Presence of serum antinuclear antibodies correlating unfavorable overall survival in patients with chronic lymphocytic leukemia

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Abstract

Background: Serum antinuclear antibodies (ANAs) are positive in some patients with chronic lymphocytic leukemia (CLL), but the prognostic value of ANAs remains unknown. The aim of this study was to evaluate the role of ANAs as a prognostic factor in CLL.

Methods: This study retrospectively analyzed clinical data from 216 newly diagnosed CLL subjects with ANAs test from 2007 to 2017. Multivariate Cox regression analyses were used to screen the independent prognostic factors related to time to first treatment (TTFT), progression free survival (PFS) and overall survival (OS). Receiver operator characteristic curves and area under the curve (AUC) were utilized to assess the predictive accuracy of ANAs together with other independent factors for OS.

Results: The incidence of ANAs abnormality at diagnosis was 13.9%. ANAs positivity and TP53 disruption were independent prognostic indicators for OS. The AUC of positive ANAs together with TP53 disruption was 0.766 (95% confidence interval [CI]: 0.657–0.826), which was significantly larger than that of either TP53 disruption (AUC: 0.706, 95% CI: 0.634–0.772, P = 0.034) or positive ANAs (AUC: 0.595, 95% CI: 0.320–0.668, P < 0.001) in OS prediction. Besides, serum positive ANAs as one additional parameter to CLL-international prognostic index (IPI) obtained superior AUCs in predicting CLL OS than CLL-IPI alone.

Conclusion: This study identified ANAs as an independent prognostic factor for CLL, and further investigations are needed to validate this finding.

Keywords: Chronic lymphocytic leukemia; Antinuclear antibody; Autoimmunity; Prognosis; Overall survival

Introduction

Chronic lymphocytic leukemia (CLL) is characterized by progressive accumulation of small, mature lymphocytes in the peripheral blood, bone marrow, and lymphoid tissues. Although considered to be rare in East Asia (CLL incidence in predominately Han Chinese was reported to be 0.05/100,000 per year),[1] increasing number of CLL cases has been identified in recent years. The clinical course of patients with CLL is highly heterogeneous, with some patients dying within rather short time, while others can even have a normal lifespan without any therapy. Therefore, investigators have made a great effort to elucidate the heterogeneity of CLL, and have found many prognostic factors to predict the outcome of this disorder. These prognostic factors include serum markers like beta-2 microglobulin (β2-MG), genetic markers including immunoglobulin heavy chain variable region (IGHV) and TP53 mutational status, cytogenetic abnormalities such as del(13q), del(11q), and del(17p), as well as CD38 and ZAP-70 (ξ chain associated protein kinase 70) expression level.[2] More recently, use of next-generation sequencing has identified novel gene (eg, NOTCH1, SF3B1, EGR2, NFκBIE, and FBXW7) mutations,[3-6] which might be associated with aggressive clinical course and reduced survival.

Patients with CLL frequently present with immune disturbances. Common autoimmune diseases (AIDs) in CLL include autoimmune cytopenia such as autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), pure red cell aplasia (PRCA) and autoimmune granulocytopenia (AIG),[7-10] and non-hematological AIDs such as paraneoplastic pemphigus, neuropathies, Sjögren syndrome (SS), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), etc.[11-13] The presence of AIHA was previously demonstrated to be a poor prognostic indicator,[8,14] and our center also proved the negative survival impact of positive direct antiglobulin test on CLL patients.[15] But the role of other non-hematological AIDs in CLL for prognosis is unclear.
Antinuclear antibodies (ANAs) are directed against antigens of the cell nucleus. These autoantigens are named after their biochemical characteristics (deoxyribonucleic acid [DNA], histones, ribonucleoproteins [RNP]), the disease associated with the corresponding autoantibody (Sjögren syndrome antigen A [SS-A, also known as Ro] and SS-B [also known as La]; polymyositis, progressive systemic sclerosis [PM-Scl]) or occasionally after the patient in whom the corresponding antibody was first detected (Sm, Ro, La).\(^{[16,17]}\) ANAs can be used to diagnose different rheumatic diseases and judge disease activity, and are probably related to the pathogenesis of AIDs, such as anti-dsDNA and anti-Sm antibodies in SLE, rheumatoid factor (RF) in RA, etc.\(^{[18-21]}\)

Recently, it was found that serum ANAs were positive in some CLL patients, but the prognostic value of ANAs remains unknown. This study aimed to evaluate the role of ANAs as a prognostic factor in CLL.

