



# Intrathecal consumption of BAFF parallels local IgG synthesis in Multiple Sclerosis at clinical onset

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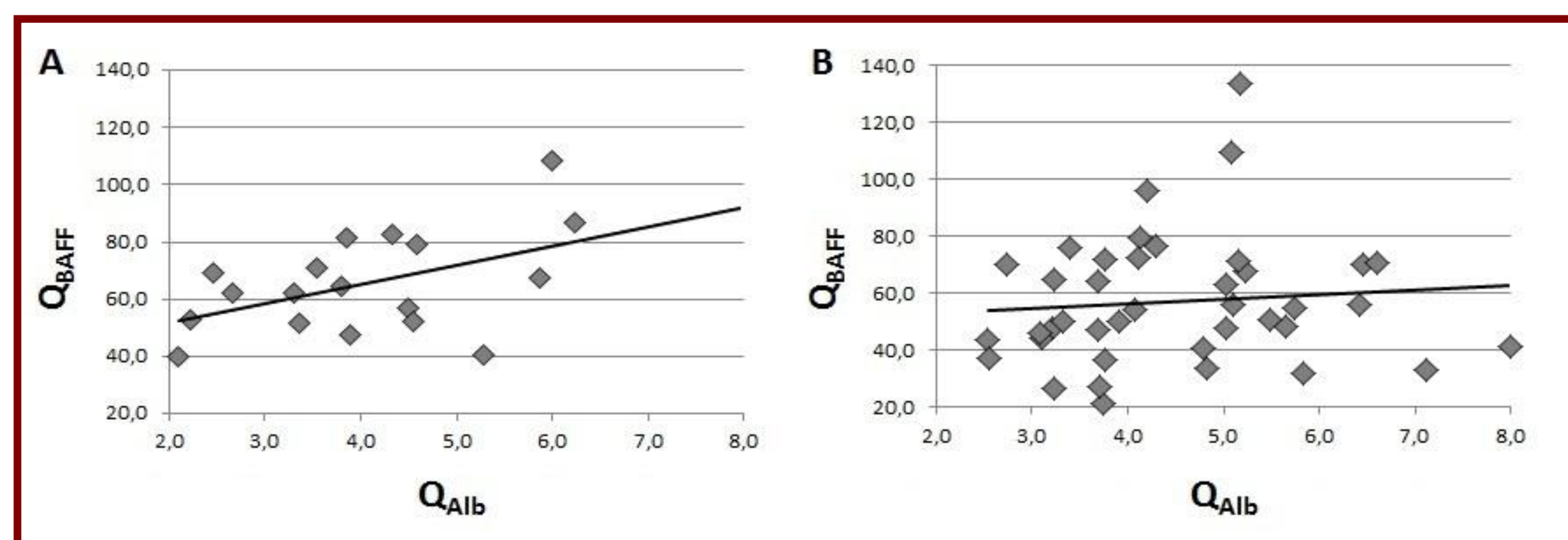
**Background.** B-cells play a relevant role in multiple sclerosis (MS) pathology. BAFF (B cell activating factor of the TNF family) is a B-cell survival factor constitutively produced inside the CNS by astrocytes.

**Objective.** We studied the correlation between the intrathecal synthesis of BAFF and locally produced IgG in patients with clinically isolated syndromes (CIS) suggestive of MS or early relapse-onset MS (eRRMS) at clinical presentation.

**Materials and Methods.** Paired serum and CSF were obtained from 40 patients with CIS/eRRMS and 18 healthy controls (HC). Routine examination of CSF and serum included cell count and differentiation, CSF-IgG/serum-IgG ratio ( $Q_{IgG}$ ), CSF-albumin /serum-albumin ratio ( $Q_{Alb}$ ), calculation of intrathecal IgG synthesis by quantitative formulae (IgG Index, IgG Reiber Hyperbolic Function for IgG intrathecal synthesis fraction (IgGIF) and Local Production (IgGLoc)) and demonstration of IgG oligoclonal bands (IgGOB). BAFF was detected by means of a highly sensitive ELISA. BAFF ratio (CSF-BAFF/serum-BAFF,  $Q_{BAFF}$ ) and Index ( $Q_{BAFF}/Q_{Alb}$ , BAFF-Index) were calculated. Patients were further classified in IgGOB+ and IgGOB-, on the base of IgGOB the detection in the CSF.

