

Expression of APRIL in Diffuse Large B Cell Lymphomas from Patients with Systemic Lupus Erythematosus and Rheumatoid Arthritis

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ABSTRACT. *Objective.* Patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) have an increased risk of diffuse large B cell lymphoma (DLBCL). The cytokine A Proliferating-Inducing Ligand (APRIL) is strongly expressed in DLBCL in the general population and is detected in high concentrations in sera from subgroups of patients with RA and SLE. To investigate a possible association between APRIL and DLBCL in RA and SLE, we examined APRIL expression in lymphoma biopsies from patients with RA and SLE and from DLBCL patients without inflammatory disease.

Methods. Lymphoma tissue from 95 RA, 12 SLE, and 63 comparator DLBCL cases were stained with anti-APRIL antibodies (Aprily-2). The percentage of positively stained cells of the comparator cases were divided into quartiles (1–4, where 4 = most stained) and compared with the results for the RA and SLE lymphomas. APRIL expression was correlated to clinical variables.

Results. The odds ratio for high expression of APRIL (quartiles 3 and 4) was elevated in the SLE DLBCL (OR 23.6, 95% CI 2.4–231.2), but not in the RA DLBCL (OR 0.8, 95% CI 0.3–2.0). RA patients in quartile 4 had higher cumulated RA disease activity than those in quartile 1 ($p = 0.013$). Epstein-Barr virus in the lymphoma tissue was associated with high APRIL expression ($p = 0.009$).

Conclusion. The high expression of APRIL in DLBCL in SLE and in an RA subset might indicate an association between APRIL and lymphoma in these subsets of rheumatic diseases, but could also reflect a dysregulation of APRIL per se in these patient groups. (J Rheumatol First Release July 1 2011; doi:10.3899/jrheum.101190)

Key Indexing Terms:

SYSTEMIC LUPUS ERYTHEMATOSUS
MALIGNANT LYMPHOMA
A PROLIFERATING-INDUCING LIGAND

RHEUMATOID ARTHRITIS
DIFFUSE LARGE B CELL LYMPHOMA
APRIL
EPSTEIN-BARR VIRUS

Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are both associated with an increased risk of lymphoma^{1,2,3,4,5,6}, and in both diseases a predominance of the aggressive subtype diffuse large B cell lymphoma (DLBCL) has been reported^{7,8}. Knowledge about mechanisms behind

the association of chronic inflammatory rheumatic diseases and lymphoma remains limited. Different treatments used for rheumatic diseases have been proposed to contribute to development of lymphoma, but despite a large number of studies there are no consistent data in favor of this connection. By contrast there is increasing support for an association between factors related to the underlying rheumatic disease and the development of lymphoma. From previous studies we have reported that inflammatory activity per se is strongly associated with risk of lymphoma in RA^{9,10}, whereas in SLE associations with certain clinical manifestations (e.g., hematological, pulmonary, sicca symptoms, and glandular swellings) have been observed^{8,11}.

RA and SLE are both characterized by a chronic activation of the immune system with autoantibody production indicating B cell activation. The driving mechanisms of B cell activation in these 2 diseases have not been clarified. In this context, 2 cytokines of the tumor necrosis factor (TNF) ligand superfamily, a proliferation-inducing ligand (APRIL) and B cell-activating factor (BAFF, also known as B lymphocyte stimulator, BLyS or TALL1), are of profound interest as both cytokines are essential for B cell survival and development. Elevated serum levels of APRIL and BAFF have been observed in patients with RA and SLE^{12,13,14}, and local pro-

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duction of these cytokines has been demonstrated in synovial fluid of inflamed joints¹⁵ and in synovial biopsies¹⁶. In the context of lymphoma development APRIL is of particular interest, as this molecule was first described in connection with tumors and its ability to potentiate growth of malignant neoplastic cells¹⁷. In a non-Hodgkin lymphoma (NHL) study, APRIL expression was preferentially high in the aggressive DLBCL subtype, but not in lower-grade NHL, and a correlation was found between high expression of APRIL and decreased patient survival in the DLBCL patients¹⁸.

