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

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

Yannick Chantran[a,b], Jean Capron[a,c], Diana Doukhi[a], Johanna Felix[a], Mélanie Féroul[a], Florian Kruse[a], Thomas Chaigneau[a], Guillaume Dorothée[a], Thibault Allou[d], Xavier Ayrignac[e], Zina Barrou[f], Thomas de Broucker[g], Corina Cret[h], Guillaume Turc[i], Roxane Peres[j], Anne Wacongne[k], Marie Sarazin[l], Dimitri Renard[k], Charlotte Cordonnier[m], Sonia Alamowitch[a,c], Pierre Aucouturier[a,b]*

[a]UMRS 938, Hôpital St-Antoine, Sorbonne Université, Inserm, Paris, France

[b]Département d'Immunologie Biologique, Hôpital Saint-Antoine, AP-HP, Paris, France

[c]Service de Neurologie et d'Urgences Neurovasculaires, Hôpital Saint-Antoine, AP-HP, Paris, France

[d]Service de Neurologie, CH Perpignan, Perpignan, France

[e]Service de Neurologie, CHU Montpellier, Hôpital Guy de Chauliac, Montpellier, France

[f]Service de Gériatrie, Hôpital Pitié Salpêtrière, AP-HP, Paris, France

[g]Service de Neurologie, Centre Hospitalier de Saint-Denis, Saint-Denis, France

[h]Service de Neurologie, Centre Hospitalier de Meaux, Meaux, France

[i]Service de Neurologie, GHU Paris Psychiatrie et Neurosciences, Université de Paris, INSERM U1266, FHU NeuroVasc, Paris, France

[j]Service de Neurologie, Hôpital Lariboisière, AP-HP, Paris, France

[11]Service de Neurologie, CHU Nîmes, Hôpital Caremeau, Nîmes, France

[12]Service de Neurologie de la Mémoire et du Langage, Centre Hospitalier Sainte-Anne, Université Sorbonne Paris Cité, Paris, France

[13]U1172 - LilNCog - Lille Neuroscience & Cognition, Inserm, CHU Lille, Univ. Lille, Lille, France

*** Correspondence:**

Pierre Aucouturier

Inserm UMRS 938, hôpital Saint-Antoine, 184 rue du Fbg Saint-Antoine, F-75012 Paris, France

pierre.aucouturier@inserm.fr

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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.

References

[1] Charidimou A, Boulouis G, Gurol ME, Ayata C, Bacskai BJ, Frosch MP, et al. Emerging concepts in sporadic cerebral amyloid angiopathy. Brain 2017a; 140: 1829–50.

[2] Béjot Y, Cordonnier C, Durier J, Aboa-Eboule C, Rouaud O, Giroud M. Intracerebral haemorrhage profiles are changing: results from the Dijon population-based study. Brain 2013; 136: 658–64.

[3] Salvarani C, Hunder GG, Morris JM, Brown RD Jr, Christianson T, Giannini C. Aβ-related angiitis: comparison with CAA without inflammation and primary CNS vasculitis. Neurology 2013; 81: 1596–603.

[4] Salvarani C, Brown RD Jr, Christianson TJH, Huston J 3rd, Giannini C, Hunder GG. Long-term remission, relapses and maintenance therapy in adult primary central nervous system vasculitis: A single-center 35-year experience. Autoimmun Rev. 2020 Apr;19(4):102497.

[5] Piazza F, Greenberg SM, Savoiardo M, Gardinetti M, Chiapparini L, Raicher I, et al. Anti-amyloid β autoantibodies in cerebral amyloid angiopathy-related inflammation: implications for amyloid-modifying therapies. Ann Neurol 2013; 73: 449–58.

[6] DiFrancesco JC, Longoni M, Piazza F. Anti-Aβ Autoantibodies in Amyloid Related Imaging Abnormalities (ARIA): Candidate Biomarker for Immunotherapy in Alzheimer's Disease and Cerebral Amyloid Angiopathy. Front Neurol 2015; 6: 207.

[7] Sperling RA, Jack CR Jr, Black SE, Frosch MP, Greenberg SM, Hyman BT et al. Amyloid-related imaging abnormalities in amyloid-modifying therapeutic trials: recommendations from the Alzheimer's Association Research Roundtable Workgroup. Alzheimers Dement 2011; 7:367–85.

[8] Neff F, Wei X, Nölker C, Bacher M, Du Y, Dodel R. Immunotherapy and naturally occurring autoantibodies in neurodegenerative disorders. Autoimmun Rev. 2008 Jun;7(6):501-7.

[9] Krestova M, Hromadkova L, Ricny J. Purification of Natural Antibodies Against Tau Protein by Affinity Chromatography. Methods Mol Biol 2017; 1643: 33-44.

[10] Folke J, Rydbirk R, Løkkegaard A, et al. Distinct Autoimmune Anti-α-Synuclein Antibody Patterns in Multiple System Atrophy and Parkinson's Disease. Front Immunol 2019; 10: 2253.

[11] Szabo P, Relkin N, Weksler ME. Natural human antibodies to amyloid beta peptide. Autoimmun Rev. 2008 Jun;7(6):415-20.

[12] Chantran Y, Capron J, Alamowitch S, Aucouturier P. Anti-Aβ antibodies and cerebral amyloid angiopathy complications. Front Immunol 2019; 10: 1534.

[13] Dorothée G, Bottlaender M, Moukari E, de Souza LC, Maroy R, Corlier F et al. Distinct patterns of antiamyloid-β antibodies in typical and atypical Alzheimer disease. Arch Neurol 2012; 69: 1181–5.

