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SYSTEMATIC ANALYSIS OF CEREBRAL AMYLOID ANGIOPATHY-RELATED INTRACEREBRAL HEMORRHAGE AND INFLAMMATION – IDENTIFYING DIAGNOSTIC AND PROGNOSTIC BIOMARKERS

PhD Thesis

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Introduction

Stroke is one of the leading causes of death and disability globally. After ischemic stroke, intracerebral hemorrhage (ICH) is the second most prevalent type (Feigin et al., 2009). The most common known risk factors of spontaneous ICHs include advanced age, chronic hypertension, cerebral amyloid angiopathy (CAA), alcohol and drug abuse, and antithrombotic medications (Aguilar et al., 2011). Based on etiological considerations, ICHs are commonly classified into deep ICHs, predominantly associated with chronic hypertensive arteriopathy, and lobar ICHs, which are most frequently related to CAA and, relatively less frequently, to other etiologies such as vascular malformations or tumors (Ikram et al., 2012). The distribution of cerebral microbleeds (CMBs) follows a similar anatomical pattern, with chronic hypertensive arteriopathy associating primarily with CMBs in deep localizations and CAA typically associating with lobar CMBs, sparing the deep structures.

In CAA, cortical and leptomeningeal small arteries/arterioles and capillaries are degenerated due to the progressive deposition of amyloid-β (Aβ) peptides. The deposition of amyloid peptides causes both CAA-related ischemic alterations (including microinfarctions and leukoaraiosis) and different types of hemorrhages. These include lobar cerebral microbleeds (CMBs), lobar ICHs of various size, convexity subarachnoid hemorrhages (cSAHs) due to rupture of leptomeningeal arteries, and cortical superficial sideroses (CSSs) as chronic manifestations of cSAHs (Yamada et al., 2015; Linn et al., 2010). Typically, CAA-related ICHs are recurrent and cause various neurological deficits, headache, and epileptic seizures (Yamada et al., 2015). Patients usually develop slowly or step-wise progressive cognitive impairment and frequently experience transient ischemic attack (TIA)-like events, a.k.a. transient focal neurological episodes (TFNEs) or ‘amlyoid spells’.

Magnetic resonance imaging (MRI) sequences sensitive to susceptibility artifacts generated by hemosiderin deposits of previous micro- and macrobleeds, such as gradient echo (GRE), susceptibility-weighted imaging (SWI), or T2*sequences, in addition to a set of clinical characteristics, enable a probabilistic diagnosis of CAA *in vivo* with high diagnostic accuracy (Greenberg et al., 2018). The original Boston criteria was modified in 2010, recognizing CSS as part of CAA-related alterations (Linn et al., 2010). While deep (i.e., basal ganglionic, thalamic, and brainstem) hemorrhagic alterations preclude the diagnosis of both possible and probable CAA, cerebellar bleeds are allowed, albeit not counted for the diagnosis (Greenberg et al., 2018). Most recently, the Boston v2.0 criteria has been published, updating the definition of probable CAA (Charidimou et al., 2022).

Body fluid biomarkers in the cerebrospinal fluid (CSF) and, less frequently, plasma, have recently been studied in CAA patients, focusing predominantly on the core biomarkers of amyloid metabolism (i.e., $A\beta_{1-42}$ and $A\beta_{1-40}$), neurodegeneration (total Tau), and neurofibrillary tangle (NFT) pathology (phosphorlyated Tau (pTau)), with the aim of offering complimentary approaches (Charidimou et al., 2018).

It is estimated that CAA pathology is present in 2.3% of people between the age of 65 and 74, in 8.0% of those who are between 75 and 84, and in 12.1% of the patients over 85 y (Biffi et al., 2011).

