, and vascular Malformation), confirmed that CAA was the second most common cause of spontaneous ICH (after hypertension) in our population. It was the suspected cause in 31% of the studied cases: probable in 20%, possible in 10.5%, and definite in 0.5% [37].

This lobar location of the CAA-related ICH reflects the underlying distribution of the vascular amyloid deposits, which affects cortical vessels and largely spare white matter, deep gray matter, and the brainstem. Because of their superficial location, CAA-related ICH often extends into the subarachnoid space, and less frequently ruptures into ventricles. CAA-related ICH are also more likely to arise in posterior brain regions [38]. The explanation for the posterior brain clustering of CAA-related ICH is undetermined, but it may be related to as yet unknown characteristics of posterior circulation vessels that influence Aß peptide elimination, or to increased vulnerability of these brain regions to minor trauma [38,39].

The clinical presentation of CAA-related ICH varies with the size and location of the lesion. The overall mortality of CAA-related ICH is in the range of 10-30% [40,41]. However CAA is associated with a substantially higher risk of lobar ICH recurrence in patients who survive the initial bleed. It has been shown that areas of high Aß deposition in amyloid imaging appear to predict sites for future ICH [42]. The history and number of previous lobar ICH, increasing number of lobar microbleeds [43], anticoagulant or antiplatelet use [44], hypodense lesions on





computerized tomography (CT) scan and, as mentioned above, the presence of the ε2 or ε4 *APOE* alleles confer an increased risk of recurrence. Age, sex, and arterial hypertension are not well established predictors of recurrence [45,46].

### *Cerebral lobar microbleeds*

Cerebral microbleeds are identified as small, rounded, homogeneous, hypointense lesions visible on paramagnetic-sensitive MRI sequences, such as gradient-echo T2* (T2*-GRE) or susceptibility weighted imaging (SWI) (Figure 3H) [47,48]. Pathologically, they correspond to clusters of hemosiderin-laden macrophages within the perivascular space due to blood extravasation without disruption of the surounding tissue [30,49]. Cerebral microbleeds in general are not patognomonic of CAA, however when they follow a strictly lobar distribution, they strongly predict CAA pathology [50]. Topographically, in addition to the lobar distribution, they show a predilection for the parietal lobes, while their location in deep regions (basal ganglia, thalamus, and brainstem) suggest a hypertensive angiopathy [48,51]. Cerebral lobar microbleeds are one of the diagnostic markers of the Boston criteria for CAA (Table 1) and are strongly associated with the *APOE*-ε4 genotype [52].

The MRI acquisition settings, such as the sequence used and the field strength, are strongly associated with the prevalence of microbleeds [53]. When using T2*-GRE MRI, the most sensitive technique, lobar microbleeds are found in around 5-6% of the general population [54,55] and in 16-32% of patients with AD [56,57]. Although the exact mechanism of cerebral lobar microbleeds occurrence in CAA is not well established, they appear to be spatially correlated with areas of Aß deposition in positron emission tomography (PET) studies using amyloid tracers [58]. New hemorrhages occur preferentially at the sites of increased Aß deposits, supporting the idea that amyloid imaging may be useful to predict future CAA-related hemorrhages [19,42]. Nonetheless, a recent histopathological work examining nine cases with CAA found that the majority of the vessels involved in microhemorrhages did not contain Aß. This study suggests that microhemorhages may not be a direct consequence of severe local CAA pathology [59]. Thus, further studies are needed to determine the exact mechanisms by which vascular Aß leads to microbleed formation in CAA.

### *Cortical superficial siderosis / Convexal subarachnoid hemorrhage*

cSS has attracted growing interest in recent years and has been suggested as the third cardinal haemorrhagic neuroimaging signature of CAA, alongside symptomatic CAA-related ICH and lobar microbleeds [60]. Imaging features and suggested criteria for cSS identification are shown in table 2.

**TABLE 2: RECOMMENDED CRITERIA FOR IDENTIFICATION OF CORTICAL SUPERFICIAL SIDEROSIS AND ACUTE CONVEXAL SUBARACHNOID HEMORRHAGE (ACUTE CSAH) IN THE CONTEXT OF CAA** [61]**.**

- Well-defined, homogeneous hypointense curvilinear signal intensity (black) on T2*-GRE or SWI MRI in the superficial layers of the cerebral cortex, within the subarachnoid space, or both.
- Blooming effect on T2*-GRE and SWI compared to T1 or T2-weighted sequences.
- If there is corresponding signal hyperintensity in the subarachnoid space on proton density-weighted of Fluid Attenuated Inversion Recovery (FLAIR) sequences (or hyperdense on CT if available), the term 'acute cSAH' is recommended.
- Axial T1-weighted or FLAIR images should be used for anatomical confirmation of the gyral location of the signal hypointensities identified on T2*-GRE or SWI sequences.
- Absence of infratentorial (brainstem, cerebellum, spinal cord) siderosis.
- Ensure exclusion of potential hemorrhagic and non-hemorrhagic mimics (e.g. vessels flow voids, thrombosed vessels, petechial hemorrhagic transformation of infarcts, calcium deposits).
- Consider all potential non-CAA secondary etiologies of cSS and acute cSAH.

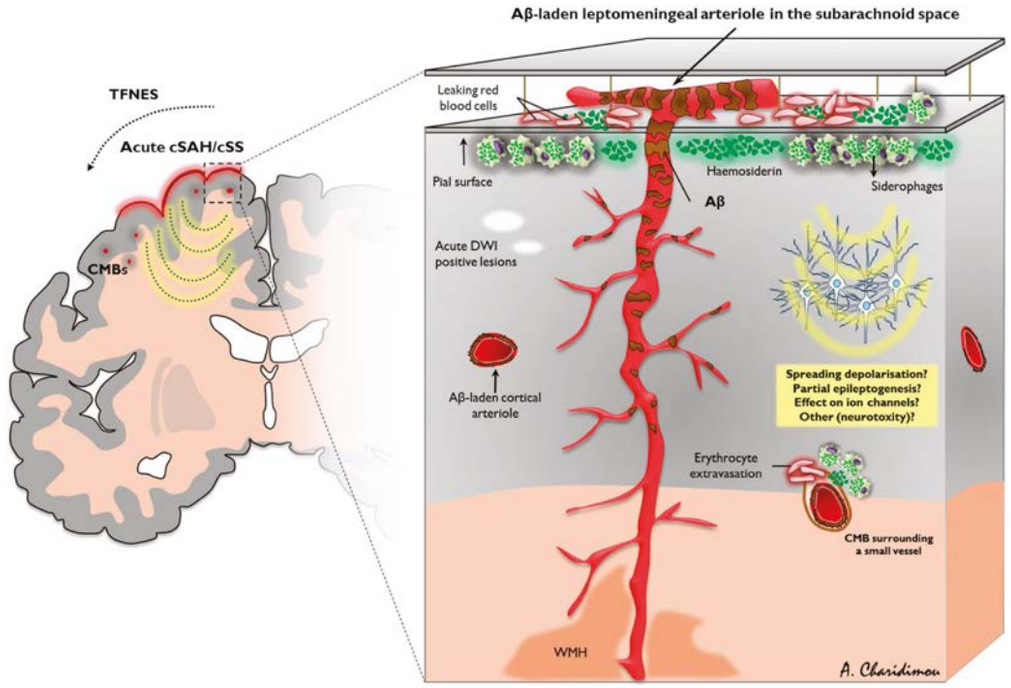
cSS reflects chronic deposits of blood breakdown residues, including haemosiderin and hemosiderin-laden macrophages (siderophages), within the subarachnoid space, in a curvilinear pattern following the cortical surface. The current hypothesis is that cSS (Figure 3B) is a result of repeated episodes of blood leakage originating from fragile leptomeningeal or very superficial cortical CAA-affected vessels into the sub-arachnoid space or superficial subpial cortical layers, such as acute cSAH (Figure 3A), Those blood products are degraded over time and blood residues are deposited in superficial cortical layers leading to chronic cSS [62]. However, the exact pathophysiologic mechanism has yet to be fully elucidated. Acute cSAH and cSS could trigger transient focal neurological episodes (TFNE), could induce acute microinfarcts on diffusion-weighted (DWI) MRI,





could be associated with future risk of ICH, or could be independent risk factors for new onset dementia after ICH. Figure 4 shows a schematic diagram of the key features of acute cSAH and cSS.

**FIGURE 4: SCHEMATIC DIAGRAM OF LEPTOMENINGEAL AND SUPERFICIAL PARENCHYMAL ARTERIOLES SHOWING KEY FEATURES OF CONVEXAL SUBARACHNOID HEMORRHAGE IN SPORADIC CEREBRAL AMYLOID ANGIOPATHY** [63]**.**




Leptomeningeal and perforating arterioles of a brain section showing Aß deposits. Note the reducing vascular Aß severity moving from the cortical surface into the cerebral white matter. Diffusion-weighted (DWI), magnetic resonance imaging (MRI), cerebral lobar microbleeds (CMB), Transient focal neurological episodes (TFNEs), white matter hyperintensities (WMH).

In population-based studies, cSS has been reported with a prevalence of 0.4% [61] and it has been found to be around 2.7-3.5% in memory clinic settings [60,64,65]. In these studies, the presence of cSS was strongly associated with the presence of lobar microbleeds and white matter hyperintensities. Another study found cSS in 60.5% of patients with neuropathologically-proven CAA, but in

none of the controls with neuropathologically-proven non-CAA-related ICH [13]. These studies support the hypothesis in which CAA is the prevailing underlying pathology for cSS. Nevertheless, other authors still take this assertation with caution [66].

cSS can improve the diagnosis of CAA-related ICH. It is associated with a high risk of future symptomatic CAA-related lobar ICH, often at the location of pre-existing cSS [13,67,68]. This association is independent of lobar microbleeds burden [69]. Moreover, disseminated cSS is more common in patients with neuropathological CAA-related ICH than in those with CAA without ICH. Genetic studies have associated cSS with the APOE-ε2 allele, but not with *APOE*-ε4 [70].

***Transient focal neurologic episodes***

TFNE, also called "amyloid spells", are the second most common clinical presentation of sporadic CAA after CAA-related ICH [71]. They consist of brief, transitory, stereotypic episodes of paresthesias, weakness, or numbness, with an onset ranging from seconds to minutes, usually resolving in less than 30 minutes [71]. The underlying mechanisms remain unclear, but could be related to epileptic or migraine aura-like cortical spreading depression mechanisms [72,73].

Presentation with TFNE can be used as a clinical marker of hemorrhagic components of CAA and the is the strongest clinical marker of cSS [74]. An anatomical correlation between clinical symptoms of TFNE and lobar microbleeds or cSAH/cSS location has been described [69].

The diagnosis of CAA-related TFNE should be considered a warning sign of future symptomatic CAA-related ICH [67] and, consequently, has potential implications for antiplatelet or anticoagulant treatment decisions when they are misdiagnosed with transient ischaemic attacks [61], thus highlighting the clinical importance of their diagnosis.





## Non- hemorragic manifestations of cerebral amyloid angiopathy

***White matter hyperintensities***

White matter hyperintensities (WMH) are detected as a hyperintense signal in subcortical or periventricular white matter, typically sparing subcortical U fibres, in T2-weighted or fluid-attenuated inversion recovery (FLAIR) MRI sequences (Figure 3G). Pathological substrates include demyelination, axon loss, and mild gliosis, probably due to chronic hypoperfusion of the white matter and disruption of the BBB due to Aß deposition in cortical small vessels and/or as a result of the accumulation of silent microinfarcts.

The presence and severity of WMH strongly correlate with CAA and are typically more severe in CAA patients than in patients with AD [75]. Patients with CAA-related ICH exhibit occipital dominant WMH in MRI studies [76]. This topography is consistent with the predilection of the CAA pathology for posterior brain regions [77]. This posterior distribution of WMH has been associated with the presence of CAA pathology and, the WMH volume correlates with higher total Aß load on 11C-Pittsburgh Compound B- PET (PET-PIB) [78] and low CSF- Aß42 levels [79] [79].

***Cerebral cortical microinfarcts***

Cerebral microinfarcts are unapparent on gross pathologic examination or conventional structural MRI, and only visible on microscopic tissue examination [31]. However, the accompanying cytotoxic edema can be detected through MRI diffusion weighted imaging (DWI) within a time window of approximately 10 days (Figure 3C). DWI lesions are detected in approximately 15% of patients with CAA and ICH [80](Auriel Neurology 2012), and cerebral cortical microinfarcts in up to 100% of CAA patients using ultra-high field strength MRI [81]. CAA-related ischemic infarcts are more often located in the cerebral cortex.

Cerebral microinfarcts are more commonly found in patients with CAA than in subjects without CAA [82] and are more frequent after acute CAA-related ICH than after other spontaneous bleeds [83]. They have been associated with the severity of WMH and lobar microbleeds, suggesting that they are due to a CAA-related arteriopathy. Cerebral microinfarcts could have a substantial impact on

cognitive function and independently contribute to brain atrophy [84]. The exact pathogenic mechanism underlying these microinfarcts remains unknown.

***MRI- visible dilated perivascular spaces***

Dilated perivascular spaces, or Virchow-Robin spaces, visible on MRI represent another non-hemorrhagic biomarker of CAA [47] due to its association with the number of lobar microbleeds [85] and cSS [86]. They are identified on MRI-T2 sequences as round, ovoid, or linear structures with CSF-like signal, no larger than 2 mm in diameter, and located in territories supplied by perforating arteries (Figure 3F) [47,85].

Severe dilated perivascular spaces in the centrum semiovale have been proposed as a promising new neuroimaging marker to improve the sensitivity of CAA *in vivo* diagnosis [87]. Its severity is strongly associated with cognitive impairment [88]. Recently, the perivascular space severity in the centrum semiovale has been associated with cortical amyloid burden in PET in probable CAA-related ICH patients and healthy elderly subjects [89].

The relationship between CAA and dilated semiovale perivascular spaces detected by MRI could reflect the failure of protein elimination implicated in disease pathogenesis, suggesting that interstitial fluid drainage impairment within perivascular spaces is caused by cumulative leptomeningeal and superficial cortical vascular Aß deposition [85].

***Cognitive impairment***

CAA is an important contributor to cognitive impairment, although it is hard to dissect its specific contribution in the elderly due to the overlap with other age-related brain pathologies. It is thought that CAA independently contributes to cognitive impairment and can worsen the severity of cognitive dysfunction in AD. The proposed mechanism involves the potentiation of neurodegeneration in the AD pathophysiological process, the predisposition to microhemorrhagic and microischemic injury to the brain parenchyma, the alteration of structural connectivity, and the interference with the autoregulation of central nervous system blood flow [90]. The concomitant occurrence of CAA and AD pathology has been associated with significantly worse cognitive performance compared with AD





pathology alone, even after controlling for age, neurofibrillary tangles, amyloid plaque number, infarctions and *APOE* genotype [5].

The prevalence of CAA is consistently higher in patients with dementia vs. non-demented subjects in several population-based studies, independent of vascular events [91]. Furthermore, moderate and severe CAA is associated with lower performance in perceptual speed or episodic memory in community-dwelling patients, independent of the effect of AD pathology [6,92]. Mild CAA and capillary CAA have no clear neuropsychiatric correlates, suggesting they are either physiologic or pre-clinical conditions.

A recent study revealed that there is a substantial risk of incident dementia in dementia-free survivors of spontaneous ICH and found that underlying CAA is a contributing factor to the occurrence of new-onset dementia in this population [93]. Other studies have estimated that over 40% of patients with CAA-related ICH suffer cognitive decline during their life [94]. Overall, CAA is now recognized as an important variable in the pathophysiology of AD [90], in fact, vascular cognitive impairment and AD are now conceptualized as a continuum [12].

***Cerebral amyloid angiopathy-related inflammation***

Cerebral amyloid angiopathy-related inflammation (CAA-ri) appears to represent a rare distinct manifestation of CAA. Clinically, it manifests as a meningoencephalitis presenting with acute or subacute cognitive decline, mental status disturbances, seizures, headache, and occasionally focal neurologic deficits [95]. The neuroimaging features are unifocal or patchy multifocal WMH, typically asymmetric and extending to the subcortical white matter, which can be detected in FLAIR sequences. Other CAA related neuroimaging features such as hemorrhagic CAA-related findings in blood-sensitive MRI sequences, like previous lobar ICH, multiple lobar microbleeds, or cSS are frequently found. It is important to recognize CAA-ri in clinical practice, as early immunosuppressive treatment can lead to a clinicoradiological response [96]. Recurrences are rare [97] and *APOE*-ε4 genotype is overrepresented in CAA-ri patients.

There are no data currently available to determine the precise prevalence of CAA-ri. Spontaneous vasogenic edema and inflammation, attributed to CAA, in

patients with AD are rarely present, reportedly found in 2 out of more than 3000 subjects clinical trial screenings [98], thus CAA-ri seems to be an infrequent condition.

Diagnostic criteria for CAA-ri were initially suggested by Chung *et al* in 2011 (Table 3A) [99]. These criteria required a pathological confirmation to reach the category of 'definite CAA-ri'. However, new validated clinicoradiological criteria have been recently proposed and are listed in table 3B. CSF studies are not included in those diagnostic criteria.

CAA-ri can be the first presentation of CAA or a trigger for rapidly progressive dementia within the course of prodromal and established AD [98,101] in autosomal dominant forms of AD (*APP* duplication carriers and in two *Presenilin 1* mutations) [102–104].

Over the last few years, CAA-ri has received much attention because of its notable clinical and radiological similarities to the ´amyloid related imaging abnormalities´ (ARIA), the most frequent adverse event in anti-amyloid therapies. ARIA is the term used to define the clinical-radiological side effects reported within the anti-amyloid clinical trials for patients with AD: ARIA-E is characterized by the evidence of vasogenic edema on FLAIR-MRI sequences, as hallmarks of inflammation while ARIA-H consists of microbleeds and/or cSS on T2*-GRE or SWI, as hallmarks of CAA. Reported ARIA frequencies differ according to the immunotherapy strategy, reaching 56% of patients treated with aducanumab. Moreover, ARIA appeared at a higher frequency in the high-dose group and in *APOE*-ε4 carriers [105].

The commonalities between CAA-ri and ARIA are evident. In both, the clinical presentation can consist of symptomatic or midly symptomatic acute/subacute neurological signs, that are accompanied by bilateral and asymmetrical vasogenic edema involving the posterior cortical/subcortical whtite matter, and by diffuse microbleeds or signs of cSS. Additionally, in both entities, *APOE*-ε4 allele is overrepresented and they typically resolve either spontaneously or after immunosuppressive therapy.





**TABLE 3. PROPOSED DIAGNOSTIC CRITERIA FOR CEREBRAL AMYLOID ANGIOPATHY-RELATED INFLAMMATION**

| A. PREVIOUS CAA-RI CRITERIA [99] | B. CURRENT CAA-RI CRITERIA [100] |
|---|---|
| - | **Possible CAA-ri**<br>**1.** Age≥40 years<br>**2.** Presence of ≥1 of the following clinical features: headache, decrease in consciousness, behavioral change, or focal neurological signs and seizures; the presentation is not directly attributable to an acute ICH<br>**3.** MRI shows WMH lesions that extend to the immediately subcortical white matter<br>**4.** Presence of ≥1 of the following corticosubcortical hemorrhagic lesions: cerebral macrobleed, cerebral microbleed, or cortical superficial siderosis<br>**5.** Absence of neoplastic, infectious, or other cause |
| **Probable CAA-ri**<br>All of the following:<br>**1.** Acute or subacute onset of symptoms<br>**2.** Age≥40 years of age or older<br>**3.** At least one of the following clinical features: headache, mental status or behavioural change, focal neurological signs and seizures<br>**4.** MRI shows patchy or confluent T2 of FLAIR hyperintensity which is:<br>**a)** Usually asymmetric<br>**b)** With or without mass effect<br>**c)** With or without leptomeningeal or parenchymal enhancement<br>**5.** Evidence of pre-existing CAA on SWI-MRI sequences<br>**a)** Multiple lobar hemorrhages (ICH or microbleeds)<br>**b)** Recent or past lobar hemorrhage<br>**6.** Absence of neoplastic, infectious or other cause | **Probable CAA-ri**<br>**1.** Age ≥40 years<br>**2.** Presence of ≥1 of the following clinical features: headache, decrease in consciousness, behavioral change, or focal neurological signs and seizures; the presentation is not directly attributable to an acute ICH<br>**3.** MRI shows unifocal or multifocal WMH lesions (corticosubcortical or deep) that are asymmetric and extend to the immediately subcortical white matter; the asymmetry is not due to past ICH<br>**4.** Presence of ≥1 of the following corticosubcortical hemorrhagic lesions: cerebral macrobleed, cerebral microbleed, or cortical superficial siderosis<br>**5.** Absence of neoplastic, infectious, or other cause |
| **Definite CAA-ri**<br>All of the above plus histopathological confirmation with:<br>**1.** Perivascular, transmural and/or intramural inflammation<br>**2.** Amyloid deposition within vessels of affected area in the cortex and leptomeninges | - |

To date, there are no early biomarkers able to predict the occurrence of ARIA, however patients with AD and ≥5 microbleeds and/or any evidence of cSS or prior ICH are systematically excluded from anti-amyloid clinical trials [106,107]. The discovery that elevated CSF- anti-Aß autoantibodies in CAA-ri could play a role in CAA-ri pathophysiology has increased the understanding of the etiological mechanisms of ARIA and CAA-ri is now considered to be a spontaneous model of ARIA [108–110]. It has been suggested that immunotherapy-related movement of Aß from brain parenchyma into and out of the cerebral vasculature may lead to accumulation of Aß in perivascular drainage pathways, and, in consequence, to an increase in CAA and cerebral microbleeds [111]. This disruption in vascular integrity would increase vascular permeability resulting in ARIA-E from leakage of proteinaceous fluid, and ARIA-H from leakage of blood products and thus hemosiderin deposititon. A similar reaction could spontaneously occur in CAA-ri, mediated by intrathecally synthetized autologous anti-Aß autoantibodies. Consequently, increased levels of CSF- anti-Aß autoantibodies in acute phase of CAA-ri are considered specific markers of CAA-ri.

Increased levels of CSF anti-Aß autoantibodies are key to the manifestation of ARIA, causing the inflammatory event at the site of greater Aß removal [107,112]. In CAA-ri, levels of CSF- anti-Aß autoantibodies have also been associated with a massive release of soluble Aß during the inflammatory phase, which is followed by reduced retention of amyloid tracer in the affected region. The inflammatory reaction, in both cases, might be then considered as an indirect sign of Aß removal. The publication of a few cases of patients with ARIA and available PET with amyloid tracer support this theory, but, the only three published cases with CAA-ri and PET with amyloid tracer did not show differences in tracer retention in areas that suffered from a recent inflammation [97,113].





## 2.3. BIOMARKERS OF CEREBRAL AMYLOID ANGIOPATHY

A biomarker is a parameter that can be objectively measured *in vivo* and gives information about a biological or pathogenic process [114]. To understand the underlying biology and neurological effect of the disease, an ideal biomarker for CAA would be one that is clinically meaningful, representative of the disease's underlying biological progression, efficient at detecting changes in response to treatment and a reliable and reproducible measure that can be easily generalized across different sites [115]. The most accepted biomarkers for CAA and CAA-related disorders are listed in table 4. In this section, we describe some of the techniques used for the obtention of those and other measures that, although currently are not well-established biomarkers for CAA diagnosis, are being studied and considered as potential candidates.

**TABLE 4: BIOMARKERS FOR CEREBRAL AMYLOID ANGIOPATHY AND CAA-RELATED DISORDERS.** (MODIFIED FROM YAMADA *ET AL.* [116]).

| |
|---|
| **BIOMARKERS SUGGESTIVE OF CAA:** |
| Amyloid imaging with greater occipital uptake |
| Decreased CSF-Aß40 levels |
| **BIOMARKERS SUGGESTIVE OF CAA-RELATED DISORDERS:** |
| Symptomatic lobar ICH on CT/MRI |
| Lobar microbleeds on MRI, GRE-T2* or SWI sequences |
| cSS, cSAH on MRI, GRE-T2* or SWI sequences |
| Cortical microinfarcts on MRI |
| Posterior distribution of WMH on MRI |
| Severe enlargement of perivascular spaces in the centrum semiovale on MRI |
| Subacute leukoencephalopathy compatible with CAA-ri |
| Presence of anti-Aß antibodies in CSF for CAA-ri |
| Perceptual speed and episodic memory impairment in cognitive testing |

### a) Imaging biomarkers of cerebral amyloid angiopathy

The most widely studied imaging markers in CAA are measures of brain structure and amyloid deposition obtained through MRI or PET, respectively. These

techniques can simultaneously give mechanistic and topographical information. Although conventional CAA neuroimaging biomarkers are currently used for diagnosis and clinical monitoring, none has yet emerged as a valid surrogate biomarker for clinical trials.

With respect to structural measures, cranial CT is able to detect lobar ICH. However, MRI techniques, such as T2*-GRE or SWI sequences are also useful for detecting microbleeds, cSS, and cSAH. From a diagnostic standpoint, these sequences have emerged as the most important diagnostic aid for CAA identification during life [117]. SWI is a 3D sequence and has a higher spatial resolution that generates images with improved sensitivity to microbleeds than conventional T2*-GRE. The typical spatial resolution of SWI is 0.5 x 0.5 x 2 mm, whereas GRE sections are up to 5 mm thick with intersection gaps up to 2 mm [53]. Imaging of hemorrhagic components of CAA also benefits from increased magnetic field strength.

Other MRI sequences, such as FLAIR or DWI, are useful for detecting WMH and acute cortical microinfarcts, respectively, although the latter can occasionally go undetected due to their limited size. Ultra high strength clinical MRI scanners with a higher spatial resolution may be required to enable their detection. FLAIR sequences are also able to detect inflammatory reactions occurring in CAA-ri. As mentioned above, MRI-visible dilated perivascular spaces in the centrum semiovale have also been suggested as an imaging marker of CAA.

Two studies have provided evidence of CAA-related cortical atrophy independent of AD [118,119]. The atrophy pattern was predominant in medial frontal and posterolateral brain regions, in contrast to the regions known to develop the earliest cortical thinning in AD [120]. The clinical implications of those findings and their relation to CAA-related clinical manifestations deserve further study.

Microstructural changes detected by diffusion tensor imaging and microinfarcts can also be found in CAA. Altered network structure in CAA subjects compared to controls has been found in CAA, particularly in occipital, posterior parietal, and posterior temporal regions in a pattern resembling the distribution of the CAA pathology [121]. Network disturbances were associated with worse cognitive functioning and amyloid load on PET, providing links between vascular





Aß and impairment in white matter connectivity. Acute microinfarcts detected by DWI in subcortical white matter produce focal changes in mean diffustivity and fractional anisotropy [122] suggesting that altered diffusivity might partially reflect the cumulative effect of cerebral microinfacts or other focal lesions on cortical microstructure.

Altered structural connectivity can also influence cognitive impairment. In support of this hypothesis, a recent study examined brain networks in CAA as a surrogate measure of global small-vessel pathology and showed that lower global network efficiency was independently related to a worse performance in tests of processing speed and executive functioning [121].

PET imaging with amyloid tracers (PiB, Florbetapir, Florbetaben, and Flutemetamol) can be used to detect amyloid deposition within the brain. In patients with symptomatic CAA-related ICH, PiB-PET has low specificity for CAA due to the frequent occurrence of high C-PiB uptake in healthy elderly subjects reflecting preclinical AD, which might also be present in suspected CAA. However, a negative PiB-PET rules out CAA with excellent specificity. This has clinical implications for the prognosis and selection of candidates for drug trials. Lobar microbleeds are associated with amyloid tracer retention that labels vascular Aß [123].

As previously mentioned, there are only three cases of CAA-ri with an available PiB-PET reported in the literature. All were performed several months after remission and no changes in tracer retention were found in the previously affected regions [97,113].

**b) Markers of cognitive decline**

After symptomatic ICH, cognitive impairment is the most clinically prominent manifestation of CAA. Previous studies have reported a worse perceptual speed and episodic memory in individuals with moderate-to-severe CAA abnormalities at autopsy than those with none-to-minimum CAA, independent of AD pathology, cerebral infarcts, Lewy bodies, age at death, sex and education [92]. The mechanisms by which advanced CAA leads to cognitive impairment remain

unknown, but could reflect the cumulative effects of the various tissue injuries previously discussed, as well as the contribution from coexistent AD pathology. The non-specificity of any cognitive test battery for CAA, the absence so far of a sensitive and specific cognitive profile to discriminate CAA from other age-related disorders (episodic memory and perceptual speed overlap with domains affected in AD and other dementias), and the fact that those measures are not able to provide pathophysiological information on the biological effects limit the use of cognitive testing as a useful biomarker of CAA [115].

### c) Markers of vascular physiology

CAA appears to have substantial effects on vascular physiology. Amyloid deposition in the vessel wall may cause gliovascular unit impairment, endothelial dysfunction, impaired autoregulation, and BBB disruption. The best established physiological change in individuals with advanced CAA is reduced vasodilation following physiological stimuli. Studies with transcranial Doppler ultrasound and functional MRI measuring blood oxygen level-dependent have reported impaired vascular reactivity in response to visual stimulation [124,125]. The functional MRI response to a visual task seems to be associated with markers of CAA severity, such as lobar microbleed count and WMH volume, suggesting that these parameters might reflect the underlying extent of the disease and represent a potentially important mechanism for its pathogenesis [115].

### d) Biochemical markers

Biochemical markers that can be measured in accessible biological fluids, such as plasma or CSF, offer another window to study CAA pathophysiology, and enable CAA diagnosis.

Regarding plasma biomarkers, one study reported higher Aß40 and Aß42 concentrations in subjects with probable CAA compared with healthy controls [126]. However, these differences have not been replicated by other studies. Other plasma biomarker candidates include the MMP family (MMP-2 and MMP-9 in particular), whose deregulation, as previously mentioned, have been suggested as





an important contributor to CAA-related ICH [32]. Nevertheless, to our knowledge, no study has reported increased levels of MMP in plasma of patients with CAA.

CSF has been used for over two decades as an accessible biological source to study neurodegenerative diseases. Unlike plasma, CSF is in direct contact with the extracellular space of the brain and might more accurately reflect the pathophysiological processes that take place in CAA. The most studied CSF biomarkers in CAA are Aß40, which has been proposed to be specific for CAA, and the "core" AD biomarkers: Aß42, total-tau (tTau), and phosphorylated Tau at threonine-181 (pTau).

As previously mentioned, while AD senile plaques in the brain parenchyma consist mainly of Aß42, vascular deposits contain predominantly Aß40. Decreased levels of Aß42 but not Aß40 in CSF are found in AD, while both Aß42 and Aß40 levels are decreased in CAA with respect to healthy controls and AD patients [127]. These findings have also been reported in preclinical CAA in a form of hereditary CAA, suggesting that CSF-Aß42 and CSF-Aß40 could also be useful as preclinical biomarkers [128]. CSF-tTau and CSF-pTau concentrations in CAA have been found to be increased compared to healthy controls but decreased compared to AD patients [129].

CSF studies in the various CAA-related disorders or manifestations are scarce and most studies consider them within the context of AD.

There are no established CSF biomarkers for lobar microbleeds. In the context of AD, patients with lobar microbleeds have lower levels of CSF- Aß42 than those without them [130–132]. Similarly, a higher number of lobar microbleeds has been associated with lower Aß42 levels in AD and mild cognitive impairment [79]. Data with respect to tTau and pTau levels and Aß40 levels in patients with AD with and without microbleeds seem contradictory [130,131,133]. In respect to CSF biomarkers for cSAH/cSS, there is also a lack of evidence. The few published studies on this topic suggest that low levels of Aß42 and Aß40 with high levels of tTau could be indicative of cSAH or cSS [64,134,135]. However, more studies are, needed to establish reliable biomarkers for cSAH/cSS.

There are few and conflicting studies evaluating the CSF biomarker profile in CAA-ri. Both increased [109,136] and decreased [101] levels of CSF-Aß42 and CSF-Aß40 have been described in the acute phase of CAA-ri.

A few years ago, the discovery that typical MRI findings characterizing the acute phase of CAA-ri -vasogenic edema, multiple lobar microbleeds and/or cSS - represent a variation of anti-amyloid therapies-induced ARIA has generated great interest for the field of immunotherapy and CAA pathophysiology [109]. Since then, the iCAß International Network has described and emphasized the role of CSF anti Aß-autoantibodies as a specific biomarker for the diagnosis of CAA-ri [109,137]. Although the validation of cut-off points for clinical diagnostic purposes is still ongoing, it has been proposed that in the clinical setting, determining the dosage of CSF anti Aß-autoantibodies in CAA-ri could be useful for adjusting immunosuppressive treatment, monitoring the response and even avoiding a brain biopsy [138]. The temporal relationship between anti-Aß autoantibodies and clinico-radiological improvement of CAA-ri, the transient massive drainage of Aß from brain and vascular deposits, the specificity of anti-Aß autoantibodies for CAA-ri, and their possible role in predicting CAA-ri recurrence make the presence of anti-Aß autoantibodies a promising candidate biomarker for the diagnosis, monitoring and management of ARIA [139].

Classically, BBB integrity has been studied through the CSF/serum albumin ratio, which is reported to be higher in AD and multiple CMBs and in CAA-ri than in AD or mild cognitive impairment [79,140].





## 2.4. CEREBRAL AMYLOID ANGIOPATHY AND ALZHEIMER'S DISEASE

### 1.4.1 Cerebral amyloid angiopathy and sporadic Alzheimer's disease

AD is the most common form of dementia. Most AD cases are sporadic, a result of complex interactions between genetic and environmental factors. The disease is generally diagnosed in subjects over the age of 65, and the highest prevalence is found in the elderly. Despite a great deal of investment in research and clinical trials spanning several decades, there is currently no effective treatment for AD. While the main pathological hallmark of the disease is the accumulation of amyloid plaques in the brain, the mechanisms by which the formation of these senile plaques leads to neurodegeneration and cognitive symptoms is still poorly understood.

A widely accepted theory for AD pathogenesis is the amyloid cascade hypothesis, which postulates an imbalance between Aß production and clearance that leads to a conformational change in Aß that makes it prone to aggregation. The initial formation of soluble oligomers is followed by insoluble larger fibrils that accumulate into diffuse and neuritic plaques. This leads to the impairment of synaptic function and chronic neurodegeneration, cognitive impairment and, finally, dementia [141]. It is believed that cognitive impairment is due to the inhibition that Aß oligomers exert on hippocampal long term potentiation and impaired synaptic function, as well as to other factors such as oxidative stress, neuroinflammation, and synaptic and neuronal degeneration. In that hypothesis, Tau pathology and tangle formation is regarded as a downstream event that contributes to cognitive symptoms (Figure 5). In addition to this excessive deposition of Aß, its clearance is also impaired at the prodromal stage of sporadic AD, particularly in carriers of the *APOE*-ε4 allele, and is exacerbated by the deposition of immune complexes, and age. Impaired clearance leads to elevated levels of soluble Aß in the brain that can accumulate in arterial walls thus blocking perivascular pathways thereby further exacerbating the reduced Aß clearance in AD and CAA forms [26] (Figure 5).

CAA is, therefore, a major contributor to AD pathogenesis and has been considered as a pathological hallmark for AD in addition to its role in the devel-

opment of hemorrhages in elderly patients [142,143], although its exact role remains unknown. Patients who initially have AD with no or mild CAA may present with increased vascular Aß deposition during the clinical course, which might lead to development of CAA-related cerebrovascular disorders. As previously mentioned, CAA is reported in more than 85% of AD cases. Likeiwse, patients who initially present with symptomatic CAA-related ICH without AD may present increased parenchymal Aß deposition during the clinical course, leading to development of dementia [93,144]. Moreover, CAA has been associated with an earlier dementia onset in AD [145]. The fact that CAA and AD are two sides of a single condition- cerebral Aß amyloidosis- explains the high prevalence of cerebral Aß parenchymal deposition in patients with CAA as well as the high frequency of CAA in patients with AD.

