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Serum Antinuclear Antibodies as Indicator of Relapse in Patients with Non-Hodgkin's Lymphoma

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Abstract

Background: The association of antinuclear antibodies (ANA) has been reported in Non-Hodgkin's lymphoma (NHL). This study was conducted to assess the serum levels of ANA in NHL patients and its relationships with prognosis.

Subjects and methods: This study included 64 patients with NHL and 30 healthy controls. Enzyme-linked immunosorbent assay (ELISA) was performed to determine serum levels of ANA.

Results: There were significant differences in the serum ANA between NHL patients and controls (P<0.001). ANA positivity was detected in 19 NHL patients (29.6%). Positive ANA levels were observed in 17 patients in relapse of 19 (89.4%). No significant association was found between serum ANA levels and various clinicopathological and hematological features. There was a significant association between ANA levels and leukocyte common antigen (LCA) (P=0.010). ROC curve was applied to assess the diagnosis of relapse in NHL with cutoff >0.8 (AUC: 0.876).

Conclusion: Our data argue that positive ANA levels can be useful for diagnosis of relapse in NHL patients.

Keywords: Antinuclear antibody; Non-Hodgkin's lymphoma

Introduction

Non–Hodgkin Lymphoma (NHL) are a group of lymphoproliferative disorders with different biology and prognosis [1,2]. NHL comprises a group of cancers, which have both indolent and aggressive types [3]. In spite of NHL treatment advances, many patients are immune to treatments or relapse.

Antinuclear antibodies (ANA) are antibodies that bind to several nuclear antigens. ANA have been reported in cancers, suggesting that they may have a role in the pathogenesis of malignancy [4,5]. Many studies have reported that NHL patients could display ANA antibodies [6,7]. This study assessed serum levels of ANA in NHL patients and examined its association with hematological, clinicopathological, and immunophenotyping parameters of NHL and assessed whether high ANA titres are related to relapse in NHL.

Subjects and Methods

The present work was carried out on 64 NHL patients aging from 13 to 75 years old and thirty healthy controls were included in the analysis for ANA. NHL patients were selected from the Minia Oncology Center in the period from October 2015 to March 2016. Minia Oncology Center committee gave approval and informed consent, was obtained from all patients in this study.

Laboratory Investigations

Complete blood picture was assessed by Cell Dyn 1700 (USA). Serum levels of creatinine, blood urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), Lactate dehydrogenase (LDH) and uric acid were assessed using ACE automated chemistry Analyzer (Schiaparelli Biosystems. INC; USA).

Measurement of antinuclear antibodies

An ELISA assay was used for the detection of ANA in the serum of all patients and controls (Sunred Biological Technology Co., Ltd, Shanghai). ELISA reader was used at 450 nm for reading. ANA index ≥ 1.2 was considered positive. Detection of ANA by ELISA is used as a method for different diseases [8].

Statistical methods

Data analyses were performed using SPSS program (SPSS–20, Chicago, IL, USA). Data were expressed as mean ± SD. Mann-Whitney test, Chi-square test, ROC curve and the Fisher Exact test were done for analysis of the data. Spearman's and Pearson's correlation coefficient were calculated to determine the relations between the variables. P values ≤ 0.05 was considered significant.

Results

Serum levels of ANA in NHL

Serum ANA was detected in 19 NHL patients, and the mean was 2.07 ± 2.56 (range 0.2-8.5) which was significantly higher than that of controls (P <0.001) (Table 1). A total of 19 patients out of 64 (29.6%) showed a positivity for ANA antibodies (Table 2).

	NHL (n=64)	Control (n=30)	P value
ANA			
Range	(0.2-8.5)	(0.1-0.8)	**<0.001****
Mean ± SD	2.07 ± 2.56	0.41 ± 0.21	
Highly statistically significant differences (P ≤ 0.001) are indicated with asterisks (**). NHL: Non-Hodgkin's Lymphoma; N: Number; ANA: Antinuclear Antibodies.			

Table 1: ANA levels in NHL patients. Mann-Whitney tests for nonparametric quantitative data between the two groups.

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	Descriptive statistics
ANA	(n=64)
-Ve	45 (70.3%)
+Ve	19 (29.7%)
NHL: Non-Hodgkin's Lymphoma; N: Number; ANA: Antinuclear Antibodies.	

Table 2: Prevalence of antinuclear antibodies in 64 NHL patients.

	ANA		P value
	Negative ANA (n=45)	Positive ANA (n=19)	
Treatment outcome			
No treatment	23 (51.1%)	0 (0%)	**<0.001****
Complete remission	8 (17.8%)	1 (5.3%)	**0.26**
Partial remission	9 (20%)	1 (5.3%)	**0.258**
Relapse	5 (11.1%)	17 (89.5%)	**<0.001****
Negative ANA was <1.2; high, ≥ 1.2. Highly statistically significant differences (P<0.001) are indicated with asterisks (**). NHL: Non-Hodgkin's Lymphoma; N: Number; ANA: Antinuclear Antibodies.			