In addition to the common phenotype of sporadic CAA described above, a subgroup of CAA patients present with subacute cognitive/behavioral decline, focal neurological symptom(s), headache, and/or seizure(s) (Kinnecom et al., 2007). These symptoms are associated with the typical CAA imaging alterations as well as the MRI appearance of asymmetric and confluent white matter hyperintensities (WMHs) on T2/fluid-attenuated inversion recovery (FLAIR), representing vasogenic edema, similar to the clinical-radiological presentation of a subtpye of primary angiitis of the central nervous system (PACNS) (Omisade et al., 2013; Hainline et al., 2017). In this inflammatory form of CAA, the CAA-affected vessels are infiltrated by inflammatory cells (Dumitrascu et al., 2018). The first systematic clinical definition of this syndrome was given by Eng *et al.* and was termed *'CAA-related inflammation'* (CAA-RI) (Eng et al., 2004). The clinical-radiological criteria were defined in 2011 by Chung et al. (Chung et al., 2011) and have been improved and validated by the Boston group (a.k.a., the Auriel criteria) (Auriel et al., 2016). Most patients with this phenotype respond to immunosuppression, with corticosteroids in the first line (Regenhardt et al., 2020).

Though curative therapy in CAA is lacking, the clinical relevance of the diagnosis is high. Indeed, the use of anticoagulants is contraindicated in CAA according to current guidelines (Kernan et al., 2014; Heidbuchel et al., 2015) and the use of antiplatelet therapy should also be carefully considered in CAA (Biffi et al., 2010; Gregoire et al., 2010). In addition, a >10 CMB number *per se* has recently been introduced as a contraindication of systemic thrombolysis in acute ischemic stroke due to uncertain benefit (Powers et al., 2018). In CAA-RI the potential reversibility of inflammation-related symptoms also necessitates an increased survaillence.

Objectives

Based on the discrepancy between the expected frequency of CAA among the elderly and the experienced occurrence of CAA diagnosis in routine clinical practice, our aim was to assess the frequency of the different types of spontaneous ICHs in our stroke center, with special focus on estimating the underlying prevalence of CAA, by a retrospective re-evaluation of written and imaging documentation. Emphasis was given on the analysis of the predictive value of putative risk factors for ICH location, probable/definite CAA diagnosis, and fatal outcome.

Our second study aimed to identify and profile published probable/definite CAA-RI cases (including and presenting 7 new cases) and to perform an in-depth systematic analysis of subject-wise collected neuropathological, radiological, clinical, and laboratory variables to provide insights into previously unrevealed associations and identify diagnostic/prognostic biomarkers.

Methods

In the CAA-related ICH study, via screening the electronic database of our center, patients who received acute in-patient care between 01/07/2014 and 01/07/2018 with any of the intracranial hemorrhage-related International Classification of Diseases (ICD) diagnosis codes were identified. Reviewing the imaging scans and medical records, spontaneous ICHs were separated from intracranial hemorrhages with traumatic etiology, cases with basal SAH, primary intraventricular hemorrhage, and hemorrhagic transformation of ischemic stroke, and from cases with inadequate coding. Spontaneous ICHs were further classified according to hematoma localization as deep ICHs (basal ganglia, thalamus, or brainstem) and lobar/cerebellar ICHs (regions compatible with the diagnosis of probable CAA, enabling the estimation of the prevalence of underlying CAA). The prevalence of different etiologies behind lobar/cerebellar ICHs was assessed in a subpopulation who underwent ‘complete’ clinical work-up, defined as being subjected to computed tomography angiography (CTA) or MR angiography (MRA) as well as SWI (if structural etiology was not identified by the above modalities) and/or *post mortem* neuropathological work-up. Definite CAA, probable CAA, and possible CAA diagnoses were retrospectively established or revised as per the Modified Boston criteria (Linn et al., 2010; Charidimou et al., 2022). An ICH was considered CAA-related if met the criteria for probable and/or definite CAA.

