Letter to the Editor : Serum anti-Aβ antibodies in cerebral amyloid angiopathy

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Dear Editor,

Sporadic cerebral amyloid angiopathy (CAA) relates to cerebrovascular accumulation of amyloid fibrils made of amyloid-β peptide $A\beta_{40}$ [1]. CAA is very frequent in Alzheimer's disease (AD) but also in non-AD aged subjects. It may associate with vascular cognitive impairment, hemorrhagic features (CAA-he) [2] and, in rare cases, with corticosensitive Aβ-related CNS vasculitis (CAA-related inflammation, CAA-ri) [3,4].

Pathophysiological mechanisms leading to CAA-he and CAA-ri are poorly understood. Anti-Aβ antibodies of IgG class were shown to increase in the CSF during CAA-ri [5,6], which suggests an autoimmune factor in this condition. Moreover, AD subjects treated by infusions of monoclonal anti-Aβ antibodies developed dose-dependent hemorrhagic and vasculitis features termed amyloid-related Imaging abnormalities (ARIAs) [7]. Anti-Aβ antibodies belong to the natural autoantibody repertoire, as other natural auto-antibodies involved in neurodegenerative disorders [8-11]. Gross serum analyses in AD led to inconsistent conclusions [12], whereas more refined analyses such as anti-Aβ IgG subclasses, discriminate between atypical (focal) and classical forms of AD [13]. To our knowledge, there is no published data regarding serum anti-Aβ antibodies in spontaneous (non-AD) CAA-ri and CAA-he.

This case-control prospective study enrolled 105 participants: 46 CAA-he inpatients fulfilling the modified Boston criteria for probable or definite CAA [14] ; 18 CAA-ri inpatients fulfilling the criteria for non-invasive diagnosis of CAA-ri [15] ; 41 healthy aged controls with normal MRI diffusion sequences and normal cognitive status. Ages and gender ratios were not different between groups.

For ELISA analyses of serum anti-Aβ antibodies dilution curves, dried synthetic $A\beta_{1-40}$ and $A\beta_{1-42}$ aliquots were dissolved in 10 µL DMSO, sonicated to yield monomeric forms, and, for fibrillar $A\beta_{1-}$

$_{42}$ (f-Aβ_{42}) and A$\beta_{1\text{-}40}$ (f-Aβ_{40}) respectively, incubated at 37°C during 72h or 15 days to allow fibril formation. Freshly prepared antigenic preparations were diluted to 15 µg/mL in coating buffer 30mM HEPES 160mM NaCl for A$\beta_{1–40,}$ or 10mM NaCl for A$\beta_{1–42}$, with 10Eq Cu^{2+} for soluble A$\beta_{1\text{-}42}$ (s-Aβ_{42}) and A$\beta_{1\text{-}40}$ (s-Aβ_{40}) respectively, and allowed to bind in 96-wells plates for 16 hours at 4°C. Serial dilutions of serum samples from 1:50 to 1:12800 were incubated 40 min at 20°C in 0.1M Glycine-HCl buffer pH 3.0 to dissociate pre-existing immune complexes, then neutralized in the same volume of 2xPBS 4% BSA 0.02N NaOH, immediately distributed into coated pates and left 1h at 20°C. Bound IgG, IgA, or IgM were detected using appropriate antisera, and IgG subclass were revealed with selected monoclonal antibodies. Values from uncoated wells were subtracted in order to retain signals specific for anti-Aβ. Of note, IgG2 detection did not yield measurable results.

Experimental dilution curves were fitted to sigmoid models [16] that allowed defining 3 parameters: 1. the *maximum* signal in antibody excess, reflecting the amount of antigen binding sites, hence the diversity of epitope recognition; 2. the *titer*, corresponding to the dilution at half-maximum signal, depending on concentration and avidity of polyclonal antibodies; and 3. the *steepness* of the curve at half-maximum, varying with cooperativity phenomena between distinct antibody binding sites. The apparent *avidity* constant was calculated through a linearization procedure [17]. The sigmoid modeling of experimental curves showed excellent goodness-of-fit (mean $R^2 = 0.97$), and internal control mean coefficients of variation were inferior to 20% for all four parameters.

Table I presents the anti-Aβ serologic parameters independently associated with CAA, CAA-he and CAA-ri, as compared to controls, according to multivariable logistic regression models. Fig 1 presents individuals predicted response resulting from the CAA-, CAA-he-, and CAA-ri models considering individual anti-Aβ profiles.