The aim of our study was to pursue the observations of elevated levels of APRIL in sera of patients with RA and SLE, and to investigate a potential association between APRIL and lymphoma in RA and SLE. From studies on lymphomas in RA¹⁰ and SLE patients⁸ we have access to a large collection of unique data on lymphoma specimens as well as detailed clinical information about the patients' rheumatic diseases and lymphomas. We analyzed APRIL expression in DLBCL tissue from the RA and SLE patients and used DLBCL patients without chronic inflammatory disease as comparator cases. Further, we correlated the results to clinical data and presence of Epstein-Barr virus (EBV) in the lymphoma tissue.

MATERIALS AND METHODS

Selection of DLBCL lymphoma cases. DLBCL in RA. We combined RA discharge diagnoses (ICD-7: 722; ICD-8: 712.38-9; ICD-9: 714A-C, W) in the Swedish Hospital Discharge Register, January 1964 to December 1994, with diagnosis of incident malignant lymphoma (ICD-7: 200-2) reported to the Swedish Cancer Register, January 1964 to December 1995; 424 RA-lymphoma patients were identified. The RA diagnosis was verified by scrutinizing medical records and the lymphoma diagnosis was verified by reviewing and reclassifying the lymphoma tissues according to the 2001 WHO classification¹⁹. After exclusions due to medical records missing, incorrect RA diagnosis according to the American College of Rheumatology (ACR) 1987 RA classification criteria²⁰, and missing/poor quality of lymphoma tissues, 343 reclassified cases of RA-lymphoma were left, of whom 165 were DLBCL. Of these DLBCL specimens, there was enough lymphoma tissue left for tissue microarray (TMA) construction in 111 patients.

DLBCL in SLE. A similar linked register study (Hospital Discharge Register-Cancer Register, 1964-1995) of SLE (ICD-7: 456.20; ICD-8: 734.10; ICD-9: 710A) and cancer risk yielded a national cohort of SLE patients who had developed malignancy²¹. A subsequent case-control study of risk factors for development of NHL in SLE identified 42 SLE-NHL cases in this cohort⁸. SLE diagnosis was verified from medical records using the ACR criteria for SLE²² and the lymphoma tissues were reviewed and classified according to the 2001 WHO classification¹⁹. Medical records from all 42 patients were reviewed, and after exclusions, mostly due to not fulfilling ≥ 4 ACR criteria, 16 SLE-NHL cases (10 DLBCL) remained. Another 3 (2 DLBCL) of the initial 42 cases in this study did not strictly fulfill ≥ 4 SLE ACR criteria, but as a clinical SLE diagnosis was obvious, they were also included in the study. Of the original cohort of SLE patients with malignancy 8 cases were reported to the cancer register as Hodgkin's lymphoma. They were also reviewed and one additional case was reclassified as DLBCL and was included in the study. In total, the study comprised 13 SLE-DLBCL cases, 12 with lymphoma tissue in TMA blocks and one with whole sections from paraffin-embedded lymphoma tissues.

Apart from DLBCL, lymphoma tissues from other lymphoma subtypes among the patients in the RA and SLE cohorts were also included in the TMA blocks.

DLBCL in the general population. We identified 85 cases of DLBCL, diag-

nosed between 1984 and 2002, from the files of the Department of Pathology, Uppsala University Hospital, Uppsala. These cases had previously been included in a study of DLBCL subtypes²³. Of these, 74 cases had specimens remaining that were suitable for TMA construction. From their medical records it was established that none of them had any concomitant inflammatory, autoimmune, or rheumatic disease. Even if the comparison cases were not identified from the Hospital Discharge Register, a majority had been hospitalized before the diagnosis of lymphoma.