In our CAA-RI study, we systematically collected and reviewed previously published cases in PubMed, using the terms ‘ABRA’, ‘amyloid beta-related angiitis’, ‘CAA-RI’, ‘cerebral

amyloid angiopathy-related inflammation', or 'inflammatory cerebral amyloid angiopathy'. Non-English papers were considered and data used if deemed unambiguous by using an online translator software. In addition to cases collected from the literature, 7 cases diagnosed with CAA-RI (including 2 definite CAA-RI) in our center until the completion of the study, were included in the analysis. The reports were prepared in line with the CARE guidelines (Kimura et al., 2013). Definite CAA-RI was considered in the presence of either perivascular or transmural/intramural inflammatory infiltrates associated with CAA vessels (Chung et al., 2011), with a modification that meeting the clinical-radiological criteria was not a prerequisite of definite diagnosis (hence allowing the measurement of their sensitivity). Probable CAA-RI was considered by adopting the Auriel criteria (Auriel et al., 2016), with slight modifications in wording.

The statistical analysis was performed by the SPSS 20.0 and 22.0 software for the first (CAA-related ICHs) and second (CAA-RI) study, respectively. The level of significance was $p<0.05$.

Results

A. Results of the CAA-related ICH study

1. Revision of diagnoses, estimation of CAA-related ICH prevalence

A total of 324 patients having received any intracranial hemorrhage-related ICD codes as leading diagnosis in the given period were identified. After exclusions, 213 spontaneous ICHs were identified. Among spontaneous ICHs, 121 deep ICHs and 92 lobar/cerebellar ICHs (85 localized to any cerebral lobe and 7 to the cerebellum) were detected. Out of lobar/cerebellar ICHs, 47 had 'complete' clinical work-up, of whom 2 proved to be definite CAA *post mortem* and 14 were consistent with the diagnosis of probable CAA clinically (one of them also became definite *post mortem*), rendering 34.0% of all 'completely' worked-up lobar/cerebellar ICHs and an estimated 14.7% of all spontaneous ICHs to be CAA-related. CMBs were present in 92.9% of probable CAA cases, with 42.9% having >10 CMBs, whereas CSS was present in 78.6%. In addition, 10 patients met the criteria for possible CAA. Out of the 14 probable CAA cases identified, originally only 4 had received CAA as suspected diagnosis (28.6%).

2. Analysis of possible discriminators of ICH subgroups

The median age of the 213 patients with spontaneous ICH was 69.1 y, with the lobar/cerebellar ICH group being significantly older compared to deep ICHs (74.5 vs. 64.7

years; p<0.001). The distribution of sex was significantly different between deep and lobar/cerebellar ICH groups (p=0.029), with a remarkable male preponderance in deep ICHs (66.9%) and a close to even ratio in lobar/cerebellar ICHs. Analyzing the risk factors with significant between-group difference (age, sex, antiplatelet use, combined antithrombotic treatment, and hypertensive excess at presentation) in a multivariable binary logistic regression model revealed advanced age (p=0.014; odds ratio (OR)=1.03) and antiplatelet use (p=0.043; OR=1.96) to be statistically significant independent predictors of a lobar/cerebellar ICH, and hypertensive excess to be a strong significant independent predictor of deep ICH (p=0.002; OR=0.39).

3. Case fatality of ICH patients

The 1-m case fatality of ICH patients was 33.8%, with no significant difference between deep and lobar/cerebellar ICH groups. Significant determinants of 1-m case fatality in ICHs as a whole were age, current anticoagulant use and INR>1.4 in univariable comparisons, with only advanced age (p=0.003; OR=1.04) and INR>1.4 (p=0.035; OR=2.51) proven to be independent predictors of case fatality in multivariable analysis.

4. Analysis of factors to predict CAA

The probable/definite CAA subgroup had the highest mean age at ICH presentation (75.9±2.3 y). This was associated with a significant female predominance in probable/definite CAA (62.5%) as opposed to the male predominance (64.5%) in the comparator (p=0.035).Some 31.3% of probable/definite CAA cases had prior clinical event(s) of intracranial hemorrhage and exactly the same rate had prior TIA/TFNE, significantly higher than in the non-probable CAA group. Multivariable analysis of factors significant in the univariable comparative analyses revealed older age (p=0.012; OR=1.08), prior intracranial hemorrhage (p=0.005; OR=8.53), and antiplatelet use (p=0.042; OR=3.45) as independent significant predictors of definite/probable CAA diagnosis.