Analyses of blood anti-Aβ antibodies demonstrate complex serological profiles in CAA, with distinctive features in CAA-he and CAA-ri, and suggests the existence of defined circulating anti-Aβ antibody species associated with distinct pathological phenotypes. Such anti-Aβ antibody species could enhance CAA and/or trigger hemorrhagic or inflammatory manifestations, as suggested in experimental mouse models [18,19], and in AD patients treated with monoclonal anti-Aβ antibodies [7]. This study drives attention towards potentially relevant anti-Aβ antibody patterns in CAA. Despite a huge sequence overlap, multivariable statistical analysis pinpoints the relevance of both $A\beta_{1-40}$- and $A\beta_{1-42}$-related parameters. Regarding the isotypes reacting with $A\beta_{1-40}$, i.e. the main component of CAA vascular deposits, our results suggest preferential involvement of IgG3 and IgG4 antibody responses in CAA. Lower diversity of anti-soluble $A\beta_{1-40}$ IgM was also a common characteristic of both CAA-he and CAA-ri profiles, which could indicate a driven IgM response toward some particular pathogenic $A\beta_{1-40}$ epitopes in response to cerebrovascular deposits.

The causal relevance of serological differences in spontaneous CAA manifestations remains to be further elucidated. It is not known whether they relate to peculiar natural antibody repertoires that might favor CAA and its complications or if they rather emerge from B-cell selection processes induced by Aβ pathological species, or both. Indeed, the link between self-replicating proteins, inflammation and active auto-immune processes has been underlined [20,21].

In conclusion, this correlative study demonstrates distinct serum anti-Aβ antibody patterns in CAA and resulting hemorrhagic and inflammatory manifestations. Larger prospective and experimental studies should elucidate the triggering role of anti-Aβ antibodies in spontaneous or immunotherapy-induced CAA manifestations, and provide appropriate biomarkers of these conditions.

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Figure legend

Figure 1. Serological differences associated with CAA clinical phenotypes. A, CAA-model predicted value using the logistic multivariable regression model presented in Table 1, upper part. B, CAA-he model predicted values using the logistic multivariable regression model presented in Table 1, middle part. C, CAA-ri model predicted values using the logistic multivariable regression model presented in Table 2, lower part. *:$p<0.05$; **: $p<0.01$; ***: $p<0.001$. Wilcoxon's test.