### 1.4.2 Cerebral amyloid angiopathy and genetically determined Alzheimer's disease

In both AD and CAA, amyloid derives from the processing of amyloid precursor protein (*APP*) to produce Aß fragments. The presence of mutations in *APP* or in one of the two presenilin genes (*PSEN1* and *PSEN2*) cause a shift in the processing of Aß favouring the production of Aß species that are more prone to aggregation. These mutations are transmitted with an autosomal dominant pattern of inheritance and will lead to early onset AD, generally between 30 and 50 years of age. Autosomal dominant forms of AD (ADAD) account for a small proportion of all AD cases, estimated to be around 1% [146].

As previously mentioned, brain Aß deposition in AD results from the chronic imbalance between Aß production and clearance. Pathophysiologically, from a simplistic point of view, a chronic increase in the absolute or relative production of Aß over time could be the prevailing mechanism in ADAD [146] while a an increased and altered production together with a reduced clearance of Aß could be the mechanisms involved in sporadic AD [147].

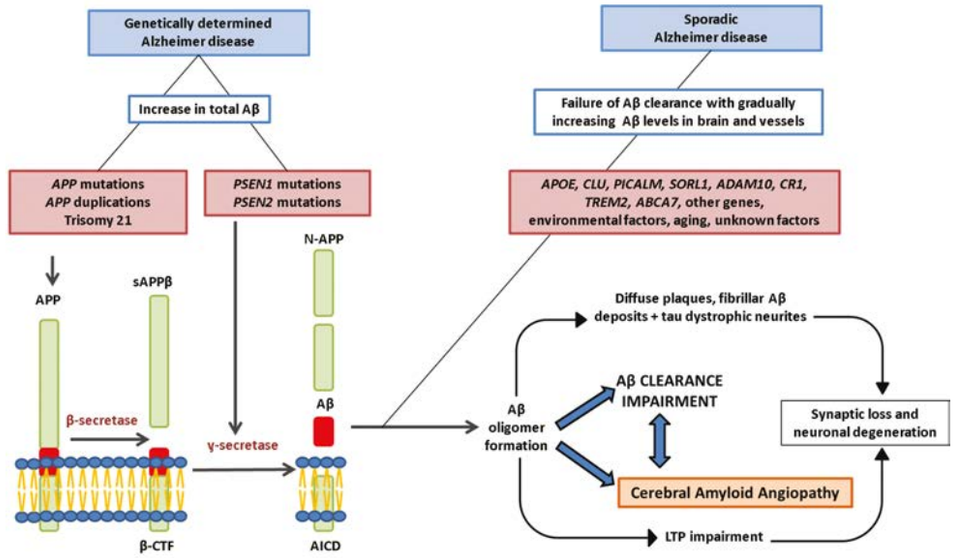
Different mutations in *PSEN1* and *PSEN2* affect the structure or function of ɤ-secrease in several ways but are aimed at increasing the available amount of





Aß42 relative to Aß40. However, this increase in the Aß42/Aß40 ratio is carried out by different mechanisms, depending on the type of mutation. Some mutations increase the production of Aß42, others decrease the production of Aß40 and others lead to a qualitative change in the monomers of Aß42, which makes them more likely to adopt a fibrillar conformation. All this leads to an increase in the deposit of Aß42 at a relatively young age. At the pathological level, ADAD has more amyloid and neurofibrillary pathology than both sporadic AD and CAA. For some *APP* mutations (mainly those located within the coding sequence), or *PSEN1* (those that occur beyond codon 200), the CAA even drives the clinical picture.

**FIGURE 5: THE BALANCE BETWEEN APP PROCESSING AND CLEARANCE IN A MODEL OF ALZHEIMER'S DISEASE PATHOGENESIS.**




The amyloid precursor protein (APP) is cleaved by ß-secretase and then again by γ-secretase to form Aß as part of the amyloidogenic pathway. According to the amyloid cascade hypothesis, an imbalance between Aß production and clearance plays a critical role in progression of AD. In ADAD, mutations in APP, PSEN1 and PSEN2, or cases with an extra copy of APP on chromosome 21 in Down syndrome, are thought to result in increased APP processing. On the other hand, the current studies suggest that in sporadic AD, there are other genes as *APOE* among others that may be involved in reducing Aß clearance [148].

Aß: Amyloid protein; ABCA7: ATP-Binding Cassette Sub-Family A Member 7 gene; ADAM10: ADAM metallopeptidase domain 10; AICD: Amyloid intracellular domain; APOE: Apolipoprotein E; APP: Amyloid precursor protein, ß -CTF: ß-cleaved carboxy-terminal fragment of APP; CAA: Cerebral amyloid angiopathy; CLU: Clusterin; CR1: complement C3b/C4b receptor 1; LTP: Hippocampal long term potentiation; N-APP: N-terminal fragment of APP; PICALM: phosphatidylinositol binding clathrin assembly protein; PSEN1: Presenilin 1; PSEN2: Presenilin 2; sAPPß: soluble fragment of APP; SORL1: Sortilin-related receptor; TREM2: triggering receptor expressed on myeloid cells 2.

Down syndrome (DS) is now also recognized as a form of genetically determined AD caused by the triplication of the *APP* gene, among others [149]. At age 40 all individuals with DS have the typical AD neuropathological hallmarks [150], and the prevalence of dementia increases exponentially thereafter, reaching 80% in the seventh decade [151]. By contrast, although most post mortem examinations of people with DS over the age of 50 show moderate or severe CAA [152–155], CAA is not a universal finding in people with DS [152]. In fact, individuals with DS appear to have a lower risk for developing CAA-related ICH and to develop less severe CAA compared to families with APP duplications, despite the fact they also have three copies of the *APP* gene [156]. This could be due to the overall protective effect of the other genes located on chromosome 21. Difference in the clearance of Aß and other factors such as the lower prevalence of atherosclerosis, lower blood pressure, or differences in the oxidative stress, or neuroinflammation could also play a role. There are limited data in respect to the importance of *APOE* genotype as a risk factor for CAA-related ICH in DS or ADAD [156–159].

Despite the different genetic background, the AD neuropathological findings in sporadic AD, ADAD and DS are very similar [160,161]. Previous neuropathological studies have suggested that CAA might be more severe in ADAD than in sporadic AD [161–163], however, to our knowledge, a systematic assessment of the modified Boston criteria in ADAD and DS has not been reported. The role of CSF biomarkers and *APOE*-ε4 allele on CAA in genetically determined forms of AD has yet to be established.





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Chapter 2

# Outline and introduction

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## GENERAL HYPOTHESES

Cerebral amyloid angiopaghy (CAA) is a polymorphic entity that underlies a diverse range of clinical conditions beyond the well-reported association with spontaneous intracerebral hemorrhage. While a major cause of cognitive impairment in the elderly, very frequently in association with Alzheimer's disease, CAA can also produce distinct and rare clinical entities that should be recognized in clinical practice.

## HYPOTHESES

**1.** CAA is the main underlying pathophysiological substrate of convexal subarachnoid hemorrhage in the elderly

**2.** CAA-related inflammation occurs as an immune reaction against vascular ß-amyloid deposits. Cerebrospinal fluid biomarkers and amyloid positron emission tomography can inform on the underlying pathophysiological process

**3.** Genetically determined forms of Alzheimer's disease have more extended CAA than sporadic early onset Alzheimer's disease. These differences are modulated by *APOE* genotype and reflected by different cerebrospinal fluid profiles

## OBJECTIVES

The general objective of this thesis is to study the CAA pathophysiology and CAA clinical entities through biomarkers. Specifically the objective of the three articles included in this thesis were:

**1.** To characterize the clinical spectrum, prognosis, and pathophysiologic mecanisms of CAA-related convexal subarachnoid hemorrhage

**2.** To study cerebrospinal fluid core Alzheimer's disease biomarkers, cerebrospinal fluid anti-Aß autoantibodies and amyloid positron emission tomography in subjects with CAA-related inflammation

**3.** To assess CAA neuroimaging features, *APOE* genotype, and cerebrospinal fluid biomarkers (Aß42, Aß40) in sporadic early onset Alzheimer's disease, autosomal dominant Alzheimer's disease, and Down syndrome.

Chapter 3

# Cerebral amyloid angiopathy-related atraumatic convexal subarachnoid hemorrhage: an ARIA before the tsunami

Chapter 3

# Cerebral amyloid angiopathy-related atraumatic convexal subarachnoid hemorrhage: an ARIA before the tsunami

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## ABSTRACT

Atraumatic convexal subarachnoid hemorrhage (cSAH) in elderly patients is a rare entity that has been associated with cerebral amyloid angiopathy (CAA) and intracerebral hematomas (ICH). To characterize this entity and to study these associations, 22 patients over 60 with cSAH were included in a multicenter ambispective cohort study. Clinical data, magnetic resonance imaging (MRI) studies, APOE genotyping, and cerebrospinal fluid (CSF) biomarkers were evaluated. Results were compared with data from healthy controls (HC), non-cSAH CAA patients (CAAo), and Alzheimer disease patients. Convexal subarachnoid hemorrhage presented with transient sensory or motor symptoms. At follow-up (median 30.7 months), 5 patients had died, 6 survivors showed functional disability (modified Rankins Scale (mRS)>2), and 12 cognitive impairment. Four patients had prior ICH and six had an ICH during follow-up. CSF-Aß40 and Aß42 levels were lower in cSAH and CAAo compared with HC. Convexal subarachnoid hemorrhage presented an APOE-ε2 overrepresentation and CAAo had an APOE-ε4 overrepresentation. On MRI, all patients fulfilled CAA-modified Boston criteria; nine showed cortical ischemia in the surrounding cortex or the vicinity of superficial siderosis. The neuropathologic study, available in one patient, showed severe CAA and advanced Alzheimer-type pathology. Convexal subarachnoid hemorrhage in the elderly is associated with cognitive impairment and lobar ICH occurrence. Our findings support the existence of an underlying CAA pathology.



## KEY WORDS

*APOE*; biomarkers; cerebral amyloid angiopathy; lobar hemorrhage; subarachnoid hemorrhage.

## 1. INTRODUCCION

Atraumatic convexal subarachnoid hemorrhage (cSAH) is a rare type of cerebrovascular disease. Bleeding in cSAH is located in one or several adjacent sulci in the convexity of the brain. Two subtypes of cSAH have been proposed. The first subtype typically presents in young patients and has been associated with cerebral reversible vasoconstriction syndrome, while the second is usually observed in older patients and has been associated with cerebral amyloid angiopathy (CAA) and future lobar spontaneous intracerebral hemorrhage (ICH) [1,2].

The term CAA is used to describe the pathologic changes occurring in cortical and leptomeningeal cerebral vessels as the result of the deposition of ß-amyloid proteins (Aß). The term ARIA (amyloid-related imaging abnormalities) refers to a spectrum of imaging abnormalities in the setting of amyloid-modifying therapeutic approaches. It includes signal changes thought to represent 'vasogenic edema', microhemorrhages, or cortical superficial siderosis (cSS) [3]. The shift of amyloid in the perivascular spaces of small vessels is the common pathophysiologic mechanism underlying these phenomena [3,4]. Vasogenic edema may occur rarely in the natural history of AD, and more commonly, in patients with presumed CAA [3].

The diagnostic criteria for CAA and ARIA rely on magnetic resonance imaging (MRI) findings [3–5]. Nonetheless, few recent studies have used other biomarkers [6–9]. The *APOE* genotype is central in the pathogenesis of CAA and ICH, and also in Alzheimer's disease (AD) [10–13].

Convexal subarachnoid hemorrhage has been associated with lobar ICH in previous studies [2,14,15]. It has been proposed that the bleeding begins in the subarachnoid space in a subset of ICH [16,17]. The rupture of arteries in the subarachnoid space would cause an initial bleeding contained by the surrounding cortex, which might develop ischemia. Subsequently, the hemorrhage would extend to the brain parenchyma. Convexal subarachnoid hemorrhage itself has been proposed as supportive of this theory [18].

The objectives of this multicentric study were therefore threefold. First, to bet-

ter characterize the clinical spectrum and prognosis of CAA-related cSAH in the elderly. Second, to show its association with CAA in a neuroimaging, genetic, and cerebrospinal fluid (CSF) biomarker study. Third, to study its association with ICH and to find evidence of acute or chronic cortical ischemia associated with the cSAH, which would support the aforementioned theory.





# 2. MATERIALS AND METHODS

## 2.1. STUDY PARTICIPANTS AND DATA COLLECTION

Ambispective observational study of a convenient cohort from five tertiary care hospitals in Barcelona, Spain. Patients were recruited between 2008 and 2013. Radiologic reports were reviewed to screen those with a potential cSAH.

We included patients over 60 years who met the Kumar description for cSAH, in which the bleeding localized in the convexities of the brain without involvement of the adjacent parenchyma or extension into the interhemispheric fissures, basal cisterns, or ventricles.1 Medical records, laboratory data, and imaging studies were sent to the coordinating center for evaluation—(HSP (Hospital de la Santa Creu i Sant Pau))—where a centralized readout of the computed tomography (CT) or MRI images, blinded to clinical, genetic, and CSF data, was performed. Follow-up information was obtained by outpatient consultation or telephone interview.

We suggested the cSAH patients to undergo a formal cognitive evaluation, a lumbar puncture, and *APOE* genotyping. For biochemical studies, CSF samples from non-cSAH CAA patients (CAAo), AD patients, and healthy controls (HC) were used. For genetic studies, DNA samples from CAAo patients, AD patients, and HC were selected from the Memory Unit of HSP.

We reviewed CAA patients from our Memory Unit cohort with available echo gradient or susceptibility MRI images without cSAH to select CAAo patients. We selected patients who met the modified Boston criteria for probable CAA and with genetic and CSF data available. Twelve patients were identified. They all were assessed because of cognitive impairment. In addition, 6/12 had presented with focal neurologic symptoms (visual disturbances in 3, seizures in 2, and a frontal syndrome in 1). More information is provided in Supplementary Material.

Patient outcome at follow-up was graded following the modified Rankins Scale (mRS).

The ethics committee from Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) approved the study.

## 2.2. COGNITIVE ASSESSMENT

We suggested every cSAH patient to complete the study with a formal cognitive evaluation (Supplementary Table 1).

## 2.3. NEUROIMAGING DATA

All cSAH patients underwent a brain CT at admission in the emergency room and an MRI sometime during the process. All MRI scans included fluid attenuated inversion recovery, T2, T2*-GRE, and diffusion-weighted images (DWI). Some of the patients had also an angiographic study.

We assessed the modified Boston criteria for possible or probable CAA [5] in every patient as well as other CAA-associated features. The systematic evaluation of the MRI scans is detailed in Supplementary Material.

## 2.4. CEREBROSPINAL FLUID BIOMARKERS AND GENETICS

In cSAH patients who consented to a lumbar puncture, core CSF (AD) biomarkers (Aß42, total-Tau-t-Tau- and phosphorylated Tau at threonine-181-p-Tau-) and Aß40 were determined. The CSF samples from 44 AD patients, 12 CAAo, and 20 HC were used for comparison.

*APOE* genotype was determined in cSAH patients who consented. Blood samples from 539 AD patients, 12 CAAo, and 324 HC selected from our Memory Unit were used for comparison.

Methods for CSF acquisition and biomarker measurement are detailed in Supplementary Material.

## 2.5. NEUROPATHOLOGIC STUDY

Postmortem neuropathologic brain examination of one patient was performed, according to standardized protocols [19].





## 2.6. STATISTICAL METHODS

Statistical analyses were performed with the software Statistical Package for the Social Sciences v19 (http://www-01.ibm.com/software/es/analytics/spss/). Allele frequencies (proportion of chromosomes in which the allele was present) were compared using $2 \times 2$ tables with Fisher's exact test for significance. To study the differences of CSF biomarker levels between groups, we used the Mann–Whitney U test. Statistical significance for all the analyses was set at 5% ($\alpha = 0.05$).

# 3. RESULTS

## 3.1. CLINICAL FINDINGS

Twenty-two patients with cSAH were included. Table 1 contains available data and Table 2 contains demographic data and neuroimaging findings.

**TABLE 1. DATA OF CSAH PATIENTS**

| CHARACTERISTICS | VALUES |
|---|---|
| **IMAGING AVAILABLE** | |
| CT | 22 |
| MRI (images available/only reports) | 20/2 |
| Conventional angiography/ Angio CT/MRA, No. | 3/4/10 |
| **CSF BIOMARKERS (NUMBER OF SUBJECTS)** | |
| cSAH group | 7 |
| HC group | 20 |
| AD group | 44 |
| **APOE GENOTYPE (NUMBER OF SUBJECTS)** | |
| cSAH group | 13 |
| HC group | 324 |
| AD group | 539 |

AD, Alzheimer's disease; cSAH, convexal subarachnoid hemorrhage; CSF, cerebrospinal fluid; CT, computed tomography; HC, healthy control; MRI, magnetic resonance imaging.

The most frequent clinical presentation consisted of transient episodes of acute sensory and motor symptoms. In most cases they lasted less than 30 minutes. The episodes were usually repetitive and stereotyped (16 patients) and remitted completely. One patient died during admission because of an ICH 2 days after the cSAH.





Symptoms were related to cSAH topography, being negative ('stroke-like') in 6, positive in 4 ('prolonged aura-like'), and combined (positive and negative symptoms) in 12 patients. The most common positive symptoms were paresthesias (12 patients), affecting mouth and hand in all cases, and dysarthria (11 patients). Other less common symptoms included aphasia (5 patients), right cortical signs (2 patients), limb-jerking episodes (2 patients), and arm stiffness sensation (1 patient). None of our patients had thunderclap headache (2 presented non-specific headache). One patient had a generalized tonicoclonic seizure. Surface electroencephalography was performed in 11 patients. Epileptiform activity was only found in 1 patient.

At least one vascular risk factor was present in all patients (77.3% had hypertension, 54.5% had dyslipidemia, and 18.2% had diabetes mellitus). Five patients had a history of heart attack, and six of them had a history of previous stroke (four with lobar hematomas, all of them symptomatic). Eleven patients were taking antithrombotic drugs at the time of cSAH (nine antiplatelets and two anticoagulant drugs, both in the normal range of international normalized ratios—between 2 and 3).

## 3.2. NEUROPSYCHOLOGICAL FEATURES

Ten cSAH patients consented to a neuropsychologic assessment.

At the time of the cSAH, 6 patients had a diagnosis of mild cognitive impairment (MCI) and 2 of mixed dementia. During the follow-up, 6 previously unimpaired patients were diagnosed with MCI and 1 with dementia. Four further MCI patients progressed to dementia. From the 10 patients formally evaluated, 1 was cognitively spared, 8 had memory impairment (6 with involvement of additional cognitive domains), and 1 had only executive dysfunction (details in Supplementary Table 1). The neuropsychologic profile was heterogeneous as was the degree of cognitive impairment (Clinical Dementia Rating –sum of boxes ranging from 0.5 to 12). In 4 of the 10, there were no reports of cognitive impairment before the cognitive evaluation.

## 3.3. IMAGING FINDINGS

Each patient had an MRI sometime during the process; 16/22 were performed in the acute phase before discharge (median time 4 days; range 0 to 11 days). MRI was deferred in three patients, specifically for this study (0.16, 0.63, and 2.40 years after the cSAH). Three patients had an MRI before the cSAH and had already been diagnosed with CAA-related ICH (3.5 years, 2 years, and 7 days before the cSAH).

Conventional angiography was performed in 3 patients, MR angiography in 10, CT angiography in 4, or both MR and CT in 1. The angiographic study was negative in all cases except in 2 patients who had an aneurysm affecting the contralateral vertebral artery and the posterior cerebral artery respectively, interpreted as incidental.

Neuroimaging data are summarized in Table 2 and Supplementary Table 2. CT detected the acute cSAH in all but 1 subject, who was later diagnosed by MRI fluid attenuated inversion recovery images.

**TABLE 2. GLOBAL DEMOGRAPHICS AND NEUROIMAGING FINDINGS IN THE COHORT OF CSAH PATIENTS.**

| CHARACTERISTICS | NO. OF PATIENTS |
|---|---|
| No. of cSAH patients | **22** |
| Median age, y (Range) | 79.1 (69.7-92.2) |
| Gender (men) | 13 |
| **VASCULAR RISK FACTORS** | |
| Arterial hypertension | 17 |
| Dyslipidemia | 12 |
| Diabetes Mellitus | 4 |
| Previous acute myocardial infarction | 5 |
| Previous stroke (lobar hematoma) | 6 (4) |
| **PREVIOUS ANTITHROMBOTIC TREATMENT** | |
| Antiplatelet drugs | 9 |
| Anticoagulant drugs | 2 |
| **FOLLOW-UP INFORMATION AVAILABLE** | **21** |
| Median of follow-up, range | 30,7 months (0,07-49,25) |
| Recurrent cSAH | 0 |
| MCI | 8 |
| Dementia | 7 |
| Intracerebral lobar hemorrhage | 6 |
| Functional dependence (mRS≥4) | 11 |
| Deaths | 5 |
| **CSAH** | **22** |
| Affecting 1 sulcus | 18 |
| Affecting ³2 sulci | 4 |
| **CEREBRAL MICROBLEEDS** | **8** |
| Location Lobar | 8 |
| Lobar and deep | 1 |
| Number 1-5 | 5 |
| 5-10 | 0 |
| >10 | 3 |
| **CORTICAL SUPERFICIAL SIDEROSIS** | **19** |
| Focal (<4 sulci) | 3 |
| Disseminated (≥4 sulci) | 16 |
| **HISTORY OF STROKE** | **6** |
| Lobar intracerebral hemorrhage | 4 |
| Lacunar stroke | 3 |

| | |
|---|---|
| Large vessel ischemic stroke | 2 |
| **LEUKOARAIOSIS** | **21** |
| Fazekas score 1 | 4 |
| Fazekas score 2 | 7 |
| Fazekas score 3 | 10 |
| **MEDIAL TEMPORAL LOBE ATROPHY** | **3** |
| MTA ≤2 | 15 |
| MTA >2 | 3 |

cSAH, convexal subarachnoid hemorrhage; MCI, mild cognitive impairment, mRS, modified Rankin Scale; MTA, medial temporal lobe atrophy.

## 3.4. MODIFIED BOSTON CRITERIA FOR CEREBRAL AMYLOID ANGIOPATHY AND SYSTEMATIC EVALUATION OF THE MAGNETIC RESONANCE IMAGING

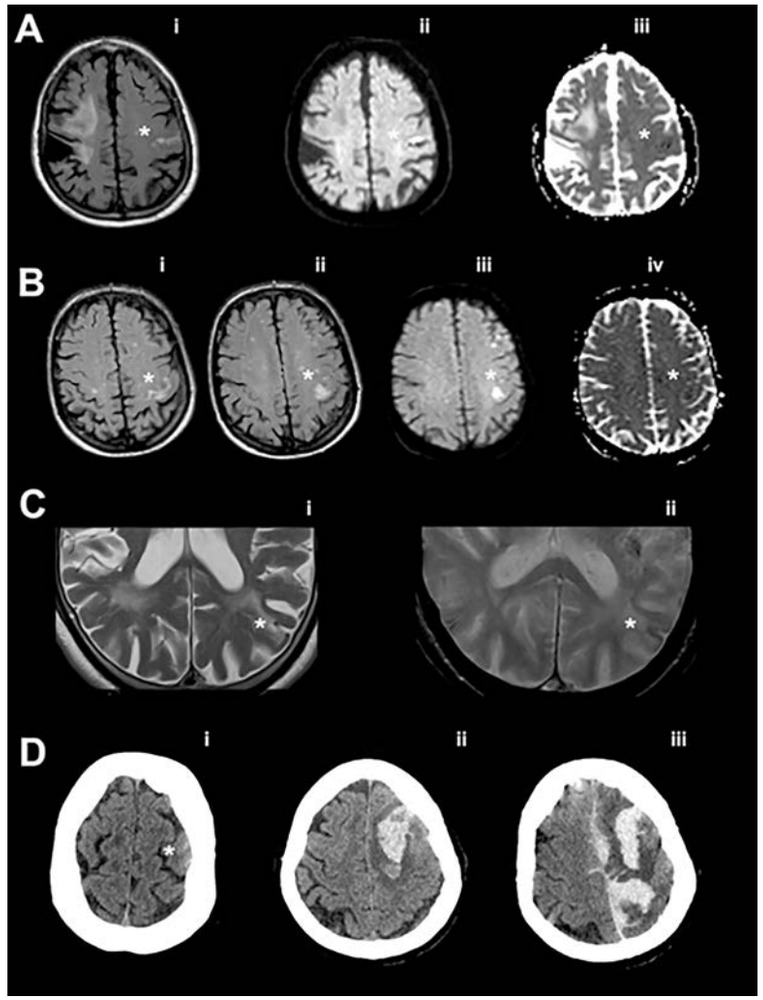
Thirteen patients met the diagnosis of possible CAA and nine probable CAA. Radiologic findings are shown in Table 2.

Sixteen patients underwent an MRI during the acute phase before discharge that included DWI sequences. The DWI showed restricted diffusion involving the adjacent cortex surrounding the cSAH in 7 patients (Figure 1, Supplementary Figure 1). Moreover, 2 patients had chronic cortical infarcts in the vicinity of cSS or cSAH.





**FIGURE 1: ATRAUMATIC CONVEXAL SUBARACHNOID HEMORRHAGE (CSAH)-RELATED ISCHEMIA AND INTRACEREBRAL HEMATOMAS IN DIFFERENT PATIENTS.**




**(A)** Magnetic resonance imaging (MRI) performed 3 days after the onset of the symptoms showing the cSAH on the left precentral sulcus and a chronic right fronto-parietal ischemic stroke (fluid attenuated inversion recovery sequence) (i). Diffusion-weighted image (DWI) (ii) and aparent diffusion coefficient (iii) sequences show acute ischemia that colocalizes with the cSAH.

**(B)** MRI performed 1 week after the onset of the symptoms showing the cSAH and subacute ischemia on the underlying cortex. Fluid attenuated inversion recovery sequences show cSAH on left precentral sulcus (i) and a hyperintense lesion located on the underlying cortex (ii). This area shows restricted diffusion on DWI (iii) and hypointense signal on apparent diffusion coefficient (iv).

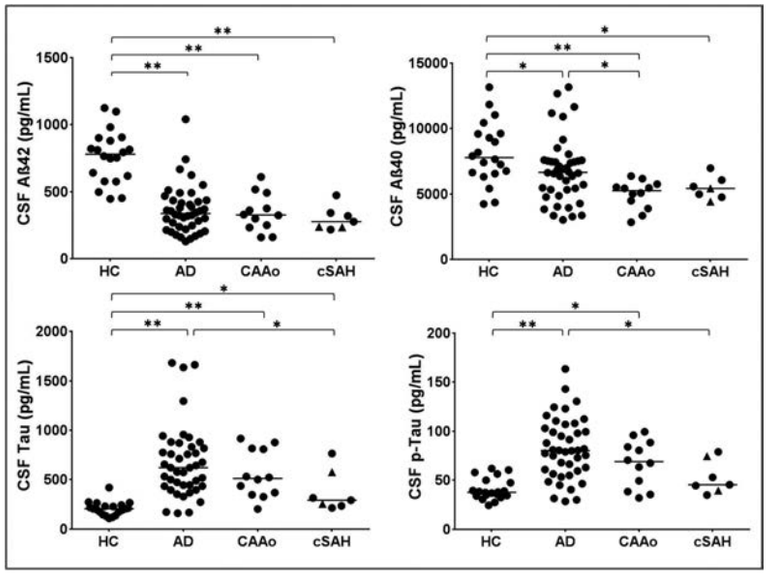
**(C)** MRI demonstrating a pure chronic cortical infarction affecting left posterior parietal cortex. T2-weighted sequence (i) shows the presence of a focal laminar cortical lesion (star). This region colocalizes with an area of cortical superficial siderosis (cSS), as shown in T2*-GRE weighted sequence (ii).

**(D)** cSAH-related intracerebral hemorrhages. (i) Computed tomography (CT) showing an acute cSAH on the superior frontal sulcus. (ii) Three months later, the patient presented a frontal ICH that colocalized with the previous cSAH (iii). Sixteen hours later, the patient's clinical state worsened due to an ICH expansion that led to death after 2 days.

## 3.5. CEREBROSPINAL FLUID BIOMARKERS AND APOE GENOTYPE

Cerebrospinal fluid was available in 7 cSAH patients. The median time between the vascular event and the lumbar puncture was 16 months (range 2 to 33 months). The results are shown in Figure 2 and Table 3.

**FIGURE 2. CEREBROSPINAL FLUID (CSF) STUDY.**




Healthy controls (HC), Alzheimer disease (AD) patients, cerebral amyloid angiopathy non-cSAH patients (CAAo), cSAH patients (cSAH). P-values o0.05 are marked with a star (*); P-valueso0.001 are marked with double star (**). cSAH patients with a previous intracerebral hemorrhage (ICH) are highlighted using a triangled symbol. cSAH, convexal subarachnoid hemorrhage.





**TABLE 3. DEMOGRAPHICS, CSF, AND APOE GENOTYPE DATA OF THE DIFFERENT CLINICAL GROUPS.**

| | cSAH | CAAo | AD | | HC | |
|---|---|---|---|---|---|---|
| | | | CSF | *APOE* genotype | CSF | *APOE* genotype |
| **N** | 22 | 12 | 42 | 539 | 20 | 324 |
| **MEAN AGE, YEARS (RANGE)** | 79.1 (69.7- 92.2) | 69.8 (52.1-83.7) | 67.6 (50.6-79.8) | 79 (30.7-93.7) | 66.5 (55.7-77.5) | 72.3 (45-89) |
| **GENDER (%MALES)** | 59 | 33 | 71 | 33.8 | 45 | 65.7 |
| **CSF Aß42** | 276 (•) (107) | 325.75 (225.5) | 336.25 (406.13) | *N/A* | 779.5 (308.6) | *N/A* |
| **CSF Aß40** | 5422.28 (•) (1307.5) | 5247.1 (1927.6) | 6656.1 (2329.5) | *N/A* | 7775.5 (3041.1) | *N/A* |
| **CSF Tau** | 292.5 (•) (340) | 512 (461.25) | 620.75 (406.13) | *N/A* | 205 (87) | *N/A* |
| **CSF p-Tau** | 45.5 (•) (35) | 69 (46.13) | 80.25 (45.25) | *N/A* | 37.75 (15.25) | *N/A* |
| ***APOE*-E2** | 19.2 (••) | 4.2 | *N/A* | 3.4* | *N/A* | 5.4* |
| ***APOE*-E3** | 69.2 (••) | 37.5* | *N/A* | 71.1 | *N/A* | 84 |
| ***APOE*-E4** | 11.5 (••) | 58.3* | *N/A* | 25.5 | *N/A* | 10.6 |

AD, Alzheimer's disease; cAAo, cerebral amyloid angiopatthy non-cSAH; cSAH, atraumatic convexal subarachnoid hemorrhage; CSF, cerebrospinal fluid; HC, healthy controls. - CSF study, median values (IQ range) in pg/mL. Performed on 7/22 ((•)). - APOE genotype study, allele frequency (%). Performed on 13/22 ((••)). P-valueso0.05 are marked with a star (*) when compared with cSAH group. Main results for *APOE*-ε2 are cSAH versus AD (P=0.002); cSAH versus HC (P= 0.01); cSAH versus CAAo (P= 0.192). Main results for APOE-ε4 are cSAH versus CAAo (P= 0.001); CAAo versus AD (P=0.001); CAAo versus HC (Po0.001); and AD versus HC (Po0.001).

The CSF profile was similar in cSAH and CAAo patients. We found significant reductions in CSF Aß42 and Aß40 levels, elevations in CSF t-Tau, and a trend in p-Tau with respect to HC. The AD patients showed significantly higher levels of t-Tau and p-Tau than cSAH and CAAo patients but no differences in CSF Aß42 and Aß40.

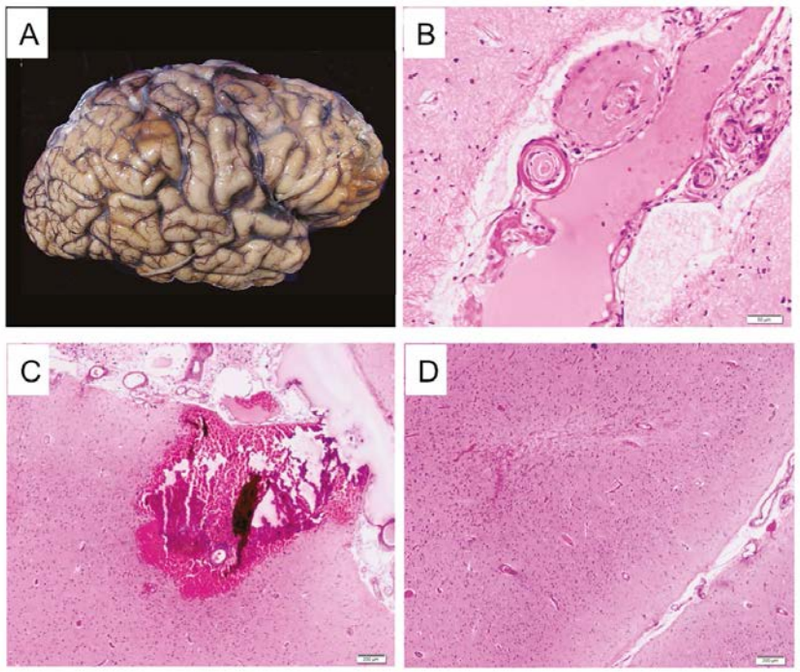
*APOE* genotype determination was performed in 13 cSAH patients. The *APOE* genotypes are summarized in Table 3. *APOE*-ε2 (allelic frequency of 19.2%) was significantly overrepresented in the cSAH group with respect to HC (5.4%; odds

ratio 4.17). *APOE*-ε4 was significantly overrepresented in the CAAo group. There was no association of cSAH with *APOE*-ε4 genotype. The AD patients showed the expected underrepresentation of APOE-ε2 (3.4%) and overrepresentation of APOE-ε4 (25.5%) compared with HC.

### 3.5. NEUROPATHOLOGIC STUDY

Pathologic brain examination was conducted in 1 cSAH patient. It showed neuropathologic changes associated with severe AD, severe amyloid angiopathy with capilar involvement, extense leptomeningeal siderosis, cortical microbleeds, and multiple intracortical microinfarcts (Figure 3) (Clinical and neuropathologic data are available in Supplementary Material).

**FIGURE 3: NEUROPATHOLOGIC FINDINGS.**




(A) External view of the fixed right cerebral hemisphere showing a yellow-brownish discoloration of basal temporal lobe and frontopolar and parietal region. (B) Prominent alterations of the vascular wall of large leptomeningeal vessels with concentric hyaline thickening and double barrel morphology (H&E). (C and D) Parenchymal lesions consisting in small fresh cortical superficial bleedings (C) and intracortical microinfarcts (D) in areas underlying prominent leptomeningeal amyloid angiopathy (H&E).





## 4. DISCUSSION

To our knowledge, this is the first study evaluating genetic and CSF biomarkers in cSAH in elderly people. Our main findings were as follows. First, the clinical presentation in CAA-related cSAH was fairly homogenous and associated with poor prognosis because of its frequent association with ICH and cognitive impairment in the follow-up. Second, our CSF results, neuroimaging data, the association with *APOE*-ε2 and lobar ICH suggest that CAA is the underlying cause of cSAH in our patients, as confirmed by postmortem brain examination in one subject. And third, the evidence for acute cortical ischemia in the cortex adjacent to the cSAH could support the theory that ICH in some of these patients might originate in the subarachnoid space and progress through an ischemic cortex into the brain.