B. Results of the CAA-RI study

1. The CAA-RI cases diagnosed in our center

Seven patients diagnosed with probable CAA-RI (2 with definite histopathological confirmation) in our center until the completion of our second study were included in the

analysis. Their courses ranged from minimally symptomatic through keenly steroid-responsive to non-responsive, fatal cases.

2. Nomenclature analysis of the literature in definite CAA-RI

The nomenclature of definite CAA-RI varied substantially. The Chung (Chung et al., 2011) and the Auriel (Auriel et al., 2016) criteria used the umbrella term CAA-RI to cover cases with perivascular-only and transmural inflammation, similarly to the largest case series (Kinnecom et al., 2007). Some authors differentiate two types of CAA-RI using the terms vasculitic (transmural) as opposed to perivasculitic, non-vasculitic, or non-destructive (Kimura et al., 2013; Rastogi et al., 2015; Rempe et al., 2020; Hagiwara et al., 2014). Many others use different nomenclatures, either using 'inflammatory CAA' or similar terms to cover the two subtypes distinguished as ABRA (for transmural) and CAA-RI (for perivascular) (Salvarani et al., 2013; Castro Caldas et al., 2015; Chen et al., 2022; Maddox et al., 2022), or using CAA-RI to cover the ABRA (for transmural) and inflammatory CAA (for perivascular) (Dumitrascu et al., 2018; Poli et al., 2020; Martin-Jimenez et al., 2021; Chu et al., 2016; Du et al., 2019).

3. Epidemiologic analysis of the literature in definite CAA-RI

Including our 2 cases, our analysis detected 205 definite CAA-RI cases for descriptive analysis, 200 including a report of the conditions of obtaining the histopathological specimen. The mean age at diagnosis was 67.2±0.6 y, without sex preference (51.7% males).

4. Neuropathology of definite CAA-RI cases in the literature analysis

Some 71.7% of definite CAA-RI cases with sufficient data (132/184) had transmural inflammation (ABRA), the remaining 28.3% being consistent with perivascular CAA-RI. Our presented perivascular CAA-RI case is one of the 7 similar cases published with autopsy confirmation (Dewitte et al., 2019; Anders et al., 1997; Annweiler et al., 2008). The co-localization of CMBs with confluent WMHs were more common in ABRA (87.0%) than in perivascular CAA-RI (52.6%; p=0.020) implicating the pathogenic role of angiodestructive inflammation in the development of hemorrhagic alterations. Among the 138 cases where cellular components were described or well presented, lymphocytes were almost unequivocally demonstrated (97.1%), macrophages/histiocytes (72.5%) and multinucleated giant cells (69.6%) were frequent, whereas eosinophil granulocytes were seldom reported (8.0%). Regarding concomitant Alzheimer's disease (AD) pathology, Aβ plaques and NFTs were described in 81.3% and 48.3% of cases where addressed, frequently described as mild,

especially regarding NFTs. Changes corresponding to WMHs comprised tissue rarefaction, myelin pallor, spongy vacuolation, and astrogliosis (Scolding et al., 2005; Munoz et al., 1995).

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5. Clinical and radiological presentation of definite CAA-RI

Regarding core clinical signs, the prevalence of headache, focal neurological sign(s), seizure(s), and altered higher mental state were 39.3%, 58.1%, 42.9%, and 77.6%, respectively. Of the 170 definite cases with sufficient data, 98.2% met the clinical part of the present criteria.

Regarding core radiological features, 76.6% of analyzable cases had asymmetric confluent WMHs and 82.3% had lobar CMBs. Of cases with sufficient data, 67.3% met the present radiological criteria of probable CAA-RI. In patients with sufficient clinical and radiological data for decision-making, this yielded a 65.7% sensitivity of the present criteria for probable CAA-RI to diagnose definite CAA-RI.