Clinical information. Detailed clinical information about all the patients had previously been collected from medical records^{8,10,23}. Information about the rheumatic diseases covered the period from onset of the disease until diagnosis of lymphoma. In RA, disease activity levels were scored based on swollen and tender joint counts, erythrocyte sedimentation rate, and the treating physician's global assessment of disease activity. Cumulative disease activity was assessed as the duration (months) of absence of low, medium, or high disease activity from the onset of RA until lymphoma diagnosis. Values were calculated as the area under the curve (AUC), as described¹⁰. Treatment was defined as 4 or more consecutive weeks receiving a specific drug during the entire RA/SLE disease course. All patients were followed up for survival until death or September 15, 2009.

DLBCL subtyping and Epstein-Barr virus testing. All DLBCL in the study were subject to DLBCL subtyping into germinal center (GC)-like and non-GC-like subtypes with immunohistochemical staining for CD-10, Bcl-6, and MUM1/IR-4 according to the model of Hans, *et al*²⁴. Presence of EBV had previously been analyzed in the RA and SLE DLBCL^{8,10} using *in situ* hybridization for EBV-related RNA (EBER)²⁵.

Tissue microarray blocks. TMA blocks were constructed from the formalin-fixed paraffin-embedded lymphoma tissues. Morphologically representative areas were carefully chosen and core biopsies of 0.5 μm diameter were punched out, 2 for each lymphoma specimen, and were transferred to microarray paraffin blocks.

Immunohistochemistry. A Ventana XT module (Ventana Medical Systems, Tucson, AZ, USA) was used for the immunohistochemical staining procedure. Briefly, paraffin-embedded TMA sections (4 μm thick) were deparaffinized and were subjected to heat-induced antigen retrieval programs. A primary antibody directed against the c-terminal TNF homology domain of APRIL (clone Aprily-2; Enzo Life Sciences AG, Lausen, Switzerland) was incubated 32 min at 37°C. Immunoreactivity was visualized with the Iview DAB detection kit (Ventana) and counterstained with hematoxylin blueing reagent kit (Ventana). The Aprily-2 antibody detects secreted APRIL.

Two investigators (BL, CS) blinded for all clinical data, independently estimated the staining results using a conventional light microscope. The number of stained cells in each specimen was counted in 2 ways. In the first, the number of stained cells among 100 cells in one representative area was counted. In the second, an ocular with 25 lattice squares was used. From 2 different representative parts of the lymphoma specimen, of each 25 squares, the number of stained/not stained cells in the 50 squares was counted. The final value was the mean percentage of stained cells out of the total counted cells from the 2 investigators.

APRIL staining in other lymphoma subtypes than DLBCL was not investigated in detail, but it was noted if APRIL was detectable or not.

Additional immunohistochemistry was performed to identify the APRIL-positive cell types.

Statistics. Statistical Package for the Social Sciences (SPSS) was used for all statistical analyses. Staining results (percentage Aprily-2 stained cells) of the DLBCL of the general population (comparator cases) were divided in quartiles and compared with results of the RA and SLE cases using the Kruskal-Wallis test. Quartile 1-2 is referred to as "APRIL low," quartile 3-4 as "APRIL high." The Mann-Whitney test was performed for calculating differences in clinical characteristics between groups, and Kaplan-Meier and Cox regression methods for survival analysis. Survival was measured from date of lymphoma diagnosis according to the Cancer Register until the date of death. Odds ratio for APRIL expression was measured by logistic regression and adjusted for sex, age at lymphoma diagnosis (categorized as < 60, 60-75, >

75 years), calendar year of lymphoma diagnosis, DLBCL subtype, and lymphoma stage at diagnosis.

The study was approved by the local ethics committees.