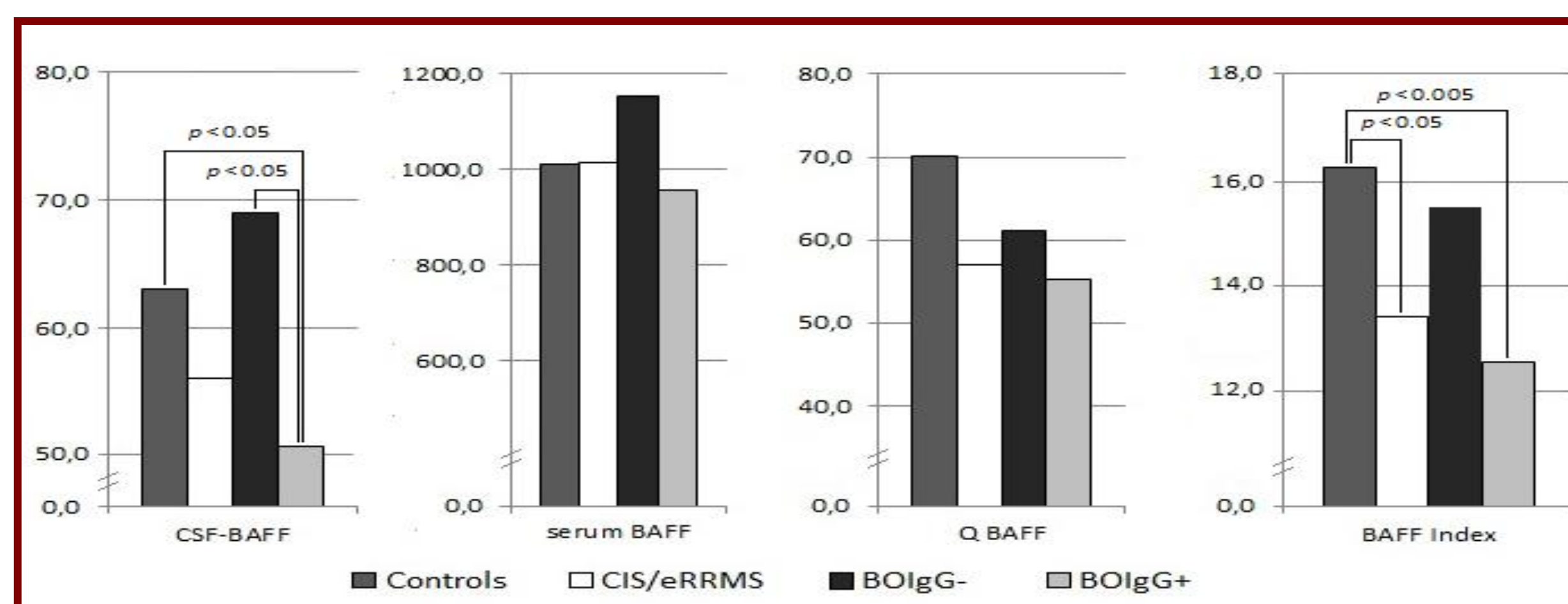
**Results. 1. Study Population** The 40 CIS/eRRMS (26 females, 14 males; mean age  $36.6 \pm 10.9$  years) and the 18 controls (HC, 13 F, 5 M; mean age  $41.9 \pm 9.0$  years) included in the study did not differ in age ( $p=0.1$ ) and gender ( $p=0.8$ ). For CIS/eRRMS, mean disease duration was  $0.4 \pm 1.0$  year.

**2. CSF/serum IgG and BAFF in HC and CIS/eRRMS** As expected, CSF IgG concentration ( $p<0.05$ ),  $Q_{IgG}$  ( $p<0.05$ ), IgG Index ( $p<0.01$ ), IgGLoc ( $p<0.001$ ), IgGIF ( $p<0.005$ ) and IgGOB frequency ( $p<0.001$ ) were significantly higher in CIS/eRRMS compared to controls.  $Q_{IgG}$  strongly correlated to  $Q_{Alb}$  in controls ( $r:0.9$ ,  $p<0.0001$ ), while the correlation was milder in all the CIS/eRRMS group ( $r:0.5$ ,  $p<0.005$ ). BAFF levels in serum and CSF, and  $Q_{BAFF}$  did not differ between CIS/eRRMS and controls, while BAFF Index was significantly lower in CIS/eRRMS patients ( $13.5 \pm 5.7$  vs  $17.3 \pm 5.2$ ,  $p<0.05$ ). Furthermore,  $Q_{BAFF}$  slightly correlated to  $Q_{Alb}$  in controls ( $r: 0.5$ ,  $p<0.05$ , Figure 1A), but not in CIS/eRRMS ( $r:0.1$ , Figure 1B) even when age or leucocyte counts were considered as co-variants. This finding suggests that in the presence of a normal BBB function the CSF concentrations of BAFF are partly determined by serum filtration at the choroid plexus levels, and partly supported by its constitutive intrathecal production by astrocytes, thus supporting histological findings.