Regarding additional radiological features not part of the present criteria, 61.7% of cases with contrasted MRI demonstrated enhancement (48.4% leptomeningeal, 5.5% parenchymal, 5.5% both). Notably, leptomeningeal enhancement (LE) was present in 20 cases with isolated leptomeningeal involvement. In a subgroup with subject-wise available data on WMH, hemorrhagic, and enhancement profiles, adding *LE* to the criteria as an alternative (AND/OR) to *asymmetric confluent WMH(s)* (still in the presence of appropriate hemorrhagic profile) increased the sensitivity from 71.4% to 82.5%.

Based on a prior observation that sulcal non-nulling (SNN) (a.k.a. sulcal non-attenuation, hypoattenuation, hyperintensity, or effusion) on FLAIR can co-localize with LE (Salvarani et al., 2016) and that it is an established component of amyloid-related imaging abnormality-edema (ARIA-E) (Sperling et al., 2011), we analyzed 70 definite CAA-RI cases with FLAIR image(s) of sufficient quality published and additional 2 where SNN was recognizably described. We found that SNN not only co-occurred with an isolated LE in 100.0%, but it was in fact present in 80.8% of definite CAA-RI cases with LE regardless of the presence/pattern of WMH. Moreover, SNN was detectable in 50.0% of definite CAA-RI cases without LE (rendering the association between LE and SNN significant; p=0.022). These yield a 59.7% overall prevalence of SNN within definite CAA-RI cases with sufficient data. In certain cases, the evolution of isolated SNN into confluent WMHs was observed on disease progression/recurrence, suggesting that SNN might be an early manifestation of CAA-RI. The subgroup analyses revealed that adding *SNN on FLAIR* to the criteria as an alternative (AND/OR) to *asymmetric confluent WMH(s)* (still with appropriate hemorrhagic profile) increased the sensitivity of from 72.2% to 81.5%. Furthermore, adding *LE and/or SNN* as an

alternative to *asymmetric confluent WMH(s)* increased the sensitivity of the present criteria from 70.0% to 82.5%.

6. Laboratory biomarkers in definite CAA-RI

The prevalence of CSF pleocytosis and elevated total protein concentration were 44.2% and 79.8%, respectively, with 82.8% being pathological in at least one. Of clinical interest, CSF pleocytosis (p=0.029) and either CSF pleocytosis/elevated protein concentration (p=0.014) were significantly associated with headache, implicating the role of focal meningitis underlying the headache. On larger grounds, CSF pleocytosis and elevated protein levels followed the clinical course (improvement/deterioration) in 45.5% and 85.7%, suggesting a role for elevated protein concentration as a surrogate biomarker of clinical change.

The findings of AD core CSF biomarker studies (Szalardy et a., 2016) showed decreased $A\beta_{1-42}$ in 80.0%, increased total Tau in 33.3%, and increased pTau in 28.6%. Of pathogenic relevance, CSF levels of anti-Aβ autoantibodies were found increased in definite CAA-RI cases during an acute phase compared to spontaneous (Piazza et al., 2013) or corticosteroid-induced remission (Kimura et al., 2013; Piazza et al., 2013; Boncoraglio et al., 2015) or compared to non-CAA-RI (Piazza et al., 2013; Boncoraglio et al., 2015).

The *APOE* ε genotype, an established risk factor for AD and a possible risk factor for CAA or CAA-related ICH (Greenberg et al., 1998), was reported in 28 definite CAA-RI cases. At least 1 *APOE* ε4 allele was carried by 60.7%, which is remarkably high compared to control populations, but comparable to that in AD and CAA as a whole (Eng et al., 2004; Mayeux et al., 1993; Vemuri et al., 2010; Ringman et al., 2014; Premkumar et al., 1996). Notably, the carrier rate for *APOE* ε2 was also high (32.1%), higher than in most studies with CAA (Makela et al., 2016; Ringman et al., 2014; Premkumar et al., 1996; Rannikmae et al., 2014; Charidimou et al., 2016).