RESULTS

Clinical characteristics. APRIL expression could be evaluated in 95 of 111 RA cases, 12 of 13 SLE cases, and 63 of 74 DLBCL cases without inflammatory disease. Clinical data of the 3 groups are summarized in Table 1. Sex distribution was similar in RA and comparator cases, whereas all SLE patients were females, with a lower mean age at the start of disease than the RA patients, reflecting the typical epidemiology of the 2 diseases. The mean age at lymphoma diagnosis was similar in RA and comparator cases, but was significantly lower among the SLE patients. Only a minority of the SLE and RA patients had been treated with potent immunosuppressive/cytotoxic drugs, but glucocorticoid treatment was common, in particular in the patients with SLE. Lymphoma stage at diagnosis and DLBCL subtype distribution were not significantly different among the 3 groups. Presence of EBV was similar in the SLE and RA lymphoma tissues. SLE patients had the best outcome, but after adjustment for sex and

age at diagnosis of lymphoma, overall survival was similar in the SLE and comparator cases, but remained worse in RA (data not shown).

APRIL expression in DLBC lymphoma tissue. Most of the APRIL-positive cells were dendritic cells and histiocytes staining positively for CD68, S100, or CD35, and only a minority of the APRIL-positive cells was morphologically assessed as tumor cells (Figure 1). The Aprily-2 antibody predominantly stained coarse granular structures in the cytoplasm of the cells.

There was a difference in the expression of APRIL in the DLBCL tissue among patients with SLE and RA and the non-inflammatory cases. The mean percentage of cells expressing APRIL was significantly higher in the SLE patients (21%) than in the RA patients (11%) and the comparator cases (10%). Similarly, dividing the percentage APRIL-positive cells into quartiles based on the distribution among the comparator cases (quartile 4 including the cases with the highest percentage of stained cells), the quartile distribution was significantly different from the comparator cases in patients with SLE ($p = 0.007$) but not in those with RA ($p = 0.14$). A major

Table 1. Clinical data of patients with systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) complicated by diffuse large B cell lymphoma (DLBCL) and comparator cases with DLBCL but no inflammatory disease. Information about Ann Arbor stage, extranodal involvement, and DLBCL subtype was available in 90 of the RA patients and 61, 58, and 60 of the comparator cases, respectively. Epstein-Barr virus (EBV) was analyzed in 91 of the RA patients.

Characteristic	SLE, n = 12	RA, n = 95	Comparator Cases, n = 63
No. female (%) / no. male	12 (100) / 0	53 (56) / 42	31 (49) / 32
Age, mean (SD) yrs	45.8 (14.6)	50.8 (13.7)	—
Age			
Age, mean (SD) yrs at diagnosis of lymphoma	60 (15.0)	71 (9.6)	70 (13.0)
Treatment for SLE/RA [†]			
Ever (%)			—
Immunosuppressive drug	4* (33)	17** (18)	
Oral glucocorticoids	11 (92)	41 (45)	
At lymphoma diagnosis (%)			
Immunosuppressive drug	0	10*** (11)	
Oral glucocorticoids	10 (83)	20 (22)	
Lymphoma stage, Ann Arbor (%)			
I	—	13/90 (14)	16/61 (26)
II	2 (17)	15 (17)	11 (18)
III	4 (33)	17 (19)	10 (16)
IV	6 (50)	45 (50)	24 (39)
Extranodal involvement	6 (50)	59/90 (66)	27/58 (47)
EBV ^{††}	1 (8)	10/91 (11)	Not assessed
DLBCL subtype [#]			
GC	2 (17)	26/90 (29)	26/60 (43)
Non-GC	10 (83)	64 (71)	34 (57)
Overall survival from diagnosis of lymphoma, mean yrs (range)	8.4 (0–26)	2.0 (0–22)	5.1 (0–18)

[†] Defined as regular treatment ≥ 4 weeks; * AZA (n = 4); ** AZA (n = 6), POD (n = 6), CYC (n = 4), MTX (n = 3), CHL (n = 1); 3 patients > 1 immunosuppressive drug. *** POD (n = 5), MTX (n = 3), AZA (n = 1), CHL (n = 1). ^{††} Analyzed by EBV-related RNA *in situ* hybridization in lymphoma tissue. [#] According to the model by Hans, *et al*²⁴. AZA: azathioprine; POD: podophyllotoxin; CYC: cyclophosphamide; MTX: methotrexate; CHL: chlorambucil; GC: germinal cell.

