RESEARCH ARTICLE

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Impact of Anti-nuclear Antibody Seropositivity on Clinicopathological Parameters, Treatment Response, and Survival in Lymphoma Patients

Mahendra Kumar[1], Gaurav Prakash[2]*, Amanjit Bal[3], Anu Kumari[2], Yashwant Kumar[1], Ranjeet Bhardwaj[1], Pankaj Malhotra[2], Ranjana W Minz[1]

Abstract

Purpose: Lymphoproliferative disorders and autoimmune diseases both are interrelated. The high incidence of lymphoma in autoimmune diseases and frequent antinuclear antibody (ANA) positivity in lymphoma patients have been observed. But the impact of ANA positivity on various clinical parameters and responses to therapy has not been elucidated properly. **Methods:** In the present study, 73 treatment-naive lymphoma patients were recruited prospectively and samples were collected at baseline and after completion of therapy for evaluation of ANA. Comparative analysis was performed for various parameters between ANA-positive and ANA-negative groups. **Results:** The prevalence of ANA at baseline was 27% in lymphoma patients which further increased to 35% after chemotherapy. The ANA-positive group had a significantly higher mean age (58±14.7 vs 47±19.9; p=0.01), early stage (77% vs 38%; p=0.02,) and infrequent B-symptoms (25% vs 52%; p=0.03) as compared to ANA-negative group. No significant difference was observed in the response to therapy and survival (both event-free and overall survival). The most frequent ANA pattern was speckled (50%) at baseline, and homogenous (42%) after the therapy. **Conclusion:** ANA is more frequent in lymphoma and increases further after chemotherapy. Higher mean age, early stage, and infrequent B symptoms were found to be significantly more frequent in ANA-positive lymphoma patients; however, only limited evidence supports its role as a prognostic marker or response to therapy. A wider study with appropriate follow-up data and molecular assay could shed light on the immunobiology of ANA production and its more defined clinical utility in lymphoma.

Keywords: Antinuclear antibody- lymphoma- diffuse large B-cell lymphoma- Autoimmune diseases

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Introduction

Association between autoimmune diseases (AD) and malignancy including lymphoma has been described in various studies [1, 2]. Regarding cancers, antinuclear antibodies (ANA) are considered to be an epiphenomenon; however, some evidence suggests that the presence of ANA may represent a part of anti-tumor immunity [3, 4]. There is evidence of the prognostic impact of these autoantibodies on a few types of cancers [3]. In lung and colorectal cancers, the presence of ANA is a good prognostic factor. In contrast to it, in patients with breast cancer, it has been associated with a higher risk of recurrence and metastasis [3]. Regarding lymphoproliferative disorders, ANA are detected at a higher frequency, but, neither the definite role nor the cause of occurrence of these antibodies has been clearly understood in these patients. Researchers have tried to correlate the presence of antinuclear antibodies with various clinical parameters in lymphoma patients as well but the findings have been inconsistent [5, 6]. To study the impact of the presence of ANA in patients with lymphoma the present study examines the correlation of various clinic-pathological features and responses to chemotherapy with the presence of antinuclear antibodies in lymphoma patients.

Materials and Methods

Study design and sample collection

It is a prospective observational study on consecutive patients with malignant lymphoma undergoing treatment at our center. Following approval from Institute Ethical Committee and consent from all the participants (in accordance with Helsinki), we prospectively recruited 73 treatment-naive lymphoma patients in the study. Baseline laboratory and clinical data including demographic details

*[1]Department of Immunopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India. [2]Department of Clinical Hematology and Medical Oncology, Post Graduate Institute of Medical Education and Research, Chandigarh, India. [3]Department of Histopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India. *For Correspondence: drgp04@gmail.com*

were noted down and staged according to the Cotswold modification of the Ann-Arbor Staging System. All the patients received treatment as per the departmental protocols. Bedside treatment modifications were permitted according to the advanced age, comorbidities, and performance status of a patient.