7. Analysis of therapy, responsiveness, dosing, and course of definite CAA-RI cases

A total of 152 definite CAA-RI cases were eligible for the analysis of treatment, 149 with unequivocal data on the type of immunosuppression. Some 91.4% received some form of therapy, 3.9% were treated only with surgical resection/lobectomy and 87.5% with immunosuppression. Among the immunosuppressed with sufficient data, 99.2% received corticosteroids, 56.2% as monotherapy and 43.1% where it was combined/replaced with other immunosuppressant(s). Only 1 patient received cyclophosphamide monotherapy.

Definite CAA-RI patients not receiving immunosuppression were older than the treated (72.3±2.8 y vs. 66.2±0.8 y; p=0.031), suggesting a decision bias based on age and possibly concomitant diseases. Among immunosuppressed definite CAA-RI cases, 78.8% showed clinically meaningful improvement and radiological improvement was observable in 89.7%. Though being significantly lower than the treatment effect (p=0.0007), the spontaneous clinical remission rate was considerably high (30.8%). The spontaneous radiological remission rate was even more marked (57.1%; p=0.042). The predictor analysis identified LE as a predictor of clinical improvement (66.2% of the improved vs. 16.7% of the not improved; p=0.003).

The rate of positive outcome at 6 m after initiating immunosuppression was 61.0%, significantly higher than in spontaneous improvers at 6 m (25.0%; p=0.028). LE was associated with positive outcomes at 6 m (p=0.005) and 1 y (p=0.003). CSF pleocytosis at presentation was significantly associated with adverse outcome at 6 m (p=0.015) with trends at 1 y. Regarding all-cause mortality, 16.7% and 25.4% of immunosuppressed definite cases died within 6 m and 1 y, respectively, trending to be less than those not receiving immunosuppression. Advanced age at presentation was associated with higher all-cause 6-m (p=0.043) and 1-y mortality (p=0.024) in the total cohort. Regarding predictors of future lobar ICH(s), while immunosuppression itself tended to influence the occurrence of ICH(s) within 1 y, clinical improvement (spontaneous and/or treatment-related) significantly decreased their incidence within 1 y in the total cohort (p=0.026), with a trend in the treated.

Analysis of treatment regimens in definite cases revealed that among patients treated exclusively by corticosteroids until evaluation, low-dose therapy was non-inferior to high-dose therapy regarding clinical improvement (81.8% vs. 82.7%), positive outcome (66.7% vs. 64.3% at 6 m; 58.3% vs. 33.3% 1 y), all-cause mortality (26.7% vs. 16.7% at 6 m; 33.3% vs. 37.5% at 1 y), and relapse (43.8% vs. 18.2% at 6 m; 53.8% vs. 42.9%; all without signficance).

8. Expansion with probable CAA-RI cases

Previous horizontal associations between explanatory variables remained significant, such as between SNN and LE (p=0.024) and between CSF pleocytosis or CSF either alteration and headache (p=0.017; p=0.002). Additionally, the association between CSF elevated protein levels and headache (p=0.009) as well as between CSF pleocytosis and LE (p=0.042) became significant. A total of 88.8% of the cases received immunosuppressive therapy in the probable/definite CAA-RI cohort. Age was not a significant predictor of treatment in the expansion; however, altered mental state was a decisive trigger for immunosuppression (p=0.010). Expectedly, clinical improvement occurred more frequently in the treated (85.4%,

p=0.005); however, spontaneous improvement was strikingly frequent (62.5%). Treatment itself, however, only tended to influence longer-term outcomes, with no significant influence on mortality. Keeping in mind that the add-on probable CAA-RI cases by definition lacked patients with strictly leptomeningeal process, LE remained only a marginally significant predictor of clinical improvement (p=0.046) and did not remain significant for other outcomes. CSF pleocytosis at presentation remained a significant predictor of no clinical improvement (in total: p=0.004; in the treated: p=0.011) and unfavorable outcome at 6 m (in total: p=0.0004, in the treated: p=0.001). CSF either alteration showed significant associations at 6 m (p=0.034 for unfavorable outcome; p=0.032 for mortality). Lobar ICH within the respective period still significantly associated with unfavorable outcome at 6 m (in total: p=0.0007, in the treated: p =0.0008) and 1 y (in total: p=0.006, in the treated: p=0.009), with strong associations with 6-m m (in total: p<0.0001, in the treated: p<0.0001) and 1-y mortality (in total: p=0.0002, in the treated: p=0.0005). Similarly, relapse within the respective periods strongly associated with unfavorable outcomes at 6 m (in total: p=0.0003, in the treated: p=0.0005) and 1 y (in total: p=0.0008, in the treated: p=0.001; table not shown), but not with mortality. In the expansion, relapse among improvers to immunosuppression occurred in 21.6% and 37.5% within 6 m and 1 y, respectively.