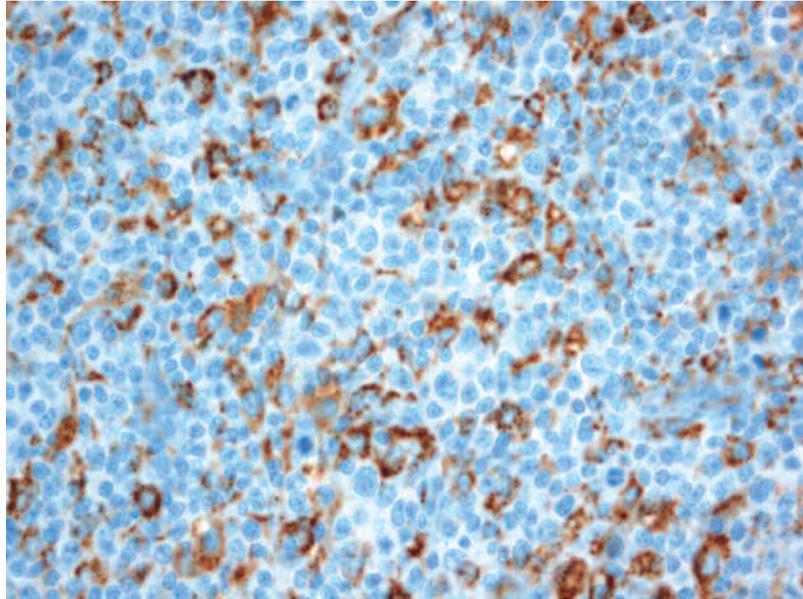


Figure 1. DLBCL tumor lesion from a patient with SLE stained with Aprily-2 showing high APRIL expression. Most of the APRIL-positive cells were dendritic cells and histiocytes staining positively for CD68, S100, or CD35, and only a minority of the APRIL-positive cells were morphologically assessed as tumor cells.

ity (75%) of the SLE-DLBCL were strongly positive for APRIL (quartile 4) compared to 29% of the RA-DLBCL (Table 2).

To account for differences in patient characteristics, the odds ratio for high APRIL expression (quartile 3-4) was analyzed and adjusted for sex, age at lymphoma diagnosis, calendar year of lymphoma diagnosis, DLBCL subtype, and lymphoma stage at diagnosis. The unadjusted and adjusted odds ratios for high APRIL expression were increased in SLE (adjusted OR 23.6, 95% CI 2.4–231.2) but not in RA (adjusted OR 0.8, 95% CI 0.3–2.0) compared with the comparator cases (Table 2).

APRIL expression in other lymphoma subtype tissues. Next to

DLBCL the most common lymphoma subtypes in the TMA were follicular lymphoma (n = 20) and small lymphocytic lymphoma/chronic lymphocytic leukemia (n = 11). There was a striking difference between the DLBCL cases and the lymphomas of these subtypes as APRIL-positive cells were non-existent or almost so in the follicular and small lymphocytic lymphomas.

APRIL expression in relation to characteristics of the rheumatic disease and the lymphoma. In RA, there was a correlation between APRIL expression in the lymphoma tissue and severity of RA measured as area under the curve of cumulative disease activity from onset of RA until lymphoma diagnosis. The RA patients with the highest percentage of APRIL-

Table 2. Quartile distribution of APRIL-positive cells in DLBCL tissue in SLE, RA, and comparator cases based on results in the comparator group. Odds ratio (OR) for APRIL high (quartile 3 and 4) expression in DLBCL tissue in SLE and RA patients, unadjusted and adjusted for sex, age at and calendar year for lymphoma diagnosis, DLBCL subtype, and lymphoma stage at diagnosis with comparator cases as reference.