**Methods**

**Ethical approval**

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital. Informed written consent was obtained from all patients prior to their enrolment in this study.

**Subjects**

Two hundred and sixteen consecutive CLL patients were retrospectively enrolled from May 2007 to December 2017 at Department of Hematology, the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital. Baseline characteristics including gender, age, Binet stage, absolute lymphocyte count (ALC), hemoglobin (Hb), platelet (PLT), lactate dehydrogenase (LDH), albumin (ALB), β2-MG, and AIDs history were collected. Patients’ survival data were further investigated to explore the prognostic value of ANAs. Diagnosis of CLL was based on criteria of the International Workshop on CLL-National Cancer Institute (IWCLL-NCI).\(^{[22]}\) All the samples were collected prior to treatment.

Overall survival (OS) was defined as time from diagnosis until death. Time to first treatment (TTFT) was calculated as time from diagnosis to first treatment. Progression free survival (PFS) was referred to time from diagnosis until disease progression (PD). Patients without a documented event (for OS was death; for TTFT was start of CLL treatment; for PFS was disease progression) were censored at the date of last observation or February 26, 2018.

**Cytogenetics**

Fluorescence in situ hybridization (FISH) analysis was performed on most subjects to detect del(11q22.3) and del (17p13) using standard protocols as previously described.\(^{[23]}\) The cut-off levels for positive values (mean of normal control ± 3 standard deviation [SD]), determined from samples of eight cytogenetically normal persons, was 7.7% and 5.2% for del(11q22.3) and del (17p13), respectively.

**Detection of ZAP-70 and CD38**

Flow cytometry was used to detect ZAP-70 and CD38 expression on fresh samples of CLL cells as previously described.\(^{[24]}\) Data acquisition and analysis were performed using a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA) and Cell Quest software (BD Biosciences). Cut-off points of 30% and 20% were used to define positivity for CD38 and ZAP70, respectively.

**Analyses of immunoglobulin heavy chain variable gene and TP53 mutations**

IGHV sequencing was performed as described before,\(^{[25]}\) and unmutated-IGHV (U-IGHV) was defined as ≥98% germline homology. Genomic DNA isolation and direct Sanger sequencing was performed for exon 4 to 9 of TP53 as stated before.\(^{[26]}\) This study referred the cohort with TP53 mutation and/or del(17p13) as TP53 disruptions.

**Antinuclear antibody testing and antinuclear antibody profile (IgG) testing**

ANAs in patients’ sera were tested after diagnosis and before any treatment by indirect immunofluorescence test (IIFT), the gold standard for the determination of ANAs, with human epithelial (HEp-2) cells as substrate (EUROMUN, Lubeck, Germany). In this study, only patients with ANA titers of ≥1:100 were considered to be positive. Both of the sensitivity and specificity of the ANAs test are 100%, compared with enzyme-linked immuno sorbent assay (ELISA) and immunoblotting methods reported by the manufacturer. ANA profiles: anticientromere antibody (ACMA), anti-proliferating cell nuclear antigen (PCNA) antibody, nRNP/Sm, 5m, PO, SS-A, Ro-52, SS-B, Scl-70, Jo-1, PM-Scl, centromere protein-B (CENP-B), nucleosome, histones, anti-mitochondrial antibody (AMA)-M2, and ds-DNA (IgG) were further tested in ANAs positive patients using the EUROBLOT technology (EUROIMUN). All the tests were performed in the Laboratory of Rheumatology, the First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, with the recommended protocol by the manufacturer.

**Statistical analysis**

Categorical variables were compared using Chi-square test or Fisher exact test. Survival curves were plotted using Kaplan-Meier method and log-rank test was used for comparisons. Univariate and multivariate analyses were performed by Cox proportional-hazard regression, and hazards ratios (HR) were also calculated. Binary Logistic regression, receiver-operator characteristic (ROC) curve and corresponding area under the curve (AUC) were constructed to assess the predictive accuracy of positive ANAs together with other independent factors for OS or CLL-international prognostic index (IPI),\(^{[27]}\) and the differences in AUCs were tested by a nonparametric
The induction treatment included: (1) FC(M)R (fludarabine, cyclophosphamide, mitoxantrone, rituximab; n = 32, 25.2%); (2) F/F/C/FM (fludarabine-containing chemotherapy; n = 13, 10.2%); (3) bendamustine (n = 17, 13.4%); (4) bendamustine plus rituximab (n = 1, 0.8%); (5) chlorambucil and rituximab (n = 3, 2.4%); (6) chlorambucil (n = 22, 17.3%); (7) CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) or cyclophosphamide single drug (n = 4, 3.1%); (8) CHOP or EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) plus rituximab (n = 3, 2.4%); (9) rituximab-fresh frozen plasma (n = 17, 13.4%); (10) ibrutinib (n = 5, 3.9%); (11) other regimens and supportive care (n = 10, 7.9%). We defined the above therapies numbered (1)–(4), (7), (8) and (10) as intensive treatments, and the remaining regimens as less intensive treatments. These strategies were chosen due to patients’ age, performance status, and cytogenetics abnormalities.