[14] Linn J, Halpin A, Demaerel P, Ruhland J, Giese AD, Dichgans M et al. Prevalence of superficial siderosis in patients with cerebral amyloid angiopathy. Neurology 2010; 74: 1346–50.

[15] Auriel E, Charidimou A, Gurol ME, Ni J, Van Etten ES, Martinez-Ramirez S, et al. Validation of Clinicoradiological Criteria for the Diagnosis of Cerebral Amyloid Angiopathy-Related Inflammation. JAMA Neurol 2016; 73: 197–202.

[16] Cheung YB, Xu Y, Remarque EJ, Milligan P. Statistical estimation of antibody concentration using multiple dilutions. J Immunol Methods. 2015;417:115-23.

[17] Orosz F, Ovádi J. A simple method for the determination of dissociation constants by displacement ELISA. J Immunol Methods 2002; 270: 155–62.

[18] Pfeifer M, Boncristiano S, Bondolfi L, Stalder A, Deller T, Staufenbiel M et al. Cerebral hemorrhage after passive anti-Abeta immunotherapy. Science. 2002; 298: 1379.

[19] Thakker DR, Weatherspoon MR, Harrison J, Keene TE, Lane DS, Kaemmerer WF, et al. Intracerebroventricular amyloid-beta antibodies reduce cerebral amyloid angiopathy and associated micro-hemorrhages in aged Tg2576 mice. Proc Natl Acad Sci USA 2009; 106: 4501–06.

[20] Butnaru D, Chapman J. The impact of self-replicating proteins on inflammation, autoimmunity and neurodegeneration-An untraveled path. Autoimmun Rev. 2019 Mar;18(3):231-40.

[21] Bonam SR, Muller S. Parkinson's disease is an autoimmune disease: A reappraisal. Autoimmun Rev. 2020 Dec;19(12):102684.

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

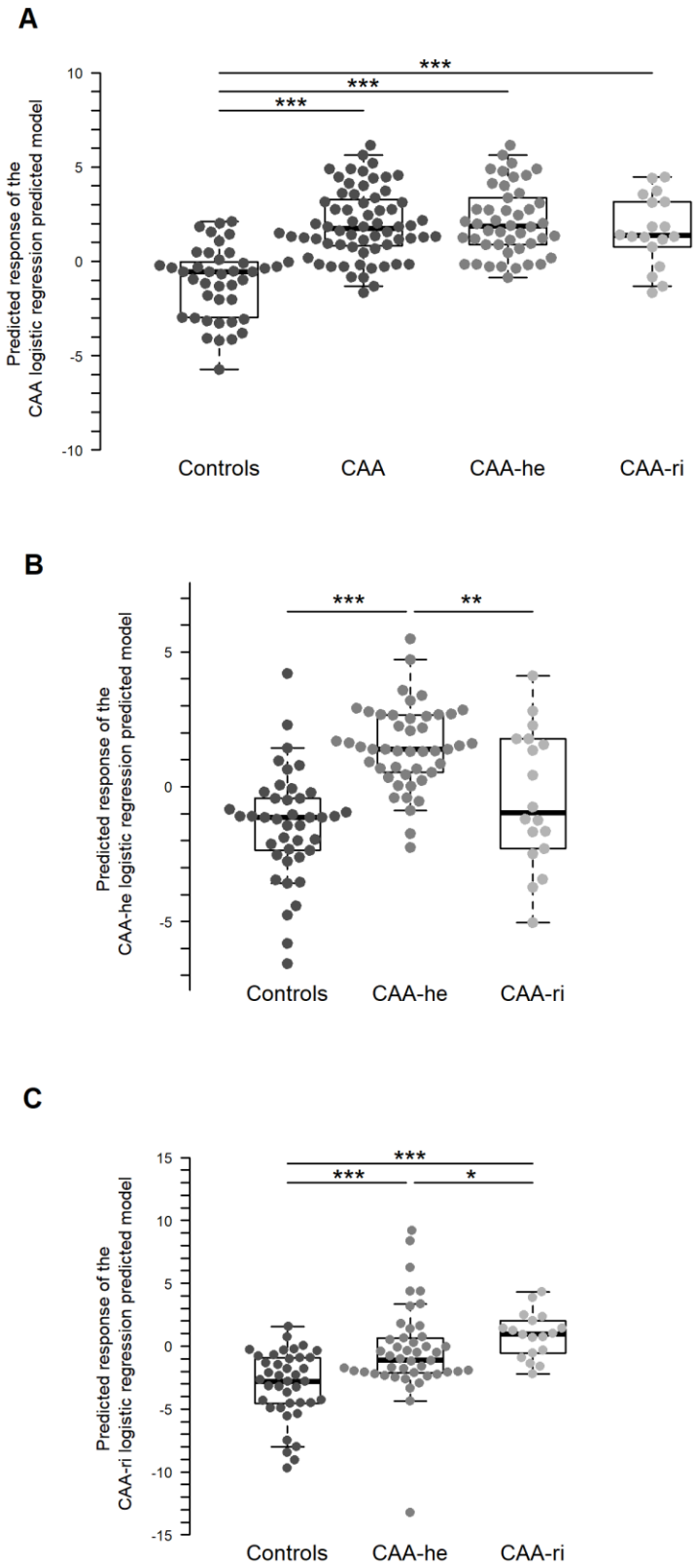
A
Predicted response of the CAA logistic regression predicted model
10
5
0
-5
-10
***
***
***
Controls
CAA
CAA-he
CAA-ri
B
Predicted response of the CAA-he logistic regression predicted model
5
0
-5
***
**
Controls
CAA-he
CAA-ri
C
Predicted response of the CAA-ri logistic regression predicted model
15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