We collected a post-treatment blood sample one month after the completion of the last chemotherapy dose and then followed up all the patients for 6 months. We also enquired about the history of symptoms suggestive of rheumatologic disorders from all participants and asked about any history of joint pain, swelling, morning stiffness, rashes, and dryness of mouth at the time of recruitment and during follow-up. As the later part of the study lies during the COVID-19 pandemic, we excluded the patients who had confirmed SARS-CoV-2 infection or clinical features specific to COVID-19. To compare the incidence of ANA in the healthy population, fifty healthy controls were also evaluated for ANA.

Evaluation for antinuclear antibody (ANA)

The serum was separated soon after the collection of the blood sample and stored at -200 C. ANA-screening has been done through indirect immunofluorescence (IIF) on the Hep2 cell line (NOVA Lite Hep-2 ANA Kits, San Diego). Screening-positive cases were further subjected to immunoblot testing for the characterization of antigen targets (HOB-LIA-ANA, China). ANA was reported as per recent guidelines provided by the "International Consensus on ANA Patterns" [7].

Statistical analysis

The statistical analyses were conducted using STATA9.0 software from Stata-corps. Descriptive statistics were used to present proportions and ratios. Mean with standard deviation, and median with range was used for continuous data, and frequencies were used for categorical data. Fisher Exact test and Mann-Whitney U test were applied to compare the two groups as the majority of the variables were not normally distributed. A P-value of less than 0.05 was considered statistically significant. Survival analysis was done using Kaplan-Meier survival curves and the log-rank test was used to assess predictors of survival.

Results

Enrolled patients had a mean age of 50 years with a male: female ratio of 1.8:1. The distribution of 3 major subtypes of lymphoma in the study cohort was: diffuse large B-cell lymphoma (DLBCL - 54.43%), Hodgkin lymphoma (HL-16.46%), and T-cell non-Hodgkin lymphoma (TNHL - 8.87%). Other minor subtypes follicular lymphoma, small marginal zone lymphoma, mantle cell lymphoma/B-NHL-NOS (not otherwise specified), B cell chronic lymphoproliferative disorder, and primary central nervous system lymphoma constituted 20.24% together. ANAs were found in 27% (20/73) of the patients which was quite higher than the healthy controls at 4% (02/50).

ANA-positive vs ANA-negative group

Comparative evaluations of patients with and without ANA have been discussed in the Table 1. The ANA-positive group had a significantly higher mean age (58±14.7 vs 47±19.9; p=0.01), early stage (77% vs 38%; p=0.02), and infrequent B-symptoms (25% vs 52%; p=0.03) as compared to ANA-negative group (Table 1). There was no significant difference in response to chemoimmunotherapy between ANA positive and negative groups. The patients with ANA-positive status had a lesser frequency of hepatomegaly and bulky disease. However, there was no difference between these two groups concerning splenomegaly, raised lactate dehydrogenase (LDH), primary extranodal disease, low albumin, low vitamin D, event-free-survival (EFS), and overall survival (OS) (Table1 and Figure 1). In the ANA-positive group, 30% (06/20) of patients had one or more rheumatological symptom/s and ANA could be a component of the rheumatological disease. Moreover, 22.6% (12/53) of patients in the ANA-negative group also reported symptoms alluding to rheumatologic illnesses which may be attributed to non-specific lymphoma-related symptoms.

Change in ANA status after anti-lymphoma therapy

Out of the 73 patients recruited in the study, 14 died before the completion of the planned number of chemotherapy courses. Two patients left the treatment in between and shifted to other centers. Of the remaining 57 patients, five refused a follow-up blood testing of ANA. At the time of the study, COVID-19-related restrictions started and hence we could collect post-therapy serum samples from 40 patients.

We observed that the ANA positivity rate increased after chemo-immunotherapy from 27% (20/73) to 35% (14/40). During follow-up, 10 patients' ANA status changed from negative to positive, and two changed from positive to negative (Table 2). No specific characteristics were observed in these patients except relatively higher frequency of advanced-stage (07/10) and B-symptoms (08/10) in patients whose ANA status changed from negative to positive (Table 2). Four out of these 10 patients, also reported the development of rheumatological symptoms like joint pain and rash.