The clinical presentation in our series was congruent with previous descriptions; consisted of transient focal neurologic symptoms related to the topography of the cSAH [20]. These symptoms were mostly recurrent, stereotyped, and brief. This transient presentation probably contributes to making cSAH an underdiagnosed entity [2]. There are discrepancies in the literature regarding the pathophysiology of the symptoms. Epileptic and migraine aura-like cortical spreading depression mechanisms have been proposed, but other mechanisms are possible [15,20]. However, the lack of abnormal electroencephalography findings in our study and the finding of cSAH-related acute cortical ischemia—which has been associated with spreading depression in aneurysmatic SAH and stroke—broadly favor the nonepileptic mechanism[21].

Cerebral amyloid angiopathy can be associated with both MCI and full-blown dementia [22,23]. The pattern of cognitive impairment is related to the underlying mechanisms (CAA-related ICH or infarcts, leukoaraiosis, Alzheimer-type pathology, or CAA by itself) [22]. We were able to identify all these mechanisms in our patients, explaining both the very high prevalence and incidence of cognitive impairment and the different patterns and degrees observed. No imaging follow up was performed in non-(new) symptomatic patients. Although cSAH did not

recur, the frequent occurrence of ICH and cognitive impairment explains the poor outcome in our patients. Therefore, while previous literature suggested that cSAH had a favorable prognosis the only study with a long follow-up reported a poor outcome in cSAH, especially in those patients with neuroimaging findings compatible with CAA [2,15,24].

Our neuroimaging, genetic, and CSF biomarker findings support the hypothesis that CAA is the underlying cause of cSAH in our patients. On MRI, 40.9% of patients fulfilled the modified Boston Criteria for probable CAA and 51.1% for possible CAA. Microbleeds and cSS might be related to different pathophysiologies [25]. We found a high frequency of disseminated cSS beyond the cSAH location, in agreement with previous studies in which transient focal neurologic episodes were the strongest clinical marker for cSS [5,26]. These figures, however, greatly differ from epidemiologic studies of AD in which cSS is found in only 1% of cases [27,28]. Microbleeds were found in 40%, in contrast to a prevalence of 68% in ICH series and 25% in AD series [28,29]. These observations could be contextualized within the idea highlighted by Charidimou and Jäger, in which several different endophtenotypes of CAA may exist. Our patients would fit in the group of 'macrobleeders', where there is a low microbleed count and cSS would be associated itself with high risk of hemorrhage [25,30].

The CSF profile in cSAH patients characterized by reductions in Aß40 and Aß42 is consistent with the profile reported in CAA [7,8]. This pattern was previously found in two cSAH patients and in four cSS patients [8,9]. The mild elevation in CSF t-Tau and p-Tau levels (significantly lower than in AD patients) is consistent with a lower level of tau-containing pathology in CAA. However, in the patient with neuropathologic study, extensive tau-positive neurofibrillary pathology was observed. We also found an overrepresentation of the *APOE*-ε2 allele in cSAH but not of *APOE*-ε4. Although there are no previous genetic studies in cSAH, APOE-ε2 and APOE-ε4 are independent risk factors for ICH [12,13]. The fact that the CSF profile is similar in cSAH and CAAo patients indicates that there is an underlying CAA but also raises the possibility that mechanisms other than amyloid burden may have a role in cortical and leptomeningeal vessel fragilty leading to cSAH and cSS.





*APOE*-ε2 and *APOE*-ε4 might promote CAA-related hemorrhage through separate mechanisms: APOE-ε4 would enhance amyloid deposition, whereas *APOE*-ε2 would favor amyloid-laden vessels to undergo the vasculopathic changes that lead to rupture [31]. This hypothesis would explain the influence of *APOE*-ε2, but not of *APOE*-ε4 on ICH expansion [12].

The high association of cSAH with ICH in our series together with the observation of acute cortical ischemia in the vicinity of cSAH (which would enable the extension of the hematoma to the brain parenchyma) could be an in vivo evidence for the hypothesis of Takeda et al [16,17] concerning CAA-related ICH pathogenesis [32]. Our results are also compatible with the 'tsunami hypothesis' to explain hematoma growth. According to this theory, the brain blood vessels surrounding the initial bleeding in *APOE*-ε2 carriers are more prone to bleeding themselves, and contribute to hematoma enlargement [12,33,34]. In our study, the ICH was located in the vicinity of a previous cSAH (four patients) or cSS (two patients), a colocalization that has also been found in cSS [35]. Therefore, cSS might be a marker of vessel fragilty [26]. In fact, cSS, particularly if disseminated, has also been associated with an increased risk of symptomatic lobar ICH [26]. The chronic cortical infarct found in the vicinity of cSS also supports Takeda's theory. Of note, focal laminar cortical lesions after aneurysmatic SAH in the absence of macroscopic vasospasm are more common than territorial infarcts [36,37].

The present study has some limitations. First of all, we used a convenient sample. This could affect the generalizability of the findings because our sample might not be representative of the general population. All patients who presented with cSAH in the emergency room or in the clinics between 200 and 2013 were included, so our sample equates to a great extent of the elder population in Barcelona. Second, the sample sizes in the biomarker subgroups were small. However, significant differences were found in the CSF and in the genetic analyses. Third, although the mean follow-up was 2 years, some patients had a shorter follow-up. However, cSAH was clearly associated with poor prognosis. Fourth, regarding the areas of restricted diffusion in close proximity to cSAH, one concern is that they might not necessarily reflect acute microinfarction, but instead

restricted diffusion due to the presence of acute blood products [38]. The DWI signal intensity changes should be interpreted in light of the T2 signal intensity changes, especially at the earliest time point, when both, hematoma and acute ischemia, are hyperintense on DWI images. At the acute stage, hematomas are hypointense on T2- weighted images [38]. The fact that our cSAH was hyperintense on T2-weighted images suggests the presence of acute cortical ischemia in the vicinity of cSAH, more than restricted diffusion due to the presence of acute blood products. Furthermore, in some of the cases, chronic infarctions were found in the vicinity of SS both in the MRI and in the pathologic examination. On the other side, the association between cSAH or cSS and ischemic lesions might just indicate focally active severe CAA-related disease and the observation of cortical ischemia could be due to microvascular spasm, cerebral autoregulation impairment or other mechanisms. Those findings should be taken with caution and require further study to be confirmed. Finally, definite histologic CAA diagnosis, was only available in one patient.

In conclusion, our biomarker and genetic study supports an underlying CAA pathology for cSAH in elderly patients. Convexal subarachnoid hemorrhage might be considered a warning sign for poor prognosis because of its frequent association with cognitive impairment and ICH. The frequent lobar ICH in these patients might originate in the subarachnoid space and progress through an ischemic cortex into the brain. However, further studies with neuropathologic confirmation are needed to confirm these hypotheses.





## 5. DISCLOSURES AND ACKNOWLEDGMENTS

All authors report no biomedical financial interests or potential conflicts of interest related to this work.

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# 7. SUPPLEMENTAL MATERIAL

## 7.1. SUPPLEMENTAL METHODS

**Study participants and data collection:**

Healthy controls (HC), Alzheimer´s disease (AD) and non-cSAH CAA (CAAo) patients were recruited at the Memory Unit in HSP as part of our biomarker research program. The program has enrolled over 400 HC and patients with core CSF biomarkers and *APOE* genotype. All participants were evaluated by neurologists with expertise in neurodegenerative diseases, and all underwent formal cognitive evaluation using a previously published neuropsychological battery [1].

- ***AD patients:*** met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [2].
- ***HC:*** Subjects were classified as HC when they did not have cognitive complaints and the results of the neuropsychological evaluation were in the normal range for age and education.
- ***CAAo patients:*** Subjects that fulfilled modified Boston criteria for probable CAA with available genetic and CSF data were selected from our memory unit cohort. Twelve patients were included; all of them had cortical microbleeds and two, in addition, cortical superficial sierosis (cSS) in the echo-gradient or susceptibility MRI images. All of them had lobar microbleeds and two had, in addition, cortical superficial siderosis on MRI. Three of them had had a symptomatic ICH.

**Methods for MRI systematic evaluation:**

The evaluation included the exact location and the extension of cSAH (Supplementary table 2), as well as the presence of cSS disseminated from the cSAH. cSAH and cSS were further classified as focal (limited to less than 4 sulci) or disseminated (affecting 4 or more sulci). The presence of previous ICH and microbleeds was assessed in the T2*-GRE sequences. Low microbleed-count was defined as 1 to 5 microbleeds. Lacunar infarcts or previous large vessel ischemic strokes were assessed on T1-weighted or FLAIR images. Leukoaraiosis and medial temporal lobe atrophy were semi-quantitatively assessed according to the Fazekas scale in the FLAIR sequences and the visual rating assessment protocol described by Scheltens (MTA) in the T1 weighted images, respectively [3, 4].

In order to study the possible presence of ischemia in the surrounding cerebral cortex in cSAH, the MRI in the acute phase were further analysed for signs of ischemia in the DWI sequences. Moreover, the T2-weighted images were reviewed to search for small cortical infarcts in the vicinity of the cSAH or cSS.

The MRI parameters for T2*-GRE MRI were:

- Field strength: 1,5T in 4 centers, 3T in 1 center.
- Slice thickness was 5mm in each center.
- Echo time: 15ms in 1 center; 23 ms in two centers and 26ms in two centers.

**Methods for CSF acquisition and biomarker measurement:**

The AD and HC subset of subjects were age-matched and had been previously used in a receiver operating characteristic curve (ROC) to determine the internal cut-offs in our laboratory. The CAAo were specifically selected for this study.

CSF samples were collected following international consensus recommendations [5]. We used commercially available ELISA kits to determine levels of Aß42 (Innotest™ ß-Amyloid$_{1-42}$, Innogenetics), Aß40 (Millipore),t-Tau (Innotest™ hTAU Ag, Innogenetics) and p-Tau$_{181p}$ (Innotest™ Phospho-Tau$_{181P}$, Innogenetics). Our laboratory has experience in CSF biomarker determination and participates in the Alzheimer's Association quality control program for CSF biomarkers [6]. Our internal cut-off values to distinguish between HC and AD patients are 550 pg/ml for Aß42, 350 pg/mL for t-Tau and 61 pg/mL for p-Tau [7].

*APOE* genotyping was determined by allelic discrimination of rs429358 and rs7412 polymorphisms using the real-time polymerase chain reaction as previously described [8].

**Neuropathological study:**

*Clinical data*

We performed a neuropathological study in one of the deceased patients. In order to anonymise the patient's identity, identifiable details as age, gender and ethnicity are not detailed. The patient was visited in the Memory Unit during 16 months because of progressive cognitive impairment affecting mainly memory and language. The patient also presented involuntary movement episodes compatible with myoclonic jerks. The neuropsychological assessment showed memory impairment with involvement of other cognitive domains (language fluency,visuoperceptive and ejecutive impairment).

The MRI, performed during the rapidly progression of his cognitive decline, showed a subacute subarachnoid haemorrhage in the right central and precentral sulci, a significant diseminated cSS and a large number of yuxtacortical microbleeds (without deep microbleeds). No cortical infarcts were appreciated. According to the Modified Boston Criteria he was diagnosed with probable CAA.

The CSF biomarker determinations were Aß42=276 pg/mL; Aß40=4753.6 pg/mL; t-Tau= 315 pg/mL; p-Tau= 45.5 pg/mL. The *APOE* genotype was ε3/ε3.

Besides the complete study, we also performed an EEG that did not show periodic patterns nor epileptic graphoelements, and a 14-3-3 protein determination in CSF, that was negative.

*Neuropathological findings*

Postmortem brain examination was performed in one patient, histological images are represented in Supplemental Figure 2 (Supp-Fig.2). Unfixed brain weight was 1245 g. Macroscopically, a yellow-brownish discoloration of leptomeninges overlying fronto and temporobasal regions (Supp-Fig. 2A) and parietal convexity (Supp-Fig. 2B) was observed, along with several small millimetric cortical microbleeds on coronal sections. Histologically, there was a prominent congophilic angiopathy involving leptomeningeal (Supp-Fig. 2C) and cortical vessels (Supp-Fig. 2D) as well as capillaries, the latter mainly in parieto-occipital regions. There were also frequent hemosiderin-laden macrophages in leptomeninges. The





underlying cortical areas showed several intracortical fresh microhemorrhages (Supp-Fig. 2I), some foci with hemosiderin deposits, as well as multiple small intracortical microinfarcts (Supp-Fig. 2 J) along with a prominent diffuse subpial gliosis (Supp-Fig. 2 I). There was also a diffuse white matter rarefaction and gliotic areas accompanied by small vessel disease. Immunohistochemistry revealed intense betaA4 amyloid deposits in the walls of vessel of all calibers and in brain parenchyma (Supp-Fig 2 F, L) in form of diffuse and moderate mature plaques, involving cortical areas, limbic system, basal ganglia, brainstem and cerebellum. Moreover, abundant hyperphosphorylated tau immunoreactive neurofibrillary tangles, neuropil threads and dystrophic neurites were detected in limbic (Supp-Fig. 2 M) and neocortical areas, corresponding to a high level of Alzheimer disease neuropathologic change A3, B3, C2.[9] There were additional alpha-synuclein aggregates in form of Lewy-bodies and Lewy neurites restricted to the olfactory system.

## 7.2. SUPPLEMENTAL TABLES

**SUPPLEMENTAL TABLE 1. NEUROPSYCHOLOGICAL DETAILS.**

A neuropsychological assessment was available in 10 patients. Two neuropsychological batteries, that included tests for each cognitive domain, were administred, depending on the hospital of origin of each evaluated patient. Protocol A was administred on 8 patients, protocol B was performed on 2. Tests included were:

**Protocol A(e1):** MMSE, Interview for Deterioration in Daily Living Activities in Dementia (IDDD), Geriatric Depresion Scale (GDS), Spanish Word Accentuation Test (SWAT), Rey-Osterrieth Complex figure (Rey), CERAD Figures (CERAD-F), Poppelreuter Test, VOSP number location (VOSP), Boston Naming Test (BNT), WMS III digit-span (Digit-span), FCSRT-Buschke (FCSRT), CERAD Word List (CERAD-W), category fluency, Trail Making Test A (TMT-A), Trail Making Test B (TMT-B), Clock Drawing Test (Clock test), Global Deterioration Scale- Functional Assessment Staging (GDS-FAST), Clinical Dementia Rating (CDR).

**Protocol B:** MMSE, Lawton-Brodie Scale, Barthel index, WMS III word list (WMS-IIIw), WMS III auditive span (Auditive Span), WMS III verbal span (Verbal span), WMS III- family pictures (WSM-IIIp), category fluencies, TMT-A, Stroop Test, BNT, Clock Drawing test, Rey, VOSP silhouettes Test and VOSP-N.

| | MEMORY | EXECUTIVE FUNCTION | PRAXIS | GNOSIS | LANGUAGE | FUNCTIONAL INTERFERENCE |
|---|---|---|---|---|---|---|
| **PROTOCOL A** | **FCSRT<br>CERAD-w<br>CERAD-f<br>REY<br>Digit-span** | **Category fluency<br>TMT-A<br>TMT-B<br>Clock test** | **Rey<br>CERAD-f** | **Poppelreuter<br>VOSP** | **BNT** | **IDDD** |
| **Patient 1** | i (2) | I (1) | S | I (1) | S | I |
| **Patient 2** | i (3) | I (3) | S | I (2) | S | I |
| **Patient 3** | i (4) | I (4) | I (2) | I (2) | I (1) | I |
| **Patient 4** | i (4) | I (4) | I (1) | I (1) | S | I |
| **Patient 5** | i (3) | S | S | S | S | I |
| **Patient 6** | s | S | S | S | S | S |
| **Patient 7** | i (1) | I (3) | S | S | S | S |
| **Patient 8** | i (4) | I (3) | S | S | S | I |
| **PROTOCOL B** | **WMS III-W<br>WMS III-P<br>VERBAL SPAN<br>AUDITIVE SPAN** | **CATEGORY FLUENCY<br>TMT-A<br>STROOP TEST<br>CLOCK TEST** | **REY** | **VOSP** | **BNT** | **LAWTON-BRODIE BARTHEL INDEX** |
| **Patient 9** | S | I (2) | S | I (1) | S | S |
| **Patient 10** | I (3) | I (4) | I (1) | N/A | I (1) | I |

I: Impaired domain (number of abnormal tests).S: Spared domain. N/A: Not available data.

**SUPPLEMENTAL TABLE 2: EXACT SULCAL LOCATION OF CSAH.**

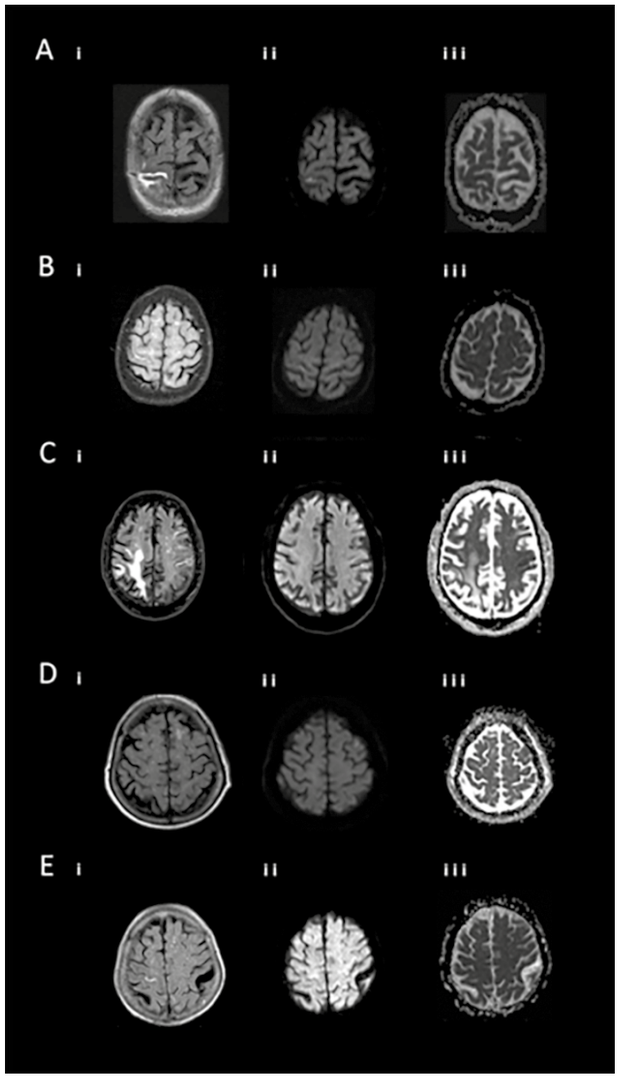
| LOCATION | LEFT HEMISPHERE | RIGHT HEMISPHERE |
|---|---|---|
| Precentral sulcus | 6 | 5 |
| Central sulcus | 2 | 2 |
| Postcentral sulcus | 2 | 0 |
| Precentral and central sulci | 0 | 1 |
| Central and postcentral sulci | 0 | 1 |
| Frontal superior sulcus | 2 | 0 |
| Temporal medial sulcus | 1 | 0 |





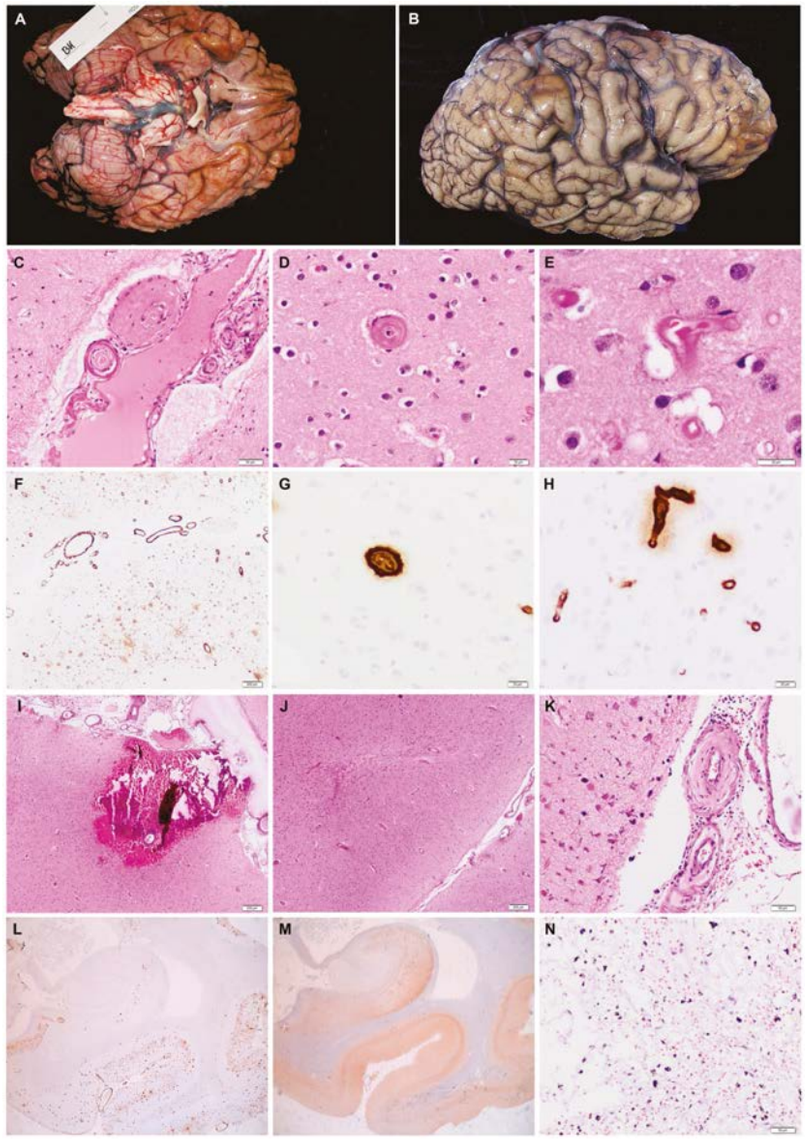
## 7.3. SUPPLEMENTAL FIGURES

**SUPPLEMENTAL FIGURE 1: ATRAUMATIC CONVEXAL SUBARACHNOID HEMORRHAGE (CSAH) RELATED ISCHEMIA. EACH LETTER REPRESENTS A DIFFERENT PATIENT.**




**(i)** The FLAIR (Fluid attenuated inversion recovery sequence) shows the cSAH.
**(ii)** The DWI (Diffusion weighted images) sequence shows the acute ischemia that colocalizes with the cSAH.
**(iii)** The ADC (Apparent diffusion coefficient) sequence demonstrates the restricted diffusivity compatible with acute ischemia.

**SUPPLEMENTAL FIGURE 2: NEUROPATHOLOGICAL FINDINGS.**




A, B: Macroscopic images. External view of the basis of the unfixed brain (A) and fixed right cerebral hemisphere (B) showing a yellow-brownish discoloration of both basal temporal lobes and orbitofrontal cortex (A) and of frontopolar and parietal region (B).

C-N representative histological images of underlying pathology: C-E: prominent alterations of the vascular wall of large leptomeningeal vessels with concentric hyaline thickening and double barrel morpholgy (C, H&E), of middle sized parenchymal vessels (D; H&E) and capillaries (E; H&E). F-H: immunohistochemistry applying anti-betaA4 amyloid antibodies reveals intense beta-amyloid angiopathy involving leptomeningeal (F) and parenchymal vessels (G) as well as capillaries (H). I-K: parenchymal lesions consisting in small fresh cortical superficial bleedings (I; H&E), intracortical microinfarcts (J; H&E) and prominent subpial gliosis (K; H&E) in areas underlying prominent leptomeningeal amyloid angiopathy. L-M: abundant parenchymal beta-amyloid deposits in hipocamppus and parahippocampal regions (L; Abeta IHC) and tau-immunoreactive neurofibrillary tangles, neuropil threads and dystrophic neurites (M; AT8 IHC). N: hemosiderin pigment in the areas of superficial siderosis (H&E).





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Chapter 4

# Cerebrospinal Fluid Anti-Amyloid-ß Autoantibodies and Amyloid PET in Cerebral Amyloid Angiopathy-Related Inflammation

Chapter 4

# Cerebrospinal Fluid Anti-Amyloid-ß Autoantibodies and Amyloid PET in Cerebral Amyloid Angiopathy-Related Inflammation

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## ABSTRACT

We report a biomarker and genetic evaluation of four patients with cerebral amyloid angiopathy-related inflammation (CAA-ri) treated with corticosteroids. Patients presented with focal symptomatology and cognitive impairment. MRI revealed cortical microbleeds and asymmetrical hyperintense white matter lesions (WML). Cerebrospinal fluid (CSF) biomarker analyses showed increased anti-Aß autoantibodies, t-Tau, and p-Tau and decreased Aß40 and Aß42. After treatment, focal symptomatology disappeared, and WML and anti-Aß autoantibodies decreased. The *APOE*-ε4 allele was overrepresented. Florbetapir-PET showed cortical deposition with lower retention in swollen areas. In the case of suspected CAA-ri, both CSF anti-Aß autoantibodies levels and Florbetapir-PET could provide highly useful data to guide the correct diagnosis.



## KEY WORDS

Biomarkers, cerebral amyloid angiopathy, cerebrospinal fluid, Florbetapir-PET, inflammation.

## 1. INTRODUCTION

Cerebral amyloid angiopathy-related inflammation (CAA-ri) is a rare type of meningoencephalitis that affects a subgroup of CAA patients who develop vascular inflammation. Clinically, CAA-ri usually presents with cognitive decline and focal neurological symptoms, white matter abnormalities on T2-weighted images, and multiple microhemorrhages on T2*-GRE weighted images [1]. In some cases, reaching a diagnosis is complex, and invasive procedures, such as a cerebral biopsy, are required. This complexity, together with the variable clinical course and the usual responsiveness of CAA-ri to immunosuppressive treatment [2], highlights the need of biomarkers to allow early diagnosis.

The CAA-ri pathogenesis remains unknown. The genetic studies have found an association with the *APOE* -ε4 allele [3]. The better characterization of CAA-ri through biomarkers has allowed the development of diagnostic criteria for probable CAA-ri based on typical clinico-radiological findings without requiring a biopsy [2,3].

Cerebrospinal fluid (CSF) and amyloid positron emission tomography (PET) are increasingly recognized as markers of CAA, however, there are few studies evaluating biomarkers in CAA-ri [4–6]. It is worthy to mention that increased CSF anti-A_ autoantibodies in the acute phase of CAA-ri were first reported by DiFrancesco et al. [7], while Piazza et al. recently suggested that they might be a specific marker of CAA-ri [8].

We describe a case series of four CAA-ri patients in whom CSF AD biomarkers, anti-Aß autoantibodies, and amyloid PET biomarkers were analyzed.

# 2. METHODS

## 2.1. STUDY PARTICIPANTS AND PROCEDURES

A convenient sample of four patients with focal neurological symptoms due to probable CAA-ri were evaluated. Diagnosis of probable CAA-ri was performed based on the proposed diagnostic clinical criteria [3]. All participants provided written informed consent approved by the local ethics committee.

We performed a pre and post-treatment brain MRI and lumbar puncture for CSF core Alzheimer's disease (AD) biomarkers (Aß42, Tau, p-Tau), Aß40, and anti-Aß autoantibodies determination [8]. Lumbar puncture was performed in standardized conditions during the acute phase of the disease in every patient. CSF samples were collected and stocked following international recommendations [9]. CSF cell count, proteins and glucose were determined. CSF levels of Aß40 (Millipore, Billericca, MA); Aß42, total Tau (t-Tau) and 181-phosphorylated Tau (p-Tau) (Innogenetics, Ghent, Belgium) were measured using commercially available ELISA kits according to manufacturer's instructions. Our internal cut-off points have been already published [10]. Anti-Aß autoantibodies dosage in CSF was determined as previously reported [8].

MRI studies were obtained using 1.5 T and 3T facilities, and a designated, protocol including FLAIR, T2, T2*-GRE and diffusion weighted images.

*APOE* genotyping was determined by allelic discrimination of rs429358 and rs7412 polymorphisms using the real-time polymerase chain reaction as previously described [11].

PET data were acquired using a Philips Gemini TF scan 50 minutes after injection of 370mBq of Florbetapir. After transmission data was obtained, brain PET dynamic acquisition was performed (2x5min frames). The reconstruction method was iterative (LOR RAMBLA, 3 iterations and 33 subsets) with a 128x128 image size, 2mm pixel size and slice thickness. Florbetapir images were normalized to the MNI space using the SPM8 T1 template. Mean florbetapir uptake for the regions of interest defined in the Automated Anatomic Labeling atlas (AAL) was computed and normalized by the mean cerebellar uptake.

## 2.2. STATISTICAL ANALYSIS

Non-parametric tests were used to calculate the differences between CSF biomarkers before and after the treatment.

# 3. RESULTS

## 3.1. STUDY PARTICIPANTS

Table 1 summarizes the demographic, clinical, and biomarker data of the four patients.

**Patient 1**. A 73-year-old man with a 2-year history of amnestic mild cognitive impairment (MCI) and arterial hypertension presented with an acute left frontal syndrome and cognitive impairment. Brain MRI showed hyperintense white matter lesions (WML) predominantly in left frontal region and multiple diffuse lobar/cortical microbleeds (Fig. 1A,B). Intravenous corticosteroids were administered. The patient's frontal syndrome resolved, and his cognitive status promptly improved. Follow-up MRIs post-treatment showed a reduction of the WML (Fig. 1C), new cortical microbleeds, superficial siderosis, and further cortical atrophy. During the follow-up, he developed dementia.

**Patient 2**. A 74-year-old man with a history of cerebellar hemorrhage and amnestic MCI was referred because of left arm paresis and epileptic seizures. The initial MRI showed hyperintense asymmetrical WML, involving frontal and parieto-occipital regions, lobar microbleeds, and old hemorrhages (cerebellum and right putamen). Cognitive assessment was impracticable. Intravenous corticosteroids were administered, resulting in cognitive improvement. Two months later, he presented a bronchopneumonia and died.

**Patient 3**. A 68-year-old woman with a history of dyslipidemia and multidomain MCI was referred because of subacute aphasia and cognitive impairment in the two previous months. The initial MRI showed hyperintense, asymmetrical, and confluent WML, affecting predominantly the left temporoparietal region, with lobar/cortical microbleeds located predominantly in those areas (Fig. 1E,F). Intravenous corticosteroids were administered, resulting in cognitive improvement and a reduction of the WML in the post-treatment MRI (Fig. 1G). During follow-up, her cognition worsened and the follow-up MRI showed new parieto-occipital lobar microbleeds and medial temporal atrophy.





**Patient 4**. A 68-year-old woman with a history of dyslipidemia and arterial hypertension was referred because of oscilopsia and stereotyped, repetitive, and self-limited visual hallucinations. The EEG was normal. Initial MRI showed hyperintense asymmetrical cortico-subcortical WML in both occipital lobes, and multiple bilateral occipital lobar microbleeds. After receiving IV corticosteroids, visual symptoms partially improved. After 6 months of follow-up, she died with a spontaneous lobar frontal intracerebral hemorrhage.

**TABLE 1. CHARACTERISTICS OF 4 PATIENTS WITH CAA-RI.**

| | PATIENT 1 | | PATIENT 2 | PATIENT 3 | PATIENT 4 |
|---|---|---|---|---|---|
| **AGE, Y/SEX** | 73/M | | 74/M | 78/F | 68/F |
| **PREVIOUS COGNITIVE STATUS** | MCI<br>MMSE 29/30 (4 months before) | | MCI<br>MMSE 22/30 (11 months before) | MCI<br>MMSE 22/30 (9 months before) | Normal<br>MMSE 29/30 |
| **FOCAL SYMPTOMS** | Frontal syndrome<br><br>MMSE 24/30 | | Left arm paresis<br>tonico-clonic seizures<br><br>MMSE not applicable | Aphasia<br><br>MMSE 7/30 | Visual disturbances (palinopsia, oscilopsia)<br><br>MMSE 28/30 |
| **PRE-TREATMENT MRI** | Hyperintense WML and swelling affecting predominantly left frontal region<br>Multiple and diffuse, but predominantly left frontal CMB<br><br>Cortical temporal, frontal and parietal bilateral cSS<br>(Fig. 1A, 1B) | | Hyperintense right fronto-parieto-occipital swelling WML<br><br>Multiple and predominantly right frontal CMB and deep microbleeds<br><br>Cerebellar cSS | Hyperintense left temporo-parietal WML causing mass effect<br>Multiple predominantly left temporal CMB<br><br>No cSS<br>(Fig. 1E, 1F) | Hyperintense bilateral occipital swelling WML<br><br>Multiple global, but predominantly right occipital, CMB<br>Frontal and parietal bilateral cSS |
| **DURATION OF FOCAL SYMPTOMS AT THE TIME OF PRE-TREATMENT MRI** | 4 months | | 1 month | 2 months | 5 months |
| **PRE-TREATMENT CSF STUDY** | | | | | |
| **CORE AD CSF BIOMARKERS (PG/ML)** **Aß40**<br>**Aß42**<br>**t-Tau**<br>**p-Tau** | 5750<br>249.5<br>368.5<br>70.5 | | 2835.7<br>158.5<br>523<br>35.5 | 6365.9<br>323<br>915.5<br>99.5 | 4480<br>298.5<br>326.5<br>49.5 |
| **ANTI-Aß AUTOANTIBODIES (NG/ML)** | 40.3 | | 68.8 | 50.4 | 53.4 |
| **DURATION OF FOCAL SYMPTOMS AT THE TIME OF PRE-TREATMENT CSF STUDY** | 0.5 months | | 2 months | 2 months | 8 months |
| **CORTICOSTEROIDS THERAPY** | Metilprednisolone 1000 mg/d x 3 days + decreasing dose 5 months | | Metilprednisolone 1000 mg/d x 3 days + decreasing dose 2 months | Metilprednisolone 1000 mg/d x 5 days + decreasing dose 3 months | Metilprednisolone 1000 mg/d x 3 days + decreasing dose 3 months |
| **DURATION OF FOCAL SYMPTOMS AT THE TIME OF CORTICOSTEROIDS** | 4 months | | 2 months | 2 months | 11 months |
| **CLINICAL RESPONSE TO TREATMENT AFTER BOLUS** | MMSE 26/30<br>Total frontal syndrome resolution | | MMSE 17/30<br>Partial improvement of the paresis | MMSE 14/30<br>Partial language improvement | MMSE 30/30<br>Partial visual improvement |
| **POST-TREATMENT MRI** | Partial reduction of WML, remission of swelling<br>(Fig. 1C) | | ND | Persistence of WML, reduction of swelling and mass effect<br>(Fig. 1G) | Partial reduction of WML, remission of swelling |
| **TIME BETWEEN CORTICOSTEROIDS AND POST-TREATMENT MRI. POST-TREATMENT CSF STUDY** | 2 months | | ND | 1 month | 1 month |

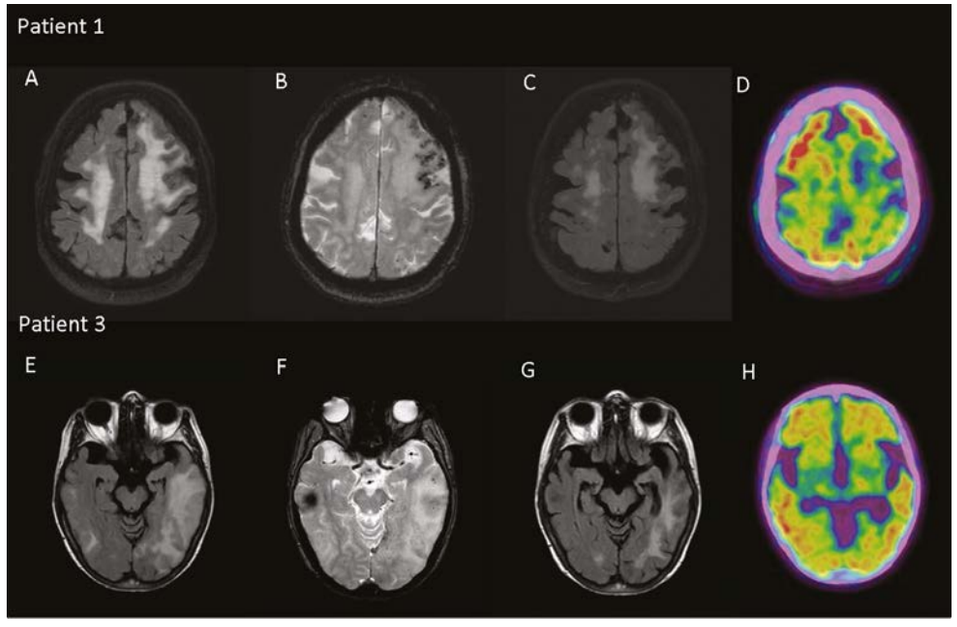
| | | | | | |
|---|---|---|---|---|---|
| **CORE AD CSF BIOMARKERS (PG/ML)**<br>**Aß40**<br>**Aß42**<br>**t-Tau**<br>**p-Tau** | 5497.4<br>223<br>271<br>50.5 | | ND<br>ND<br>ND<br>ND | 5436.6<br>325.5<br>528<br>73 | 3667.1<br>238.5<br>175.5<br>31 |
| **ANTI-Aß AUTOANTIBODIES (NG/ML)** | 23.78 | | ND | 29.99 | 33.23 |
| **TIME BETWEEN CORTICOSTEROIDS AND POST-TREATMENT CSF STUDIES** | 13 months | | ND | 10 months | 2 months |
| **CLINICAL FOLLOW-UP** | MMSE19/30; mRS4 | | mRS6 | MMSE11/30; mRS4 | mRS6 |
| **TIME OF CLINICAL FOLLOW-UP AFTER CORTICOSTEROIDS** | 28 months | | 2 months | 16 months | 6 months |
| **FOLLOW-UP MRI** | Increased number of CMB<br>New areas of cSS<br>Greater cortical atrophy | | ND | Increased number of CMB<br>No cSS<br>Greater cortical atrophy | ND |
| **TIME BETWEEN CORTICOSTEROIDS AND FOLLOW-UP MRI** | 24 months | | ND | 10 months | ND |
| ***APOE* GENOTYPE** | 4/4 | | 4/4 | 4/3 | 3/3 |
| **AMYLOID PET (FLORBETAPIR)** | Positive (Fig. 1D) | | ND | Positive (Fig. 1H) | ND |
| **TIME BETWEEN CORTICOSTEROIDS AND AMYLOID-PET** | 19 months | | ND | 13 months | ND |

Abbreviations: Aß = Amyloid ß; CAA = Cerebral amyloid angiopathy; CMB= Cortical microbleeds; cSS= Cortical superficial siderosis; F = female; M = male; MCI= mild cognitive impairment; MMSE = Mini-mental state exam; MRI = Magnetic resonance imaging; ND = Not done; WML = white matter lessions. mRS= modified Rankin Scale.