	Stained Cells			
	Quartile 1, 0–5%	Quartile 2, 6–10%	Quartile 3, 11–15%	Quartile 4, ≥ 16%
Comparator cases, n = 63 (%)	19 (30)	13 (21)	16 (25)	15 (24)
SLE, n = 12 (%)	0	1 (8)	2 (17)	9 (75)
RA, n = 95 (%)	37 (39)	19 (20)	11 (12)	28 (29)
Unadjusted OR (95% CI), Quartiles 3 and 4				
Comparator cases	1			
SLE	11.4 (1.4–93.3)			
RA	0.7 (0.4–1.4)			
Adjusted OR (95% CI), Quartiles 3 and 4				
Comparator cases	1			
SLE	23.6 (2.4–231.2)			
RA	0.8 (0.3–2.0)			

positive cells (quartile 4) had significantly higher cumulated disease activity than those with the lowest number of stained cells (quartile 1) ($p = 0.013$). We found no correlation between APRIL expression and any clinical manifestations of SLE that were associated with increased lymphoma risk in our previous study of NHL in SLE (hematological, pulmonary, sicca symptoms, and glandular swellings)⁸. Overall, there was no correlation between the treatment in RA and SLE patients and APRIL expression.

EBV positivity was associated with higher APRIL expression ($p = 0.009$). Of the 11 cases positive for EBV (one SLE and 10 RA cases), 7 (64%) were those with the highest APRIL expression (quartile 4). Patients with EBV-positive lymphomas did not differ from those with EBV-negative lymphomas regarding treatment with glucocorticoids or cytotoxic drugs.

Neither the stage (extent of dissemination) of the lymphoma disease at diagnosis (Ann Arbor stage III and IV) nor the prognostically unfavorable non-GC DLBCL subtype was associated with higher APRIL expression.

APRIL expression and survival. There was no difference in survival between patients with high or low APRIL expression among the RA patients or in the comparator group (Figure 2A, 2B). Survival in relation to APRIL expression could not be analyzed in the SLE group because almost all SLE patients had APRIL high expression.

DISCUSSION

Patients with RA and SLE share an increased risk of developing lymphoma, and this risk is mainly due to excess of DLBCL^{7,8}. In our study of lymphoma tissue from patients with RA or SLE or with no inflammatory disease we found that expression of the B cell-stimulating factor APRIL was strongly associated with DLBCL, whereas it was hardly detected in other lymphoma subtypes (follicular lymphoma and chronic lymphocytic leukemia). Second, there was a differential expression in the patient population investigated. Higher APRIL expression was detected in lymphoma tissue from SLE patients and a subset of RA patients with high cumulative RA disease activity, compared with DLBCL patients without inflammatory disease or RA patients with low to moderate disease activity. Further, EBV positivity correlated strongly with high APRIL expression. These observations indicate that APRIL could play a role in the development of distinct subsets of lymphoma and in lymphoma development in subgroups of patients with SLE and RA.

The most striking finding was the consistent expression of APRIL in the DLBCL from all SLE patients, while the APRIL-staining results were more complex for the RA-DLBCL patients, with a subset of patients with almost no presence of APRIL in the lymphoma tissue. An association with the clinical features was evident in both SLE and RA patients. Almost all the SLE patients had systemic manifestations at the time of diagnosis of lymphoma, possibly as a

