Abstract of Dissertation submitted by HOANG THI THU TRANG

Measurement of anti-suprabasin antibodies, multiple cytokines and chemokines as potential predictive biomarkers for neuropsychiatric systemic lupus erythematosus

抗 suprabasin 抗体、サイトカインおよびケモカインによる神経精神ループスの診断予測バイオマーカーの可能性

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Introduction: Neuropsychiatric SLE (NPSLE) varies in presentation and is one of the leading causes of morbidity and mortality among patients with SLE. Distinguishing NPSLE from other neuropsychiatric conditions with different etiologies is challenging. The pathogenesis of NPSLE is multifactorial and involves diverse cytokines, autoantibodies and immune complexes inducing blood-brain barrier (BBB) dysfunction, neuroendocrine-immune imbalance, vascular occlusion, tissue, and neuronal damage. Previously, we have shown that the titer of anti-SBSN antibodies in CSF of NPSLE patients was significantly higher than in SLE, MS and NPH groups. An *in vitro* study indicated that anti-SBSN antibodies in CSF bound directly to the astrocytes and activated the senescence and autophagy pathways.
This analysis aimed to elucidate the predictive values of serum anti-SBSN antibodies and cytokines/chemokines for the development of NPSLE as this may have clinical utility prior to the onset of neuropsychiatric symptoms.

Materials and Methods: We retrospectively analyzed 35 NPSLE patients, 34 SLE patients, 20 viral meningitis (VM) patients, and 16 relapsing-remitting multiple sclerosis (MS) patients who were admitted to Nagasaki University Hospital from 2014 to 2020. We used serum of 38 healthy individuals (HC) as the non-autoimmune, non-inflammatory control. We measured anti-SBSN antibodies concentrations in serum by using Luciferase immunoprecipitation system (LIPS) assay. The serum concentrations of cytokines/chemokines were measured by using Multiplex cytokines and chemokines magnetic bead assay. All the clinical information and laboratory tests were collected at the time of admission.

Results: The median [IQR] of anti-SBSN antibody relative concentration (SRC) in the NPSLE, SLE, VM and MS groups were 2.70 [2.10; 3.37], 2.23 [1.72; 2.93], 2.06[1.51; 2.47] and 2.20[0.86; 2.58], respectively. The SRC of NPSLE group was significantly higher than VM and MS groups (p=0.011 and p = 0.039, respectively). There was no significant difference in SRC between the NPSLE and SLE groups (p= 0.22). The posterior mean of the cut-off of SRC was 5.26 with the 95% probability (highest posterior density interval (HPDI)) of the cut-off falls between the range from 3.68 to 7.17. The Bayesian posterior mean and 95% HPDI for PPV and 1-NPV were 0.87, (0.72; 1.0) and 0.44, (0.36; 0.5), respectively. We found serum FGF-2 level was significantly higher in the NPSLE group compared to the SLE group and the healthy control group (pairwise p = 0.035 and p= $1.4x10^{-8}$, respectively). The level of VEGF in serum of the NPSLE is significantly higher than in the SLE group but no different with the HC group (pairwise p = 0.015 and p= 0.15, respectively). Among analyzed biomarkers, VEGF had the highest sparsity-oriented important learning (SOIL) importance, followed by SRC, sCD40L, IL-10, GRO, MDC, IL-8, IL-9, TNFα, MIP-1α.

Discussion: Our results provide convincing evidence that serum anti-SBSN antibodies is one of the predictive markers for the development of NPSLE. The presence of autoantibodies in the serum is not thought to reliably predict the development of NPSLE. It has been suggested that additional 'hits' such as excessive stress or underlying infections may cause temporary destruction of the BBB, thereby promoting brain damage caused by serum-derived autoantibodies. In the condition that BBB is damaged, serum anti-SBSN antibodies may pass through the BBB to enter the brain and induce pathogenesis. Additionally, leukocytes secrete pro-inflammatory cytokines infiltrate the central nervous system, promoting B cells survival and local antibodies production. In other words, we believe that anti-SBSN antibodies in serum, not alone but in combination with multiple biomarkers, can be crucial in discriminating between NPSLE and SLE. Based on these results, the significance of anti-SBSN antibodies in serum and CSF as biomarkers is currently different and should be considered separately.

Among studied biomarkers, the ten highest SOIL variable importance biomarkers are VEGF, SRC, sCD40L, IL-10, GRO, MDC, IL-8, IL-9, TNF-α, MIP-1α, appeared in order.

One of the drawbacks of our study is that we were not able to produce a complete predictive model for NPSLE because we had available only small sample size. Instead, we chose to generate data to understand variable importance of the biomarkers to avoid the over-fitting model problem and to have reliable information that can be included in prediction of NPSLE. The variable volume gives researchers and clinicians a comprehensive understanding of the possible roles of serum biomarkers in predicting NPSLE other than trusting a single predictive model. Our findings are helpful for further research by providing a ranking of biomarkers that can be used as a reference for variable selection for the predictive model. In the clinical setting, they may assist clinicians to change or replace biomarkers for prediction and prognosis purposes in response to clinical considerations. On the other hand, the role of cytokines/chemokines and anti-SBSN antibodies appears to be inconsistent in relation to the heterogeneous manifestations of NPSLE. A larger patient group is required to establish a reliable predictive model for NPSLE, particularly in determining the relationship between specific clinical manifestations and biomarkers, and in term of predicting the progress of the disease.