**Figure 1.** Correlation between  $Q_{Alb}$  and  $Q_{BAFF}$  in controls (A) and CIS/eRRMS (B).  $Q_{Alb}$  correlates to  $Q_{BAFF}$  in the control group ( $r: 0.9$ ,  $p<0.0001$ ), but not in CIS/eRRMS ( $r: 0.1$ ). Abbreviation as in Table 1.

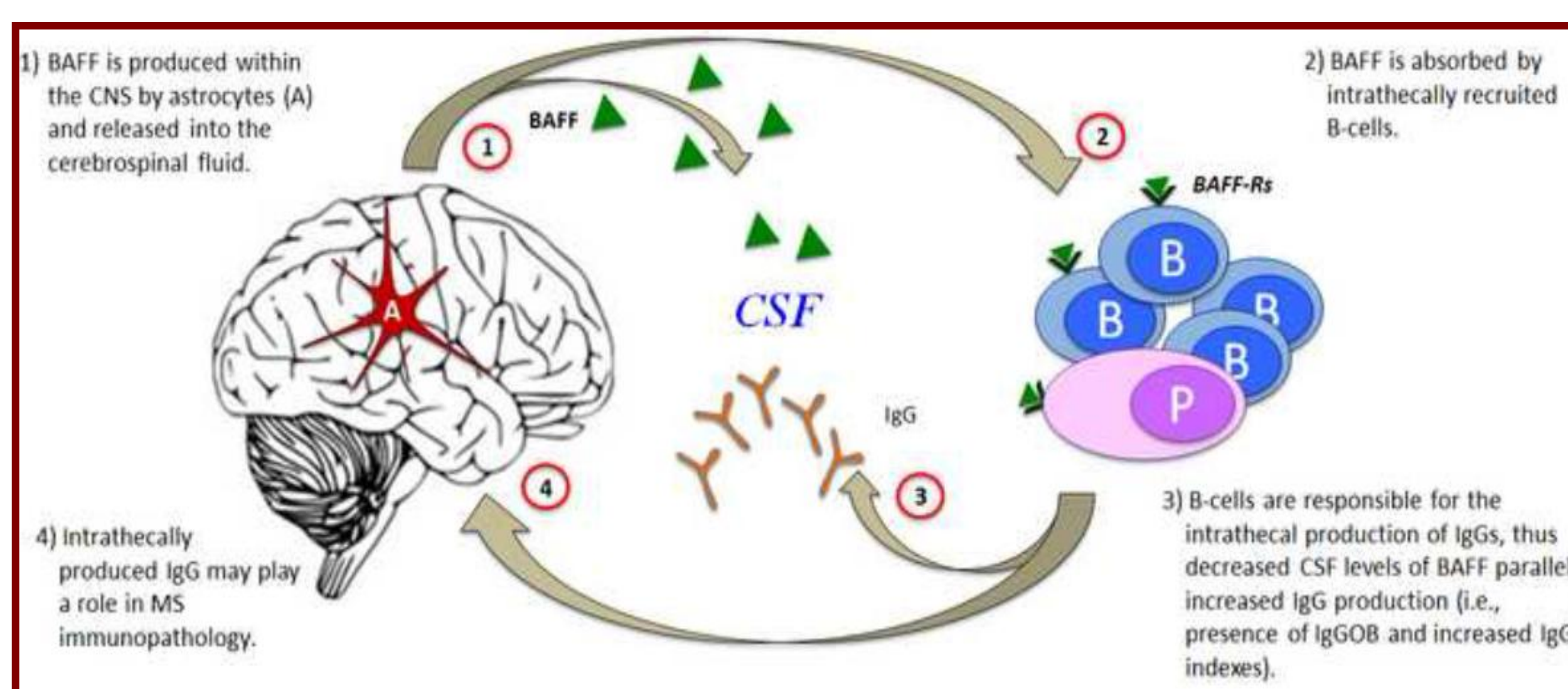
**3. IgGOB in CIS/eRRMS** Compared to both BOIgG- and HC, IgGOB+ presented lower CSF-BAFF concentration ( $p<0.05$ ) and BAFF Index ( $p<0.01$ ) (Figure 2). No differences in standard CSF parameters and BAFF concentrations were disclosed between BOIgG- and HC, while IgGOB+ presented higher CSF-IgG concentration,  $Q_{IgG}$ , IgG Index, IgG Loc, IgGIF and Leucocyte counts compared to HC and BOIgG- (all p-value almost  $< 0.05$ ). Finally, a significant inverse correlation between  $Q_{IgG}$  and  $Q_{BAFF}$  ( $r: -0.4$ ,  $p<0.05$ ) and between BAFF index and IgGIF ( $r: -0.4$ ,  $p<0.05$ ) or IgG Index ( $r: -0.3$ ,  $p=0.05$ ) was found only in IgGOB+.