Prognostic value of ANA in CLL

During the follow-up, 45 (20.8%) patients developed PD, and 36 (16.7%) patients passed away due to disease-related death. In all of CLL patients, median TTFT was 35 months (range: 0–110 months), median PFS was 76 months (range: 0–110 months), and median OS was not reached (range: 0–115 months). The 5-year PFS rate was 67.1%, and 5-year OS rate was 73.7%. Ten of deaths (27.8%) owned positive ANAs, suggesting the potential prognostic value of ANAs.

We further studied the prognostic impact of ANAs on TTFT, PFS and OS. As demonstrated in Figure 1, the median TTFT for patients with positive ANAs was significantly shorter than those with negative ANAs (13 vs. 40 months, P = 0.049, Figure 1A). A similar result was also indicated as to OS with 54 months for ANAs positive group, while the median OS was not reached for ANAs negative group (54 months vs. not reached, P = 0.017, Figure 1C). However, ANA status showed no impact on PFS (56 vs. 75 months, P = 0.988, Figure 1B).

Next, we performed the univariate and multivariate analyses by Cox proportional-hazard regression to detect the independent prognostic factors related to TTFT, PFS, and OS of CLL. We included the classical factors like age, gender, Binet stages, ALC, Hb, PLT, del(11q22.3), TP53 disruption status, IGHV mutational status, immunophenotyping markers (CD38 and ZAP-70), serological examinations (LDH, albumin, and β2-MG) as well as ANAs test [Table 2]. In univariate analyses, Binet stage B/C, ALC≥50×10^9/L, Hb<100g/L, PLT<100×10^12/L, LDH>ULN, β2-MG>3.5mg/L, TP53 disruption, and unmutated-IGHV were significantly associated with shorter TTFT. Subsequently, these 8 parameters were included in multivariate Cox regression analyses. Finally, Binet stage B/C (HR: 2.190, 95% confidence interval [CI]: 1.415–3.389, P < 0.001), along with ALC≥50×10^9/L (HR: 2.254, 95% CI: 1.434–3.540, P < 0.001) and β2-MG>3.5mg/L (HR: 1.818, 95% CI: 1.220–2.709, P = 0.003) were independent predictors of shorter TTFT. As to PFS, Binet stage B/C, Hb<100g/L, PLT<100×10^12/L, LDH>ULN, β2-MG>3.5mg/L, TP53 disruption, and...
Table 1: Clinical characteristics of CLL patients with or without positive ANAs.