ANA staining pattern: Details of the ANA patterns have been described in Figure 1. The most common pattern before therapy was speckled but post-therapy was homogenous (Figure 2). At baseline, the specific antigen targets in positive cases were dsDNA (02), histone (05), SSA (05), SSB (01), PCNA (01), and Jo-1(01), however, five cases couldn't highlight any antigen-target coated on immunoblot.

Discussion

Autoimmune diseases and lymphoma appear to have a bidirectional relationship as patients with autoimmune diseases have a higher prevalence of lymphoma and ANA positivity rates are more frequently observed in lymphoma patients [1, 2, 4]. AD as a risk factor for the development of lymphoma is relatively better established; yet, the

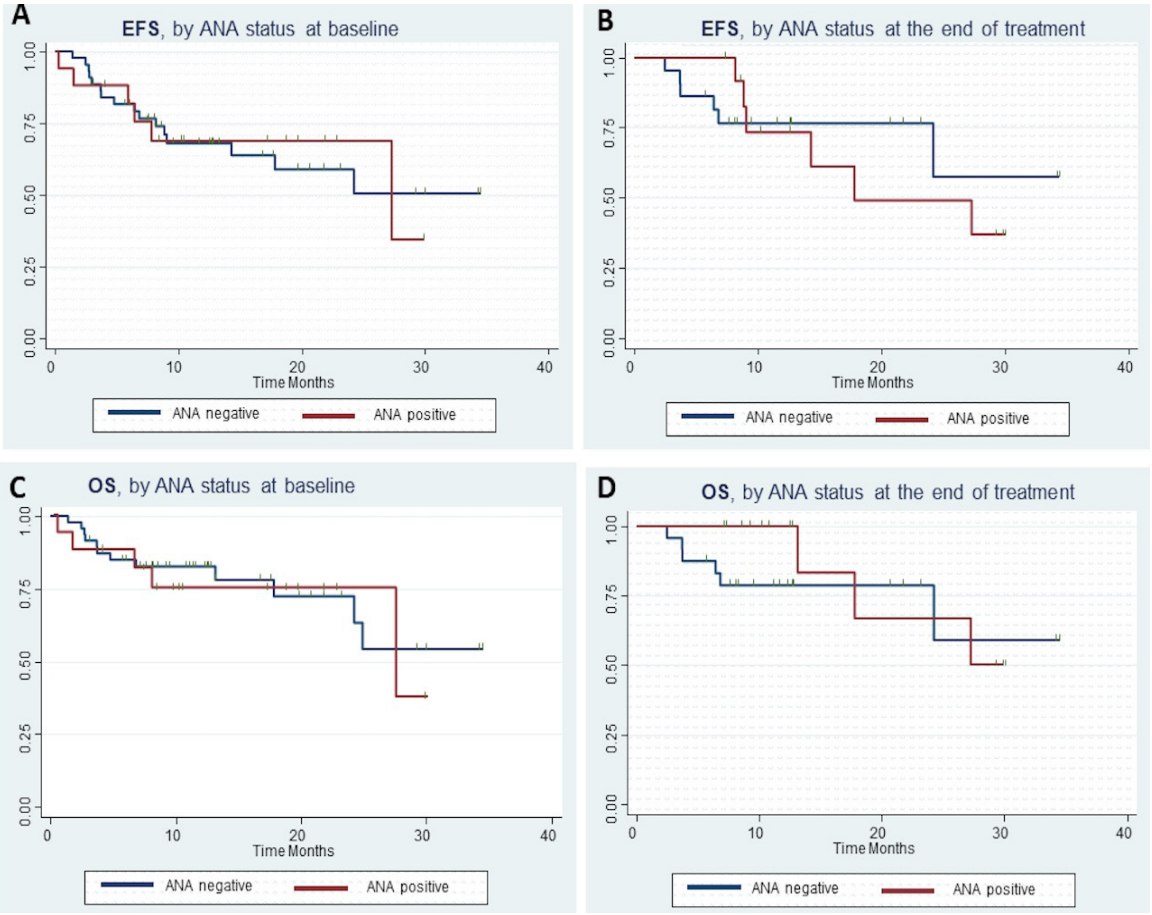

Figure 1. Event-Free Survival (EFS) and Overall Survival (OS) of ANA-Positive and ANA-Negative Groups at Baseline (A & C) and at the End of the Therapy (B & D)

