BTK Overexpression Is Associated with the Risk of Lymphoma in Primary Sjögren’s Syndrome: Data from Whole Blood Transcriptome of 346 Patients Followed-up Prospectively for 10 Years

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Background/Purpose: To identify a molecular signature associated with lymphomagenesis in primary Sjögren’s Syndrome (pSS).

Methods: Whole peripheral blood samples were collected from 346 well-phenotyped pSS patients enrolled in the ASSESS prospective cohort. A transcriptomic analysis was performed using Clariom S Human Arrays (Affymetrix). Patients with a pSS-related Non-Hodgkin lymphoma (NHL) (historic or incident NHL) were compared with 3 control populations: i) Patients without lymphoma and without any risk factor, among 9 validated predictive factors of developing lymphoma (systemic complications, parotid swelling, purpura, lymphocytopenia, CD4/CD8≤0.8, rheumatoid factors, cryoglobulinemia, monoclonal component and low C4 levels) (this comparator limits the risk of including patients who might develop lymphoma later during follow-up); ii)
Patients without lymphoma but with moderate or high systemic disease activity at enrolment (ESSDAI≥5) (this comparator allows to identify relevant predictors in an at-risk population); iii) All patients without lymphoma. We focused on genes that were significantly differentially expressed in patients with lymphomas compared to each of these control populations.

**Results:** At enrolment, 13 patients had a history of lymphoma. During the 10-year follow-up, 9 patients developed an incident lymphoma. A total of 324 pSS patients had no lymphoma, including 110 patients with an ESSDAI≥5 and 61 patients without any risk factor of lymphoma. Gene Set Enrichment Analysis (GSEA) identified an over-expression of B-cell activation related genes in NHL-pSS, such as BAFF (p=0.007), APRIL (p=0.0009), BCMA (p=0.02) or BTK (p=0.0003) compared to patients without any risk factor of NHL. APRIL and BCMA were significantly up regulated when NHL-pSS patients were compared to all patients without NHL (p=0.002, p=0.04, respectively) but not BAFF (p=0.1). However, the gene expression profile of NHL-pSS patients compared to patients with an ESSDAI ≥ 5 did not show significant over expression of BAFF (p=0.3), APRIL (p=0.2) and BCMA (p=0.2). Conversely, the Bruton Tyrosine Kinase (BTK) gene was over expressed at enrolment, before the occurrence of lymphoma in patients with an incident NHL, in patients with a history of NHL, and in all NHL-pSS patients (either history of or incident) when compared to patients without any risk factor of developing lymphoma (p=0.006, p=0.005 and p=0.0003, respectively), to patients without lymphoma but with an ESSDAI ≥ 5 (p=0.03, p=0.02, p=0.003, respectively) and to all patients without lymphoma (p=0.02, p=0.01, p=0.0008 respectively).

**Conclusion:** BTK, a pivotal transducer of B-cell receptor, is over expressed in the peripheral blood of pSS patients before the occurrence of lymphoma. Conversely to BAFF, APRIL, and BCMA, BTK over expression is not related to a higher disease activity since BTK is up regulated in patients with lymphoma even when compared to patients with a moderate or high systemic disease activity and no lymphoma. BTK might therefore represent a pivotal pathogenic player in the transition from B-cell polyclonal activation to a monoclonal malignant proliferation. The present results suggest that BTK might represent a therapeutic target of interest in pSS.

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