| Characteristics | Total (n=216) | Patients with positive ANAs (n=30) | Patients with negative ANAs (n=186) | \( \chi^2 \) values | P     |
|-----------------|---------------|-----------------------------------|-------------------------------------|----------------------|-------|
| Gender          |               |                                   |                                     |                      |       |
| Male            | 141           | 19 (63.3)                         | 122 (65.6)                          | 0.058                | 0.810 |
| Female          | 75            | 11 (36.7)                         | 64 (34.4)                           |                      |       |
| Age             |               |                                   |                                     |                      |       |
| \( \leq 65 \) years | 133          | 17 (56.7)                         | 116 (62.4)                          | 0.355                | 0.552 |
| >65 years       | 83            | 13 (43.3)                         | 70 (37.6)                           |                      |       |
| Rai Stage       |               |                                   |                                     | 2.087                | 0.352 |
| 0 (Low risk)    | 42            | 6 (20.0)                          | 36 (19.3)                           |                      |       |
| I–II (Intermediate risk) | 110 | 12 (40.0)                         | 98 (52.7)                           |                      |       |
| III–IV (High risk) | 64          | 12 (40.0)                         | 52 (28.0)                           |                      |       |
| Binet Stage     |               |                                   |                                     | 1.277                | 0.259 |
| A               | 85            | 9 (30.0)                          | 76 (40.9)                           |                      |       |
| B/C             | 131           | 21 (70.0)                         | 110 (59.1)                          |                      |       |
| CLL-IPI         |               |                                   |                                     | 2.854                | 0.091 |
| 0 (Low risk)    | 42            | 6 (20.0)                          | 36 (19.3)                           |                      |       |
| I–II (Intermediate risk) | 110 | 12 (40.0)                         | 98 (52.7)                           |                      |       |
| III–IV (High risk) | 64          | 12 (40.0)                         | 52 (28.0)                           |                      |       |
| ALC             |               |                                   |                                     |                      |       |
| <50 \times 10^9/L | 176         | 25 (83.3)                         | 151 (81.2)                          | 0.079                | 0.778 |
| \( \geq 50 \times 10^9/L \) | 40          | 5 (16.7)                          | 35 (18.8)                           |                      |       |
| Hb              |               |                                   |                                     | 3.878                | 0.091 |
| <100 g/L        | 32            | 8 (26.7)                          | 24 (12.9)                           |                      |       |
| \( \geq 100 \) g/L | 184        | 22 (73.3)                         | 162 (87.1)                          |                      |       |
| PLT             |               |                                   |                                     | 0.025                | 0.875 |
| <100 \times 10^9/L | 48           | 7 (23.3)                          | 41 (22.0)                           |                      |       |
| \( \geq 100 \times 10^9/L \) | 168       | 23 (76.7)                         | 145 (78.0)                          |                      |       |
| LDH\(^*\)      |               |                                   |                                     | 1.338                | 0.247 |
| <ULN            | 175           | 22 (73.3)                         | 153 (82.3)                          |                      |       |
| >ULN            | 41            | 8 (26.7)                          | 33 (17.7)                           |                      |       |
| ALB             |               |                                   |                                     | <0.001               | 0.991 |
| <40 g/L         | 79            | 11 (36.7)                         | 68 (36.6)                           |                      |       |
| \( \geq 40 \) g/L | 137       | 19 (63.3)                         | 118 (63.4)                          |                      |       |
| B2-MG           |               |                                   |                                     | 8.587                | 0.003 |
| \( \leq 3.5 \) mg/L | 116         | 10 (33.3)                         | 106 (62.0)                          |                      |       |
| \( >3.5 \) mg/L | 85           | 20 (66.7)                         | 65 (38.0)                           |                      |       |
| TP53 disruption |               |                                   |                                     | 0.365                | 0.546 |
| Presence        | 35            | 6 (24.0)                          | 29 (18.8)                           |                      |       |
| Absence         | 144           | 19 (76.0)                         | 125 (81.2)                          |                      |       |
| Del (11q22.3)   |               |                                   |                                     | 3.769                | 0.107 |
| Presence        | 24            | 6 (26.1)                          | 18 (11.4)                           |                      |       |
| Absence         | 157           | 17 (73.9)                         | 140 (88.6)                          |                      |       |
| IGHV gene       |               |                                   |                                     | 0.090                | 0.765 |
| Unmutated       | 77            | 11 (45.8)                         | 66 (42.6)                           |                      |       |
| Mutated         | 102           | 13 (54.2)                         | 89 (57.4)                           |                      |       |
| CD38            |               |                                   |                                     | 4.890                | 0.027 |
| <30%            | 145           | 15 (57.7)                         | 130 (77.8)                          |                      |       |
| \( \geq 30% \)  | 48            | 11 (42.3)                         | 37 (22.2)                           |                      |       |
| ZAP-70          |               |                                   |                                     | 0.875                | 0.350 |
| <20%            | 105           | 16 (69.6)                         | 89 (59.3)                           |                      |       |
| \( \geq 20% \)  | 68            | 7 (30.4)                          | 61 (40.7)                           |                      |       |
| Treatments      |               |                                   |                                     | 0.338                | 0.561 |
| Intensive       | 76            | 15 (65.2)                         | 61 (58.7)                           |                      |       |
| Treatments      | 51            | 8 (34.8)                          | 43 (41.3)                           |                      |       |

