CONNECTION BETWEEN LEPTOMENINGEAL CONTRAST ENHANCEMENT FORMATION AND DISTURBANCES IN PERIPHERAL BLOOD B-CELLS' SUBSETS

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Introduction. B-cells play a pivotal role in the multiple sclerosis (MS) pathogenesis. Of particular interest is the involvement of B cells in the formation of ectopic lymphoid follicles (ELFs) within the subarachnoid space. These ELFs have been associated with cortical demyelination, neurodegeneration and disease progression. Recent studies have shown that ELFs may be recognized on magnetic resonance imaging (MRI) as foci of leptomeningeal contrast enhancement (LMCE). Thus, LMCE could be a noninvasive, in vivo biomarker of ELF formation.[1] The aim of our research was to study if LMCE formation is associated with disturbances in peripheral blood B-cells' subsets.

Materials and methods. 32 MS cases and 20 age-matched neurologically healthy controls were included into the study. Characteristics of the studied population are presented in the Table 1. Examples of LMCE are presented in the Figure 1.

LMCE was detected with a 3 Tesla 3D FLAIR post-contrast sequence in the subarachnoid space as areas of increased signal, that weren't detected on pre-contrast sequence. Blood was taken within 3-6 months after the MRI. To analyze the distribution of B-lymphocytes subsets flow cytometry was performed according to Bm1-Bm5 classification (cell surface IgD, CD38 co-expression).

Results. ● ***In the LMCE + subgroup we found several B-cell subset disturbances:***

● Increased levels of immature Bm2, Bm2' subsets compared to controls **(Bm2: p=0.0357; Bm2': p=0.0030).**

● Bm3-Bm4 subset didn't differ from controls **(p=0.3337)**, but were significantly lower than in LMCE-negative peers **(p=0.0434)**.

● The analysis revealed decreased mature eBm5, Bm5 subset in LMCE-positive subgroup compared to controls (eBm5: **p=0.0492**; Bm5: **p=0.0329**) and LMCE-negative patients (**Bm5: p=0.0396**). The results are presented in details in the Table 2.

Table 1. **Main characteristics of the study groups**

	Control group n=(20)	LMCE-negative subgroup n=(15)	LMCE-positive subgroup n=(17)	p-value
n (%) female n (%) male	8 (40,0%) 12 (60,0%)	8 (53,3%) 7 (46,7%)	10 (58,8%) 7 (41,2%)	>0,05
Age at MRI, years (mean±SD)	37,1±9,0	38,1±12,1	42,8±14,9	>0,05
Disease duration, months (mean±SD)	-	84,9±65,4	162,1±104,6	***0,0198***
MS phenotype, n (%): -RRMS -PMS	-	11 (73,3%) 4 (26,7%)	16 (94,1%) 1 (5,88%)	0,1609
Duration of therapy, month (mean±SD)	-	40,1±44,4	65,6±60,1	0,1908

Figure 1. Examples of LMCE detected in different patients

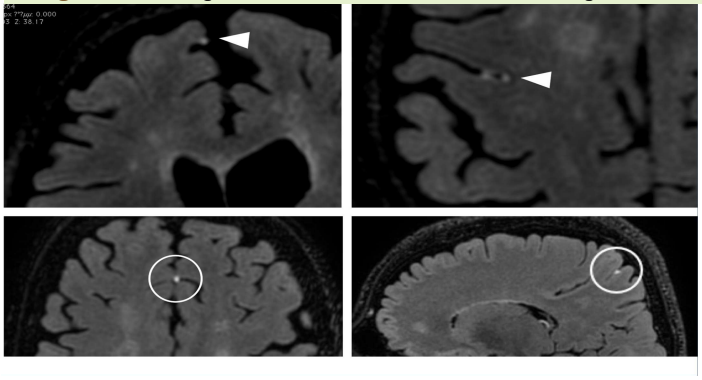

Table 2. **B-lymphocytes analysis according to the LMCE status**

	Control group n=(20)	LMCE-negative subgroup n=(15)	LMCE-positive subgroup n=(17)	p-value
Bm1 (cells/μl)	27,097 (29,10)	24,19 (15,86)	19,87 (48,06)	0,3861* 0,2301✚ 0,7198 ‡
Bm2 (cells/μl)	91,99 (51,88)	132,54 (66,03)	129,57 (84,40)	0,0891* ***0,0357*** ✚ 0,7770 ‡
Bm2' (cells/μl)	11,84 (12,04)	18,82 (25,07)	25,54 (38,59)	***0,0303**** ***0,0030*** ✚ 0,6641 ‡
Bm3+Bm4 (cells/μl)	1,59 (0,97)	4,47 (4,15)	2,32 (1,79)	***0,0003**** 0,3337 ✚ ***0,0434*** ‡
eBm5 (cells/μl)	27,44 (17,68)	25,65 (13,33)	15,03 (21,52)	0,8939* ***0,0492*** ✚ 0,0670 ‡
Bm5 (cells/μl)	19,49 (11,48)	23,51 (16,26)	9,77 (19,18)	0,5485* ***0,0329*** ✚ ***0,0396*** ‡

Data presented as median (IQR)

p-value: *-control-vs-LMCE- ✚-control-vs-LMCE+ ‡ -LMCE- vs LMCE+;

Discussion. LMCE-positive patients showed significant disturbances in the peripheral blood B cell subpopulations compared with LMCE-negative patients and healthy donors. Such altered proportions might be a consequence of increased activation, disruption in peripheral differentiation and/or trafficking of B cells. **Altered** proportions of B cells were obtained with other immunological diseases (rheumatoid arthritis, Sjögren's syndrome).[2] More studies are needed to define the pathogenesis of LMCE formation and role of B cells in this process. The key to understanding these phenomena can be the flow cytometry of the CSF and the study of the chemokines contained in it.

Literature. [1] - Gleb Makshakov, Evgeniy Magonov, Natalia Totolyan, Vladimir Nazarov, Sergey Lapin, Alexandra Mazing, Elena Verbitskaya, Tatiana Trofimova, Vladimir Krasnov, Maria Shumilina, Alexander Skoromets, and Evgeniy Evdoshenko, «Leptomeningeal Contrast Enhancement Is Associated with Disability Progression and Grey Matter Atrophy in Multiple Sclerosis», *Neurology Research International,* Volume 2017 (2017), Article ID 8652463, 7 pages. [2] - Janne Ø. Bohnhorst, Marie B. Bjørgan, Jørn E. Thoen, Jacob B. Natvig and Keith M. Thompson, «Bm1−Bm5 Classification of Peripheral Blood B Cells Reveals Circulating Germinal Center Founder Cells in Healthy Individuals and Disturbance in the B Cell Subpopulations in Patients with Primary Sjögren's Syndrome», *J Immunol* 2001; 167:3610-3618; doi: 10.4049/jimmunol.167.7.3610

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