**Figure 2.** BAFF in patients and controls. BAFF concentrations in CSF and serum,  $Q_{BAFF}$  (C) and BAFF Index in controls, CIS/eRRMS, IgGOB+ and IgGOB-. BAFF levels in CSF and BAFF Index were significantly lower in IgGOB+ compared to both controls and IgGOB-. Abbreviations as in Table 1.

	Controls	CIS/eRRMS All patients	CIS/eRRMS IgGOB+	CIS/eRRMS IgGOB-
CSF-Alb (mg/dL)	17.6 ± 5.7	19.2 ± 5.9	20.2 ± 6.3	16.7 ± 3.9
serum-Alb (mg/dL)	4387 ± 374.4	4246.5 ± 330.4	4293.9 ± 344.1	4135.8 ± 277.9
$Q_{Alb}$ ( $10^{-3}$ )	4.0 ± 1.3	4.5 ± 1.3	4.7 ± 1.4	4.1 ± 1.0
CSF-IgG (mg/dL)	2.2 ± 0.8	3.9 ± 2.9*	4.7 ± 3.1***	2.1 ± 0.6##
serum-IgG (mg/dL)	1129.9 ± 214.2	1052.5 ± 200.9	1088.4 ± 212.6	975.6 ± 133.1
$Q_{IgG}$ ( $10^{-3}$ )	2.0 ± 0.6	3.8 ± 3.2*	4.5 ± 3.6***	2.2 ± 0.6#
IgG Index	0.5 ± 0.1	0.8 ± 0.5**	0.9 ± 0.5****	0.5 ± 0.1#
IgG Loc (mg/L)	0.0 ± 0.0	10.3 ± 23.4****	14.6 ± 27.1****	0.4 ± 0.8*
IgGIF (%)	0.0 ± 0.0	15 ± 20***	21 ± 22****	2 ± 3####
Leucocyte counts	2.1 ± 1.1	8.5 ± 11.2*	10.7 ± 12.7**	3.4 ± 3.1
IgGOB (%)	0 (0/18)	70 (28/40)****	100 (28/28)****	0 (0/12)####
CSF-BAFF (pg/mL)	66.6 ± 21.1	56.1 ± 23.4	50.5 ± 19.8*	68.9 ± 26.7#
serum-BAFF (pg/mL)	1063.4 ± 379.1	1015.4 ± 334.1	956.5 ± 345.8	1152.7 ± 269.8
$Q_{BAFF}$ ( $10^{-3}$ )	65.3 ± 17.6	57.0 ± 23.4	55.3 ± 23.0	61.1 ± 22.2
BAFF Index	17.3 ± 5.2	13.5 ± 5.7*	12.6 ± 5.7**	15.5 ± 5.2

**Table 1.** CSF and serum parameters and BAFF concentrations in the study group. CIS/eRRMS: Clinical Isolated Syndrome or early Relapsing Remitting Multiple Sclerosis patients; IgGOB+: patients with IgG Oligoclonal Bands in the CSF; IgGOB-: patients without IgG Oligoclonal Bands in the CSF; CSF: cerebrospinal fluid; Alb: albumin; IgGOB: IgG Oligoclonal Bands;  $Q_{Alb}$ : Albumin ratio;  $Q_{IgG}$ : IgG ratio;  $Q_{BAFF}$ : BAFF ratio; IgGLoc: IgG Local Production; IgGIF: IgG intrathecal synthesis fraction. \*:  $p<0.05$  compared to controls; \*\*:  $p<0.01$  compared to controls; \*\*\*:  $p<0.005$  compared to controls; \*\*\*\*:  $p<0.001$  compared to controls; #:  $p<0.05$  compared to IgGOB+; ##:  $p<0.01$  compared to IgGOB+; ###:  $p<0.005$  compared to IgGOB+; ####:  $p<0.001$  compared to IgGOB+.



**Figure 3.** The decreased CSF-BAFF in IgGOB+ at clinical onset may be explained by its absorption by intrathecally recruited B cells.

**Conclusions.** The decreased intrathecal BAFF levels in IgGOB+ strongly suggest the absorption of this factor by the target cells and indicate that B-cells are early recruited in MS CNS (Figure 3). Whether locally produced BAFF contributes to FLS formation and/or to the chronic evolution of MS-related inflammation deserves further investigation.

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