\(^*\)The ULN of LDH in this study was 271 U/L. The data are shown as n or n (%) ; β2-MG: Beta-2 microglobulin; ALB: Albumin; ALC: Absolute lymphocyte count; ANA: Antinuclear antibody; CLL: Chronic lymphocytic leukemia; Hb: Hemoglobin; IGHV: Immunoglobulin heavy chain variable region; IPI: International Prognostic Index; LDH: Lactate dehydrogenase; PLT: Platelet; ULN: Upper limits of normal.
unmutated-IGHV were strongly associated with inferior PFS in univariate analyses. Hb<100 g/L (HR: 3.618, 95% CI: 1.486–8.812, P=0.005), TP53 disruption (HR: 3.908, 95% CI: 1.730–8.830, P=0.001) and unmutated-IGHV (HR: 3.033, 95% CI: 1.444–6.372, P=0.001) were independent PFS prognostic factors. Further, Hb<100 g/L, β2-MG>3.5 mg/L, TP53 disruption, and positive ANAs were significantly correlated with shorter OS in univariate analyses. While TP53 disruption (HR: 6.483, 95% CI: 3.285–12.801, P<0.001) and positive ANAs (HR: 2.237, 95% CI: 1.058–4.729, P=0.035) showed independent prognostic impact on OS in multivariate analyses. In conclusion, serum ANAs level was independent predictor for OS in CLL, but not for TTFT or PFS.

Binary Logistic regression and ROC curves were consequently constructed to analyze the ability of ANAs and TP53 in predicting OS in CLL patients [Figure 2]. The AUC of positive ANAs together with TP53 disruption was 0.766 (95% CI: 0.697–0.826), which was significantly superior to mere positive ANAs (AUC: 0.595, 95% CI: 0.520–0.668, P<0.001) or TP53 disruption (AUC: 0.706, 95% CI: 0.634–0.772, P=0.034). Therefore, patients with disrupted TP53 as well as positive ANAs might have a worse prognosis than those with either one of these factors.

Positive ANA improves the risk stratification of CLL-IPI

Since CLL is a heterogeneous disease, recently, a large-scale meta-analysis containing data from 3472 patients...
proposed a new international prognostic index for CLL (CLL-IPI) to replace traditional Rai and Binet staging system. Five independent prognostic factors were included in the CLL-IPI: TP53 status, IGHV mutational status, serum β2-MG concentration (≤3.5 vs. >3.5 mg/L), clinical stage (Binet A or Rai 0 vs. Binet B–C or Rai I–IV), and age (≤65 vs. >65 years). Thus, we next used our data of Chinese CLL patients to validate CLL-IPI.

As is shown in Figure 3, the survival curves of TTFT and PFS were significantly distinguished in the 4 subgroups [Figure 3A and 3B], while the curves of OS in low-, intermediate- and high-risk subgroups were not well stratified (low vs. intermediate: P = 0.616; intermediate vs. high: P = 0.221; low vs. high: P = 0.078, Figure 3C). Given that serum ANAs, together with TP53 status were independent prognostic factor for OS in the multivariate analyses, we proposed that combination of positive ANAs and CLL-IPI (which contains TP53 status) might improve the prognostic capacity of CLL-IPI. We thus defined a novel prognostic index (CLL-PI) by the sum of CLL-IPI and one point for the presence of serum ANA. We discovered that positive ANA as 1 additional point to CLL-IPI obtained larger AUCs in OS prediction than CLL-IPI alone, although the difference was not statistically significant (AUC: 0.781 vs. 0.769, P = 0.431) [Figure 4].

Further validation of CLL-PI was conducted by segregating the CLL cohort into four risk grades: low-risk group (CLL-PI score: 0–2), intermediate-risk group (CLL-PI score: 3–5), high-risk group (CLL-PI score: 6–8) and very-high-risk group (CLL-PI score: 9–11). And pairwise comparisons for CLL-PI demonstrated more obvious differences in OS between each two specific groups (low vs. intermediate: P = 0.090, intermediate vs. high: P = 0.039, low vs. high: P = 0.001, high vs. very high: P = 0.063, Figure 5C). While the similar result was not reproducible for TTFT or PFS, due to ANA not being an independent prognostic factor for TTFT [Figure 5A] or PFS [Figure 5B]. Collectively, positive ANA may improve the risk stratification of CLL-IPI in OS prediction, and larger cohort should be included to validate this finding.
Discussion
ANAs have been well-described to be associated with diagnosis and prognosis in many rheumatic diseases, including SLE and RA. To our knowledge, this is the first study providing evidence of ANAs as a strong predictive marker for adverse prognosis in CLL survival. Although the pathophysiologic correlation between positive ANAs and malignancies is not fully elucidated, serum ANAs as a simple and easily measured parameter may be a good candidate for predicting prognosis in future clinical practice of CLL.