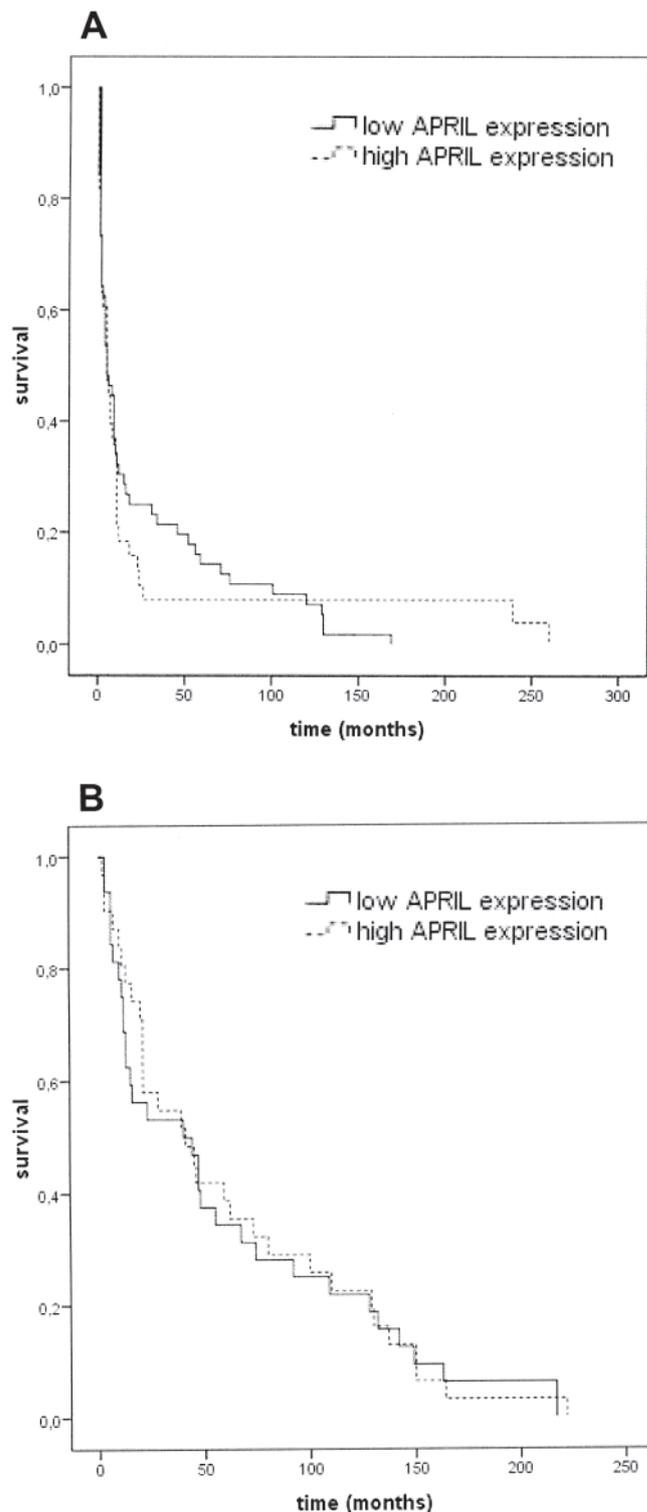


Figure 2. A. Survival in DLBCL patients with RA, divided by high and low APRIL expression in the DLBCL tissue. B. Survival in DLBCL patients without inflammatory disease, divided by high and low APRIL expression in the DLBCL tissue.

result of B cell activation, that motivated therapy with oral glucocorticoids⁸. However, hampered by the low numbers of

each separate manifestation we could not establish any statistical correlation between APRIL and a specific clinical or laboratory SLE manifestation. RA patients with the highest percentage of APRIL-positive cells had a high level of cumulated RA disease activity, that is, clinical and laboratory signs of ongoing inflammation, that could also indicate high B cell activity.

Earlier studies of APRIL levels in serum or as local expression in different organs in patients with RA or SLE have been contradictory concerning associations to clinical variables. Thus one study reported no correlation between serum levels of APRIL and the SLE Disease Activity Index²⁶, while other studies described elevated intrathecal levels of APRIL and BAFF in neuropsychiatric SLE²⁷ and correlations between musculoskeletal SLE manifestations and elevated serum levels of APRIL¹⁴. In general, serum levels of APRIL seem to vary between patients and there are discrepancies between tissue expression and serum levels²⁸.