Our internal cut-off values are 550 pg/ml for Aß42, 350 pg/mL for t-Tau and 61 pg/mL for p-Tau, as cited in supplemental material [9]. Anti-Aß autoantibodies dosage observed in healthy controls is 18.1±5.7 ng/mL [8].

**FIGURE 1. NEUROIMAGING FINDINGS IN 2 PATIENTS WITH CAA-RI.**

Neuroimaging findings in patient 1 (A–D) and patient 3 (E–H). A) Magnetic resonance imaging (MRI) demonstrating subcortical asymmetric white matter lesions affecting predominantly left frontal lobe on a fluid-attenuated inversion recovery sequence (FLAIR) sequences. B) MRI T2*-GRE weighted sequences showing extensive lobar microbleeds that colocalized with the area of the edema. C) FLAIR sequence MRI performed two months after corticosteroid treatment, showing reduction of cerebral edema. D) PET imaging with Florbetapir showing retention of the amyloid tracer in frontal and parietal regions. Note the swollen region, where PET shows a lower Florbetapir uptake (SUVr = 1.08 in left frontal inferior gyrus, SUVr = 1.05 in left precentral gyrus, SUVr = 1.08 in left postcentral gyrus) than in the homonym contralateral areas (SUVr = 1.10 in right frontal inferior gyrus, SUVr = 1.06 in right precentral gyrus, SUVr = 1.18 in right postcentral gyrus). E) MRI performed to patient 3, showing subcortical asymmetric white matter lesions affecting left temporal occipital lobes on FLAIR sequence. F) MRI T2*-GRE weighted sequences indicating the presence of lobar microbleeds on the affected areas (arrows). G) One month after corticosteroid treatment the FLAIR MRI image showed reduction of the white matter lesions. H) PET imaging with Florbetapir shows cortical retention of the amyloid tracer. Note the swollen region, where the Florbetapir uptake (SUVr = 1.85 in left temporal middle gyrus, SUVr = 1.89 in left occipital middle gyrus) is lower than in homonym contralateral regions (SUVr = 1.98 in right temporal middle gyrus, SUVr = 1.97 in right occipital middle gyrus).








## 3.2. BIOCHEMICAL AND NEUROIMAGING FINDINGS

CSF analyses showed low Aß40 and Aß42 levels in all patients. T-Tau and p-Tau levels were increased in three and two patients, respectively [10].AD biomarkers did not significantly change after treatment (Table 1).

Anti-Aß autoantibody titers were elevated in all four patients pre-treatment. After corticosteroids, the titer returned to normal levels in the three patients with available follow up CSF ($p = 0.034$) [8].

The APOE-ε4 allele frequency was 62.5%.

Florbetapir-PET was performed in patients #1 and #3 (Fig. 1D, H), 19 and 13 months after corticosteroid treatment, respectively. The visual assessment showed A_ deposition. The quantified analysis (both by lobes or Automated Anatomic Labeling atlas, however, showed lower cortical tracer uptake in the areas in which inflammation had developed than in contralateral homonymous regions (Table 2) (Fig. 1D,H).

**TABLE 2: FLORBETAPIR SUVR IN PATIENTS #1 AND #3.**

| | | PATIENT 1 | PATIENT 3 |
|---|---|---|---|
| **OVERALL MEAN** | | **1,21** | **1,70** |
| Frontal | Right | 1,21 | 1,75 |
| | Left | 1,71 | 1,72 |
| Parietal | Right | 1,26 | 1,80 |
| | Left | 1,26 | 1,78 |
| Temporal | Right | 1,16 | 1,57 |
| | Left | 1,12 | 1,49 |
| Occipital | Right | 1,34 | 1,78 |
| | Left | 1,31 | 1,74 |

## 4. DISCUSSION

The present study analyses CSF anti-Aß autoantibodies and amyloid PET in CAA-ri. This pilot study suggests that CSF analysis and amyloid PET might add diagnostic specificity to the proposed clinical criteria for CAA and that CSF anti-Aß autoantibodies might help diagnose and monitor the response to treatment in CAA-ri [8].

The clinical presentation consisted of focal neurological symptoms and rapidly progressive cognitive decline. MRI examinations showed, besides CAA-related findings, asymmetrical and confluent WML, indicating vasogenic edema that remitted shortly after corticosteroids. However, clinical prognosis was poor. Two patients died during the follow-up and one developed dementia.

The CSF data showed a pattern compatible with an underlying CAA [12]. Low levels of Aß40 and Aß42 in CSF could be an alternative diagnostic marker of CAA [4,7,13,14]. Some [4,8], but not all [5], previous works have reported increased CSF Aß42 and Aß40 levels in the acute phase of CAA-ri. We did not find this elevation, but the antecedent MCI (prodromal AD) in three out of four patients, which might have affected CSF AD biomarker levels, should be noted.

Florbetapir-PET showed widespread cortical amyloid deposition. Regions presenting with inflammation in the acute phase seemed to present lower retention. This pilot study might suggest a reduction of Aß uptake after remission that must be confirmed with further dedicated studies. There is only one recent case report with Pittsburg Compound B PET in CAAri [15]. This finding suggests a relationship between inflammation and amyloid clearance, a liaison that has been described in the bapineuzumab and gantenerumab studies [16–18]. Beyond the mechanistic importance of amyloid PET, amyloid imaging might aid in the diagnosis of patients with focal syndromes and suspected CAA in the acute phase given the aforementioned conflicting results of CSF Aß40 and Aß42 levels at this stage [6,15].

The genetic analyses showed that the *APOE*-ε4 allele was overrepresented (when compared to Spanish population)[19]. This has been already reported in





CAA-ri [2,3]. Of note, beyond the clinical similarities between CAA-ri and the amyloid related imaging abnormalities observed in AD patients treated with anti-amyloid therapies [8,16–18], were more frequent in the higher-dose groups of patients carrying the *APOE*-ε4 allele and with a major vascular amyloid burden [20].

Autoantibody titers were high during the acute phase and decreased to normal values after corticosteroids [4,5,7,8]. To our knowledge this is the first study replicating the findings of Piazza et al. on the utility of anti-Aß autoantibodies titer to diagnose CAA-ri. We suggest that CSF anti-Aß autoantibodies should be added to the diagnostic criteria for CAA-ri in order to reduce the number of cerebral biopsies. Moreover, CSF anti-Aß autoantibodies might be used to monitor treatment response. This is usually done with MRI, but although clinical response to immunosuppressive therapy is associated with lesion volume reductions, CAA-ri is also associated with irreversible WML [2,20].

The main limitation of the study is the absence of neuropathological confirmation in our patients. Nonetheless, our findings support the hypothesis that anti-A autoantibodies play a role in the pathophysiological process of CAA-ri. Other limitations include the absence of a control group of sporadic CAA cases or AD that should be explored in future works. Finally, brain atrophy might have affected the quantification of Aß deposition.

In conclusion, our results support the utility of CSF core AD biomarkers, CSF anti-Aß autoantibodies, and amyloid PET in CAA-ri. However, further studies with larger samples and neuropathological confirmation are needed to confirm these findings.

## 5. DISCLOSURES AND ACKNOWLEDGMENTS

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Chapter 5

# Cerebral amyloid angiopathy in Down syndrome and sporadic and autosomal dominant Alzheimer's disease

Chapter 5

# Cerebral amyloid angiopathy in Down syndrome and sporadic and autosomal dominant Alzheimer's disease

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## ABSTRACT

Introduction: We aimed to investigate if cerebral amyloid angiopathy (CAA) is more frequent in genetically determined than in sporadic early onset forms of Alzheimer disease (EOAD).

Methods: Neuroimaging features of CAA, *APOE*, and cerebrospinal fluid-Aß40 levels were studied in subjects with Down syndrome (DS, n=117), autosomal dominant AD (ADAD, n=29), sporadic EOAD (n=42), and healthy controls (n=68).

Results: CAA was present in 31%, 38% and 12% of cognitively impaired DS, symptomatic ADAD, and sporadic EOAD subjects, and in 13% and 4% of cognitively unimpaired DS individuals and healthy controls, respectively. *APOE*-ε4 genotype was borderline significantly associated with CAA in sporadic EOAD ($p$=0.06), but not with DS or ADAD. There were no differences in Aß40 levels between groups or between subjects with and without CAA.

Discussion: CAA is more frequently found in genetically determined AD than in sporadic EOAD. Cerebrospinal fluid-Aß40 levels are not a useful biomarker for CAA in AD.

## KEY WORDS

Cerebral amyloid angiopathy, sporadic early onset Alzheimer disease, autosomal dominant Alzheimer disease, Down syndrome, neuroimaging, cerebrospinal fluid biomarkers.

## 1. INTRODUCTION

Most cases of Alzheimer's disease are sporadic, and caused by complex interactions between genetic and environmental factors. In approximately 5% of cases, Alzheimer's disease can present clinically before the age of 65 (early-onset Alzheimer's disease-EOAD) [1]. These patients frequently present with non-amnestic phenotypes and faster clinical decline than older sporadic Alzheimer's disease cases [1]. In 0.1-0.5% of cases, Alzheimer's disease is transmitted with an autosomal dominant pattern of inheritance (ADAD) due to the presence of mutations in presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), or amyloid precursor protein (*APP*) genes [2]. Down syndrome (DS) is also recognized as a form of genetically determined AD, mainly caused by the *APP* gene triplication [2]. Despite the different genetic background, the Alzheimer's disease neuropathological findings in sporadic EOAD, ADAD and DS are very similar [3,4].

Cerebral Amyloid Angiopathy (CAA) is a major cause of lobar intracerebral hemorrhage (ICH) in the elderly and is present in up to 90% of Alzheimer's disease brains at autopsy [3]. Previous neuropathological studies have suggested a more severe CAA in ADAD than in sporadic Alzheimer's disease [4]. CAA in some APP mutations or duplication carriers drives the clinical presentation [4], and is also consistently observed in subjects with DS [5]. The modified Boston criteria for CAA-related hemorrhage (mBCAA) have been validated to attribute *in vivo* an ICH to CAA based on several neuroimaging features, and are frequently used in clinical practice [6]. There are no previous studies systematically assessing the CAA neuroimaging features in DS and ADAD.

Aß40 is the major form of Aß deposited in the vessel walls in individuals with CAA. Low levels of Aß40 and Aß42 have been found in the CSF of subjects with sporadic CAA [7]. However, scarce and contradictory data are available about the CAA CSF biomarker profile in sporadic Alzheimer's disease patients [8–10], and no previous studies have assessed this profile in DS or ADAD. Moreover, the *APOE*-ε4 genotype is a risk factor for both sporadic Alzheimer's disease and sporadic CAA [11], as it increases Aß deposition in both the parenchyma and blood vessels [12]. However, the effect of the *APOE* genotype in Alzheimer's disease dementia within DS and ADAD is controversial, and there are no studies assessing the influence of the *APOE* genotype on CAA in ADAD or DS [13].

The differences in the CAA neuroimaging features and CSF biomarkers profile in the different forms of Alzheimer's disease are thus, not established. Our primary objective was to determine the CAA presence assessing the fulfillment of the mBCAA and the CSF Aß40 levels in three different Alzheimer's disease populations: DS, ADAD and EOAD. We hypothesized that patients with genetically determined Alzheimer's disease would have more CAA neuroimaging and biochemical features than EOAD.

# 2. MATERIAL AND METHODS

## 2.1. STUDY DESIGN AND PARTICIPANTS

A total of 256 subjects were recruited from 5 centers: Hospital of Sant Pau, Hospital Clínic de Barcelona, and Barcelona Down Medical Center in Barcelona, Spain; and the Sanders-Brown Center on Aging in Kentucky and the Down Syndrome Biomarker Initiative (DSBI) project in San Diego, United States of America. Four study groups were evaluated: EOAD, ADAD, DS, and healthy controls (HC).

**EOAD (N=42):** patients were recruited at the Memory Unit of Hospital de Sant Pau from the Sant Pau Initiative on Neurodegeneration (Barcelona SPIN cohort) [14]. We used the International Working Group-2 diagnostic criteria for AD with *in vivo* evidence of AD based on CSF biomarkers [2]. This group included 19 individuals with prodromal AD (p-EOAD) and 23 subjects with probable AD dementia (d-EOAD).

**ADAD (N=29):** participants were recruited from the Genetic counseling program for familial dementias (PICOGEN) at the Hospital Clínic de Barcelona [15]. Fifteen symptomatic carriers (CDR≥ 0.5) carrying 9 different *PSEN*1 mutations (M139T, S169P, L173F, G209E, L235R, K239N, L282R, L286P, I439S) and one symptomatic carrier of the *APP* I716T mutation were included. The symptomatic carriers were further classified as prodromal ADAD (pAD-ADAD, *n*=5) and ADAD dementia (dADAD, *n*=11). Twelve pre-symptomatic mutation carriers (CDR= 0) carrying 7 different *PSEN*1 mutations (M139T, S169P, L173F, R220G, K239N, L282R, I439S) and one pre-symptomatic carrier of *APP* I716T were labeled as asymptomatic ADAD. We used the IWG2 diagnostic criteria for AD [2].

**DS (N=117):** adults with DS were recruited from three centers, the Down Alzheimer Barcelona Neuroimaging Initiative (DABNI) in the Barcelona Down Medical Center [16]; the Sanders-Brown Center on Aging; and the DSBI pilot project [17]. Adapted neuropsychological batteries (detailed in the Appendix section), covering all cognitive domains classified DS subjects into "without cognitive decline" (asymptomatic DS, N=91), prodromal AD (pAD-DS, N=13), and AD dementia (dAD-DS, N=13). pAD-DS and dAD-DS were also labeled as symptomatic DS.

**Healthy controls (N=68):** participants were recruited at Hospital de Sant Pau (n=60) and Hospital Clínic de Barcelona (n=8) enrolled among patients' caregivers. They did not have cognitive complaints, scored 0 on CDR, had normal neuropsychological evaluation, and normal core Alzheimer's disease CSF biomarkers [18,19].

## 2.2. PROCEDURES

Medical records were reviewed for potential confounders and effect modifiers: age, sex, and presence of arterial hypertension, neuropsychological information on disease severity (MMSE for EOAD and ADAD and the Cambridge Examination for mental Disorders of Older People with DS and Others with Intellectual Disabilities-Cognitive Scale - CAMCOG-DS scale for DS) were recorded and sent to the coordinating center (Hospital de Sant Pau) with the CSF biomarkers (Aß42, Aß40) and neuroimaging data.

The study was approved by the local Ethics Committees following the ethical standards recommended by the Helsinki Declaration. All participants and/or their caregivers gave their written informed consent.

## 2.3. NEUROIMAGING ASSESSMENTS

The inclusion criteria for all participants included a 1.5 or 3T MRI scan including T2*-GRE or SWI, axial fluid attenuated inversion recovery (FLAIR) and coronal T1-weighted sequences in the five centers involved GRE or SWI sequences were assessed for the presence of the main CAA neuroimaging features: localization and number of lobar microbleeds, presence of cortical superficial siderosis (cSS), and lobar ICH. We evaluated the fulfillment of mBCAA in all participants regardless of the age criterion included in the criteria set (>55 years) [6]. White matter hyperintensites (WMH) were semi-quantitatively assessed in FLAIR sequences according to the Fazekas score [20]. MTA was evaluated in coronal T1-weighted

images trough the Scheltens scale [21]. MTA was scored bilaterally and the highest score was considered for the analyses.

The radiological evaluation was performed by two raters (either MCI or MB; neurologists with expertise in cognitive disorders and SG; neuroradiologist) blinded to the clinical data. Inter-rater reliability was above 90% and discrepancies within ratings were solved by consensus.

### 2.4. CSF BIOMARKERS AND APOE GENOTYPE

The inclusion criteria for EOAD and healthy controls included CSF data, but not for ADAD and DS. Details of analysis are described elsewhere [14,18]. In short, commercially available ELISA kits were used to determine CSF-Aß40 and CSF-Aß42 levels (Millipore and Fujirebio-Europe, respectively), following the manufacturers' recommendations.

*APOE* genotyping was performed by PCR amplification of the exon four fragment containing the two polymorphisms (rs429358 and rs7412) that encode the three common *APOE* isoforms. The following oligonucleotides: APOE-F: 5'-ACTGGAGGAACAACTGACCC-3' and APOE-R: 5'-CTGCCCATCTCCTCCATC-3', were used and final PCR products were purified and Sanger sequenced using BigDye terminator chemistry (Applied Biosystems). Sequences were run on an Applied Biosystems® 3130 Genetic Analyzer and resulting elecropherograms were visually inspected using Sequencher (version 4.1, Gene Codes Corp.).

### 2.5. STATISTICAL ANALYSIS

Statistical analyses were performed with the Statistical Package for the Social Sciences v19 software (IBM corp. http://www-01.ibm.com/software/es/analytics/spss/). The primary objectives of this study were to compare across groups the frequency of the mBCAA and the CSF-Aß40 levels and were analyzed with the exact Fisher and Mann-Whitney test respectively.





The secondary objectives were to assess the white matter lesions measured by the Fazekas scale and the hippocampal atrophy measured by the Scheltens scale and were analyzed with the exact Fisher test. Spearman correlation coefficients were calculated between age, clinical stage, hippocampal atrophy and white matter lesions and the different study groups. With the purpose of improving the statistical power, prodromal and demented groups in EOAD, ADAD and DS were merged when analyzed. All significance tests were two-sided with the statistical significance set at 5%.

## 3. RESULTS

Table 1 displays the demographics, clinical features, CSF data, and *APOE* genotype of the participants. CSF data were available in 71% (N=182) of the subjects (including all healthy controls and patients with EOAD). Symptomatic ADAD and symptomatic DS subjects were younger than patients with EOAD (48.4, 54.4, 61.1 years of age respectively; $p<0.001$).

The *APOE*-ε4 allele frequency was higher in EOAD than in any other group. However, no differences were observed between symptomatic or asymptomatic subjects within the ADAD or DS groups.

Table 2 shows the neuroimaging results across the different groups. The fulfillment of the mBCAA criteria was more frequent in symptomatic DS, 31% (N=8), and in symptomatic ADAD, 38% (N=6), than in EOAD 24% (N=19) ($p=0.055$ and 0.026 respectively). When present, the most frequent CAA neuroimaging features were lobar microbleeds in 91.2% (N=31), followed by cSS in 29.4% (N=10) and ICH in 8.8% (N=3). All three features were more frequent in the symptomatic than in asymptomatic subjects within all groups (Table 2).

The symptomatic ADAD and DS groups had a higher proportion of lobar microbleeds than the EOAD group ($p=0.02$, $p=0.046$ respectively). cSS and ICH were statistically associated ($p=0.004$). In those who had cSS, 20% (N=2) had also an ICH, and cSS was present in 67% (N=2) of those with ICH. Symptomatic DS had a higher proportion of subjects with cSS and lobar ICH than the EOAD group, but this difference did not reach statistical significance ($p=0.056$). The position of the mutation (pre or post codon 200) did not significantly impact the presence of lobar microbleeds in *PSEN1* carriers (37 vs 25%, $p=0.4$). One of the asymptomatic ADAD subjects included in our study had a massive lobar ICH after recruitment into this study that lead to its dead in a stage of moderately severe dementia.

**TABLE 1: DEMOGRAPHICS AND CSF BIOMARKER CHARACTERISTICS OF THE PARTICIPANTS.**

| | HEALTHY CONTROLS | SPORADIC EOAD | | ASYMPTOMATIC ADAD § | SYMPTOMATIC ADAD | | ASYMPTOMATIC DS | SYMPTOMATIC DS | |
|---|---|---|---|---|---|---|---|---|---|
| | | p-EOAD | d-EOAD | | pAD-ADAD§ | dADAD§ | | pAD-DS | dAD-DS |
| **N** | 68 | 19 | 23 | 13 | 5 | 11 | 91 | 13 | 13 |
| **Age, years old** | 54.3 (9) | 60.9 (8) | 61.8 (7) | 33.6 (11) | 54.8 (17) | 47.3 (10) | 39.6 (16) | 51.5 (5) | 56.2 (3) |
| **Gender, % men** | 35.3 | 26.3 | 39.1 | 23.1 | 60 | 45.5 | 56 | 54 | 54 |
| **High blood pressure** | 13.2 | 21.1 | 13 | 0 | 0 | 0 | 3.1 | 0 | 15.4 |
| ***APOE*-ε4 carriers, % Ω** | 32.4 | 73.3 | 43.5 | 7.7 | 0 | 18.2 | 34.6 | 20 | 33.3 |
| ***APOE*-ε2 carriers, % Ω** | 4.4 | 5.3 | 4.3 | 7.7 | 0 | 27.3 | 19.2 | 10 | 8.3 |
| **MMSE/ Total CAMCOG ¥** | 29 (2) | 28 (2) | 20 (7) | 30 (1) | 24 (4) | 20 (7) | 80 (16) | 74 (22) | 42 (34) |
| **CSF-Aß42, pg/mL ƨ** | 779.5 (237) | 394 (242) | 360.5 (118) | 655.5 (412) | 217 (242) | 279 (201) | 724.8 (294) | 432.5 (33) | 400.8 (56) |
| **CSF-Aß40, pg/mL ƨ** | 5617.9 (2738) | 5530 (494) | 6224.5 (1923) | 4966.8 (2225) | 4837 (3602) | 5783.7 (6739) | 5559.5 (2088) | 5605 (1143) | 5763.3 (780) |
| **CSF ratio-Aß42/40 ƨ** | 0.143 (0.07) | 0.067 (0.03) | 0.060 (0.02) | 0.130 (0.14) | 0.070 (0.01) | 0.060 (0.18) | 0.106 (0.08) | 0.078 (0.02) | 0.069 (0.03) |





Unless otherwise specified, values are presented as medians (interquartilic range, IQR).

§ Median relative age (IQR) was -11.9 (14.4), 3.15 (6.5) and 3.5 (4.8) in asymptomatic ADAD, pAD-ADAD and dADAD respectively.

Ω APOE was available from 68/68 HC, 19/19 p-EOAD, 23/23 d-EOAD, 13/13 asymptomatic ADAD, 5/ pAD-ADAD, 11/ dADAD, 78/91 asymptomatic DS, 10/13 pAD-DS, and 12/13 dAD-DS.

¥ CAMCOG score was available in 55/91 asymptomatic DS subjects, 7/14 pAD-DS subjects, and 3/12 dAD-DS subjects.

ƨ CSF was available from 68/68 HC, 19/19 p-EOAD, 23/23 d-EOAD, 7/13 asymptomatic ADAD, 5/5 pAD-ADAD, 9/11 dADAD, 36/91 asymptomatic DS, 9/13 pAD-DS, and 6/13 dAD-DS.

Key: Aß42, amyloid-ß 1-42; Aß40, amyloid-ß40; APOE, Apolipoprotein E; CAMCOG-DS, Cambridge Examination for mental Disorders of Older People with Dow Syndrome and Others with Intellectual Disabilities-Cognitive Scale; CSF, cerebrospinal fluid; dADAD, dementia in autosomal dominant Alzheimer disease; dAD-DS, Alzheimer dementia in Down syndrome; d-EOAD, dementia early onset Alzheimer disease; EOAD, early onset sporadic Alzheimer disease; MMSE, minimental state examination; pAD-ADAD, prodromal Alzheimer disease in autosomal dominant Alzheimer disease; pAD-DS, prodromal Alzheimer disease in Down syndrome; p-EOAD, prodromal sporadic early onset Alzheimer disease.

**TABLE 2: NEUROIMAGING FINDINGS.**

| | HEALTHY CONTROLS | SPORADIC EOAD | | ASYMPTOMATIC ADAD § | SYMPTOMATIC ADAD | | ASYMPTOMATIC DS | SYMPTOMATIC DS | |
|---|---|---|---|---|---|---|---|---|---|
| | | p-EOAD | d-EOAD | | pAD-ADAD§ | dADAD§ | | pAD-DS | dAD-DS |
| **N** | 68 | 19 | 23 | 13 | 5 | 11 | 91 | 13 | 13 |
| **MRI (%GRE%/%SWI)** | 38.2/ 61.8 | 24.1/57.9 | 39.1/60.9 | 100/0 | 100/0 | 100/0 | 46.2/53.8 | 23.1/76.9 | 30.8/69.2 |
| **% Lobar microbleeds** | 2.9 | 10.5 | 8.7 | 0 | 20 | 45.5 | 12.1 | 23.1 | 38.5 |
| **% cSS** | 1.5 | 0 | 8.7 | 0 | 0 | 0 | 3.3 | 7.7 | 23.1 |
| **Lobar ICH** | 0 | 0 | 0 | 0 | 0 | 0 | 1.1 | 0 | 15.4 |
| **Boston criteria** | 4.4 | 10.5 | 13 | 0 | 20 | 45.5 | 13.2 | 23.1 | 38.5 |
| **Fazekas score 0** | 64.7 | 21.1 | 26.1 | 75 | 40 | 27.3 | 49.4 | 23.1 | 9.1 |
| **score 1** | 32.4 | 52.6 | 65.2 | 25 | 40 | 72.7 | 43.7 | 69.2 | 54.5 |
| **score 2** | 2.9 | 15.8 | 8.7 | 0 | 20 | 0 | 5.7 | 7.7 | 36.4 |
| **score 3** | 0 | 10.5 | 0 | 0 | 0 | 0 | 1.1 | 0 | 0 |
| **MTA score 0-1** | 97.1 | 52.6 | 56.5 | 100 | 100 | 63.6 | 68.2 | 16.7 | 0 |
| **score 2-4** | 2.9 | 47.4 | 43.5 | 0 | 0 | 36.4 | 31.8 | 3.3 | 100 |





Unless otherwise specified, values are presented as proportions.

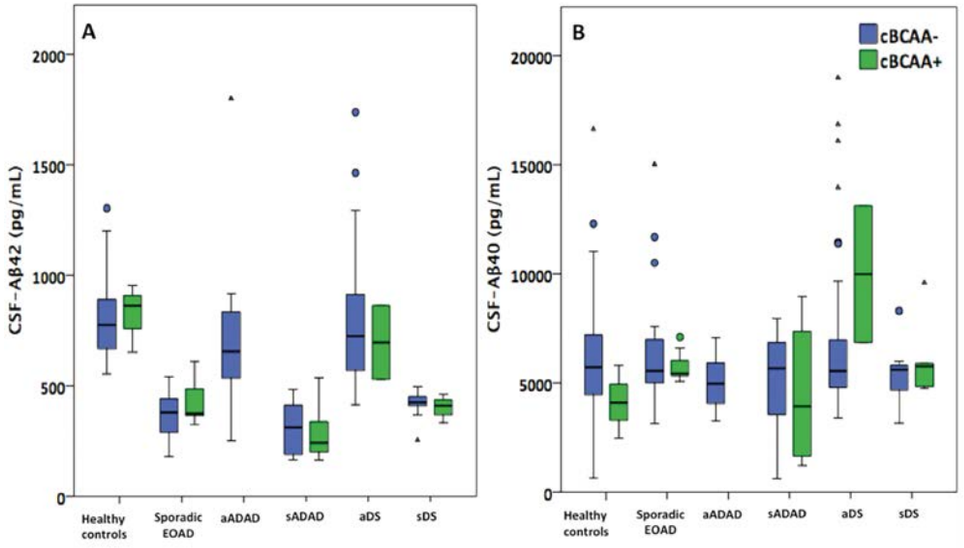
*Fazekas score was not available in 4 asymptomatic DS subjects and in 2 dAD-DS subjects.

** MTA score was not available in 1 AD subject, in 3 asymptomatic DS subjects, in 1 pAD-DS subject, and in 1 dAD-DS subject.

Key: Aß42, amyloid-ß 1-42; Aß40, amyloid-ß40, APOE, Apolipoprotein E; CAMCOG-DS, Cambridge Examination for mental Disorders of Older People with Down Syndrome and Others with Intellectual Disabilities-Cognitive Scale; CSF, cerebrospinal fluid; cSS, cortical superficial siderosis; dADAD, dementia in autosomal dominant Alzheimer disease; dAD-DS, Alzheimer dementia in Down syndrome; d-EOAD, dementia in sporadic early onset Alzheimer disease; EOAD, early onset sporadic Alzheimer disease; GRE, gradient echo sequences; HBP, high blood pressure; ICH, intracerebral hemorrhage; MMSE, minimental state examination; MRI, magnetic resonance imaging; MTA, medial temporal atrophy; pAD-ADAD, prodromal Alzheimer disease in autosomal dominant Alzheimer disease; pAD-DS, prodromal Alzheimer disease in Down syndrome; p-EOAD, prodromal sporadic early onset Alzheimer disease; SWI, susceptibility weighted imaging.

The mean time lag between MRI and CSF sampling was 5.3 months. Symptomatic participants had lower CSF-Aß42 levels than asymptomatic subjects within all groups (Figure 1A). No significant differences were detected in CSF-Aß40 levels between the different groups or between symptomatic and asymptomatic subjects within each group (Figure 1B).

**FIGURE 1: CSF BIOMARKER LEVELS IN THE CLINICAL GROUPS, ACCORDING TO MODIFIED BOSTON CRITERIA FOR CAA (cBCAA).**




Box plot displaying the distribution of CSF-Aß42 (A) and CSF-Aß40 (B) from healthy controls, sporadic EOAD, asymptomatic ADAD (aADAD), symptomatic ADAD (sADAD), asymptomatic DS (aDS), and symptomatic DS (sDS). Subgroups in blue represent those subjects who do not fulfill cBCAA, subgroups coloured in green do fulfill cBCAA for possible or probable CAA.

No differences in levels of CSF-Aß42 or in CSF- Aß40 were detected between subjects that fulfilled cBCAA and those who did not within each clinical group.

All EOAD patients fulfilling the mBCAA were *APOE*-ε4 carriers and the *APOE*-ε4 allele was significantly more frequent in EOAD subjects than in the symptomatic ADAD ($p$=0.015) or symptomatic DS ($p$=0.071) subjects fulfilling the mBCAA criteria. In sporadic EOAD, there was a trend for the association between mBCAA fulfillment and *APOE*-ε4 genotype ($p$=0.06).

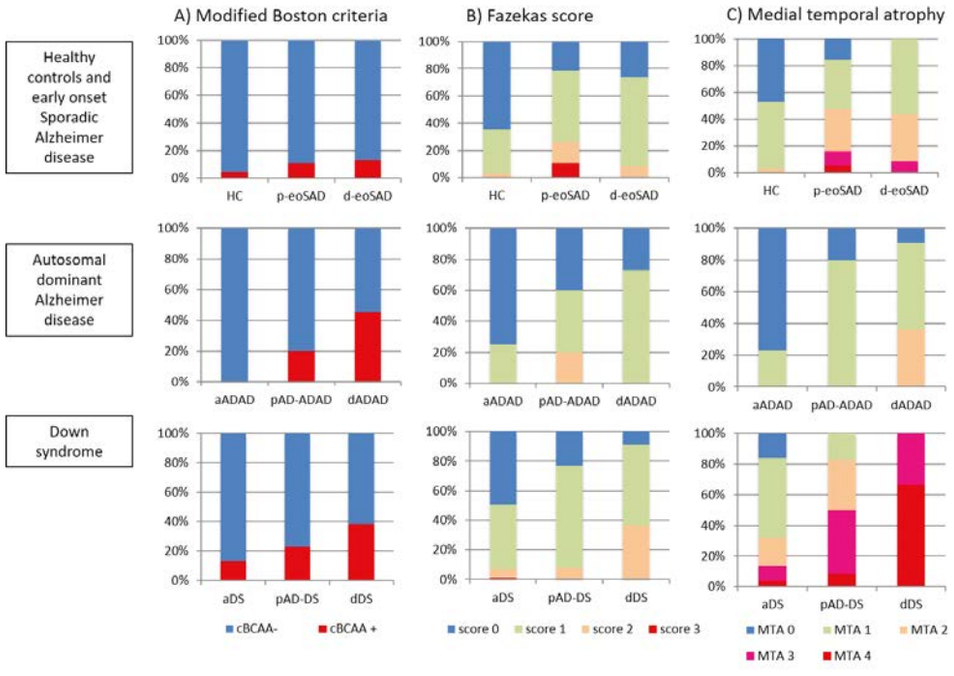




There were no differences in CSF-Aß40 levels between those subjects who fulfilled the mBCAA criteria and those who did not (or the presence of lobar microbleeds, cSS, or ICH) in any group.

Symptomatic subjects presented higher Fazekas scores than asymptomatic subjects in all groups: EOAD patients had higher Fazekas scores than healthy controls ($p$<0.001); symptomatic ADAD higher than asymptomatic ADAD ($p$=0.022) or HC ($p$=0.05); and symptomatic DS higher than asymptomatic DS ($p$=0.011) and healthy controls ($p$<0.001) (Figure 2 and table 2).