prevalence of AD diseases in patients with lymphoma and their impact on the outcome of this malignancy is relatively less explored [1, 5, 6]. Prevalence of AD in DLBCL patients has ranged from 17.3% to 22.5% as compared to the general population (03-13.8%) [5, 6, 8]. The ANA positivity rate in patients with lymphoma has

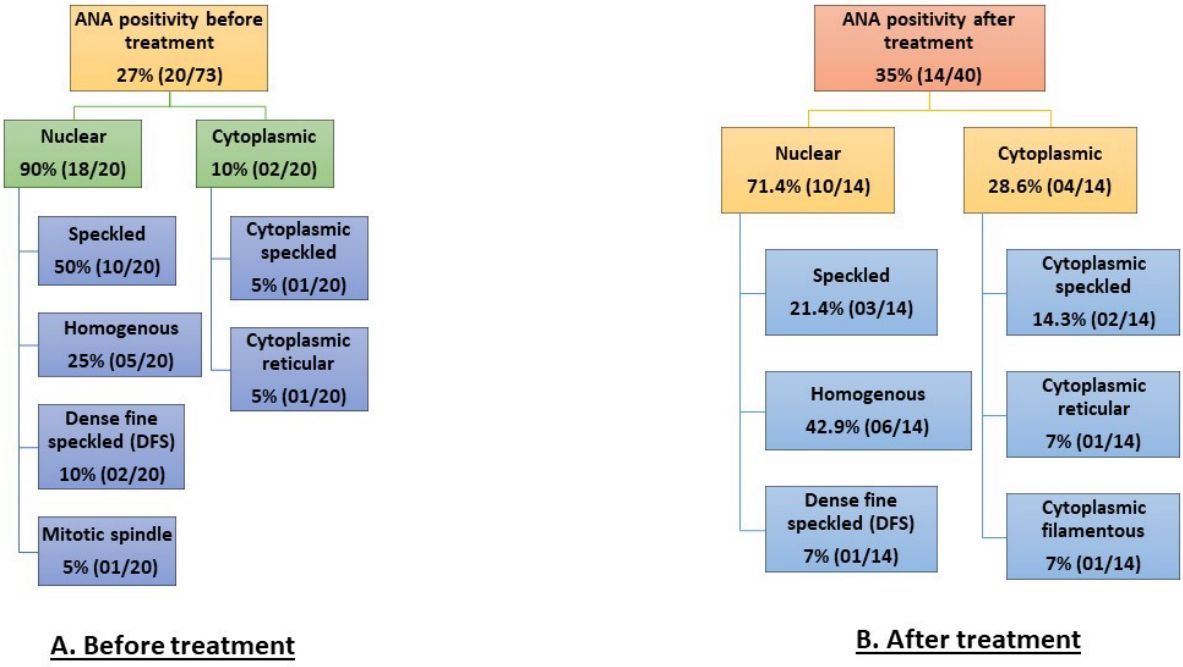

Figure 2. Distribution of ANA Patterns in Lymphoma Patients before (A) and after the Treatment (B). % Calculated out of total positive cases.