The presence of APRIL in lymphoma tissue is consistent with the current concept that inflammatory host-cell reactions are an important part in tumor development. In our study secreted APRIL was analyzed, but Schwaller, *et al*¹⁸ showed that the main cellular source of APRIL in DLBCL tissue is infiltrating inflammatory cells, mainly neutrophils and to some extent histiocytes or mesenchymal cells, and that the expression of secreted APRIL correlates with the presence of APRIL-producing cells in the tissue.

It can only be speculated that a defective immune system with dysregulation of APRIL/BAFF pathways as described in RA and SLE also could have implications for host-cell reactions in an emerging lymphoma in these patients. The exact functional role of APRIL in tumors and possibly in DLBCL remains unclear, but it has been observed that endogenous BAFF and APRIL on NHL B cells may allow these cells to escape apoptosis, and in the same study addition of neutralizing BAFF/APRIL receptors decreased NHL B cell survival²⁹.

Novel biologic therapy neutralizing BAFF or APRIL is currently of interest for clinical trials in relapsed and refractory B cell lymphomas, as well as for treatment in RA and SLE^{30,31,32,33}. Experience from such therapy may show whether RA and SLE patients in risk groups for development of DLBCL may benefit in particular from this treatment and if such therapy may influence the future risks of lymphoma in these patient groups.

Interestingly, and not previously reported, we found a strong association between APRIL expression and presence of EBV in the lymphoma tissue in the RA and SLE patients. EBV, a B-lymphotropic herpes virus, is associated with the development of B cell lymphoproliferative disorders, in particular in posttransplant patients, where EBV positivity in the tumor tissue has been reported in up to 80% of cases³⁴. A link between EBV and APRIL has been reported from cell-line studies showing that EBV triggers B cells to upregulate APRIL as well as BAFF, which in turn contributes to the per-

sistence of EBV-infected B cells³⁵. Case reports have linked EBV to lymphomas in patients with autoimmune diseases treated with immunosuppressive drugs, and with spontaneous lymphoma regression once the immunosuppressive therapy is withdrawn³⁶. However, population-based studies support that EBV-related lymphomas likely constitute only a small fraction of all RA- and SLE-associated lymphomas^{10,37}. We found no association with treatment in our study, and none of the EBV-positive lymphomas regressed spontaneously.

We could not confirm a decreased overall survival in patients with high expression of APRIL in DLBCL, as reported by Schwaller, *et al*¹⁸. It can be speculated that the discrepancies in results may reflect differences in the categorization of APRIL values and differences in the study populations. On the other hand, in accord with the study by Schwaller, *et al*, we found no association between high APRIL expression and known prognostically unfavorable factors like disseminated lymphoma at diagnosis and the non-GC DLBCL subtype, and we could also confirm their findings of high expression of APRIL in DLBCL but not in lower-grade lymphomas.

The detailed clinical information of our study made it possible to correct for differences between the patient groups. The RA and SLE cases were from population-based RA and SLE cohorts, and the study comprised a long observation time, with a followup of up to 45 years. A limitation is that the patients may not be fully representative of the rheumatic disease patient of today, considering that the treatment options for rheumatic and for malignant disease to date are more numerous and more effective in controlling the inflammation and immune response. Inclusion of patients who at some point had been hospitalized with RA or SLE may also influence the generalizability of the results to all RA and SLE patients, but these aspects do not affect our major observations of an association between APRIL expression and DLBCL in SLE and RA patients. The rather low number of SLE-DLBCL cases is explained by the relative rarity of SLE. Validated methods to quantify APRIL expression are lacking and there are no set definitions for high or low April-2 stainings. To circumvent this problem all slides were evaluated and scored independently by 2 observers, with concordant results in almost all cases, and a mean value of the readings was used.

APRIL expression was highest in the DLBCL of the SLE patients and in the RA patients with high cumulated disease activity. This observation might indicate that APRIL has a role in the development of DLBCL in these patient groups. Our findings could possibly also reflect the APRIL dysregulation per se seen in these diseases. The mechanistic role of APRIL in development of tumors including DLBCL remains to a large extent unclear and needs further investigations.

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