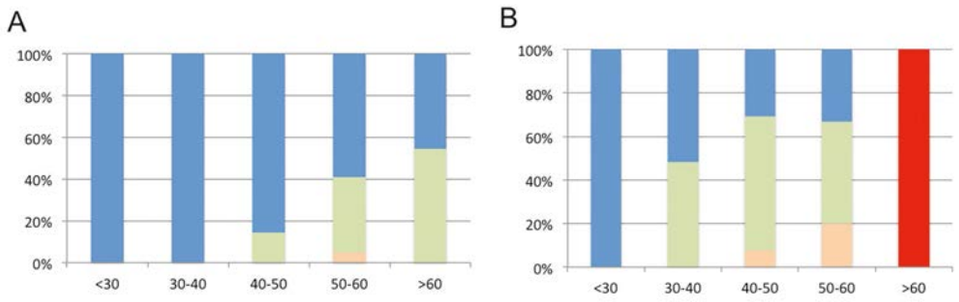
**FIGURE 2. PROPORTION OF SUBJECTS IN EACH GROUP ACCORDING MODIFIED BOSTON CRITERIA, FAZEKAS SCORE, AND MEDIAL TEMPORAL LOBE ATROPHY.**




Frequency bar graph showing: A) modified Boston criteria fulfillment, B) Fazekas score and, C) MTA score of each clinical group. Healthy controls and sporadic AD in the first row, ADAD in the second row, DS in the third row.

There was a significant positive correlation between age and Fazekas score in the whole sample (r=0.337, $p<0.001$), in ADAD (r=0.407, $p=0.031$) and in DS (r=0.506, $p=0.000$) groups. This correlation was also found in asymptomatic DS (r=0.495, $p<0.001$) and in healthy controls (r=0.323, $p=0.007$) (Figure 3).

**FIGURE 3. PROPORTION OF SUBJECTS ACCORDING TO FAZEKAS SCORE BY RANGE OF AGE.**




The frequency bar graphs showing the percentage of subjects with each Fazekas categories by age in: A) Healthy controls and, B) asymptomatic Down syndrome subjects.

Age positively correlated with Scheltens scores in the whole sample (r=0.229, $p<0.001$), healthy controls (r=0.276, $p=0.023$), ADAD (r=0.412, $p=0.027$), asymptomatic DS (r=0.341, $p=0.001$), and symptomatic DS (r=0.431, $p=0.023$). The Scheltens scores increased from asymptomatic to symptomatic subjects within each group. Symptomatic DS patients presented higher MTA scores than EOAD and symptomatic ADAD patients ($p<0.001$ in each comparison) and asymptomatic DS subjects higher than HC ($p<0.001$). There were no differences in Scheltens scores between EOAD and symptomatic ADAD.





## 4. DISCUSSION

We found that DS and ADAD have a more severe CAA than EOAD as measured by the mBCAA criteria, but CAA did not impact the CSF-A 40 levels. The *APOE*-ε4 allele might be associated with CAA in EOAD, but does not seem to have an effect in DS or ADAD.

There are previous studies assessing the prevalence of lobar microbleeds in ADAD and EOAD [22] [23,24], but, to our knowledge, none has specifically assessed and compared the mBCAA between the different AD populations. The mBCAA were more frequent in DS and ADAD, suggesting a more severe CAA, as shown in pathological studies [5,25]. The most frequent CAA neuroimaging feature was the presence of lobar microbleeds, as previously described [26]. The frequency of lobar microbleeds in ADAD (and healthy controls) was in agreement with the literature (ranging from 25% to 66%) [23,24][26], but we found a lower frequency of lobar microbleeds in EOAD (9.5%) than that reported in late onset AD (20-30%) [27]. Age might be responsible for this difference. There are no previous studies assessing the mBCAA criteria or any of its component neuroimaging features in DS. CAA is also consistently observed in DS pathological studies [5,28], but it had been proposed that other genetic factors in DS might protect these subjects from the ICH [29]. We found 38.5% of frequency for lobar microbleeds in symptomatic DS and, more importantly, a frequency of 15.4% for ICH. While this is lower than the reported 30% prevalence for symptomatic ICH in non-trisomic APP duplication carriers, it is well above the 3%-3.8% figure for symptomatic ICH in DS reported in the same study [29]. This discrepancy might be explained because many non-fatal ICH might be unnoticed in DS with AD. Of note, both subjects with DS and ICH on the MRI did not present with ICH-related clinical symptoms.

A significant percentage of symptomatic subjects, nonetheless, did not meet the mBCAA and were free of the CAA associated neuroimaging features. This is in contrast with pathological studies, where CAA is found in up to 90% of AD brains, suggesting that the available MRI sequences only identify a subset of AD-CAA

subjects [23]. The CAA neuroimaging features might thus detect only the most severe cases or, alternatively, they might select different subgroups of patients [23]. In this respect, cSS, although less frequent than lobar microbleeds, was strongly associated with lobar ICH. cSS might be a particular important marker for severe CAA in AD, as it has been suggested in sporadic CAA [7]. More work in longitudinal studies is needed to confirm the higher risk conferred by cSS for future ICH and cognitive decline.

We also assessed other neuroimaging features associated with CAA, but not included in the mBCAA criteria. Both WMH and MTA are increasingly recognized as core Alzheimer's disease features and as a manifestation of CAA [30]. We found a gradient in WMH extension in all groups [31], but we also found more extended WMH in those subjects fulfilling the mBCAA criteria. WMH also increased with age and in relation with vascular risk factors. We found this correlation also in healthy controls, even though they all had normal core Alzheimer's disease CSF biomarkers and low prevalence of HBP. However, we found a strong correlation between age and WMH in asymptomatic DS despite their younger mean age. This correlation supports the relationship between amyloid deposition and WMH. Not surprisingly, the Scheltens scores increased with symptom severity in all AD populations. Hippocampal atrophy, however, was more severe in DS, even in asymptomatic DS individuals. These results are in agreement with the notion that individuals with DS have smaller hippocampal size from birth, but also show atrophy when Alzheimer's disease develops [17].

Decreased CSF-Aß40 levels might differentiate sporadic CAA from healthy controls and Alzheimer's disease cases [7]. In our study, nevertheless, CSF-Aß40 levels did not discriminate CAA neuroimaging features in any group. This finding could be influenced by the fact that amyloid vascular burden in CAA in ADAD and DS contains not just Aß40, but also Aß42. It is difficult to sort out the contribution of vascular Aß42 deposition from parenchymal plaque deposition except with neuropathological analysis of the post-mortem brain, which was not available in this study [32,33].

To our knowledge, there are only two studies that determine CSF-Aß40 in subjects with Alzheimer's disease with and without lobar microbleeds and show conflicting findings [9,10]. In any case, our results suggest that CSF-Aß40 levels are not a sensitive biomarker to detect CAA in the context of an Alzheimer's disease process.





The *APOE*-ε4 allele confers a higher risk for CAA in the general population and in Alzheimer's disease [11,12]. We also found a trend for an association between *APOE*-ε4 genotype and CAA in sporadic early onset Alzheimer's disease. Furthermore, all EOAD subjects with CAA neuroimaging features were *APOE*-ε4 carriers. We did not find this relationship in ADAD or DS. The *APOE*-ε4 genotype might be thus associated with CAA in EOAD, but not in DS or ADAD. In ADAD and DS, other genetic factors, such as the type or position of the causing mutation in ADAD, might be more important in predicting CAA [4].

Our findings have potential clinical implications. The mBCAA criteria have not been validated in patients <55 years of age. We consider that, at least in ADAD and DS, age should not be an essential requirement for CAA diagnosis. Our results also have substantial implications in Alzheimer's disease clinical trials given the relationship between CAA and amyloid-related neuroimaging abnormalities (ARIA). Vascular amyloid may be a pathophysiological mechanism for ARIA [34,35] and recent studies have shown that after Ab immunotherapy there is an increase in CAA severity and an increase in lobar microbleeds associated with removal of plaques [36]. Trials targeting Aß commonly use lobar microbleeds and *APOE* genotype to stratify subjects [37]. However, there are no available data on the relationship of CAA neuroimaging abnormalities (and *APOE*) and ARIA in the setting of amyloid-lowering therapy in ADAD and DS. Our data emphasize heterogeneity in prevalence and possibly etiology for CAA, therefore, the recommendations on the exclusions for presence of baseline ARIA-H (microbleeds or hemosiderosis) from sporadic Alzheimer's disease should be taken with caution. On the other hand, the *APOE*-ε4 genotype is also commonly used to stratify participants given its influence on ARIA [37], however this strategy might not be as important in ADAD or DS. Finally, our data also suggest that the CSF-Ab40 levels will not be a useful biomarker in these trials.

The higher prevalence of CAA in ADAD and DS might play a role in the conversion to clinical dementia. In sporadic Alzheimer's disease, CAA is an indepen-

dent contributor to cognitive impairment and can worsen the severity of cognitive dysfunction [38]. Future longitudinal studies are needed to assess the CAA contribution to cognitive impairment in ADAD and DS.

The main strengths of the present study are the relatively large sample size of different rare populations, such as ADAD and DS, as well as the confirmation of the clinical diagnosis with genetics or CSF biomarkers. The study has some limitations. The use of two different imaging techniques is an important limitation when estimating the real prevalence of lobar microbleeds. SWI has a higher sensitivity for hemosiderin, detecting up to 50% more lobar microbleeds than conventional T2*GRE [39]. However, in our study the ADAD group was exclusively investigated using T2*GRE leading to a possible underestimation of the CAA neuroimaging features in these subjects. Finally, most of the EOAD patients were at a stage of mild dementia and we lack neuropathological data.

In conclusion, the CAA-associated neuroimaging features are more frequent in adults with DS and in patients with ADAD than in those with EOAD suggesting a more severe CAA pathology. Our study also shows that the CSF-Ab40 levels are not a reliable biomarker for CAA and that the risk factors for CAA (such as the *APOE*-ε4 genotype) might be different in EOAD and genetically determined Alzheimer's disease. These findings should be taken into account in the design of clinical trials with anti-amyloid therapies in people with ADAD or DS.





# 5. APPENDIX

Neuropsychological batteries assessed in the Down syndrome group.

Each participant received an annual physical and neurological examination. Diagnostic was established after a clinical consensus performed by the clinical neurologist and the neuropsychologist.

- Neuropsychological battery from the Down Alzheimer Barcelona Neuroimaging Initiative (DABNI), Spain.
  - Kauffman Brief Intelligence Test
  - CAMDEX-DS (informant interview) and CAMCOG-DS (subject cognitive assessment)
  - Clued recall test (Free and cued immediate and delayed recall)
  - Digit span (forward/ backward)
  - Picture cancellation task (Devenny)
  - Barcelona Test (assessment of limb apràxia-pantomime of intransitive gestures)
  - Cats and dogs tests
  - Verbal fluency (animals)
  - Abstract thinking (Barcelona test)
  - Mental State examination- DS (MEC-DS), screening instrument, not validated
  - Neuropsychiatric inventory (12 item NPI)
  - Fundació Catalana Sindrome de Down Functional Scale, not validated
  - Dementia questionnaire for people with intellectual disabilities (DMR)
- Neuropsychological battery from the Sanders-Brown Center on Aging in Kentucky, USA.
  - Kauffman Brief Intelligence Test
  - Severe Impairment Battery
  - Brief Praxis Test
  - Fuld Object Memory Evaluation

- Peabody Picture Vocabulary Test (4th ed.)
- Children's Memory Scale: Dot Locations
- Category verbal fluency
- Beery Visual Motor Integration
- Neuropsychiatric inventory (NPI)
- WISC-R Block Design
- Vineland Adaptive Behavior Scale
- Behavioral Rating of Executive Functions (BRIEF)
- Adaptive Behavior Assessment System (ABAS)
- Dementia Questionnaire for people with intellectual disabilities (DMR)

• Neuropsychological battery from the Down Syndrome Biomarker Initiative (DSBI) project in San Diego, USA is detailed in Rafii et al. Front Behav Neurosci. 2015, 14;9:239.

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Chapter 6

# General discussion, concluding remarks, and future directions

Chapter 6

# General discussion, concluding remarks, and future directions

## 1. GENERAL DISCUSSION

Sporadic cerebral amyloid angiopathy (CAA) is a common age-related form of microangiopathy that affects small cortical and leptomeningeal vessels by progressive ß-amyloid (Aß) deposition. It is a major risk factor for spontaneous lobar intracerebral hemorrhage (ICH), transient focal neurological episodes (TFNE) and an important contributor to age-related cognitive decline and dementia. Over the last few years, our understanding of CAA has greatly improved as a result of substanttial progress in neuroimaging techniques, which has allowed the characterization of the spectrum of hemorrhagic and non-hemorrhagic brain injuries associated with CAA as well as different clinical entities. CAA now is considered not only a specific cerebrovascular pathologic disorder, but also a clinical syndrome (or syndromes) with brain parenchymal lesions that can be detected by neuroimaging. It is becoming increasingly evident that CAA is not a uniform entity, but a complex and heterogenous disease that involves several pathophysiological pathways. The heterogeneity observed both by neuroimaging and in the clinical manifestations of the disease could reflect distinct neuropathological subtypes, distinct patterns of cerebrovascular amyloid deposition or the activation of diverging pathophysiological mechanisms. Sporadic CAA is commonly found in the elderly and in patients with Alzheimer's disease (AD), albeit typically mild and clinically silent. Symptomatic presentations of the disease are generally assumed to be a result of severe CAA. In the fraction of patients with CAA who do develop clinical symptoms, the clinical presentation varies and is associated with its own cluster of biomarkers.

A thorough study of the entire clinical spectrum associated with CAA, including infrequent presentations, is essential for the understanding of this heterogeneous entity. This inclusive approach would improve the characterization of the clinical manifestations and associated clusters of clinical and pathophysiologically relevant biomarkers in future CAA trials and could lead to improved patient management.

In this thesis, we took advantage of several cerebrospinal fluid (CSF), neuroi-

maging, and genetic biomarkers to study CAA from a translational point of view. We tried to go beyond the CAA-related ICH perspective and provide a wider framework for CAA characterization starting from other less frequent presentations.

### 1.1. UNUSUAL CEREBRAL AMYLOID ANGIOPATHY PRESENTATIONS: A DIFFERENT PERSPECTIVE ON CEREBRAL AMYLOID ANGIOPATHY

To better understand atypical CAA presentations, we first studied a cohort of elderly subjects with atraumatic convexal subarachnoid hemorrhage (cSAH). This is an unusual CAA presentation in the form of subarachnoid bleeding, in which the bleeding is localized to the convexities of the brain without involvement of the adjacent parenchyma or extension into the interhemispheric fissures, basal cisterns, or ventricles [1]. It is an infrequent manifestation of CAA, which might be underdiagnosed due to transient and often mild clinical manifestations. The exact prevalence is unknown. Using the largest cohort of cSAH published to date, we performed the first systematic genetic, CSF, and neuroimaging biomarker study. This was possible due to a multicenter collaborative study between 5 tertiary hospitals. In its chronic form, cortical superficial siderosis (cSS), is detected in 1% of the Alzheimer Disease Neuroimaging Initivative cohort [2], and in up to 3.5% of the subjects in memory clinic settings [3]. Although cSAH has different possible etiologies, previous data suggest that CAA is the main cause of cSAH in subjects older than 60 years of age [4], however other authors remain skeptical when other causes of bleeding are not completely ruled out [5]. An association with future lobar spontaneous ICH is apparent presumably due to the resultant fragility of leptomeningeal or superficial cortical CAA-affected vessels leading to blood leakage into the subarachnoid space, which would result in cSAH.

The three main conclusions of this work were: 1) the poor prognosis despite the initial benign presentation, 2) the confirmation of a CAA etiology linked to the Apolipoprotein E (*APOE)* ε2 allele and 3) the possibility of a new pathophysiological mechanism for a subset of lobar hemorrhages.

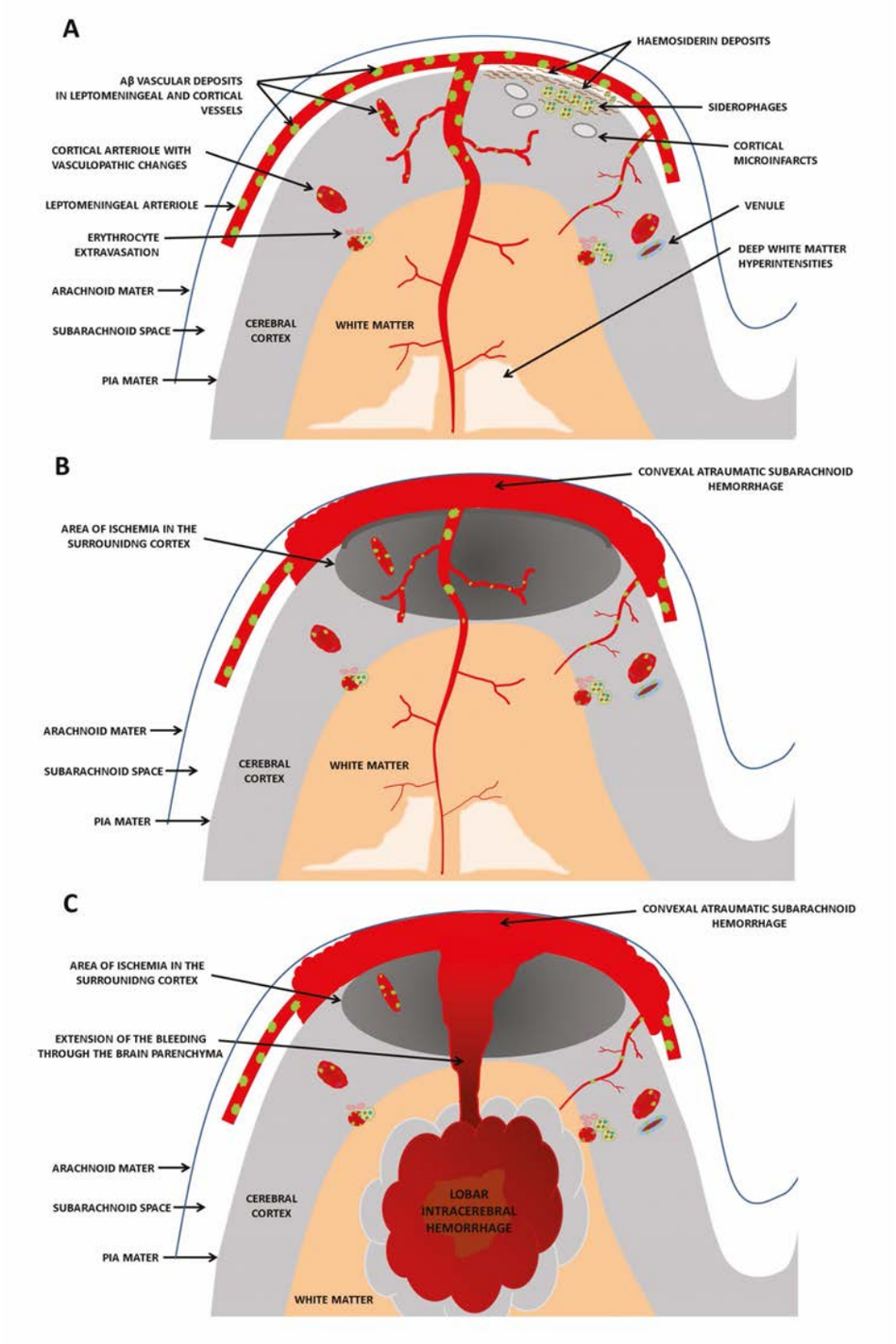
The clinical presentation of cSAH patients was fairly homogeneous, consisting of stereotyped transient focal neurologic episodes of different semiology according to the topography of the acute cSAH. However, during the follow-up, cognitive impairment or subsequent lobar ICH was frequently detected. Consequently, in spite of its apparent initial favorable prognosis, cSAH in the elderly was associated with a poor outcome.

The fulfillment of the modified Boston criteria, the very frequent coexistence of cSAH and cSS, the association found between *APOE*-ε2 allele and cSAH, and decreased levels of CSF-Aß42 and CSF-Aß40 suggested that CAA was the underlying cause of cSAH in our patients. Neuropathological study of one case confirmed severe CAA and advanced AD-type pathology.

According to previously reported studies, the *APOE*-ε2, but not *APOE*-ε4 allele confers vessel fragility, increased bleeding, and contributes to hematoma enlargement, poor functional outcome, and increased mortality [6]. The most accepted explanation for this is that while *APOE*-ε4 promotes vascular amyloid deposition, is involved in degradation systems, and alters the biochemical composition of basement membranes, *APOE*-ε2 exacerbates the vessel fragility that leads to vasculopathic changes such as fibrinoid necrosis, thereby increasing the likelihood of vessel rupture and lobar ICH [7,8].

The areas of acute ischemia in the cortex underlying the cSAH observed in some of the patients and the co-localization of ICH with cSAH or cSS could be indicative of the pathological mechanisms underlying ICH due to CAA. The results of this study provides the first in vivo evidence for a hypothesis described by Takeda based on neuropathological data, which posits that lobar ICH may originate in the subarachnoid space and progress through an ischemic cortex into the brain (Figure 1). In a subset of patients, this initial bleeding, contained by the surrounding cortex, which has the potential to develop ischemia, could extend to the brain parenchyma and lead to a lobar ICH [9–11]. In our multicentre cohort study of subjects with cSAH, described in chapter 3, we found acute ischemia in the cortex underlying the cSAH (and cerebral cortical infarcts in the vicinity of chronic cSS and a colocalization of the ICH and previous acute or subacute cSAH or cSS. These results have both clinical relevance and pathophysiological importance for the field of CAA.

**FIGURE 1. GRAPHIC DIAGRAM OF THE "TSUNAMI HYPOTHESIS" THAT COULD EXPLAIN A PATHOPHYSIOLOGICAL MECHANISM OF INTRACEREBRAL HEMORRHAGE DUE TO CEREBRAL AMYLOID ANGIOPATHY.**




A: Schematic figure representing a brain cortical section showing the main features of sporadic CAA, without ICH. Leptomeningeal and cortical vessels, that mainly follow a columnar distribution, contain ß-amyloid (Aß) deposits and show vasculopathic changes. Note the gradient towards reduced vascular amyloid severity and increased white matter hyperintensities moving from the cortical surface into the cerebral white matter. Erythrocyte extravasation is represented in the cortico-subcortical junction, where lobar microbleeds are predominantly found. Cortical superficial siderosis, as a consequence of chronic bleeding in the convexities, is represented by hemosiderin and siderophages affecting subpial and subarachnoid spaces. Cortical microinfarcts are found in an area of the cortex that underlies previous subarachnoid bleeds. B. A traumatic cortical subarachnoid hemorrhage is contained by the surrounding cortex that suffers from ischemia. C: The bleeding extends into the brain parenchyma causing a lobar intracerebral hemorrhage.





Since the publication of this article, several works have provided further evidence for the underlying CAA etiology for cSAH and its poor prognosis. Beitzke et al. [4] performed a prospective longitudinal cohort of subjects with cSAH that included a neuropathological study of one of the participants. Consistent with our study, they found that cSAH was associated with a substantial risk for future symptomatic ICH, a widespread leakage of meningeal vessels in association with intrasulcal bleeds, which appeared to add to the propagation of cSS, and direct development of lobar ICH from, or in extension to, cSAH.

cSS has gained attention in the last few years and a prospective observational multi-centre cohort study has been launched, the Superficial Siderosis in Patients with suspected CAA trial [12]. This prospective observational study aims to define its clinical features and to validate biomarkers. It will provide invaluable information for a better understanding of the pathways underlying both ischemic and hemorrhagic brain injury and their clinical consequences in CAA.

The results presented in this thesis combined with those in other published studies have lead to a redefinition of the clinical imaging spectrum of CAA. It is now recognized that cSAH and cSS should be also considered as key hemorrhagic neuroimaging signatures of CAA. There are now consensus standards for rating and reporting cSS in observational clinical studies [13]. cSAH/cSS reflect repeated episodes of blood leaking into the subarachnoid space and could be the most clinically relevant manifestation of CAA, as they can both trigger TFNE, carry a

very high risk of future lobar ICH and are related to the development of cognitive impairment [14]. The emerging key role of cSAH/cSS in neurological dysfunction in CAA might be linked with the neuropathological observation of more severe leptomeningeal CAA compared to parenchymal CAA [8,15].

The improved clinical characterization CAA allows an earlier detection of CAA avoiding the need for a major ICH and has direct implications for clinical care. Namely, strict control of blood pressure and avoidance of antithrombotic therapies should be considered. The identification of cSAH/cSS could also have implications for clinical trials using anti-amyloid therapies in patients with AD for which there are concerns regarding hemorrhagic and inflammatory complications attributed to rapid Aß shifts from brain parenchyma to the perivascular spaces of small vessels [16–18]. These complications are called amyloid-related imaging abnormalities (ARIA). Subtypes of ARIA include magnetic resonance imaging (MRI) signal abnormalities suggestive of vasogenic edema and sulcal effusions (ARIA-E) and cerebral microbleeds and hemosiderin deposits in the form of cSS (ARIA-H). Cerebral lobar microbleeds have been considered as exclusion criteria in some of these trials, but the role of cSS is still unexplored as a real risk factor for ARIA. cSS should be investigated as a potential marker for ICH or ARIA risk in future anti-amyloid trials in CAA [13] or in trials targeting lobar ICH prevention. In addition, identifying cSAH/cSS implications would be necessary for future clinical trials with antinflammatory drugs or therapies protecting the BBB.

Another infrequent, but potentially aggressive presentation of CAA is CAA-related inflammation (CAA-ri). CAA-ri is a spontaneous meningoencephalitis syndrome, characterized by rapidly progressive or subacute cognitive decline, headache, seizures and focal neurological deficits due to CAA-associated vascular or perivascular inflammation [19–21]. The unknown exact pathogenesis of CAA-ri, its variable clinical presentation, the requirement of a neuropathological confirmation for a definite diagnosis and its potential response to immunosuppressive treatment highlights the urgent need for reliable biomarkers to allow an early diagnosis of this syndrome. Recently, CAA-ri has gained interest for its clinic-radiological similarities to the ARIA, developed by a subgroup of patients





with AD receiving anti-amyloid therapies. To improve our understanding of CAA-ri pathophysiology, in chapter 4 we report an in-depth biomarker and genetic evaluation pilot study in four patients with a compatible clinico-radiological CAA-ri [21] before and after treatment with corticosteroids. We were able to measure the recently described anti-Aß autoantibodies during the acute phase of CAA-ri thanks to a collaboration with Piazza et al. Our results support its clinical utility in the context of CAA-ri. We found increased anti-Aß autoantibodies which were lowered after immunosuppressive treatment. We were able to report the CSF biomarker profile and to perform a longitudinal florbetapir-positron emission tomography (PET) study in two cases. Interestingly and similarly with what has been reported for ARIA, after the treatment we found lower retention in areas where the inflammation was present in the actue phase. The finding of a low retention of amyloid tracer during the chronic phase of the disease in regions where previously inflammation was located, could reflect a successful clearance of Aß from those locations and might reveal that inflammation is related to this correct clearance. While this has been described in ARIA [18,22], it is unclear whether this is the case for CAA-ri due to a lack of longitudinal studies in CAA-ri that include PET with amyloid tracer. That said, the mobilization of Aß could be a common pathophysiological mechanism in both CAA-ri and ARIA. While CSF biomarkers and PET with amyloid tracer are well recognized biomarkers for sporadic CAA, this is the first systematic study of CAA-ri and highlights the utility of the biomarkers in this rare form of CAA. Therefore, despite the small sample size, our data highlight the role of PET with amyloid tracer for discovering pathophysiological proccesses that may occur in this disease and provide further evidence about the utility of anti-Aß autoantibodies in CAA-ri for diagnosis and monitoring the response to treatment.

In respect to CSF biomarkers for CAA-ri, some advances have already been reported in small sized studies since the publication of our work. In agreement with our findings, decreased levels of CSF-Aß42 and CSF-Aß40 with respect to CAA, AD, and healthy controls have been described [23] and CSF- Aß42 has been proposed to be the most specific CAA-ri biomarker when differentiating CAA-ri from the other disorders. However both CSF-Aß42 and CSF-Aß40 levels showed important overlap between the different amyloid-related conditions making

diagnosis at the individual level difficult. These findings contrast with previous works that reported increased levels of those biomarkers in the acute phase of CAA-ri [24–26]. The conflicting results can be explained by the coexistence of an AD pathophysiological process since AD could interfere with the CAA-ri CSF biomarkers levels. The presence of high levels of CSF-anti-Aß autoantibodies during acute phase of CAA-ri has been replicated by other authors and are useful to distinguish CAA-ri from AD and probable CAA [26].

Coinciding with the publication of our article, the validation of the first standardized clinic-radiological criteria for possible or probable CAA-ri diagnosis was reported [27]. The criteria provide a guide for a reliable diagnosis from basic clinical and radiographic information alone, with good sensitivity and specificity, avoiding unnecessary brain biopsies. Those criteria do not incorporate data from other clinical studies, such as the response to immunosuppressive therapy or CSF biomarkers sicne these aspects have not been completely validated for CAA-ri. Our findings in chapter 4 might have a direct application for ARIA and future CAA immunotherapy trials aimed to safely clear amyloid from vessels and possibly reduce the risk of future CAA-related consequences. Since there is still an urgent need of biomarkers for CAA-ri, our results open the door to other studies that should define and validate clinical diagnostic cut-offs for CSF and PET with amyloid tracer biomarkers in CAA-ri and further explore how these can help to diagnosis and manage CAA-ri.

The Inflammatory Cerebral Amyloid Angiopathy and Alzheimer's Disease ßiomarkers International Network [28] is a world-wide consortium with the aim of discovering and validating biomarkers for ARIA in the largest cohort of CAA-ri to-date. The validation of new biomarker cut-offs, including anti-Aß autoantibodies, in association with the proper clinical and MRI features, will enable a more accurate diagnosis of CAA-ri and ARIA-like events occurring during AD clinical trials and might provide pathophysiological information of this syndrome.

Therefore, our results and the aforementioned works in infrequent CAA presentations demonstrate that the detailed study of these rare forms might provide important clues to the pathophysiology of the more frequent manifestations of CAA such as lobar ICH or the most frequent adverse event in anti-amyloid trials, ARIA.





## 1.2. CEREBRAL AMYLOID ANGIOPATHY IN THE CONTEXT OF ALZHEIMER'S DISEASE

CAA is common in AD, and considered part of the cerebrovascular amyloidosis *continuum*. Most of the available clinico-radiological knowledge in respect to CAA and AD derives from the study of sporadic late onset AD cases. However there is a lack of information regarding the frequency, neuroimaging features and biomarkers of CAA in other less frequent populations of AD e.g., sporadic early onset AD or genetically determined AD such as autosomal dominant AD (ADAD) and Down syndrome (DS). In spite of the different genetic background, the AD neuropathological findings in all these populations are very similar [29,30]. In chapter 5, we systematically evaluated for the first time the CAA neuroimaging features, *APOE* genotype, and CSF biomarkers in different populations of AD. Based on previous neuropathological data, we hypothesized that CAA would be more frequent in genetically determined AD than in sporadic early onset AD. To address this question, we carried out a multicenter study including sporadic early onset AD (EOAD), ADAD, DS, and healthy controls. We applied the autopsy-validated modified Boston criteria to assess CAA in these populations. We were able to confirm that CAA is more frequent and severe in genetically determined AD than in sporadic AD.

The genetic and biomarker study found that CSF-Aß40 levels are not a useful biomarker for CAA in the context of an AD process as opposed to sporadic CAA (chapters 3 and 4) and that the *APOE*-ε4 allele does not seem to be a risk factor for CAA in genetically determined AD. These findings suggest that the pathophysiology of CAA in genetically determined forms of AD can differ from sporadic CAA or even sporadic AD and also highlight the need of specific biomarkers able to differentiate brain Aß from vascular Aß deposition.

In addition to the pathophysiological implications of our results, these findings have impacted anti-amyloid therapies clinical trials. ARIA are the main adverse event reported in these trials and are closely related to CAA. Anti-amyloid trials commonly use lobar microbleeds as surrogate markers for CAA, but our data suggest once more that other CAA neuroimaging features, such as cSS, should also be considered due to its frequency and its relation to ICH and cognitive impairment.

On the other hand, the *APOE*-ε4 genotype is also commonly used to stratify participants, given its influence on ARIA. This strategy might not be as important in ADAD or DS since it does not seem to have such a driving effect in these populations. Finally, as we have discussed, our data suggest that the CSF-Aß40 levels would not be a useful biomarker in these trials. From a clinical point of view, based on our results we propose removing the age criteria from the modified Boston criteria for CAA, at least in the context of genetically determined Alzheimer disease forms.

In conclusion, biomarkers are powerful tools that allow an earlier and more accurate diagnosis of CAA. They also provide information about its pathophysiology that can  have direct clinical implications. The study of less common presentations of CAA enables a new perspective of the disease, leading to a better understanding of the pathophysiological processes that participate in CAA. Due to the relevance of CAA to AD clinical trials of anti-amyloid therapies and ARIA, there us an urgent need to improve our understanding of the pathophysiological pathways that play a role in CAA development.

This thesis includes a thorough study of presentations of CAA other than lobar ICH, which is the most common and studied CAA-related clinico-radiological finding. By analyzing the pathophysiological processes of these infrequent presentations through a multimodal biomarker study, the thesis provides new evidence about the processes that take place in the development of CAA-related ICH or spontaneous ARIA. This is the first study of the CAA-related neuroimaging findings and CSF biomarkers in genetically determined AD, an understudied population. A better understanding of the pathophysiology of CAA is the first step towards the development of new effective therapies, both for CAA itself and for AD.





## 2. CONCLUDING REMARKS

The main conclusions of this thesis are:

1. **Convexal subarachnoid hemorrhage in the elderly is due to underlying cerebral amyloid angiopathy pathology and is associated with future cognitive decline and lobar intracerebral hemorrhage.**
   a. The clinical presentation in CAA-related cSAH is homogeneous and seemingly benign, but it is associated with a poor prognosis at 2 years.
   b. The biomarker, genetic, and neuropathological studies provide support for the hypothesis that CAA is the underlying cause of cSAH in elderly subjects
   c. The evidence of acute cortical ischemia in the cortex adjacent to the cSAH and the localization of ICH in the site of cSAH (or cSS) support the hypothesis that a subset of ICH might originate in the subarachnoid space and progress through an ischemic cortex into the brain.

2. **Cerebral amyloid angiopathy-related inflammation can be accurately diagnosed with biomarkers.**
   a. Cerebrospinal fluid AD biomarkers and amyloid PET add diagnostic specificity to the proposed clinical criteria for CAA.
   b. Cerebrospinal fluid anti-Aß autoantibodies could  help diagnose and monitor the response to treatment in cerebral amyloid angiopathy-related inflammation.

3. **Cerebral amyloid angiopathy is more frequently found in genetically determined Alzheimer's disease than in sporadic early onset Alzheimer's disease.**
   a. Patients with autosomal dominant Alzheimer's disease and Down syndrome

fulfilled the modified Boston criteria for CAA more frequently than patients with sporadic early onset Alzheimer's disease.

b. Cerebrospinal fluid-Aß40 levels are not a useful biomarker for cerebral amyloid angiopathy in Alzheimer's disease.

c. The *APOE*-ε4 allele could be associated with CAA in sporadic early onset Alzheimer disease, but does not seem to have an effect in genetically determined Alzheimer's disease.





## 3. FUTURE DIRECTIONS AND CONSIDERATIONS

There is a growing interest in CAA as evidenced by the increase in the number of papers published over the last few years. International efforts aimed at translating mechanistic insights into novel treatment approaches (including one clinical trial), are underway. However, more research is needed to understand the CAA pathophysiology and to enable a more accurate diagnosis *in vivo.* This thesis has contributed to the study of CSF and neuroimaging biomarkers in CAA, it has answered several questions, but has undoubtedly left many more unanswered.