Figure 1

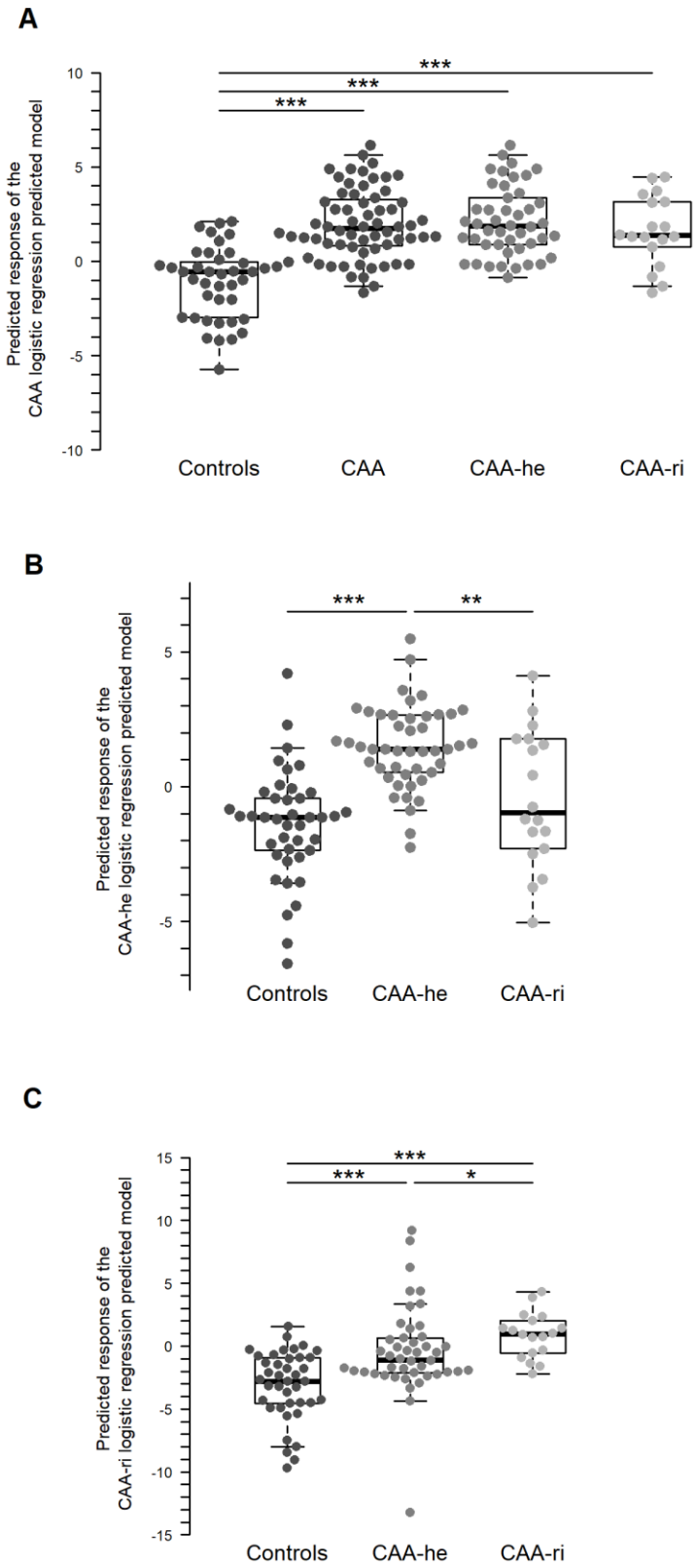

A
Predicted response of the CAA logistic regression predicted model
10
5
0
-5
-10
***
***
***
Controls
CAA
CAA-he
CAA-ri
B
Predicted response of the CAA-he logistic regression predicted model
5
0
-5
***
**
Controls
CAA-he
CAA-ri
C
Predicted response of the CAA-ri logistic regression predicted model
15
10
5
0
-5
-10
-15
***
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*
Controls
CAA-he
CAA-ri

Table I. Multivariable logistic regression models for CAA, CAA-he, and CAA-ri against healthy aged controls

CAA model

Variables	Estimates	Standard errors	Z-val.	P-val.[d]	Residual deviances	P-val.[e]
	0.13	6.57			140.5	
[a]Anti-s-Aβ_{40} IgG3 Maximum	2.89	0.92	3.14	0.004	134.0	0.011
Anti-s-Aβ_{40} IgG3 Titer	4.03	1.16	3.48	0.003	123.6	0.0013
Anti-s-Aβ_{40} IgG4 Avidity	2.29	0.94	2.44	0.016	117.6	0.014
[a]Anti-f-Aβ_{42} IgG4 Steepness	3.74	1.32	2.84	0.006	111.5	0.013
[b]Anti-s-Aβ_{40} IgM Maximum	-3.10	1.02	-3.05	0.004	102.5	0.0027
[c]Anti-s-Aβ_{42} IgA Avidity	-3.48	1.13	-3.07	0.004	92.9	0.0019
Anti-f-Aβ_{42} IgG1 Titer	-3.81	1.58	-2.42	0.016	86.5	0.012

CAA-he model

Variables	Estimates	Standard errors	Z-val.	P-val.[d]	Residual deviances	P-val.[e]
	42.14	11.84			120.32	
Anti-s-Aβ_{40} IgG4 Titer	3.30	1.11	2.97	0.009	111.57	0.0031
Anti-f-Aβ_{42} IgG4 Titer	-6.41	1.95	-3.29	0.006	98.41	0.0003
Anti-f-Aβ_{42} IgG1 Avidity	-4.65	2.04	-2.28	0.028	90.72	0.0056
[c]Anti-s-Aβ_{42} IgA Avidity	-2.77	1.06	-2.61	0.018	85.59	0.024
[b]Anti-s-Aβ_{40} IgM Maximum	-2.09	1.01	-2.06	0.039	80.95	0.031
Anti-s-Aβ_{42} IgG4 Avidity	-2.87	1.24	-2.32	0.028	74.92	0.014

CAA-ri model

Variables	Estimates	Standard errors	Z-val.	P-val.[d]	Residual deviances	P-val.[e]
	10.75	5.78			72.58	
[a]Anti-s-Aβ_{40} IgG3 Maximum	4.15	1.55	2.68	0.03	63.92	0.0032
[a]Anti-f-Aβ_{42} IgG4 Steepness	4.01	1.70	2.36	0.03	58.38	0.019
Anti-s-Aβ_{40} IgA Steepness	-18.53	8.23	-2.25	0.03	54.08	0.038
Anti-s-Aβ_{42} IgG4 Steepness	-12.19	5.25	-2.32	0.03	46.96	0.0076
[b]Anti-s-Aβ_{40} IgM Maximum	-2.40	1.21	-1.99	0.047	40.68	0.012

[a]: Variables shared between the CAA and the CAA-ri models. [b]: Variables shared between the CAA, the CAA-he and the CAA-ri models; [c]: Variables shared between the CAA and the CAA-he models; [d]: Wald's test p-values corrected by the Benjamini & Hochberg procedure. [e]: uncorrected Likelihood Ratio test p-values against the (k-1) model.