CLL patients are often accompanied with autoimmunity. Decades ago, CLL cells were found to produce antibodies (mostly IgM) against natural autoantigens such as nuclear antigens (ssDNA, dsDNA, histone proteins), as well as the Fc of IgG, vimentin, cardiolipin, cytoskeletal components, and RFs. The property of them revealed high degree of multispecificity, binding to a number of different antigens. Besides, Hamblin et al. verified that non-hematological autoantibodies were positive in 21.5% (42/195) CLL patients. More recently, a GIMEMA study containing 3, 150 CLL patients by Barcellini et al. showed that 41% (93/227) of CLL patients had at least 1 positive test for a marker of autoimmunity, including 36 ANA, 25 ACA, 23 RF, 23 anti-TPO/anti-TG, 20 SMA, and 10 miscellaneous (AMA, anti-DNA, anti-PCA), and the incidence of ANA was 15.9%, which is consistent with our results. Barcellini et al. also found that serological autoantibodies and non-hematological AIDs were mostly observed in stage A patients, while hematologic complications (AIHA and ITP) were more easily occurred in patients with advanced disease (B+C stage). However, serum ANAs did not show any preference in early Binet stage in our study. Barcellini et al. suggested different pathogenetic mechanisms underlying hematologic and non-hematological autoimmune phenomena in CLL but did not further study the prognostic value of autoantibodies, especially ANAs.

The origins of ANAs are proposed as follows. Firstly, CLL cells may act as efficient antigen presenting cells inducing a T-cell response that, in turn, induces the subsequent activation of resting normal B cells and the production of polyclonal autoantibodies. Secondly, CLL cells may also secrete inhibitory cytokines that alter immune tolerance, further facilitating the escape of self-reactive clones. Thirdly, in seldom cases, CLL cells may act as effector cells secreting a pathological monoclonal autoantibody.

The prognostic value of positive ANAs in CLL patients may be explained by autoantigens interaction with B cell receptors (BCRs). This was presumed according to the following reasons. First of all, the prognostic importance of the mutational status of IGHV genes indicates that CLL BCRs encounter antigens, which ultimately promote a certain degree of somatic hypermutations, which in turn influences the clinical behavior of the disease. In addition, the expression of quasi-identical ("stereotyped") BCRs among different patients with CLL suggests that a set of common antigens contributed to the stereotypy of the BCR in individual patients and this selective antigenic pressure may have a relevant role in the pathogenesis of the disease. In consistent with above statement, BCRs from IGHV unmutated cells are demonstrated to have low-affinity binding to a broader range of self-antigens, whereas affinity-matured BCRs from IGHV mutated cases have high-affinity binding to restricted, more specific antigens. Last but not least, GEP studies demonstrate that BCR signaling is the key regulatory pathway activated in CLL cells in lymph nodes, the sites of CLL cell proliferation, and migration. The activation of BCR is clarified to begin with antigens binding to the slg of BCR and induce downstream pathways, further inducing CLL cells proliferation and migration. Thus, we inferred that these antinuclear antigens might activate BCR and further stimulate CLL cells in this ligand dependent BCR signaling mechanism, and finally cause poorer OS in patients with positive ANAs. Of course, the pathogenesis of CLL is much more complex, resulting from the variety of underlying genetic lesions, degree of clonal evolution, epigenetic changes, activated signaling pathways and interaction with the microenvironment within lymph nodes or in the bone marrow.

We have now entered into an era of precision medicine, cytogenetics, and molecular biology examinations further deepened our knowledge of disease. Our study was not intended to replace TP53 disruption or CLL-IPI in clinical practice, but serum ANAs function should be well-assessed and managed in CLL patient care.
There were several drawbacks of our study. First, this was a retrospective study with potential selection bias. Second, the follow-up was not sufficient enough due to relatively long clinical course of CLL patients. Third, the sample size of our research was not large enough, and patients’ baseline characteristics were not perfectly matched. However, we did Chi-square test, multivariate survival analyses and ROC curves to minimize these biases. Finally, dynamic changes of ANAs titers were not observed, especially in progressed or relapsed patients. Thus, further studies should include these data.

To conclude, positive ANAs was correlated with worse TTFT and OS in CLL patients and it was an independent prognostic factor for OS. Positive ANAs could improve the prognostic capacity of TP53 disruption or CLL-IPI in OS prediction, which could be easily measured in all diagnostic laboratories. Future rigorous longitudinal investigations with larger samples and longer follow-up periods would allow us to disentangle the role of ANAs as a predictor of inferior prognosis in the CLL population.

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**Conflicts of interest**

None.

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