### Cerebral amyloid angiopathy pathophysiology

Studies that deepen our understanding of the pathophisiologic mechanisms that participate in CAA are needed. The conceptualization of CAA as a protein elimination failure angiopathy has been mentioned but has yet to be established. An understanding of Aß clearance through brain pathways for lympthatic drainage and for the convective influx/ glymphatic communication pathways could provide strategies to reduce excess Aß deposits and delay disease onset.

Brain clearance systems can be affected by several mechanisms. In CAA, perivascular drainage pathways are progressively filled of Aß allowing an increased aggregation and deposition along the brain small vessels, starting with Aß initially deposited in the basement membranes and progressively replacing all tissue elements in the artery wall [31]. This could affect the endothelium that, when activated by injury, produces pro-inflammatory cytokines that play a role in neuroinflammation, blood-brain barrier (BBB) breakdown, and alteration of vessel physiology, among others mechanisms of secondary injury. Aß has also been shown to be toxic to pericytes, enabling disturbances in angiogenesis, BBB function and neurovascular coupling. Taken together, these aggressions to the brain clearance systems could lead to multiple phenomena that progressively increase the severity of CAA e.g., tissue hypoxia and neuronal injury [32], altered metabolism of brain tissue [33] and enlargement of perivascular spaces in the underlying white matter [34].

One of the particular structures that plays a role in amyloid clearance is the BBB. Matrix metalloproteinases (MMPs) are a family of enzymes able to degrade components of the extracelular matrix, which is important for normal BBB function. Previous studies suggest that BBB function may be impaired in CAA, AD with microbleeds and vascular dementia [35–37], however studies that determine MMP in CSF or plasma of AD and CAA subjects have shown contradictory results [37–40]. We are designing a study that includes different clinical groups of the amyloidosis *continuum*: CAA (CAA-related cSAH/cSS, CAA without cSAH/cSS, CAA-ri) and AD with non-CAA related (hypertensive) ICH and healthy controls as control groups. We will assess the integrity of BBB through the CSF/serum albumin ratio and investigate the dynamics of MMPs and angiogenic factors in CSF and plasma of each clinical group.

In relation to other pathways that could play a role in CAA, the study of the complement activation is an interesting field. Complement proteins are deposited in CAA-affected vessels [41]. A polymorphism in complement receptor 1 gene-CR1 (rs6656401) influences risk and recurrence of CAA-related ICH, as well as vascular amyloid deposition severity [42]. CR1 is also considered to be one of the genetic susceptibility loci in AD. Levels of CR1 can be studied in plasma and CSF and have been found to be elevated in prodromal AD and AD dementia when compared to non-AD mild cognitive impairment [43], reflecting an impaired function of complement proteins as part of the AD process. However there are no data available in subjects with CAA or CAA-related disorders.

Another aspect that has drawn our attention is the presentation of CAA in young subjects without a familial CAA mutation. In those cases, a histological proof of vascular amyloid deposition is needed to establish the diagnosis of CAA. There are only 6 cases younger than 55 described in the literature diagnosed with CAA and with unknown genetic background of familial CAA. In 4 of them there is a history of severe traumatic brain injury in childhood [44]. We would like to determine whether CAA could be a long-term consequence of traumatic brain injury. With this aim, first of all we will study subjects with spontaneous ICH from the Vascular Unit at our center. We will focus on young patients (under 55) with CAA-related ICH following H-ATOMIC classification [45] who reported a





history of traumatic brain injury and we will determine cognitive status, CSF core AD biomarkers, CSF-Aß40, BBB markers, *APOE* genotype and MRI findings.

It is obvious that CAA is a complex entity with clinical and imaging heterogeneity and different phenotypes that might reflect distinct neuropathological subtypes or patterns of cerebrovascular Aß deposition and activation of different pathophysiological pathways. Mild or moderate CAA reflects the perivascular drainage impairment and is a common finding in elderly population and subjects with AD. Severe CAA usually presents with the clinic-radiological manifestations previously detailed in the introduction of this thesis. Nevertheless, the anatomic distribution, the degree of accumulation and the progression of Aß deposition is variable [31]. In some vessels, Aß remains focal, providing a potential site of weakness leading to hemorrhage; in other cases, Aß is deposited in the perimeter of the vessel, affecting the lymphatic drainage pathway [31]. These patterns of Aß deposition might differentially affect leptomeningeal and cortical parenchymal vessels, as well as capillaries, providing another dimension to the spectrum of the disease [8].

Several characterizations of different disease phenotypes of CAA have been proposed; however is the criteria are not uniform or firmly established and there are many patients that fall into a mixed category due to the coexistence of several phenomena. Because of their bimodal size distribution on MRI, lobar microbleeds and lobar-ICH have been proposed to be consequences of different pathophysiological mechanisms [46]. Pathologically, subjects with lobar microbleeds seem to have increased wall thickness of Aß-laden vessels compared to patients with relatively low lobar microbleed counts and lobar-ICH [46]. A recent MRI-neuropathological study reported that the former group had more common neurofibrillary tangles than the latter [47]. The same study found that disseminated cSS, which was not associated with lobar microbleeds, was more frequent in patients with lobar ICH and was associated with the *APOE*-ε2 allele. cSS could reflect a severe leptomeningeal distribution of Aß deposition rather than deeper cortical CAA, which will present with microbleeds. In addition to those theories of different patterns, there are also several aspects regarding CAA presentation to be elucidated, for example, which factors determine the phenotype, and what

are the different determinants between an inflammatory parenchymal response or a predominantly vasculopathic reaction that leads to lobar-ICH. Another issue that should be taken into account is the technical aspect, since the use of different MRI sequences and/or a different strength of field from the MRI machine directly affect sensitivity, specificity, and reproducibility of the results.

The characterization of biomarkers within each process of the CAA spectrum is useful for deepening the underlying pathophysiological mechanisms, which is the basis for developing safe clinical trials and, perhaps in the future, therapies targeting specific phenotypes.

### New biomarkers for cerebral amyloid angiopathy

This thesis has demonstrated the added value of using biomarkers in the diagnosis of the different presentations of CAA. However, we have also seen that several of the biomarkers used are not specific to CAA. Core AD CSF biomarkers and PET with amyloid tracer while useful in CAA diagnosis are, by definition, altered in AD. Even CSF-Aß40 levels, which have been described to be decreased in patients with probable CAA, as we found in chapter 3 and 4, do not discriminate between these patients with AD with and without CAA-related neuroimaging findings. This finding could be influenced by the fact that amyloid vascular burden in CAA in ADAD and DS contains not only Aß40, but also Aß42. In chapter 5, we observed that CSF-Aß40 does not seem to be a useful marker for CAA in AD, both in sporadic early onset AD and in genetically forms of AD.

The identification of biomarkers selectively involved in CAA is urgently needed for differentiating vascular Aß from brain Aß. The finding of specific biomarkers selectively involved in CAA would provide insight in the biology of CAA, would improve the performance of potentially valuable data on CAA biomarkers, and would allow the stratification of patients involved in clinical trials and future therapies.

The differences found between genetically determined AD and early onset AD, together with the different behavior of CSF Aß in sporadic CAA and CAA in the context of AD warrant further studies of sporadic late onset AD forms and in





other neurodegenerative dementias. In this respect, we are currently designing a multicenter study in a larger cohort of patients recruited from different centers in Catalunya in which we will perform a systematic evaluation with MRI, *APOE*, and CSF biomarkers (including the aforementioned potential specific new biomarkers for CAA). We aim to recruit sporadic late onset AD, dementia with Lewy bodies, frontotemporal dementia, and elderly healthy controls. The main aims are two-fold: first, to demonstrate that non-amyloidopathies do not have a biomarker profile suggestive of CAA, and second to ascertain the usefulness of CSF-Aß40 as a biomarker of CAA in patients with late onset AD.

In brief, the pathophysiological mechanisms present in CAA are complex and many aspects of them remain unknown, warranting further research in this field. This thesis is only a small step on a path that leads to further questions. The answer to those questions will be a giant leap for a better understanding of this disease.

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Chapter 7

# List of abbreviations

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| **Aß** | ß-amyloid protein |
| **ABCA7** | ATP-Binding Cassette Sub-Family A Member 7 gene |
| **AD** | Alzheimer's disease |
| **ADAD** | Autosomal dominant Alzheimer's disease |
| ***ADAM10*** | ADAM metallopeptidase domain 10 gene |
| **AICD** | Amyloid intracellular domain |
| **APOE** | Apolipoprotein E gene |
| **APP** | Amyloid precursor protein |
| **ARIA** | Amyloid-related imaging abnormalities |
| **ARIA-E** | Amyloid-related imaging abnormalities- edema |
| **ARIA-H** | Amyloid-related imaging abnormalities- hemorrhage |
| **ß -CTF** | ß-cleaved carboxy-terminal fragment of APP |
| **BBB** | Blood-brain barrier |
| **BNT** | Boston naming test |
| **CAA** | Cerebral amyloid angiopathy |
| **CAAo** | Subjects with CAA without cSAH |
| **CAA-ri** | Cerebral amyloid angiopathy-related inflammation |
| **CDR** | Clinical dementia rating scale |
| **CAMCOG-DS** | Cambridge examination for mental disorders of older people with Down syndrome and others with intellectual disabilities- cognitive scale |
| **CERAD-f** | The Consortium to Establish a Registry for Alzheimer's Disease (CERAD)- figures |
| **CERAD-w** | The Consortium to Establish a Registry for Alzheimer's Disease (CERAD)- word list |
| ***CLU*** | Clusterin gene |
| **CMB** | Cortical microbleeds |
| **CR1** | Complement C3b/C4b receptor 1 |
| **cSAH** | Convexal subarachnoid hemorrhage |

| | |
|---|---|
| **CSF** | Cerebrospinal fluid |
| **cSS** | Cortical superficial siderosis |
| **CT** | Computerized tomography |
| **DABNI** | Down Alzheimer Barcelona Neuroimaging Initiative |
| **DS** | Down syndrome |
| **DWI** | Diffusion-weighted imaging sequence |
| **dADAD** | Dementia in autosomal dominant Alzheimer's disease |
| **dAD-DS** | Alzheimer's disease dementia in Down syndrome |
| **d-EOAD** | Dementia in early onset Alzheimer's disease |
| **EOAD** | Early onset Alzheimer's disease |
| **FCSRT** | Free and cued selective reminding test |
| **FLAIR** | Fluid attenuated inversion recovery sequence |
| **GDS** | Geriatric Depresion Scale |
| **GDS-FAST** | Global Deterioration Scale- Functional Assessment Staging |
| **GRE** | Gradient echo sequence |
| **HC** | Healthy controls |
| **HSP** | Hospital de Sant Pau |
| **H&E** | Hematoxylin-Eosin stain |
| **ICH** | Intracerebral hemorrhage |
| **IDDD** | Interview for Deterioration in Daily Living Activities in Dementia |
| **IHC** | Immunohistochemistry |
| **LTP** | Hippocampal long term potentiation |
| **mBCAA** | Modified Boston criteria for cerebral amyloid angiopathy |
| **MCI** | Mild cognitive impairment |
| **MMP** | Matrix metalloproteinase |
| **MMSE** | Mini-mental state examination |
| **MRI** | Magnetic resonance imaging |
| **mRS** | Modified Ranking scale |
| **MTA** | Medial temporal atrophy |
| **N-APP** | N-terminal fragment of APP |
| **PET** | Positron emission tomography |
| **PiB** | 11C-Pittsburgh Compound B- PET |
| ***PICALM*** | Phosphatidylinositol binding clathrin assembly protein |
| ***PSEN1*** | Presenilin 1 |
| ***PSEN2*** | Presenilin 2 |
| **pTau or p-Tau** | Phosphorylated Tau at threonine-181 |
| **pAD-ADAD** | Prodromal Alzheimer's disease in autosomal dominant Alzheimer's disease |
| **pAD-DS** | Prodromal Alzheimer's disease in Down syndrome |
| **p-EOAD** | Prodromal Alzheimer's disease in early onset Alzheimer's disease |
| **Rey** | Rey-Osterrieth Complex figure |
| **sAPPß** | Soluble fragment of APP |
| **SORL1** | Sortilin-related receptor |
| **SWAT** | Spanish Word Accentuation Test |
| **SWI** | Susceptibility-weighted imaging sequence |
| **TFNE** | Transient focal neurologic episodes |
| **TMT** | Trail making test |
| **TREM2** | triggering receptor expressed on myeloid cells 2 |
| **tTau or t-Tau** | Total Tau |
| **VOSP** | Visual Object and Space Perception test |
| **WMH** | White matter hyperintensities |
| **WMS-III-w** | Wechsler memory scale III- word list |
| **WMS-III-p** | Wechsler memory scale III- family pictures |

Chapter 8

# Annexes

Chapter 8

# Annexes

# ANNEX 1:
# ORIGINAL PUBLISHED PDF CHAPTER 3

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ORIGINAL ARTICLE

# Cerebral amyloid angiopathy-related atraumatic convexal subarachnoid hemorrhage: an ARIA before the tsunami

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Atraumatic convexal subarachnoid hemorrhage (cSAH) in elderly patients is a rare entity that has been associated with cerebral amyloid angiopathy (CAA) and intracerebral hematomas (ICH). To characterize this entity and to study these associations, 22 patients over 60 with cSAH were included in a multicenter ambispective cohort study. Clinical data, magnetic resonance imaging (MRI) studies, APOE genotyping, and cerebrospinal fluid (CSF) biomarkers were evaluated. Results were compared with data from healthy controls (HC), non-cSAH CAA patients (CAAo), and Alzheimer disease patients. Convexal subarachnoid hemorrhage presented with transient sensory or motor symptoms. At follow-up (median 30.7 months), 5 patients had died, 6 survivors showed functional disability (modified Rankins Scale (mRS) > 2), and 12 cognitive impairment. Four patients had prior ICH and six had an ICH during follow-up. CSF-Aß40 and Aß42 levels were lower in cSAH and CAAo compared with HC. Convexal subarachnoid hemorrhage presented an APOE-ε2 overrepresentation and CAAo had an APOE-ε4 overrepresentation. On MRI, all patients fulfilled CAA-modified Boston criteria and 9 showed cortical ischemia in the surrounding cortex or the vicinity of superficial siderosis.
The neuropathologic study, available in one patient, showed severe CAA and advanced Alzheimer-type pathology. Convexal subarachnoid hemorrhage in the elderly is associated with cognitive impairment and lobar ICH occurrence. Our findings support the existence of an underlying CAA pathology.

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**Keywords:** *APOE*; biomarkers; cerebral amyloid angiopathy; lobar hemorrhage; subarachnoid hemorrhage

## INTRODUCTION

Atraumatic convexal subarachnoid hemorrhage (cSAH) is a rare type of cerebrovascular disease. Bleeding in cSAH is located in one or several adjacent sulci in the convexity of the brain. Two subtypes of cSAH have been proposed. The first subtype typically presents in young patients and has been associated with cerebral reversible vasoconstriction syndrome, while the second is usually observed in older patients and has been associated with cerebral amyloid angiopathy (CAA) and future lobar spontaneous intracerebral hemorrhage (ICH).[1,2]

The term CAA is used to describe the pathologic changes occurring in cortical and leptomeningeal cerebral vessels as the result of the deposition of β-amyloid proteins (Aß). The term ARIA (amyloid-related imaging abnormalities) refers to a spectrum of imaging abnormalities in the setting of amyloid-modifying therapeutic approaches. It includes signal changes thought to represent 'vasogenic edema', microhemorrhages, or cortical superficial siderosis (cSS).[3] The shift of amyloid in the perivascular spaces of small vessels is the common pathophysiologic mechanism underlying these phenomena.[3,4] Vasogenic edema may occur rarely in the natural history of AD, and more commonly, in patients with presumed CAA.[3]

The diagnostic criteria for CAA and ARIA rely on magnetic resonance imaging (MRI) findings.[3–5] Nonetheless, few recent studies have used other biomarkers.[6–9] The *APOE* genotype is central in the pathogenesis of CAA and ICH, and also in Alzheimer's disease (AD).[10–13]

Convexal subarachnoid hemorrhage has been associated with lobar ICH in previous studies.[2,14,15] It has been proposed that the bleeding begins in the subarachnoid space in a subset of ICH.[16,17] The rupture of arteries in the subarachnoid space would cause an initial bleeding contained by the surrounding cortex, which might

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develop ischemia. Subsequently, the hemorrhage would extend to the brain parenchyma. Convexal subarachnoid hemorrhage itself has been proposed as supportive of this theory.[18]

The objectives of this multicentric study were therefore threefold. First, to better characterize the clinical spectrum and prognosis of CAA-related cSAH in the elderly. Second, to show its association with CAA in a neuroimaging, genetic, and cerebrospinal fluid (CSF) biomarker study. Third, to study its association with ICH and to find evidence of acute or chronic cortical ischemia associated with the cSAH, which would support the aforementioned theory.

## MATERIALS AND METHODS

### Study Participants and Data Collection

Ambispective observational study of a convenient cohort from five tertiary care hospitals in Barcelona, Spain. Patients were recruited between 2008 and 2013. Radiologic reports were reviewed to screen those with a potential cSAH.

We included patients over 60 years who met the Kumar description for cSAH, in which the bleeding localized in the convexities of the brain without involvement of the adjacent parenchyma or extension into the interhemispheric fissures, basal cisterns, or ventricles.[1] Medical records, laboratory data, and imaging studies were sent to the coordinating center for evaluation—(HSP (Hospital de la Santa Creu i Sant Pau))—where a centralized readout of the computed tomography (CT) or MRI images, blinded to clinical, genetic, and CSF data, was performed. Follow-up information was obtained by outpatient consultation or telephone interview.

We suggested the cSAH patients to undergo a formal cognitive evaluation, a lumbar puncture, and *APOE* genotyping. For biochemical studies, CSF samples from non-cSAH CAA patients (CAAo), AD patients, and healthy controls (HC) were used. For genetic studies, DNA samples from CAAo patients, AD patients, and HC were selected from the Memory Unit of HSP.

We reviewed CAA patients from our Memory Unit cohort with available echo gradient or susceptibility MRI images without cSAH to select CAAo patients. We selected patients who met the modified Boston criteria for probable CAA and with genetic and CSF data available. Twelve patients were identified. They all were assessed because of cognitive impairment. In addition, 6/12 had presented with focal neurologic symptoms (visual disturbances in 3, seizures in 2, and a frontal syndrome in 1). More information is provided in Supplementary Material.

Patient outcome at follow-up was graded following the modified Rankins Scale (mRS).

The ethics committee from Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) approved the study.

### Cognitive Assessment

We suggested every cSAH patient to complete the study with a formal cognitive evaluation (Supplementary Table 1).

### Neuroimaging Data

All cSAH patients underwent a brain CT at admission in the emergency room and an MRI sometime during the process. All MRI scans included fluid attenuated inversion recovery, T2, T2*-GRE, and diffusion-weighted images (DWI). Some of the patients had also an angiographic study.

We assessed the modified Boston criteria for possible or probable CAA[5] in every patient as well as other CAA-associated features. The systematic evaluation of the MRI scans is detailed in Supplementary Material.

### Cerebrospinal Fluid Biomarkers and Genetics

In cSAH patients who consented to a lumbar puncture, core CSF (AD) biomarkers (Aß42, total-Tau-t-Tau- and phosphorylated Tau at threonine-181-p-Tau-) and Aß40 were determined. The CSF samples from 44 AD patients, 12 CAAo, and 20 HC were used for comparison.

*APOE* genotype was determined in cSAH patients who consented. Blood samples from 539 AD patients, 12 CAAo, and 324 HC selected from our Memory Unit were used for comparison.

Methods for CSF acquisition and biomarker measurement are detailed in Supplementary Material.

### Neuropathologic Study

Postmortem neuropathologic brain examination of one patient was performed, according to standardized protocols.[19]

### Statistical Methods

Statistical analyses were performed with the software Statistical Package for the Social Sciences v19 (http://www-01.ibm.com/software/es/analytics/spss/). Allele frequencies (proportion of chromosomes in which the allele was present) were compared using $2 \times 2$ tables with Fisher's exact test for significance. To study the differences of CSF biomarker levels between groups, we used the Mann–Whitney *U* test. Statistical significance for all the analyses was set at 5% ($\alpha = 0.05$).

## RESULTS

### Clinical Findings

Twenty-two patients with cSAH were included. Table 1 contains available data and Table 2 contains demographic data and neuroimaging findings.

The most frequent clinical presentation consisted of transient episodes of acute sensory and motor symptoms. In most cases they lasted less than 30 minutes. The episodes were usually repetitive and stereotyped (16 patients) and remitted completely. One patient died during admission because of an ICH 2 days after the cSAH.

Symptoms were related to cSAH topography, being negative ('stroke-like') in 6, positive in 4 ('prolonged aura-like'), and combined (positive and negative symptoms) in 12 patients. The most common positive symptoms were paresthesias (12 patients), affecting mouth and hand in all cases, and dysarthria (11 patients). Other less common symptoms included aphasia (5 patients), right cortical signs (2 patients), limb-jerking episodes (2 patients), and arm stiffness sensation (1 patient). None of our patients had thunderclap headache (2 presented nonspecific headache). One patient had a generalized tonicoclonic seizure. Surface electroencephalography was performed in 11 patients. Epileptiform activity was only found in 1 patient.

At least one vascular risk factor was present in all patients (77.3% had hypertension, 54.5% had dyslipidemia, and 18.2% had diabetes mellitus). Five patients had a history of heart attack, and six of them had a history of previous stroke (four with lobar hematomas, all of them symptomatic). Eleven patients were taking antithrombotic drugs at the time of cSAH (nine antiplatelets and two anticoagulant drugs, both in the normal range of international normalized ratios—between 2 and 3).

**Table 1.** Data of cSAH patients

| *Characteristics* | *Values* |
|---|---|
| *Imaging available* | |
| CT | 22 |
| MRI (images available/only reports) | 20/2 |
| Conventional angiography/Angio CT/MRA, No. | 3/4/10 |
| *CSF biomarkers (number of subjects)* | |
| cSAH group | 7 |
| HC group | 20 |
| AD group | 44 |
| *APOE genotype (number of subjects)* | |
| cSAH group | 13 |
| HC group | 324 |
| AD group | 539 |

Abbreviations: AD, Alzheimer's disease; cSAH, convexal subarachnoid hemorrhage; CSF, cerebrospinal fluid; CT, computed tomography; HC, healthy control; MRI, magnetic resonance imaging.






**Table 2.** Global demographics and neuroimaging findings in the cohort of cSAH patients

| *Characteristics* | *No. of patients* |
|---|---|
| No. of cSAH patients | 22 |
| Median age, years (Range) | 79.1 (69.7–92.2) |
| Gender (men) | 13 |
| *Vascular risk factors* | |
| Arterial hypertension | 17 |
| Dyslipidemia | 12 |
| Diabetes Mellitus | 4 |
| Previous acute myocardial infarction | 5 |
| Previous stroke (lobar hematoma) | 6 (4) |
| *Previous antithrombotic treatment* | |
| Antiplatelet drugs | 9 |
| Anticoagulant drugs | 2 |
| Follow-up information available | 21 |
| Median of follow-up, range | 30.7 months (0.1–49.3) |
| Recurrent cSAH | 0 |
| MCI | 8 |
| Dementia | 7 |
| Intracerebral lobar hemorrhage | 6 |
| Functional dependence (mRS $\geqslant$ 4) | 11 |
| Deaths | 5 |
| cSAH | 22 |
| Affecting 1 sulcus | 18 |
| Affecting $\geqslant$ 2 sulci | 4 |
| Cerebral microbleeds (total) | 8 |
| *Location* | |
| Lobar | 8 |
| Lobar and deep | 1 |
| *Number* | |
| 1–5 | 5 |
| 5–10 | 0 |
| $>$10 | 3 |
| Cortical superficial siderosis (total) | 19 |
| Focal ( $<$ 4 sulci) | 3 |
| Disseminated ($\geqslant$4 sulci) | 16 |
| History of stroke (total) | 6 |
| Lobar intracerebral hemorrhage | 4 |
| Lacunar stroke | 3 |
| Large vessel ischemic stroke | 2 |
| Leukoaraiosis (total) | 21 |
| Fazekas score 1 | 4 |
| Fazekas score 2 | 7 |
| Fazekas score 3 | 10 |
| Medial temporal lobe atrophy (total) | 3 |
| MTA $\leqslant$ 2 | 15 |
| MTA $>$ 2 | 3 |

Abbreviations: cSAH, convexal subarachnoid hemorrhage; MCI, mild cognitive impairment; mRS, modified Rankins Scale; MTA; media temporal lobe atrophy.

The median follow-up (available in 21 patients) was 30.7 months (range 0.1 to 49.3 months). Follow-up information revealed 6 patients had an ICH, located at the site of the previous cSAH in four cases; 4 of these patients died soon after the ICH and 2 required nursing care. In every case, it was a symptomatic ICH that was diagnosed with a TC scan. No follow-up imaging was performed in clinically stable patients. Regarding functional dependence, 47.6% of the patients had a favorable outcome (mRS 0 to 2) and 52.4% were disabled (mRS $\geqslant$ 3).

When focusing on the four patients with a previous ICH, 1 of them died, 3 remained functionally disabled (mRS: 4 to 5). The 3 survivals developed a dementia on the follow-up.

### Neuropsychologic Features

Ten cSAH patients consented to a neuropsychologic assessment.

At the time of the cSAH, 6 patients had a diagnosis of mild cognitive impairment (MCI) and 2 of mixed dementia. During the follow-up, 6 previously unimpaired patients were diagnosed with MCI and 1 with dementia. Four further MCI patients progressed to dementia. From the 10 patients formally evaluated, 1 was cognitively spared, 8 had memory impairment (6 with involvement of additional cognitive domains), and 1 had only executive dysfunction (details in Supplementary Table 1). The neuropsychologic profile was heterogeneous as was the degree of cognitive impairment (CDR-SOB ranging from 0.5 to 12). In 4 of the 10, there were no reports of cognitive impairment before the cognitive evaluation.

### Imaging Findings

Each patient had an MRI sometime during the process; 16/22 were performed in the acute phase before discharge (median time 4 days; range 0 to 11 days). MRI was deferred in three patients, specifically for this study (0.16, 0.63, and 2.40 years after the cSAH). Three patients had an MRI before the cSAH and had already been diagnosed with CAA-related ICH (3.5 years, 2 years, and 7 days before the cSAH).

Conventional angiography was performed in 3 patients, MR angiography in 10, CT angiography in 4, or both MR and CT in 1. The angiographic study was negative in all cases except in 2 patients who had an aneurysm affecting the contralateral vertebral artery and the posterior cerebral artery respectively, interpreted as incidental.

Neuroimaging data are summarized in Table 2 and Supplementary Table 2. CT detected the acute cSAH in all but 1 subject, who was later diagnosed by MRI fluid attenuated inversion recovery images.

### Modified Boston Criteria for Cerebral Amyloid Angiopathy and Systematic Evaluation of the Magnetic Resonance Imaging

Thirteen patients met the diagnosis of possible CAA and nine probable CAA. Radiologic findings are shown in Table 2.

Sixteen patients underwent an MRI during the acute phase before discharge that included DWI sequences. The DWI showed restricted diffusion involving the adjacent cortex surrounding the cSAH in 7 patients (Figure 1, Supplementary Figure 1). Moreover, 2 patients had chronic cortical infarcts in the vicinity of cSS or cSAH.

### Cerebrospinal Fluid Biomarkers and *APOE* Genotype

Cerebrospinal fluid was available in 7 cSAH patients. The median time between the vascular event and the lumbar puncture was 16 months (range 2 to 33 months). The results are shown in Figure 2 and Table 3.

The CSF profile was similar in cSAH and CAAo patients. We found significant reductions in CSF Aß42 and Aß40 levels, elevations in CSF t-Tau, and a trend in p-Tau with respect to HC. The AD patients showed significantly higher levels of t-Tau and p-Tau than cSAH and CAAo patients but no differences in CSF Aß42 and Aß40.

*APOE* genotype determination was performed in 13 cSAH patients. The *APOE* genotypes are summarized in Table 3. *APOE*-ε2 (allelic frequency of 19.2%) was significantly overrepresented in the cSAH group with respect to HC (5.4%; odds ratio 4.17). *APOE*-ε4 was significantly overrepresented in the CAAo group. There was no association of cSAH with *APOE*-ε4 genotype. The AD patients

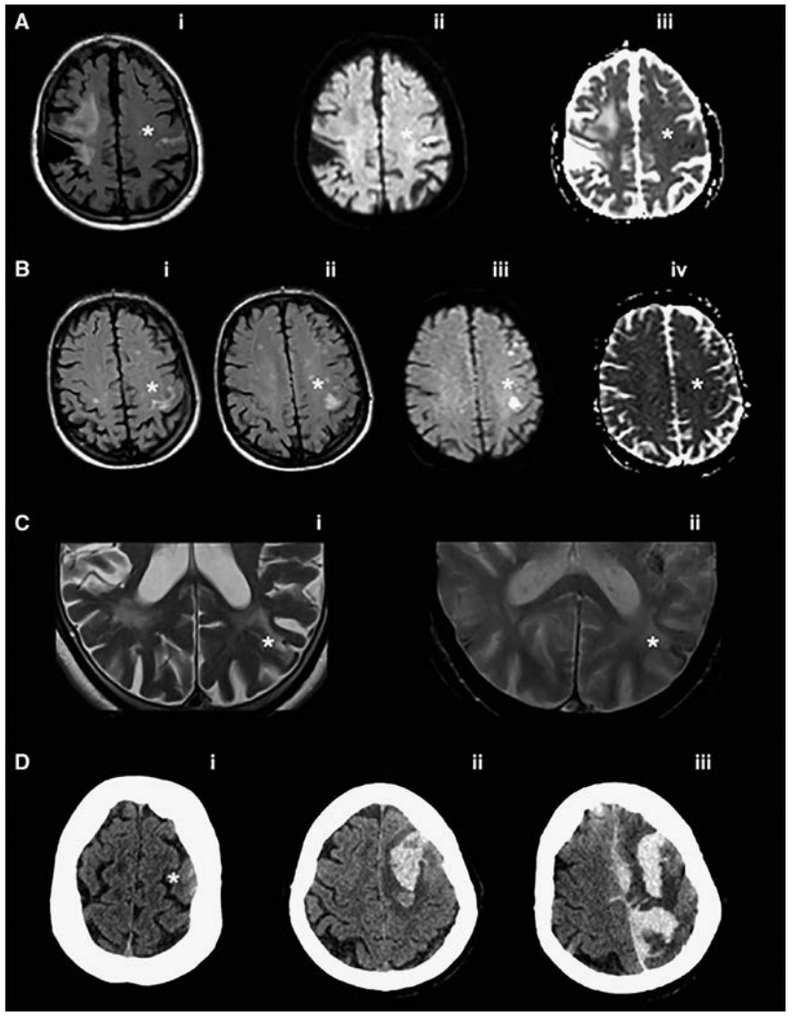


**Figure 1.** Atraumatic convexal subarachnoid hemorrhage (cSAH)-related ischemia and intracerebral hematomas in different patients. (**A**) Magnetic resonance imaging (MRI) performed 3 days after the onset of the symptoms showing the cSAH on the left precentral sulcus and a chronic right fronto-parietal ischemic stroke (fluid attenuated inversion recovery sequence) (i). Diffusion-weighted image (DWI) (ii) and apparent diffusion coefficient (iii) sequences show acute ischemia that colocalizes with the cSAH. (**B**) MRI performed 1 week after the onset of the symptoms showing the cSAH and subacute ischemia on the underlying cortex. Fluid attenuated inversion recovery sequences show cSAH on left precentral sulcus (i) and a hyperintense lesion located on the underlying cortex (ii). This area shows restricted diffusion on DWI (iii) and hypointense signal on apparent diffusion coefficient (iv). (**C**) MRI demonstrating a pure chronic cortical infarction affecting left posterior parietal cortex. T2-weighted sequence (i) shows the presence of a focal laminar cortical lesion (star). This region colocalizes with an area of cortical superficial siderosis (cSS), as shown in T2*-GRE weighted sequence (ii). (**D**) cSAH-related intracerebral hemorrhages. (i) Computed tomography (CT) showing an acute cSAH on the superior frontal sulcus. (ii) Three months later, the patient presented a frontal ICH that colocalized with the previous cSAH (iii). Sixteen hours later, the patient's clinical state worsened due to an ICH expansion that led to death after 2 days.

showed the expected underrepresentation of *APOE*-ε2 (3.4%) and overrepresentation of *APOE*-ε4 (25.5%) compared with HC.

### Neuropathologic Study

Pathologic brain examination was conducted in 1 cSAH patient. It showed neuropathologic changes associated with severe AD, severe amyloid angiopathy with capilar involvement, extense leptomeningeal siderosis, cortical microbleeds, and multiple intracortical microinfarcts (Figure 3) (Clinical and neuropathologic data are available in Supplementary Material).

## DISCUSSION

To our knowledge, this is the first study evaluating genetic and CSF biomarkers in cSAH in elderly people. Our main findings were






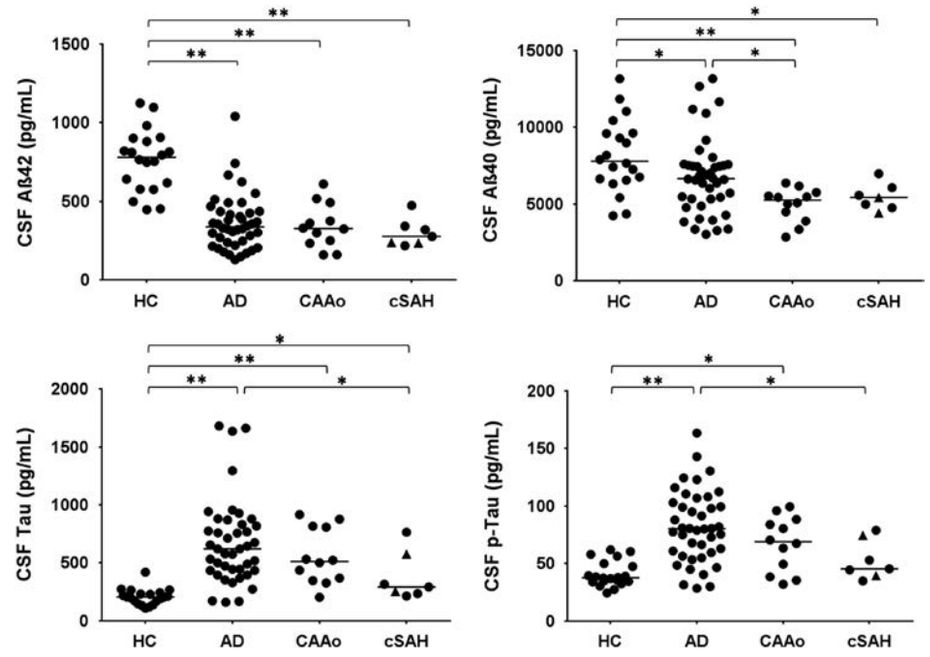


**Figure 2.** Cerebrospinal fluid (CSF) study. Healthy controls (HC), Alzheimer disease (AD) patients, cerebral amyloid angiopathy non-cSAH patients (CAAo), cSAH patients (cSAH). *P*-values $< 0.05$ are marked with a star (*); *P*-values $< 0.001$ are marked with double star (**). cSAH patients with a previous intracerebral hemorrhage (ICH) are highlighted using a triangled symbol. cSAH, convexal subarachnoid hemorrhage.