Table 1. Comparison of Baseline Characteristics of ANA-Positive and ANA-Negative Group

Features		ANA positive group (20 cases)	ANA negative group (53 cases)	p-value
1	Age Mean	58±14.7	47±19.9	0.01
	Median (IQR)	61 (26-81)	50 (15-80)	
2	Sex (M:F)	12:08	36:17:00	--
3	Hb (gm/dl)	11.9±19	12.5±2.5	NS
4	Total leukocyte count (Median with IQR)	7850 (3300-14200)	7200 (800-45,500)	NS
5	Platelets (in lakhs)	2.46 (2.20-3.45)	2.47 (2.10-2.90)	NS
6.Major Lymphoma groups	Diffuse large B cell Lymphoma (Number)	14	27	NS
	Hodgkin lymphoma (Number)	1	11	NS
	Others	5	15	--
7	B-symptom	5 (25%)	27 (52%)	0.03
8	Extra nodal Site	10 (53%)	26 (53%)	NS
9	Hepatomegaly	2 (10%)	15 (29%)	NS
10	Splenomegaly	4 (20%)	9 (17%)	NS
11. Stage	Early (I+II)	10 (77%)	17 (38%)	0.02
	Advanced (III+IV)	04 (29%)	28 (62%)	
12	Bulky	03 (16%)	10 (21%)	NS
13	Raised Lactate dehydrogenase	11 (61%)	18 (53%)	NS
14	Creatinine	1.1±0.8	0.9± 0.49	NS
15	Primary Extranodal	7 (37%)	14 (27%)	NS
16	Any rheumatological symptom/s	30% (06/20)	22.6% (12/53)	NS
17	Low Vita D (<11ng/ml)	25% (2/8)	38% (8/21)	NS
18	Performance score (PS) 234	7 (35%)	23 (45%)	NS
19	Albumin (< 4 gm)	12 (63%)	22 (46%)	NS
20	Elevated C-reactive protein (>10 mg/L)	57% (4/7)	58% (11/19)	NS
21	Event-free survival (Median)	12.1 (9.3 SD)	13.6 (9.8)	NS
22	Complete remission after therapy	11 (58%)	25 (52%)	NS
23	Duration of illness (months)	6 (1-60)	4 (6.5-48)	NS
24	Relapse	25% (03/12)	28% (10/35)	NS

*NS, Not Significant

Table 2. Cases with Change in ANA Pattern during the Course of Disease/Therapy

SN	Age	sex	Diagnosis	Stage	B-symptoms	Therapy	Pre-treatment sample	Sample after completion of therapy
Cases that turned from ANA negative to positive after the treatment								
1	70	M*	DLBCL Non-germinal center B-cell-like (GCB)	IV BE	Yes	CHOP-Mini#1, CVP1, PEPC#5	Negative	Nuclear speckled
2	62	M	DLBCL Non-GCB	II BEX	Yes	RCHOP	Negative	Nuclear Dense fine speckled
3	50	M	DLBCL Non-GCB	III BX	Yes	RCHOP	Negative	Nuclear Homogenous
4	50	F*	Hodgkin lymphoma	III B	Yes	ABVD	Negative	Cytoplasmic Reticular
5	65	F*	DLBCL Non-GCB	IV BE	Yes	RCVP#1, RCHOP#5	Negative	Nuclear Homogenous
6	45	F	NHL-Not otherwise specified	II BEX	Yes	RCVP#2, RCHOP#4	Negative	Cytoplasmic Speckled
7	38	F	Mantle cell lymphoma	III B	Yes	ABVD#2, AVD#4	Negative	Nuclear Speckled
8	70	F*	Marginal zone lymphoma	IV AE	No	BR	Negative	Nuclear Speckled
9	36	M	NHL PLASMABLASTIC	IV BX	Yes	CHOP+LEN	Negative	Cytoplasmic filamentous
10	31	F	DLBCL Non-GCB	II AX	No	RCHOP	Negative	Nuclear speckled
Cases that turned from ANA positive to negative after the treatment								
1	81	M	DLBCL Primary sinonasal	I AE	No	R-CHOP (MINI)	Nuclear speckled	Negative
2	62	M	Plasmablastic lymphoma	II E	No	LFU	Nuclear speckled	Negative