Of pathophysiological relevance, the expanded analysis tends to support an association between inflammation and lobar ICH. Indeed, clinical improvement remained significantly associated with a lower probability of lobar ICH within 1 y in the total cohort (p=0.040). Relapse (by definition not related to ICH) within 6 m after treatment was associated with the occurrence of lobar ICH within 6 m (p=0.023).

Though the use of multivariable regressions in the predictor analyses of this study was limited due to rule violations, all identified associations remained significant in valid models controlling for other significant variables in univariable analyses, except for CSF either alteration not remaining an independent predictor of adverse outcome at 6 m in the expanded cohort when the analyses included future lobar ICH as covariate.

Discussion

CAA is a largely underrecognized clinical entity in the elderly, associating with debilitating complications, including ICHs, TFNEs, progressive neurocognitive dysfunction, and, in a subgroup of patients, with subacute encephalopathy in various symptomatic presentations (i.e., CAA-RI).

Our first study identified hypertensive excess and younger age as independent predictors of deep whereas antiplatelet use of lobar/cerebellar localization, age and INR>1.4 as independent predictors of 1-m case fatality, and age, prior intracranial hemorrhage, and antiplatelet use as independent predictors of probable/definite CAA diagnosis, in addition to an estimated prevalence of CAA-related ICHs comparable to prior publications. In our study, 51.6% of spontaneous ICHs originated in the basal ganglia/thalamus, 3.3% in the cerebellum, 5.2% in the brainstem, and 39.9% were of lobar localization. The analysis of risk factors confirmed ICH as the disease of the elderly. Hypertension was by far the most common coexistent factor (~90%) irrespective of ICH localization. The use of antithrombotics were frequent (41.1%), with antiplatelet use proven to be an independent predictor of lobar/cerebellar ICH localization. The analysis of potential clinical predictors of CAA-related ICHs revealed older age (~8% increase in risk per year) and prior intracranial hemorrhage (~8.5-fold risk) as independent significant predictors of probable/definite CAA diagnosis, which is consistent with the notion that CAA is the disease of the elderly (Yamada et al., 2015) and CAA-related ICHs are often recurrent (Biffi et al., 2011). These, together with the female predominance in probable/definite CAA recapitulate key observations of a study from the U.S. comparing their probable/definite CAA-related ICH group with hypertension-related ICHs (as per SMASH-U) (Roh et al., 2018).

Antiplatelet and anticoagulant drugs are considered a risk for ICH in CAA. Our study concords with this, revealing 68.8% of ICH patients with definite/probable CAA to be under at least one type of antithrombotic medication, with the multivariable analysis identifying antiplatelet (but not anticoagulant) use as an independent predictor of probable/definite CAA diagnosis. The 3.3-times higher prevalence of probable/definite CAA diagnosis in antiplatelet users vs. non-users resembles the 2-fold prevalence of lobar CMBs in antiplatelet user ICH patients in a previous study (Gregoire et al., 2010).

As a potentially reversible manifestation of CAA, in our second study we conducted an ever detailed systematic retrospective analysis of previous case reports on CAA-RI, in addition to our case series. This study confirmed that CAA-RI associates with earlier symptomatic presentation than CAA-related ICH. Co-localization of ARIA-E and ARIA-H was the sole differentiating feature in terms of the histopathological subtype, favoring ABRA. *APOE* ε4/ε4 homozygosity was extremely prevalent, suggesting the need for an increased surveillance for CAA-RI development in possible/probable CAA patients harboring this genotype, implicating the relevance of genotyping. Current elements of the diagnostic criteria for probable CAA-RI had modest sensitivity, excluding several published cases with isolated leptomeningeal process.