**Table 3.** Demographics, CSF, and *APOE* genotype data of the different clinical groups.

| | *cSAH* | *CAAo* | *AD* | | *HC* | |
|---|---|---|---|---|---|---|
| | | | *CSF* | *APOE genotype* | *CSF* | *APOE genotype* |
| *N* | 22 | 12 | 42 | 539 | 20 | 324 |
| Mean age, years (range) | 79.1 (69.7–92.2) | 69.8 (52.1–83.7) | 67.6 (50.6–79.8) | 79 (30.7–93.7) | 66.5 (55.7–77.5) | 72.3 (45–89) |
| Gender (% males) | 59 | 33 | 71 | 33.8 | 45 | 65.7 |
| CSF Aβ42 | 276 (•) (107) | 325.75 (225.5) | 336.25 (406.13) | *N/A* | 779.5 (308.6) | *N/A* |
| CSF Aβ40 | 5422 (•) (1308) | 5247 (1928) | 6656 (2330) | *N/A* | 7776 (3041) | *N/A* |
| CSF Tau | 292.5 (•) (340) | 512 (461.3) | 620.8 (406.1) | *N/A* | 205 (87) | *N/A* |
| CSF p-Tau | 45.5 (•) (35) | 69 (46.1) | 80.3 (45.3) | *N/A* | 37.8 (15.3) | *N/A* |
| *APOE*-E2 | 19.2 (••) | 4.2 | *N/A* | 3.4* | *N/A* | 5.4* |
| *APOE*-E3 | 69.2 (••) | 37.5* | *N/A* | 71.1 | *N/A* | 84 |
| *APOE*-E4 | 11.5 (••) | 58.3* | *N/A* | 25.5 | *N/A* | 10.6 |

Abbreviations: AD, Alzheimer's disease; cAAo, cerebral amyloid angiopatthy non-cSAH; cSAH, atraumatic convexal subarachnoid hemorrhage; CSF, cerebrospinal fluid; HC, healthy controls. - CSF study, median values (IQ range) in pg/mL. Performed on 7/22 ((•)). - *APOE* genotype study, allele frequency (%). Performed on 13/22 ((••)). *P*-values $< 0.05$ are marked with a star (*) when compared with cSAH group. Main results for *APOE*-ε2 are cSAH versus AD ($P = 0.002$); cSAH versus HC ($P = 0.01$); cSAH versus CAAo ($P = 0.192$). Main results for *APOE*-ε4 are cSAH versus CAAo ($P = 0.001$); CAAo versus AD ($P = 0.001$); CAAo versus HC ($P < 0.001$); and AD versus HC ($P < 0.001$).

as follows. First, the clinical presentation in CAA-related cSAH was fairly homogenous and associated with poor prognosis because of its frequent association with ICH and cognitive impairment in the follow-up. Second, our CSF results, neuroimaging data, the association with *APOE*-ε2 and lobar ICH suggest that CAA is the underlying cause of cSAH in our patients, as confirmed by postmortem brain examination in one subject. And third, the evidence for acute cortical ischemia in the cortex adjacent to the cSAH could support the theory that ICH in some of these patients might originate in the subarachnoid space and progress through an ischemic cortex into the brain.

The clinical presentation in our series was congruent with previous descriptions, consisted of transient focal neurologic symptoms related to the topography of the cSAH.[20] These symptoms were mostly recurrent, stereotyped, and brief. This transient presentation probably contributes to making cSAH an underdiagnosed entity.[2] There are discrepancies in the literature regarding the pathophysiology of the symptoms. Epileptic and

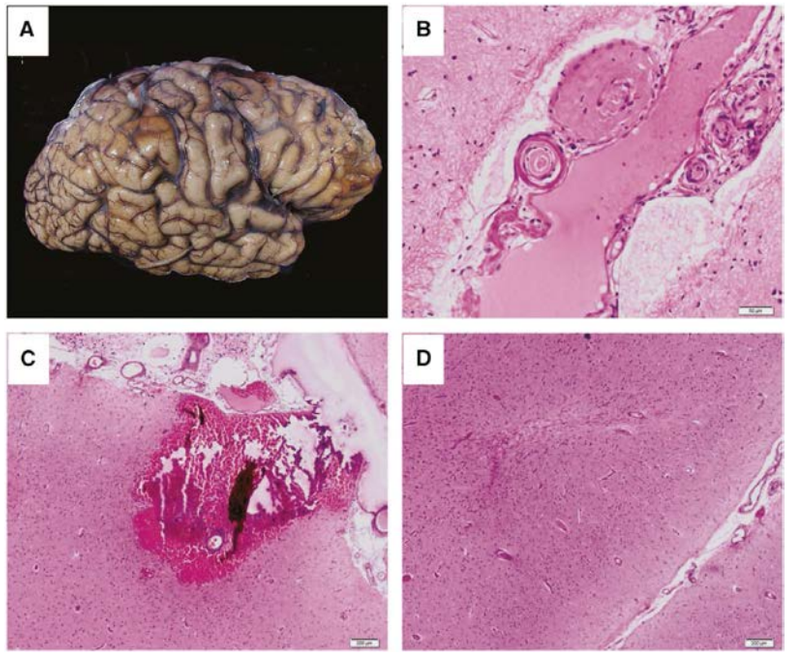


**Figure 3.** Neuropathologic findings. (**A**) External view of the fixed right cerebral hemisphere showing a yellow-brownish discoloration of basal temporal lobe and frontopolar and parietal region. (**B**) Prominent alterations of the vascular wall of large leptomeningeal vessels with concentric hyaline thickening and double barrel morphology (H&E). (**C** and **D**) Parenchymal lesions consisting in small fresh cortical superficial bleedings (**C**) and intracortical microinfarcts (**D**) in areas underlying prominent leptomeningeal amyloid angiopathy (H&E).

migraine aura-like cortical spreading depression mechanisms have been proposed, but other mechanisms are possible.[15,20] However, the lack of abnormal electroencephalography findings in our study and the finding of cSAH-related acute cortical ischemia—which has been associated with spreading depression in aneurysmatic SAH and stroke—broadly favor the nonepileptic mechanism.[21]

Cerebral amyloid angiopathy can be associated with both MCI and full-blown dementia.[22,23] The pattern of cognitive impairment is related to the underlying mechanisms (CAA-related ICH or infarcts, leukoaraiosis, Alzheimer-type pathology, or CAA by itself).[22] We were able to identify all these mechanisms in our patients, explaining both the very high prevalence and incidence of cognitive impairment and the different patterns and degrees observed. No imaging follow up was performed in non-(new) symptomatic patients. Although cSAH did not recur, the frequent occurrence of ICH and cognitive impairment explains the poor outcome in our patients. Therefore, while previous literature suggested that cSAH had a favorable prognosis the only study with a long follow-up reported a poor outcome in cSAH, specially in those patients with neuroimaging findings compatible with CAA.[2,15,24]

Our neuroimaging, genetic, and CSF biomarker findings support the hypothesis that CAA is the underlying cause of cSAH in our patients. On MRI, 40.9% of patients fulfilled the modified Boston Criteria for probable CAA and 51.1% for possible CAA. Microbleeds and cSS might be related to different pathophysiologies.[25] We found a high frequency of disseminated cSS beyond the cSAH location, in agreement with previous studies in which transient focal neurologic episodes were the strongest clinical marker for cSS.[5,26] These figures, however, greatly differ from epidemiologic studies of AD in which cSS is found in only 1% of cases.[27,28] Microbleeds were found in 40%, in contrast to a prevalence of 68% in ICH series and 25% in AD series.[28,29] These observations could be contextualized within the idea highlighted by Charidimou and Jäger, in which several different endophtenotypes of CAA may exist. Our patients would fit in the group of 'macrobleeders', where there is a low microbleed count and cSS would be associated itself with high risk of hemorrhage.[25,30]

The CSF profile in cSAH patients characterized by reductions in Aß40 and Aß42 is consistent with the profile reported in CAA.[7,8] This pattern was previously found in two cSAH patients and in four cSS patients.[8,9] The mild elevation in CSF t-Tau and p-Tau levels (significantly lower than in AD patients) is consistent with a lower level of tau-containing pathology in CAA. However, in the patient with neuropathologic study, extensive tau-positive neurofibrillary pathology was observed. We also found an overrepresentation of the *APOE-ε2* allele in cSAH but not of *APOE-ε4*. Although there are no previous genetic studies in cSAH, *APOE-ε2* and *APOE-ε4* are independent risk factors for ICH.[12,13] The fact that the CSF profile is similar in cSAH and CAAo patients indicates that there is an underlying CAA but also raises the possibility that mechanisms other than amyloid burden may have a role in cortical and leptomeningeal vessel fragilty leading to cSAH and cSS.

*APOE-ε2* and *APOE-ε4* might promote CAA-related hemorrhage through separate mechanisms: *APOE-ε4* would enhance amyloid deposition, whereas *APOE-ε2* would favor amyloid-laden vessels to undergo the vasculopathic changes that lead to rupture.[31] This hypothesis would explain the influence of *APOE-ε2*, but not of *APOE-ε4* on ICH expansion.[12]






The high association of cSAH with ICH in our series together with the observation of acute cortical ischemia in the vicinity of cSAH (which would enable the extension of the hematoma to the brain parenchyma) could be an *in vivo* evidence for the hypothesis of Takeda *et al*[16,17] concerning CAA-related ICH pathogenesis.[32] Our results are also compatible with the 'tsunami hypothesis' to explain hematoma growth. According to this theory, the brain blood vessels surrounding the initial bleeding in *APOE-ε2* carriers are more prone to bleeding themselves, and contribute to hematoma enlargement.[12,33,34] In our study, the ICH was located in the vicinity of a previous cSAH (four patients) or cSS (two patients), a colocalization that has also been found in cSS.[35] Therefore, cSS might be a marker of vessel fragilty.[26] In fact, cSS, particularly if disseminated, has also been associated with an increased risk of symptomatic lobar ICH.[26] The chronic cortical infarct found in the vicinity of cSS also supports Takeda's theory. Of note, focal laminar cortical lesions after aneurysmatic SAH in the absence of macroscopic vasospasm are more common than territorial infarcts.[36,37]

The present study has some limitations. First of all, we used a convenient sample. This could affect the generalizability of the findings because our sample might not be representative of the general population. All patients who presented with cSAH in the emergency room or in the clinics between 200 and 2013 were included, so our sample equates to a great extent of the elder population in Barcelona. Second, the sample sizes in the biomarker subgroups were small. However, significant differences were found in the CSF and in the genetic analyses. Third, although the mean follow-up was 2 years, some patients had a shorter follow-up. However, cSAH was clearly associated with poor prognosis. Fourth, regarding the areas of restricted diffusion in close proximity to cSAH, one concern is that they might not necessarily reflect acute microinfarction, but instead restricted diffusion due to the presence of acute blood products.[38] The DWI signal intensity changes should be interpreted in light of the T2 signal intensity changes, especially at the earliest time point, when both, hematoma and acute ischemia, are hyperintense on DWI images. At the acute stage, hematomas are hypointense on T2-weighted images.[38] The fact that our cSAH was hyperintense on T2-weighted images suggests the presence of acute cortical ischemia in the vicinity of cSAH, more than restricted diffusion due to the presence of acute blood products. Furthermore, in some of the cases, chronic infarctions were found in the vicinity of SS both in the MRI and in the pathologic examination. On the other side, the association between cSAH or cSS and ischemic lesions might just indicate focally active severe CAA-related disease and the observation of cortical ischemia could be due to microvascular spasm, cerebral autoregulation impairment or other mechanisms. Those findings should be taken with caution and require further study to be confirmed. Finally, definite histologic CAA diagnosis, was only available in one patient.

In conclusion, our biomarker and genetic study supports an underlying CAA pathology for cSAH in elderly patients. Convexal subarachnoid hemorrhage might be considered a warning sign for poor prognosis because of its frequent association with cognitive impairment and ICH. The frequent lobar ICH in these patients might originate in the subarachnoid space and progress through an ischemic cortex into the brain. However, further studies with neuropathologic confirmation are needed to confirm these hypotheses.

## DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.




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# ANNEX 2:
# ORIGINAL PUBLISHED PDF CHAPTER 4

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Short Communication

# Cerebrospinal Fluid Anti-Amyloid-β Autoantibodies and Amyloid PET in Cerebral Amyloid Angiopathy-Related Inflammation

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**Abstract**. We report a biomarker and genetic evaluation of four patients with cerebral amyloid angiopathy-related inflammation (CAA-ri) treated with corticosteroids. Patients presented with focal symptomatology and cognitive impairment. MRI revealed cortical microbleeds and asymmetrical hyperintense white matter lesions (WML). Cerebrospinal fluid (CSF) biomarker analyses showed increased anti-Aβ autoantibodies, t-Tau, and p-Tau and decreased $A\beta_{40}$ and $A\beta_{42}$. After treatment, focal symptomatology disappeared, and WML and anti-Aβ autoantibodies decreased. The *APOE ε4* allele was overrepresented. Florbetapir-PET showed cortical deposition with lower retention in swollen areas. In the case of suspected CAA-ri, both CSF anti-Aβ autoantibodies levels and Florbetapir-PET could provide highly useful data to guide the correct diagnosis.

Keywords: Biomarkers, cerebral amyloid angiopathy, cerebrospinal fluid, Florbetapir-PET, inflammation

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## INTRODUCTION

Cerebral amyloid angiopathy-related inflammation (CAA-ri) is a rare type of meningoencephalitis that affects a subgroup of CAA patients who develop vascular inflammation. Clinically, CAA-ri usually presents with cognitive decline and focal neurological symptoms, white matter abnormalities on T2-weighted images, and multiple microhemorrhages on T2*-GRE weighted images [1]. In some cases, reaching a diagnosis is complex, and invasive procedures, such as a cerebral biopsy, are required. This complexity, together with the variable clinical course and the usual responsiveness of CAA-ri to immunosuppressive treatment [2], highlights the need of biomarkers to allow early diagnosis.

The CAA-ri pathogenesis remains unknown. The genetic studies have found an association with the *APOE ε4* allele [3]. The better characterization of CAA-ri through biomarkers have allowed the development of diagnostic criteria for probable CAA-ri based on typical clinico-radiological findings without requiring a biopsy [2, 3].

Cerebrospinal fluid (CSF) and amyloid positron emission tomography (PET) are increasingly recognized as markers of CAA, however, there are few studies evaluating biomarkers in CAA-ri [4–6]. It is worthy to mention that increased CSF anti-Aβ autoantibodies in the acute phase of CAA-ri were first reported by DiFrancesco et al. [7], while Piazza et al. recently suggested that they might be a specific marker of CAA-ri [8].

We describe a case series of four CAA-ri patients in whom CSF AD biomarkers, anti-Aβ autoantibodies, and amyloid PET biomarkers were analyzed.

## METHODS

### *Study participants and procedures*

A convenient sample of four patients with focal neurological symptoms due to probable CAA-ri were evaluated. Diagnosis of probable CAA-ri was performed based on the proposed diagnostic clinical criteria [3]. All participants provided written informed consent approved by the local ethics committee.

We performed a pre and post-treatment brain MRI and lumbar puncture for CSF core Alzheimer's disease (AD) biomarkers ($A\beta_{42}$, Tau, p-Tau), $A\beta_{40}$, and anti-Aβ autoantibodies determination [8]. *APOE* genotype was determined. Florbetapir-PET was available in two patients. A detailed description of the different procedures is available in the Supplementary Material.

### *Statistical analysis*

Non-parametric tests were used to calculate the differences between CSF biomarkers before and after the treatment.

## RESULTS

### *Study participants*

Table 1 summarizes the demographic, clinical, and biomarker data of the four patients.

**Patient 1.** A 73-year-old man with a 2-year history of amnestic mild cognitive impairment (MCI) and arterial hypertension presented with an acute left frontal syndrome and cognitive impairment. Brain MRI showed hyperintense white matter lesions (WML) predominantly in left frontal region and multiple diffuse lobar/cortical microbleeds (Fig. 1A,B). Intravenous corticosteroids were administered. The patient's frontal syndrome resolved, and his cognitive status promptly improved. Follow-up MRIs post-treatment showed a reduction of the WML (Fig. 1C), new cortical microbleeds, superficial siderosis, and further cortical atrophy. During the follow-up, he developed dementia.

**Patient 2.** A 74-year-old man with a history of cerebellar hemorrhage and amnestic MCI was referred because of left arm paresis and epileptic seizures. The initial MRI showed hyperintense asymmetrical WML, involving frontal and parieto-occipital regions, lobar microbleeds, and old hemorrhages (cerebellum and right putamen). Cognitive assessment was impracticable. Intravenous corticosteroids were administered, resulting in cognitive improvement. Two months later, he presented a bronchopneumonia and died.

**Patient 3.** A 68-year-old woman with a history of dyslipidemia and multidomain MCI was referred because of subacute aphasia and cognitive impairment in the two previous months. The initial MRI showed hyperintense, asymmetrical, and confluent WML, affecting predominantly the left temporo-parietal region, with lobar/cortical microbleeds located predominantly in those areas (Fig. 1E,F). Intravenous corticosteroids were administered, resulting in cognitive improvement and a reduction of the WML in the post-treatment MRI (Fig. 1G). During follow-up, her cognition worsened and the follow-up MRI showed

Table 1
Characteristics of 4 patients with CAA-ri

| | | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|---|---|---|---|---|---|
| Age, y/gender | | 73/M | 74/M | 78/F | 68/F |
| Previous cognitive status | | MCI | MCI | MCI | Normal |
| | | MMSE 29/30 (4 months before) | MMSE 22/30 (11 months before) | MMSE 22/30 (9 months before) | MMSE 29/30 |
| Focal symptoms | | Frontal syndrome | Left arm paresis<br>tonico-clonic seizures | Aphasia | Visual disturbances (palinopsia, oscilopsia) |
| | | MMSE 24/30 | MMSE not applicable | MMSE 7/30 | MMSE 28/30 |
| Pre-treatment MRI | | Hyperintense WML and swelling affecting predominantly left frontal region | Hyperintense right fronto-parieto-occipital swelling WML | Hyperintense left temporo-parietal WML causing mass effect | Hyperintense bilateral occipital swelling WML |
| | | Multiple and diffuse, but predominantly left frontal CMB | Multiple and predominantly right frontal CMB and deep microbleeds | Multiple predominantly left temporal CMB | Multiple global, but predominantly right occipital, CMB |
| | | Cortical temporal, frontal and parietal bilateral cSS | Cerebellar cSS | No cSS | Frontal and parietal bilateral cSS |
| | | (Fig. 1A, 1B) | | (Fig. 1E, 1F) | |
| Duration of focal symptoms at the time of pre-treatment MRI | | 4 months | 1 month | 2 months | 5 months |
| Pre-treatment CSF study | | | | | |
| Core AD CSF biomarkers (pg/mL) | $A\beta_{40}$ | 5750 | 2835.7 | 6365.9 | 4480 |
| | $A\beta_{42}$ | 249.5 | 158.5 | 323 | 298.5 |
| | t-Tau | 368.5 | 523 | 915.5 | 326.5 |
| | p-Tau | 70.5 | 35.5 | 99.5 | 49.5 |
| Anti-Aβ autoantibodies (ng/mL) | | 40.3 | 68.8 | 50.4 | 53.4 |
| Duration of focal symptoms at the time of pre-treatment CSF study | | 0.5 months | 2 months | 2 months | 8 months |
| Corticosteroids therapy | | Metilprednisolone 1000 mg/d × 3 days+decreasing dose 5 months | Metilprednisolone 1000 mg/d × 3 days + decreasing dose 2 months | Metilprednisolone 1000 mg/d × 5 days + decreasing dose 3 months | Metilprednisolone 1000 mg/d × 3 days + decreasing dose 3 months |
| Duration of focal symptoms at the time of corticosteroids | | 4 months | 2 months | 2 months | 11 months |
| Clinical response to treatment after bolus | | MMSE 26/30 | MMSE 17/30 | MMSE 14/30 | MMSE 30/30 |
| | | Total frontal syndrome resolution | Partial improvement of the paresis | Partial language improvement | Partial visual improvement |
| Post-treatment MRI | | Partial reduction of WML, remission of swelling (Fig. 1C) | ND | Persistence of WML, reduction of swelling and mass effect (Fig. 1G) | Partial reduction of WML, remission of swelling |

*(Continued)*

Table 1
*(Continued)*

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 |
|---|---|---|---|---|
| Time between corticosteroids and post-treatment MRI | 2 months | ND | 1 month | 1 months |
| Post-treatment CSF study | | | | |
| Core AD CSF biomarkers $A\beta_{40}$ (pg/mL) | 5497.4 | ND | 5436.6 | 3667.1 |
| $A\beta_{42}$ | 223 | ND | 325.5 | 238.5 |
| t-Tau | 271 | ND | 528 | 175.5 |
| p-Tau | 50.5 | ND | 73 | 31 |
| Anti-Aβ autoantibodies (ng/mL) | 23.78 | ND | 29.99 | 33.23 |
| Time between corticosteroids and post-treatment CSF studies | 13 months | ND | 10 months | 2 months |
| Clinical follow-up | MMSE19/30; mRS4 | mRS6 | MMSE11/30; mRS4 | mRS6 |
| Time of clinical follow-up after corticosteroids | 28 months | 2 months | 16 months | 6 months |
| Follow-up MRI | Increased number of CMB<br>New areas of cSS<br>Greater cortical atrophy | ND | Increased number of CMB | ND |
| Time between corticosteroids and follow-up MRI | 24 months | ND | 10 months | ND |
| *APOE* genotype | 4/4 | 4/4 | 4/3 | 3/3 |
| Amyloid PET (Florbetapir) | Positive (Fig. 1D) | ND | Positive (Fig. 1H) | ND |
| Time between corticosteroids and amyloid-PET | 19 months | ND | 13 months | ND |

Aβ, amyloid-β; CAA, cerebral amyloid angiopathy; CMB, cortical microbleeds; cSS, cortical superficial siderosis; F, female; M, male; MCI, mild cognitive impairment; MMSE, Mini-Mental State Exam; MRI, magnetic resonance imaging; ND, not done; WML, white matter lessions; mRS, modified Rankin Scale. Our internal cut-off values are 550 pg/ml for $A\beta_{42}$, 350 pg/mL for t-Tau, and 61 pg/mL for p-Tau, as cited in the Supplementary Material [9]. Anti-Aβ autoantibodies dosage observed in healthy controls is $18.1 \pm 5.7$ ng/mL [8].



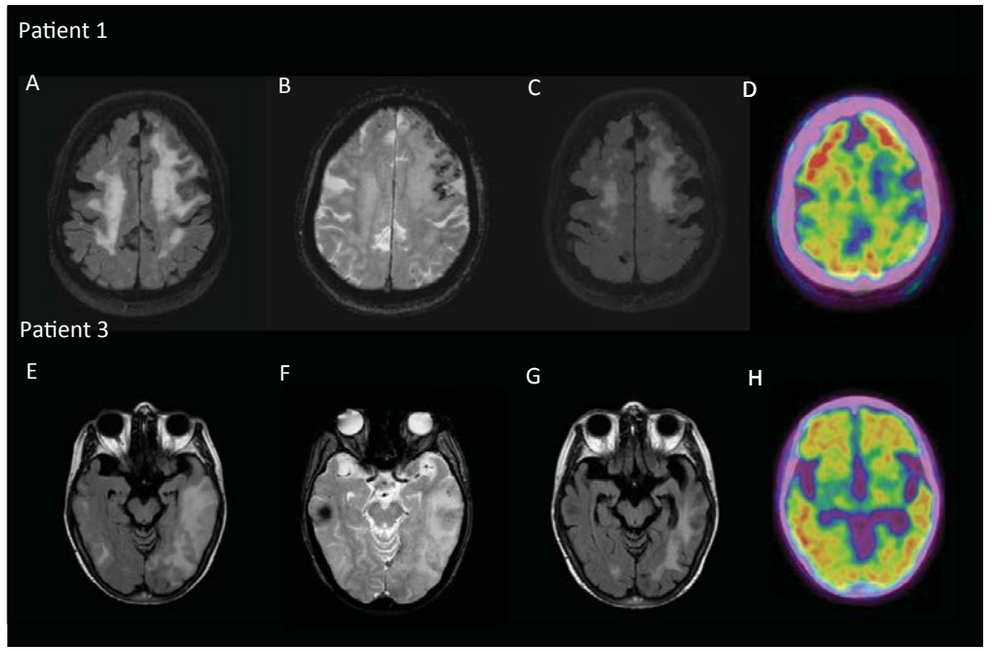


Fig. 1. Neuroimaging findings in 2 patients with CAA-ri. Neuroimaging findings in patient 1 (A–D) and patient 3 (E–H). A) Magnetic resonance imaging (MRI) demonstrating subcortical asymmetric white matter lesions affecting predominantly left frontal lobe on a fluid-attenuated inversion recovery sequence (FLAIR) sequences. B) MRI T2*-GRE weighted sequences showing extensive lobar microbleeds that colocalized with the area of the edema. C) FLAIR sequence MRI performed two months after corticosteroid treatment, showing reduction of cerebral edema. D) PET imaging with Florbetapir showing retention of the amyloid tracer in frontal and parietal regions. Note the swollen region, where PET shows a lower Florbetapir uptake (SUVr = 1.08 in left frontal inferior gyrus, SUVr = 1.05 in left precentral gyrus, SUVr = 1.08 in left postcentral gyrus) than in the homonym contralateral areas (SUVr = 1.10 in right frontal inferior gyrus, SUVr = 1.06 in right precentral gyrus, SUVr = 1.18 in right postcentral gyrus). E) MRI performed to patient 3, showing subcortical asymmetric white matter lesions affecting left temporal occipital lobes on FLAIR sequence. F) MRI T2*-GRE weighted sequences indicating the presence of lobar microbleeds on the affected areas (arrows). G) One month after corticosteroid treatment the FLAIR MRI image showed reduction of the white matter lesions. H) PET imaging with Florbetapir shows cortical retention of the amyloid tracer. Note the swollen region, where the Florbetapir uptake (SUVr = 1.85 in left temporal middle gyrus, SUVr = 1.89 in left occipital middle gyrus) is lower than in homonym contralateral regions (SUVr = 1.98 in right temporal middle gyrus, SUVr = 1.97 in right occipital middle gyrus).

new parieto-occipital lobar microbleeds and medial temporal atrophy.

**Patient 4.** A 68-year-old woman with a history of dyslipidemia and arterial hypertension was referred because of oscilopsia and stereotyped, repetitive, and self-limited visual hallucinations. The EEG was normal. Initial MRI showed hyperintense asymmetrical cortico-subcortical WML in both occipital lobes, and multiple bilateral occipital lobar microbleeds. After receiving IV corticosteroids, visual symptoms partially improved. After 6 months of follow-up, she died with a spontaneous lobar frontal intracerebral hemorrhage.

*Biochemical and neuroimaging findings*

CSF analyses showed low $A\beta_{40}$ and $A\beta_{42}$ levels in all patients. T-Tau and p-Tau levels were increased in three and two patients, respectively [9]. AD biomarkers did not significantly change after treatment (Table 1).

Anti-Aβ autoantibody titers were elevated in all four patients pre-treatment. After corticosteroids, the titer returned to normal levels in the three patients with available follow up CSF ($p = 0.034$) [8].

The *APOE* ε4 allele frequency was 62.5%.

Florbetapir-PET was performed in patients #1 and #3 (Fig. 1D,H), 19 and 13 months after

corticosteroid treatment, respectively. The visual assessment showed Aβ deposition. The quantified analysis (both by lobes or Automated Anatomic Labeling atlas, however, showed lower cortical tracer uptake in the areas in which inflammation had developed than in contralateral homonymous regions (Supplementary Table 1) (Fig. 1D,H).

## DISCUSSION

The present study analyses CSF anti-Aβ autoantibodies and amyloid PET in CAA-ri. This pilot study suggests that CSF analysis and amyloid PET might add diagnostic specificity to the proposed clinical criteria for CAA and that CSF anti-Aβ autoantibodies might help diagnose and monitor the response to treatment in CAA-ri [8].

The clinical presentation consisted of focal neurological symptoms and rapidly progressive cognitive decline. MRI examinations showed, besides CAA-related findings, asymmetrical and confluent WML, indicating vasogenic edema that remitted shortly after corticosteroids. However, clinical prognosis was poor. Two patients died during the follow-up and one developed dementia.

The CSF data showed a pattern compatible with an underlying CAA [10]. Low levels of $A\beta_{40}$ and $A\beta_{42}$ in CSF could be an alternative diagnostic marker of CAA [4, 7, 11, 12]. Some [4, 8], but not all [5], previous works have reported increased CSF $A\beta_{42}$ and $A\beta_{40}$ levels in the acute phase of CAA-ri. We did not find this elevation, but the antecedent MCI (prodromal AD) in three out of four patients, which might have affected CSF AD biomarker levels, should be noted.

Florbetapir-PET showed widespread cortical amyloid deposition. Regions presenting with inflammation in the acute phase seemed to present lower retention. This pilot study might suggest a reduction of Aβ uptake after remission that must be confirmed with further dedicated studies. There is only one recent case report with Pittsburg Compound B PET in CAA-ri [13]. This finding suggests a relationship between inflammation and amyloid clearance, a liaison that has been described in the Bapineuzumab and gantenerumab studies [14–16]. Beyond the mechanistic importance of amyloid PET, amyloid imaging might aid in the diagnosis of patients with focal syndromes and suspected CAA in the acute phase given the aforementioned conflicting results of CSF $A\beta_{40}$ and $A\beta_{42}$ levels at this stage [6, 13].

The genetic analyses showed that the *APOE* ε4 allele was overrepresented (when compared to Spanish population) [17]. This has been already reported in CAA-ri [2, 3]. Of note, beyond the clinical similarities between CAA-ri and the amyloid related imaging abnormalities observed in AD patients treated with anti-amyloid therapies [8, 14–16], were more frequent in the higher-dose groups of patients carrying the *APOE* ε4 allele and with a major vascular amyloid burden [18].

Autoantibody titer were high during the acute phase and decreased to normal values after corticosteroids [4, 5, 7, 8]. To our knowledge this is the first study replicating the findings of Piazza et al. on the utility of anti-Aβ autoantibodies titer to diagnose CAA-ri. We suggest that CSF anti-Aβ autoantibodies should be added to the diagnostic criteria for CAA-ri in order to reduce the number of cerebral biopsies. Moreover, CSF anti-Aβ autoantibodies might be used to monitor treatment response. This is usually done with MRI, but although clinical response to immunosuppressive therapy is associated with lesion volume reductions, CAA-ri is also associated with irreversible WML [2, 18].

The main limitation of the study is the absence of neuropathological confirmation in our patients. Nonetheless, our findings support the hypothesis that anti-Aβ autoantibodies play a role in the pathophysiological process of CAA-ri. Other limitations include the absence of a control group of sporadic CAA cases or AD that should be explored in future works. Finally, brain atrophy might have affected the quantification of Aβ deposition.

In conclusion, our results support the utility of CSF core AD biomarkers, CSF anti-Aβ autoantibodies, and amyloid PET in CAA-ri. However, further studies with larger samples and neuropathological confirmation are needed to confirm these findings.

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## SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: http://dx.doi.org/10.3233/JAD-150614.

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# ANNEX 3:
# ORIGINAL PUBLISHED PDF CHAPTER 5

ELSEVIER

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Alzheimer's & Dementia

Featured Article

# Cerebral amyloid angiopathy in Down syndrome and sporadic and autosomal-dominant Alzheimer's disease

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**Abstract**

**Introduction:** We aimed to investigate if cerebral amyloid angiopathy (CAA) is more frequent in genetically determined than in sporadic early-onset forms of Alzheimer's disease (AD) (early-onset AD [EOAD]).

**Methods:** Neuroimaging features of CAA, apolipoprotein (*APOE*), and cerebrospinal fluid amyloid-β (Aβ) 40 levels were studied in subjects with Down syndrome (DS, $n = 117$), autosomal-dominant AD (ADAD, $n = 29$), sporadic EOAD ($n = 42$), and healthy controls ($n = 68$).

**Results:** CAA was present in 31%, 38%, and 12% of cognitively impaired DS, symptomatic ADAD, and sporadic EOAD subjects and in 13% and 4% of cognitively unimpaired DS individuals and healthy controls, respectively. *APOE* ε4 genotype was borderline significantly associated with CAA in sporadic EOAD ($P = .06$) but not with DS or ADAD. There were no differences in Aβ040 levels between groups or between subjects with and without CAA.

**Discussion:** CAA is more frequently found in genetically determined AD than in sporadic EOAD. Cerebrospinal fluid Aβ40 levels are not a useful biomarker for CAA in AD.



*Keywords:* Cerebral amyloid angiopathy; Sporadic early-onset Alzheimer's disease; Autosomal-dominant Alzheimer's disease; Down syndrome; Neuroimaging; Cerebrospinal fluid biomarkers

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## 1. Introduction

Most cases of Alzheimer's disease (AD) are sporadic and caused by complex interactions between genetic and environmental factors. In approximately 5% of cases, AD can present clinically before the age of 65 years (early-onset AD [EOAD]) [1]. These patients frequently present with nonamnestic phenotypes and faster clinical decline than older sporadic AD cases [1]. In 0.1% to 0.5% of cases, AD is transmitted with an autosomal-dominant pattern of inheritance (autosomal-dominant AD [ADAD]) due to the presence of mutations in presenilin 1 (*PSEN1*), presenilin 2 (*PSEN2*), or amyloid precursor protein (*APP*) genes [2]. Down syndrome (DS) is also recognized as a form of genetically determined AD, mainly caused by the *APP* gene triplication [2]. Despite the different genetic background, the AD neuropathologic findings in sporadic EOAD, ADAD, and DS are very similar [3,4].

Cerebral amyloid angiopathy (CAA) is a major cause of lobar intracerebral hemorrhage (ICH) in the elderly and is present in up to 90% of AD brains at autopsy [3]. Previous neuropathologic studies have suggested a more severe CAA in ADAD than in sporadic AD [4]. CAA in some *APP* mutations or duplication carriers drives the clinical presentation [4] and is also consistently observed in subjects with DS [5]. The modified Boston criteria for CAA (mBCAA)–related hemorrhage have been validated to attribute in vivo an ICH to CAA based on several neuroimaging features and are frequently used in clinical practice [6]. There are no previous studies systematically assessing the CAA neuroimaging features in DS and ADAD.

Amyloid β (Aβ)40 is the major form of Aβ deposited in the vessel walls in individuals with CAA. Low levels of Aβ40 and Aβ42 have been found in the cerebrospinal fluid (CSF) of subjects with sporadic CAA [7]. However, scarce and contradictory data are available about the CAA CSF biomarker profile in sporadic AD patients [8–10], and no previous studies have assessed this profile in DS or ADAD. Moreover, the apolipoprotein (*APOE*) ε4 genotype is a risk factor for both sporadic AD and sporadic CAA [11], as it increases Aβ deposition in both the parenchyma and blood vessels [12]. However, the effect of the *APOE* genotype in AD dementia within DS and ADAD is controversial, and there are no studies assessing the influence of the *APOE* genotype on CAA in ADAD or DS [13].

The differences in the CAA neuroimaging features and CSF biomarkers profile in the different forms of AD are, thus, not established. Our primary objective was to determine the CAA presence by assessing the fulfillment of the mBCAA and the CSF Aβ40 levels in three different AD populations (DS, ADAD, and EOAD). We hypothesized that patients with genetically determined AD would have more CAA neuroimaging and biochemical features than EOAD.

## 2. Materials and methods

### 2.1. Study design and participants

A total of 256 subjects were recruited from five centers: Hospital de Sant Pau, Hospital Clínic de Barcelona, and Barcelona Down Medical Center in Barcelona, Spain; and the Sanders-Brown Center on Aging in Kentucky, and the Down Syndrome Biomarker Initiative project in San Diego, USA. Four study groups were evaluated: EOAD, ADAD, DS, and healthy controls (HCs).

EOAD ($N = 42$): patients were recruited at the Memory Unit of Hospital de Sant Pau from the Sant Pau Initiative on Neurodegeneration (Barcelona SPIN cohort) [14]. We used the International Working Group-2 for AD with in vivo evidence of AD based on CSF biomarkers [2]. This group included 19 individuals with prodromal EOAD (p-EOAD) and 23 subjects with probable dementia in EOAD (d-EOAD).