*Patients developed rheumatological symptoms during the course of therapy; #, Number of cycles of chemotherapy; ABVD, Doxorubicin hydrochloride (Adriamycin), Bleomycin sulfate, Vinblastine sulfate, and Dacarbazine; BR, Bendamustine and Rituximab; CHOP (MINI), Cyclophosphamide, doxorubicin hydrochloride, (hydroxydaunorubicin) Vincristine Sulfate (Oncovin), Prednisolone; CHOP+LEN- CHOP+ Lenalidomide; CVP- Cyclophosphamide, Vincristine, and Prednisone; DLBCL, Diffuse large B-cell lymphoma; GCB, Germinal center B-cell-like LFU- Lost to follow up. PEPC, Prednisolone, Etoposide, Procarbazine, and Cyclophosphamide; RCHOP- Rituximab + CHOP; RCVP- Rituximab, Cyclophosphamide, Vincristine sulfate, and Prednisone

been reported from 4.7% to 21% [9-11]. Most of the earlier reports have some limitations like the random time of recruitment (before/after therapy), retrospective data, and variable definition of AD (based only on clinical features without biological markers) [1, 5, 9, 11]. To overcome these limitations, we conducted a prospective study with two-point sampling and observed an ANA frequency of 27% in newly diagnosed lymphoma cases.

Only a handful of data tried to explore the impact of AD or ANA positivity on lymphoma [5, 10-13]. Most clinical parameters in lymphoma patients didn't exhibit a significant association with ANA positivity; however, some demonstrated it as a marker of poor prognosis and relapse [11-13]. In our cohort, the ANA-positive group has a significantly higher age, early-stage, and infrequent B-symptoms compared to the ANA-negative group (Table 1). Moreover, other clinical and laboratory data couldn't show significant associations; however, parameters related to poor outcomes (splenomegaly, raised LDH, primary extranodal disease, low albumin, low Vitamin D, and event-free-survival) were more frequent in ANA-positive patients. Charlotte Mörth et al. found a significantly increased prevalence of ANA in females, which is already established for AD in general [5]. Regarding the association of ANA with the type of lymphoma, the AABC subtype of DLBCL has a higher tendency for AD and obviously for ANA too [14, 10, 3]. Contrary to this, we noticed ANA positivity in only 33% (03/10) of DLBCL-non-GCB and 36% (13/36) of overall DLBCL, respectively.

The relationship between chemotherapy and autoimmunity in lymphoma is not well investigated. To explore this aspect and trends of ANA-positivity during the course of disease/therapy, we collected paired samples at the end of therapy and interestingly found an increased prevalence of ANA after therapy compared to baseline (27% vs 35%). One prospective study by Bilici et al. documented a mild increase in ANA titers during the course of the disease but they didn't look for the prevalence after the therapy [6]. Autoimmunity induced by chemotherapy and biological agents itself has been reported earlier but no data is available related to R-CHOP/CHOP [15-17]. Chemotherapy has an immunomodulatory effect on the immune system, even cyclophosphamide induces antibody production through the reduction of regulatory cells [17]. Another possible mechanism for autoantibody generation might include a tumor-mediated defect in tolerance, altered protein structure, and production of self-antigen or tumor-associated antigen secondary to cell death with anticancer therapy [18]. Some authors speculated that antibody repository might be a part of anti-tumor immune surveillance; however, others highlighted the role of autoreactive CD5 cells in autoantibody generation [2, 19].

Our study tried to overcome the shortcomings of the previous studies, but we still have some limitations including the low number of paired samples, the lack of quantification of ANA titer, and the evaluation of molecular assay. We could not do a detailed autoimmunity panel to further evaluate the type and severity of AD in our study cohort.

In summary, the presence of ANA is more common in lymphoma and increases further after chemotherapy. There is little evidence supporting its role as a prognostic marker for disease outcomes and response to therapy. A more holistic larger study with adequate follow-up data and molecular assessment might enlighten us regarding the immunobiology of ANA production in lymphoma and its clinical utility.

Author Contribution Statement

All authors contributed equally in this study.

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The study was performed in line with the principles of the Declaration of Helsinki and was approved by the Institute Ethical Committee (INT/IEC/2019/002235).

All authors fulfill the criteria for authorship as per the ICMJE (International Committee of Medical Journal Editors).

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