Incorporating LE and/or SNN on FLAIR to the criteria increased the sensitivity in our analysis. Keeping in mind the limitations due to our study setting, we propose our extended diagnostic criteria as a research framework that merits prospective clinicopathological validation. Among fluid biomarkers, CSF appears to be essential in the work-up of CAA-RI. Though CSF pleocytosis and elevated protein levels are non-specific to CAA-RI, the data suggest that elevated protein level might be a valuable surrogate biomarker of biological changes, including therapy responsiveness and relapse. Indeed, a group used re-emerging levels of CSF protein as a marker of breakthrough disease on deciding to add cyclophosphamide (Kimura et al., 2013). In addition, our analysis identified an association between CSF pleocytosis and an unfavorable course, which accords well with the findings of a recent prospective study (Plotzker et al., 2021). These findings expand the relevance of CSF by representing a cheap and accessible potential tool for differential diagnosis and possibly for patient selection for more potent/escalated therapies.

Among event variables, lobar ICH occurring after presenting with CAA-RI proved to be a significant determinant of morbidity/mortality during the follow-up. Though revealing a surprizing frequency of spontaneous remission, our analysis confirmed the overwhelming benefit of immunosuppressive therapy in CAA-RI (with improvements rates even higher than the ~70% reported previously (Chung et al., 2011)), at least on the short term. The data on long-term outcomes, especially on mortality, however, are less clear. Consensus guidelines for the optimal treatment approach of CAA-RI are lacking. The online available protocol of a flagship institute in CAA suggests a regimen of 0.5-1.0 g daily corticosteroids for 5 days with rapid taper as first-line, restricting other immunosuppressants to refractory cases (Viswanathan et al., 2022). Our analysis of arbitrarily dichotomized doses revealed no difference in outcomes between high-dose and low-dose corticosteroids, which urge for prospective multi-center randomized trials to elaborate an optimized regimen.

Our studies have limitations, including a) their retrospective and (for the second study) literature analytic nature inherent to observer and publication biases, b) the lack of specificity provided by targeted identification of true positive CAA-RI cases, c) the varying subject numbers, d) the lower subject numbers with longer follow-ups, and e) the surprisingly low proportion of reports with precise data on therapy dosing in the second study. Strengths include the large subject number in both studies. Furthermore, we provided an unprecedented subject number with case-wise data collection of published CAA-RI cases to allow for the evaluation of associations between several potential predictors not having been previously addressed systematically.

Conclusion

Our studies highlight the need for an increased awareness of CAA and CAA-RI. We conclude that CAA-related ICHs are at least as frequent (14.7%) in our population as reported internationally (5-20%). Notably, the world-wide low rate of clinically and radiologically established probable CAA diagnosis puts a significant percentage of the population, especially the elderly under antiplatelet therapy (as demonstrated), at a high risk of possibly lethal ICHs. Our first study set the stage for the establishment of a microcerebrovascular outpatient office in our center.

The unprecedented number of definite CAA-RI cases analyzed enabled the evaluation of the current diagnostic criteria in terms of its sensitivity, and proposals have been made to include currently unused radiological features (i.e., LE and SNN) in the diagnosis. Being the first to analyze an expanded probable/definite CAA-RI cohort, our study propose CSF pleocytosis as a negative prognostic factor, define future lobar ICH as a potentially preventable significant contributor to mortality, and implicate no evident superiority of high-dose over low-dose corticosteroids. Though confirming the short-term superiority of immunosuppression over observing a natural course, our study raises important concerns on the paucity of evidence delineating which cases would benefit the most from what strategy of immunosuppression. The identified predictors of short and long-term outcomes may aid the clinical decision-making regarding the choice on the intensity and duration of immunosuppressive therapy.

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