ADAD ($N = 29$): participants were recruited from the Genetic Counseling Program for familial dementias (PICOGEN) at the Hospital Clínic de Barcelona [15]. Fifteen symptomatic carriers (CDR $\geq 0.5$) carrying nine different *PSEN1* mutations (M139T, S169P, L173F, G209E, L235R, K239N, L282R, L286P, and I439S) and one symptomatic carrier of the *APP* I716T mutation were included. The symptomatic carriers were further classified as prodromal AD in ADAD (pAD-ADAD, $n = 5$) and dementia in ADAD (dADAD, $n = 11$). Twelve presymptomatic mutation carriers (CDR = 0) carrying seven different *PSEN1* mutations (M139T, S169P, L173F, R220G, K239N, L282R, and I439S) and one presymptomatic carrier of *APP* mutation (I716T) were labeled as asymptomatic ADAD. We used the International Working Group-2 diagnostic criteria for AD [2].

DS ($N = 117$): adults with DS were recruited from three centers—the Down Alzheimer Barcelona Neuroimaging Initiative in the Barcelona Down Medical Center [16]; the Sanders-Brown Center on Aging; and the Down Syndrome Biomarker Initiative pilot project [17]. Adapted neuropsychological batteries (detailed in the Appendix section) covering all cognitive domains classified DS participants into "without cognitive decline" (asymptomatic DS, $n = 91$), prodromal AD in DS (pAD-DS, $n = 13$), and AD dementia in DS (dAD-DS, $n = 13$). Participants with pAD-DS and dAD-DS were also labeled as symptomatic DS participants.

HCs ($N = 68$): participants were recruited at Hospital de Sant Pau ($n = 60$) and Hospital Clínic de Barcelona ($n = 8$) enrolled among patients' caregivers. They did not have cognitive complaints, scored zero on CDR, had normal neuropsychological evaluation, and normal core AD CSF biomarkers [18,19].

### 2.2. Procedures

Medical records were reviewed for potential confounders; and effect modifiers, age, sex, and presence of arterial hypertension, and neuropsychological information on disease severity (Mini–Mental State Examination [MMSE] for EOAD and ADAD and the Cambridge Examination for mental Disorders of Older People with DS and Others with Intellectual Disabilities–A Cognitive Scale for DS) were recorded and sent to the coordinating center (Hospital de Sant Pau) with the CSF biomarkers (Aβ42 and Aβ40) and neuroimaging data.

The study was approved by the local Ethics Committees following the ethical standards recommended by the Declaration of Helsinki. All participants and/or their caregivers gave their written informed consent.

### 2.3. Neuroimaging assessments

The inclusion criteria for all participants included a 1.5T or 3T magnetic resonance imaging (MRI) scan including T2*gradient echo (GRE) or susceptibility weighted imaging (SWI), axial fluid attenuated inversion recovery, and coronal T1-weighted sequences in the five centers involved. GRE or SWI sequences were assessed for the presence of the main CAA neuroimaging features: localization and number of lobar microbleeds, presence of cortical superficial siderosis (cSS), and lobar ICH. We evaluated the fulfillment of mBCAA in all participants regardless of the age criterion included in the criteria set (>55 years) [6]. White matter hyperintensities (WMHs) were semiquantitatively assessed in fluid attenuated inversion recovery sequences according to the Fazekas score [20]. Medial temporal atrophy (MTA) was evaluated in coronal T1-weighted images through the Scheltens scale [21]. MTA was scored bilaterally and the highest score was considered for the analyses.

The radiological evaluation was performed by two raters (either M.C-I or M.B.; neurologists with expertise in cognitive disorders and S.G.; neuroradiologist) blinded to the clinical data. Inter-rater reliability was above 90% and discrepancies within ratings were solved by consensus.

### 2.4. CSF biomarkers and APOE genotype

The inclusion criteria included CSF data for EOAD and HCs but not for ADAD and DS. Details of analysis are described elsewhere [14,18]. In short, commercially available ELISA kits were used to determine CSF Aβ40 and Aβ42 levels (Millipore and Fujirebio-Europe, respectively), following the manufacturers' recommendations.

*APOE* genotyping was performed by PCR amplification of the exon 4 fragment containing the two polymorphisms (rs429358 and rs7412) that encode the three common *APOE* isoforms. The following oligonucleotides, APOE-F: 5′-ACTGGAGGAACAACTGACCC-3′ and APOE-R: 5′-CTGCCCATCTCCTCCATC-3′, were used and final PCR products were purified and Sanger sequenced using BigDye Terminator Chemistry (Applied Biosystems). Sequences were run on an Applied Biosystems 3130 Genetic Analyzer, and resulting electropherograms were visually inspected using Sequencher (version 4.1; Gene Codes Corporation).

### 2.5. Statistical analysis

Statistical analyses were performed with the Statistical Package for the Social Sciences, v19 software (IBM Corp. http://www-01.ibm.com/software/es/analytics/spss/). The primary objectives of this study were to compare across

Table 1
Demographics and CSF biomarker characteristics of the participants

| | Healthy controls | Sporadic EOAD | | Asymptomatic ADAD | Symptomatic ADAD | | Asymptomatic DS | Symptomatic DS | |
|---|---|---|---|---|---|---|---|---|---|
| | | p-EOAD | d-EOAD | | pAD-ADAD | dADAD | | pAD-DS | dAD-DS |
| *N* | 68 | 19 | 23 | 13 | 5 | 11 | 91 | 13 | 13 |
| Age, y | 54.3 (9) | 60.9 (8) | 61.8 (7) | 33.6 (11) | 54.8 (17) | 47.3 (10) | 39.6 (16) | 51.5 (5) | 56.2 (3) |
| Gender, % men | 35.3 | 26.3 | 39.1 | 23.1 | 60 | 45.5 | 56 | 54 | 54 |
| High blood pressure | 13.2 | 21.1 | 13 | 0 | 0 | 0 | 3.1 | 0 | 15.4 |
| *APOE* ε4 carriers, %† | 32.4 | 73.3 | 43.5 | 7.7 | 0 | 18.2 | 34.6 | 20 | 33.3 |
| *APOE* ε2 carriers, %† | 4.4 | 5.3 | 4.3 | 7.7 | 0 | 27.3 | 19.2 | 10 | 8.3 |
| MMSE/total CAMCOG‡ | 29 (2) | 28 (2) | 20 (7) | 30 (1) | 24 (4) | 20 (7) | 80 (16) | 74 (22) | 42 (34) |
| CSF Aβ42, pg/mL | 779.5 (237) | 394 (242) | 360.5 (118) | 655.5 (412) | 217 (242) | 279 (201) | 724.8 (294) | 432.5 (33) | 400.8 (56) |
| CSF Aβ40, pg/mL | 5617.9 (2738) | 5530 (494) | 6224.5 (1923) | 4966.8 (2225) | 4837 (3602) | 5783.7 (6739) | 5559.5 (2088) | 5605 (1143) | 5763.3 (780) |
| CSF ratio-Aβ42/40 | 0.143 (0.07) | 0.067 (0.03) | 0.060 (0.02) | 0.130 (0.14) | 0.070 (0.01) | 0.060 (0.18) | 0.106 (0.08) | 0.078 (0.02) | 0.069 (0.03) |

Abbreviations: Aβ40, amyloid-β40; Aβ42, amyloid-β 1-42; APOE, apolipoprotein E; CAMCOG-DS, Cambridge Examination for Mental Disorders of Older People with Down Syndrome and Others with Intellectual Disabilities-Cognitive Scale; CSF, cerebrospinal fluid; dADAD, dementia in autosomal-dominant Alzheimer's disease; dAD-DS, Alzheimer's disease dementia in Down syndrome; d-EOAD, dementia in early-onset Alzheimer's disease; EOAD, early-onset Alzheimer's disease; MMSE, Mini–Mental State Examination; pAD-ADAD, prodromal Alzheimer's disease in autosomal-dominant Alzheimer's disease; pAD-DS, prodromal Alzheimer's disease in Down syndrome; p-EOAD, prodromal sporadic early-onset Alzheimer's disease.

NOTE. Unless otherwise specified, values are presented as medians (interquartile range). CSF was available from 68/68 HCs, 19/19 p-EOAD, 23/23 d-EOAD, 7/13 asymptomatic ADAD, 5/5 pAD-ADAD, 9/11 dADAD, 36/91 asymptomatic DS, 9/13 pAD-DS, and 6/13 dAD-DS.

*Median relative age (interquartile range) was −11.9 (14.4), 3.15 (6.5), and 3.5 (4.8) in asymptomatic ADAD, pAD-ADAD, and dADAD, respectively.

†APOE was available from 68/68 HCs, 19/19 p-EOAD, 23/23 d-EOAD, 13/13 asymptomatic ADAD, 5/ pAD-ADAD, 11/ dADAD, 78/91 asymptomatic DS, 10/13 pAD-DS, and 12/13 dAD-DS.

‡CAMCOG score was available in 55/91 asymptomatic DS subjects, 7/14 pAD-DS subjects, and 3/12 dAD-DS subjects.

groups the frequency of the mBCAA and the CSF Aβ40 levels and were analyzed with the Fisher's exact and Mann-Whitney tests, respectively.

The secondary objectives were to assess the white matter lesions measured by the Fazekas scale and the hippocampal atrophy measured by the Scheltens scale and were analyzed with the Fisher's exact test. Spearman correlation coefficients were calculated between age, clinical stage, hippocampal atrophy and white matter lesions, and the different study groups. With the purpose of improving the statistical power, prodromal and demented groups in EOAD, ADAD, and DS were merged when analyzed. All significance tests were two sided with the statistical significance set at 5%.

## 3. Results

Table 1 displays the demographics, clinical features, CSF data, and *APOE* genotype of the participants. CSF data were available in 71% ($N = 182$) of the subjects (including all HCs and patients with EOAD). Symptomatic ADAD and symptomatic DS subjects were younger than patients with EOAD (48.4, 54.4, and 61.1 years of age, respectively; $P < .001$).

The *APOE* ε4 allele frequency was higher in EOAD than in any other group. However, no differences were observed between symptomatic or asymptomatic subjects within the ADAD or DS groups.

Table 2 shows the neuroimaging results across the different groups. The fulfillment of the mBCAA was more frequent in symptomatic DS (31%, $N = 8$) and symptomatic ADAD (38%, $N = 6$), than in EOAD (24%, $N = 19$; $P = .055$ and $P = .026$, respectively). When present, the most frequent CAA neuroimaging features were lobar microbleeds in 91.2% ($N = 31$), followed by cSS in 29.4% ($N = 10$) and ICH in 8.8% ($N = 3$). All three features were more frequent in the symptomatic than in asymptomatic subjects within all groups (Table 2).

The symptomatic ADAD and DS groups had a higher proportion of lobar microbleeds than the EOAD group ($P = .02$ and $P = .046$, respectively). cSS and ICH were statistically associated ($P = .004$). In those who had cSS, 20% ($N = 2$) also had an ICH, and cSS was present in 67% ($N = 2$) of those with ICH. Symptomatic DS had a higher proportion of subjects with cSS and lobar ICH than the EOAD group, but this difference did not reach statistical significance ($P = .056$). The position of the mutation (precodon 200 or postcodon 200) did not significantly impact the presence of lobar microbleeds in *PSEN1* carriers (37% vs. 25%, $P = .4$). One of the asymptomatic ADAD participants included in our study had a massive lobar ICH after recruitment into this study that led to the participant's death in a stage of moderately severe dementia.

The mean time lag between MRI and CSF sampling was 5.3 months. Symptomatic participants had lower CSF Aβ42 levels than asymptomatic subjects within all groups (Fig. 1A). No significant differences were detected in CSF Aβ40 levels between the different groups or between symptomatic and asymptomatic subjects within each group (Fig. 1B).

Table 2
Neuroimaging findings

| | Healthy controls | Sporadic EOAD | | Asymptomatic ADAD | Symptomatic ADAD | | Asymptomatic DS | Symptomatic DS | |
|---|---|---|---|---|---|---|---|---|---|
| | | p-EOAD | d-EOAD | | pAD-ADAD | dADAD | | pAD-DS | dAD-DS |
| *N* | 68 | 19 | 23 | 13 | 5 | 11 | 91 | 13 | 13 |
| MRI (GRE/SWI), % | 38.2/61.8 | 24.1/57.9 | 39.1/60.9 | 100/0 | 100/0 | 100/0 | 46.2/53.8 | 23.1/76.9 | 30.8/69.2 |
| Lobar microbleeds, % | 2.9 | 10.5 | 8.7 | 0 | 20 | 45.5 | 12.1 | 23.1 | 38.5 |
| cSS, % | 1.5 | 0 | 8.7 | 0 | 0 | 0 | 3.3 | 7.7 | 23.1 |
| Lobar ICH | 0 | 0 | 0 | 0 | 0 | 0 | 1.1 | 0 | 15.4 |
| Boston criteria | 4.4 | 10.5 | 13 | 0 | 20 | 45.5 | 13.2 | 23.1 | 38.5 |
| Fazekas | | | | | | | | | |
| Score 0 | 64.7 | 21.1 | 26.1 | 75 | 40 | 27.3 | 49.4 | 23.1 | 9.1 |
| Score 1 | 32.4 | 52.6 | 65.2 | 25 | 40 | 72.7 | 43.7 | 69.2 | 54.5 |
| Score 2 | 2.9 | 15.8 | 8.7 | 0 | 20 | 0 | 5.7 | 7.7 | 36.4 |
| Score 3 | 0 | 10.5 | 0 | 0 | 0 | 0 | 1.1 | 0 | 0 |
| MTA | | | | | | | | | |
| Score 0–1 | 97.1 | 52.6 | 56.5 | 100 | 100 | 63.6 | 68.2 | 16.7 | 0 |
| Score 2–4 | 2.9 | 47.4 | 43.5 | 0 | 0 | 36.4 | 31.8 | 83.3 | 100 |

Abbreviations: Aβ40, amyloid-β40; Aβ42, amyloid-β 1-42; APOE, apolipoprotein E; CAMCOG-DS, Cambridge Cognition Examination for mental disorders of older people with Down syndrome and others with intellectual disabilities; CSF, cerebrospinal fluid; cSS, cortical superficial siderosis; dADAD, dementia in autosomal-dominant Alzheimer's disease; dAD-DS, Alzheimer's disease dementia in Down syndrome; d-EOAD, dementia in sporadic early-onset Alzheimer's disease; EOAD, early-onset Alzheimer's disease; GRE, gradient echo sequences; ICH, intracerebral hemorrhage; MMSE, Mini–Mental State Examination; MRI, magnetic resonance imaging; MTA, medial temporal atrophy; pAD-ADAD, prodromal Alzheimer's disease in autosomal-dominant Alzheimer's disease; pAD-DS, prodromal Alzheimer's disease in Down syndrome; p-EOAD, prodromal early-onset Alzheimer's disease; SWI, susceptibility weighted imaging.

NOTE. Unless otherwise specified, values are presented as proportions.

*Fazekas score was not available in four asymptomatic DS subjects and in two dAD-DS subjects.

†MTA score was not available in one AD subject, in three asymptomatic DS subjects, one pAD-DS subject, and one dAD-DS subject.





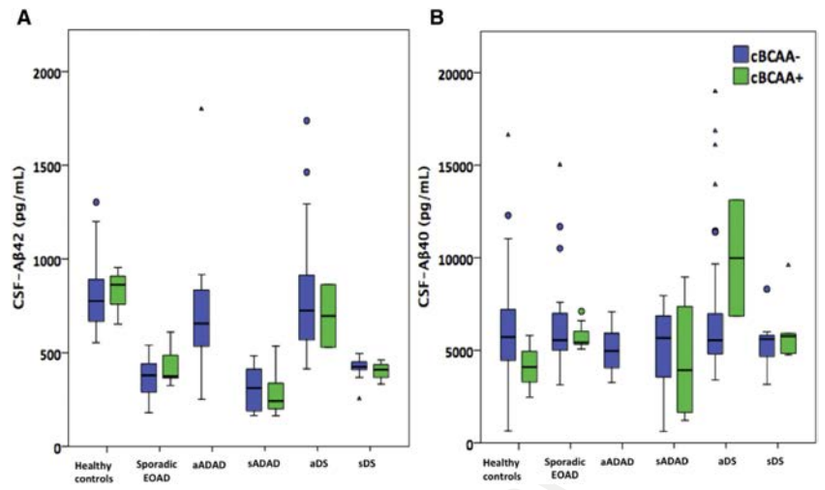


Fig. 1. CSF biomarker levels in the clinical groups, according to modified Boston criteria for CAA (cBCAA). Box plot displaying the distribution of CSF Aβ42 (A) and CSF Aβ40 (B) from healthy controls, sporadic EOAD, asymptomatic ADAD (aADAD), symptomatic ADAD (sADAD), asymptomatic DS (aDS), and symptomatic DS (sDS). Subgroups in blue represent those subjects who do not fulfill cBCAA, subgroups colored in green do fulfill cBCAA for possible or probable CAA. No differences in levels of CSF Aβ42 or Aβ40 were detected between subjects that fulfilled mBCAA and those who did not within each clinical group. Abbreviations: AD, Alzheimer's disease; ADAD, autosomal-dominant Alzheimer's disease; CAA, cerebral amyloid angiopathy; CSF, cerebrospinal fluid; DS, Down syndrome; EOAD, early-onset Alzheimer's disease.

All EOAD patients fulfilling the mBCAA were *APOE* ε4 carriers, and the *APOE* ε4 allele was significantly more frequent in EOAD subjects than in the symptomatic ADAD ($P = .015$) or symptomatic DS ($P = .071$) participants fulfilling the mBCAA. In sporadic EOAD, there was a trend for the association between mBCAA fulfillment and *APOE* ε4 genotype ($P = .06$).

There were no differences in CSF Aβ40 levels between those subjects who fulfilled the mBCAA and those who did not (or the presence of lobar microbleeds, cSS, or ICH) in any group.

Symptomatic subjects presented higher Fazekas scores than asymptomatic subjects in all groups: EOAD patients had higher Fazekas scores than HCs ($P < .001$); symptomatic ADAD higher than asymptomatic ADAD ($P = .022$) or HCs ($P = .05$); and symptomatic DS higher than asymptomatic DS ($P = .011$) and HCs ($P < .001$; Fig. 2 and Table 2).

There was a significant positive correlation between age and Fazekas score in the whole sample (r = 0.337, $P < .001$), in ADAD (r = 0.407, $P = .031$), and in DS (r = 0.506, $P = .000$) groups. This correlation was also found in asymptomatic DS (r = 0.495, $P < .001$) and in HCs (r = 0.323, $P = .007$; Fig. 3).

Age positively correlated with Scheltens scores in the whole sample (r = 0.229, $P < .001$), HCs (r = 0.276, $P = .023$), ADAD (r = 0.412, $P = .027$), asymptomatic DS (r = 0.341, $P = .001$), and symptomatic DS (r = 0.431, $P = .023$). The Scheltens scores increased from asymptomatic to symptomatic subjects within each group. Symptomatic DS patients presented higher MTA scores than EOAD and symptomatic ADAD patients ($P < .001$ in each comparison) and asymptomatic DS subjects higher than HCs ($P < .001$). There were no differences in Scheltens scores between EOAD and symptomatic ADAD.

## 4. Discussion

We found that DS and ADAD have a more severe CAA than EOAD as measured by the mBCAA, but CAA did not impact the CSF Aβ40 levels. The *APOE* ε4 allele might be associated with CAA in EOAD but does not seem to have an effect in DS or ADAD.

There are previous studies assessing the prevalence of lobar microbleeds in ADAD and EOAD [22–24], but, to our knowledge, none has specifically assessed and compared the mBCAA between the different AD populations. The mBCAA were more frequent in DS and ADAD, suggesting a more severe CAA, as shown in pathologic studies [5,25]. The most frequent CAA neuroimaging feature was the presence of lobar microbleeds, as previously described [26]. The frequency of lobar microbleeds in ADAD (and HCs) was in agreement with the literature (ranging from 25% to 66%) [23,24,26], but we found a lower frequency of lobar microbleeds in EOAD (9.5%) than that reported in late-onset AD (20%–30%) [27]. Age might be responsible for this difference.

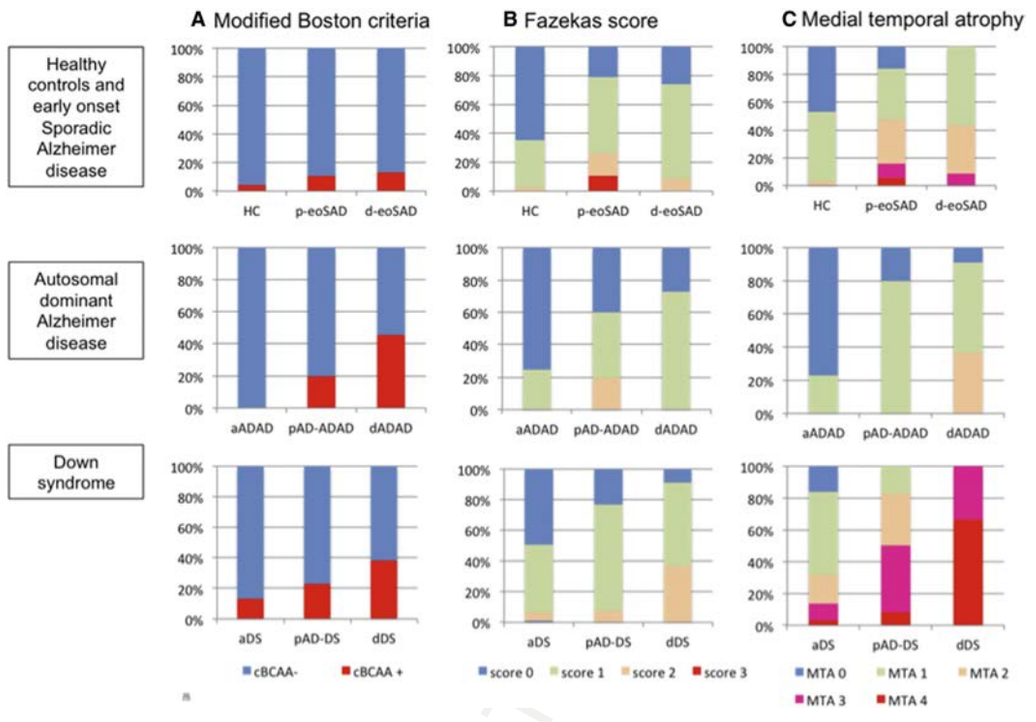


Fig. 2. Proportion of subjects in each group according to modified Boston criteria, Fazekas score, and medial temporal lobe atrophy (MTA). Frequency bar graph showing (A) modified Boston criteria fulfillment, (B) Fazekas score, and (C) MTA score of each clinical group. Healthy controls and sporadic AD in the first row, ADAD in the second row, and DS in the third row. Abbreviations: aADAD, asymptomatic autosomal-dominant Alzheimer's disease; AD, Alzheimer's disease; ADAD, autosomal-dominant Alzheimer's disease; aDS, asymptomatic Down syndrome; dADAD, dementia in autosomal-dominant Alzheimer's disease; DS, Down syndrome; pAD-ADAD, prodromal Alzheimer's disease in autosomal-dominant Alzheimer's disease; pAD-DS, prodromal Alzheimer's disease in Down syndrome. Q15

There are no previous studies assessing the mBCAA or any of its component neuroimaging features in DS. CAA is also consistently observed in DS pathologic studies [5,28], but it had been proposed that other genetic factors in DS might protect these subjects from the ICH [29]. We found 38.5% of frequency for lobar microbleeds in symptomatic DS and, more importantly, a frequency of 15.4% for ICH. Although this is lower than the reported 30% prevalence for symptomatic ICH in non–trisomic APP duplication carriers, it is well above the 3% to 3.8% figure for symptomatic ICH in DS reported in the same study [29]. This discrepancy might be explained because many nonfatal ICHs might be unnoticed in DS with AD. Of note, both subjects with DS and ICH on the MRI did not present with ICH-related clinical symptoms.

A significant percentage of symptomatic subjects, nonetheless, did not meet the mBCAA and were free of the CAA-associated neuroimaging features. This is in contrast with pathologic studies, where CAA is found in up to 90% of AD brains, suggesting that the available MRI sequences only identify a subset of AD-CAA subjects [23]. The CAA neuroimaging features might, thus, detect only the most severe cases or, alternatively, they might select different subgroups of patients [23]. In this respect, cSS, although less frequent than lobar microbleeds, was strongly associated with lobar ICH. cSS might be a particularly important marker for severe CAA in AD, as it has been suggested in sporadic CAA [7]. More work in longitudinal studies is needed to confirm the higher risk conferred by cSS for future ICH and cognitive decline.

We also assessed other neuroimaging features associated with CAA but not included in the mBCAA. Both WMHs and MTA are increasingly recognized as core AD features and as a manifestation of CAA [30]. We found a gradient in WMH extension in all groups [31], but we also found more extended WMHs in those subjects fulfilling the mBCAA. WMHs also increased with age and in relation with vascular risk factors. We found this correlation also in HCs, although





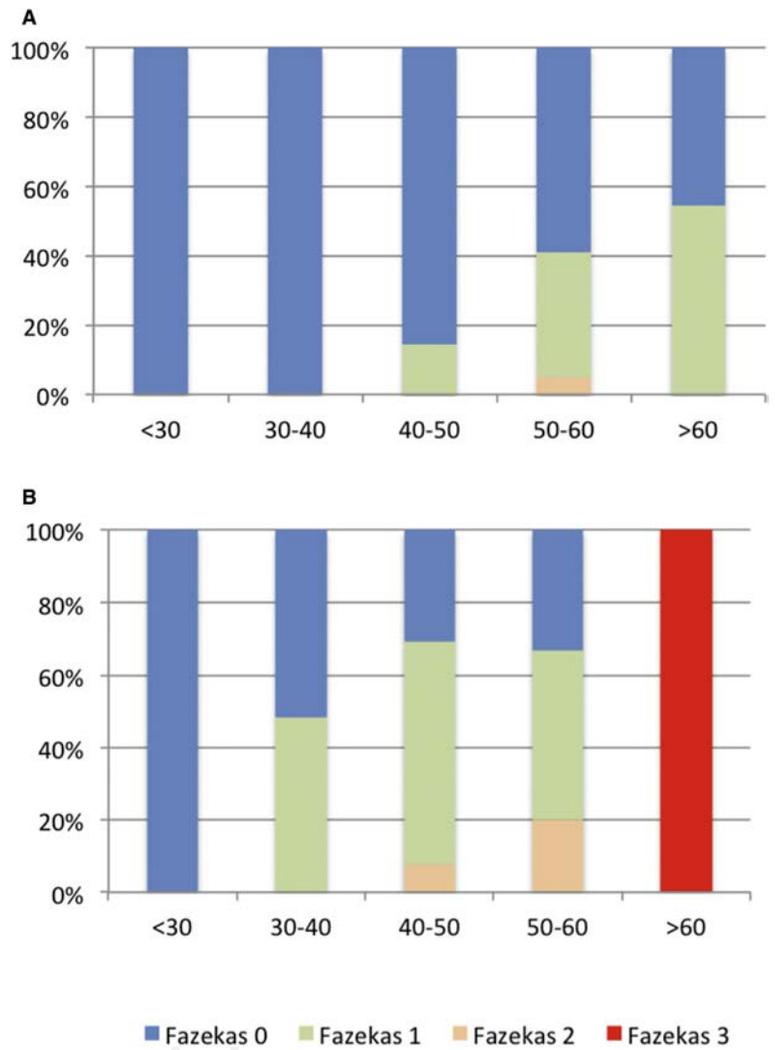


Fig. 3. Proportion of subjects according to Fazekas score by the range of age. The frequency bar graphs showing the percentage of subjects with each Fazekas categories by age: (A) healthy controls and (B) asymptomatic Down syndrome subjects.

they all had normal core AD CSF biomarkers and low prevalence of high blood pressure. However, we found a strong correlation between age and WMHs in asymptomatic DS despite their younger mean age. This correlation supports the relationship between amyloid deposition and WMHs. Not surprisingly, the Scheltens scores increased with symptom severity in all AD populations. Hippocampal atrophy, however, was more severe in DS, even in asymptomatic DS individuals. These results are in agreement with the notion that individuals with DS not only have smaller hippocampal size from birth but also show atrophy when AD develops [17].

Decreased CSF Aβ40 levels might differentiate sporadic CAA from HCs and AD cases [7]. In our study, nevertheless, CSF Aβ40 levels did not discriminate CAA neuroimaging features in any group. This finding could be influenced by the fact that amyloid vascular burden in CAA in ADAD and DS contains not just Aβ40, but also Aβ42. It is difficult to sort out the contribution of vascular Aβ42 deposition from parenchymal plaque deposition except with neuropathologic

analysis of the postmortem brain, which was not available in this study [32,33].

To our knowledge, there are only two studies that determine CSF Aβ40 in participants with AD with and without lobar microbleeds and show conflicting findings [9,10]. In any case, our results suggest that CSF Aβ40 levels are not a sensitive biomarker to detect CAA in the context of an AD process.

The *APOE* ε4 allele confers a higher risk for CAA in the general population and in AD [11,12]. We also found a trend for an association between *APOE* ε4 genotype and CAA in sporadic EOAD. Furthermore, all EOAD subjects with CAA neuroimaging features were *APOE* ε4 carriers. We did not find this relationship in ADAD or DS. The *APOE* ε4 genotype might be thus associated with CAA in EOAD but not in DS or ADAD. In ADAD and DS, other genetic factors, such as the type or position of the causing mutation in ADAD, might be more important in predicting CAA [4].

Our findings have potential clinical implications. The mBCAA have not been validated in patients <55 years of age. We consider that, at least in ADAD and DS, age should not be an essential requirement for CAA diagnosis. Our results also have substantial implications in AD clinical trials given the relationship between CAA and amyloid-related neuroimaging abnormalities (ARIAs). Vascular amyloid may be a pathophysiological mechanism for ARIAs [34,35], and recent studies have shown that after Aβ immunotherapy, there is an increase in CAA severity and an increase in lobar microbleeds associated with removal of plaques [36]. Trials targeting Aβ commonly use lobar microbleeds and *APOE* genotype to stratify subjects [37]. However, there are no available data on the relationship of CAA neuroimaging abnormalities (and *APOE*) and ARIAs in the setting of amyloid-lowering therapy in ADAD and DS. Our data emphasize heterogeneity in prevalence and possibly etiology for CAA; therefore, the recommendations on the exclusions for presence of baseline ARIA-H (microbleeds or hemosiderosis) from sporadic AD should be taken with caution. On the other hand, the *APOE* ε4 genotype is also commonly used to stratify participants given its influence on ARIAs [37]; however, this strategy might not be as important in ADAD or DS. Finally, our data also suggest that the CSF Aβ40 levels will not be a useful biomarker in these trials.

The higher prevalence of CAA in ADAD and DS might play a role in the conversion to clinical dementia. In sporadic AD, CAA is an independent contributor to cognitive impairment and can worsen the severity of cognitive dysfunction [38]. Future longitudinal studies are needed to assess the CAA contribution to cognitive impairment in ADAD and DS.

The main strengths of the present study are the relatively large sample size of different rare populations, such as ADAD and DS and the confirmation of the clinical diagnosis with genetics or CSF biomarkers. The study has some limitations. The use of two different imaging techniques is an important limitation when estimating the real prevalence of lobar microbleeds. SWI has a higher sensitivity for hemosiderin, detecting up to 50% more lobar microbleeds than conventional T2*GRE [39]. However, in our study, the ADAD group was exclusively investigated using T2*GRE, leading to a possible underestimation of the CAA neuroimaging features in these subjects. Finally, most of the EOAD patients were at a stage of mild dementia, and we lack neuropathologic data.

In conclusion, the CAA-associated neuroimaging features are more frequent in adults with DS and in patients with ADAD than in those with EOAD suggesting a more severe CAA pathology. Our study also shows that the CSF Aβ40 levels are not a reliable biomarker for CAA and that the risk factors for CAA (such as the *APOE* ε4 genotype) might be different in EOAD and genetically determined AD. These findings should be taken into account in the design of clinical trials with antiamyloid therapies in people with ADAD or DS.

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## Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jalz.2017.03.007.





**RESEARCH IN CONTEXT**

- To our knowledge, cerebral amyloid angiopathy (CAA) neuroimaging features have not been systematically assessed in subjects with sporadic early-onset Alzheimer's disease (AD), autosomal-dominant AD, or Down syndrome. Only the frequency of lobar microbleeds in autosomal-dominant AD (25%–66%) has been reported. Regarding the CAA characterization from a cerebrospinal fluid (CSF) biomarker profile or *APOE* genotype point of view, there are no published data in these three populations of AD.
- This is the first study that includes a systematic assessment of the modified Boston criteria for CAA and other related features in sporadic early-onset AD, autosomal-dominant AD, and Down syndrome. This is also the first study that determines CSF amyloid-β Aβ40 or Aβ42 levels and *APOE* genotype in those populations of AD within the study of CAA. Our results show that CAA is more frequent in genetically determined cases of AD than in sporadic early-onset AD; however, these differences are not reflected in CSF Aβ40 or Aβ42 levels.

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Chapter 9

# Acknowledgments/ Agradecimientos

Chapter 9

# Acknowledgments/ Agradecimientos

Una tesis doctoral sirve para demostrar que una persona es capaz de llevar a cabo una investigación científica. Durante su preparación, una tiene la oportunidad de aprender muchas cosas, pero la más importante de ellas tal vez sea que la ciencia avanza gracias al esfuerzo combinado de muchas personas, al intenso trabajo de equipos multidisciplinares. Nadie puede hacer ciencia solo encerrado en su casa.

Este libro que tienes en las manos condensa cinco años de aprendizaje, ilusión y esfuerzo. El esfuerzo de un magnífico equipo, la unidad de memoria del Hospital de Sant Pau a la que tengo la enorme fortuna de pertenecer.

Sintetizar en unas pocas líneas mi gratitud hacia todas las personas que han contribuido a la realización de este trabajo me resulta más difícil que escribir la discusión o contestar a las exigentes demandas de los correctores de las revistas en las que, no sin mucho esfuerzo, pudimos publicar los artículos. Como si me acabaran de dar un Oscar, tengo miedo de olvidarme de alguien fundamental, de ser demasiado cursi o parca. Pero no es momento de flaquear, así que vamos a ello.

En primer lugar quiero dar las gracias a mis tres directores de tesis, Rafa, Alberto y Juan por haber creído en mí más que yo misma. Cada uno a su manera me ha guiado hasta aquí.

Me gustaría agradecer también a todas las personas que componen la unidad de memoria del Hospital de Sant Pau, un gran familia cuyo talento científico es solo superado por su incomparable calor humano. También a la unidad de Alzheimer y Down, compañeros de fatigas, que con la perseverancia de las hormiguitas poco a poco están construyendo un gran imperio. A todos los pacientes y a sus familiares, especialmente a aquellos que se ofrecen como voluntarios para que la ciencia pueda avanzar. Ellos son la razón de todo esto y su generosidad es el motor de nuestro trabajo. Su coraje ante la adversidad es la lección de toda una vida.

A mi familia, a mi cuadrilla y a mi maji. Por su apoyo constante, ánimos e infinita paciencia. Porque como canta Amaral, sin ellos no soy nada.

A Mikel, porque sin haber nacido aún, ha trabajado conmigo en que esta tesis sea una realidad ayudándome a terminarla a tiempo.

De todo corazón, gracias. Moltes gràcies.

Chapter 10

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DOCTORAL THESIS

# Sporadic cerebral amyloid angiopathy, beyond lobar intracerebral hemorrhage: multimodal biomarker studies of atypical presentations

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