Table 3: Correlation of ANA with relapse in NHL patients.

	ANA	
	r	P value
(1)HB	0.135	0.288
(1)WBCs	-0.038	0.769
(1)PLT	-0.101	0.428
(1)AST	-0.096	0.452
(1)ALT	-0.016	0.898
(1)LDH	-0.159	0.211
(1)Urea	0.085	0.503
(1)Creatinine	-0.095	0.454
(2)Hepatmegaly	-0.045	0.727
(2)Splenomegaly	0.071	0.578
(1) Pearson's correlation, (2) Spearman's rho correlation, r=0. 75-1 (strong correlation), r=0.5-0.74 (moderate correlation), r=0.25-0.49 (fair correlation), r=0.1-0.24 (weak correlation). NHL: Non-Hodgkin's lymphoma; ANA: Antinuclear Antibodies; Hb: Haemoglobin; WBCs: White Blood Cells; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; LDH: Lactate Dehydrogenase.		

Table 4: Correlation of ANA levels with features in NHL patients.

Correlation between ANA levels and relapse in NHL patients

Among ANA antibody-positive patients, Relapse occurred in 17 of 19 patients with a positive ANA level (89.4%) (P <0.001) (Table 3). Complete remission occurred in 1 of 19 (5.2%); 1 patients with partial remission (5.2%). and no antibodies detected in patients with no treatment (P<0.001). The correlation between ANA levels and clinical parameters No significant correlation was found between ANA levels and various clinicopathologic parameters (age, sex, histologic type, hepatomegaly, splenomegaly, or laboratory profile; P>0.05) (Table 4). There was a significant association between ANA and LCA (P=0.010) (Table 5), among 5 patients with positive LCA, positive ANA was detected in 4 of 5 patients (80%). In 59 LCA-negative patients, negative ANA was found in 44 of 59 patients (74.6%) but there was no significant association with other immunophenotyping markers (Table 5).

Diagnostic performance of serum ANA in NHL

For differentiating NHL patients in relapse, ROC curves of ANA were constructed (Figure 1A). The sensitivity of serum ANA levels was 77.27%, and the specificity was 95.24% at a cutoff value of >0.8. The AUC value was 0.876 (P <0.001) (Figure 1B).

In newly diagnosed patients, the sensitivity of serum ANA levels was 86.96%, and the specificity was 53.66% at a cutoff value of ≤ 0.7. The area under the curve for serum ANA was 0.668 (P=0.04) the discriminative cutoff of serum ANA in complete remission was ≤ 0.5, with AUC 0.670. The cutoff of serum ANA in partial remission was ≤ 0.8, with AUC value of 0.659.

Discussion

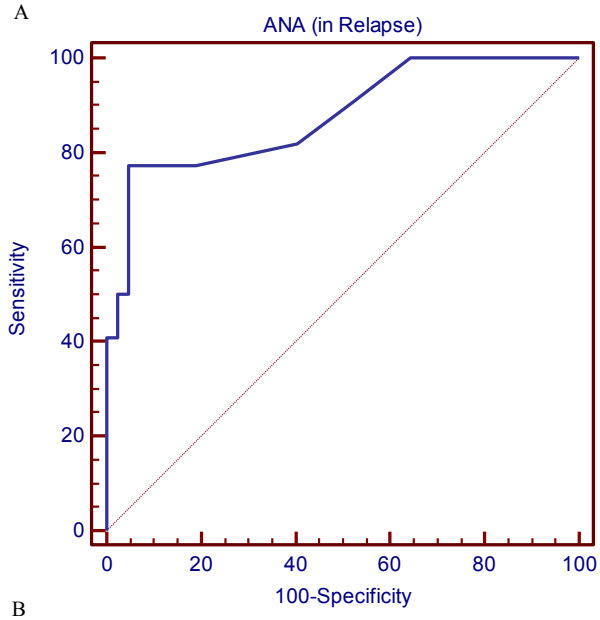
In spite of advances in NHL therapy, many NHL patients are not completely cured with chemotherapy. Thus, use of a biomarker to identify relapse may be able to stratify patients, allowing improved treatment outcome.

Serum antinuclear antibodies have been investigated in NHL. Here, we found a prevalence of ANA (29.6%) in patients with NHL, which confirms data from other studies [9,10]. Other studies reported that ANA positivity was not observed in NHL patients, this may be because of performing the study on only newly diagnosed, untreated NHL patients [11].

Zhou et al. [9] had reported that 30.9% of patients with NHL were positive for ANA antibodies. Billici et al. [10] found that 4.7% of their

	LCA		P value
	(n=59) -Ve	(n=5) +Ve	
ANA			
<1.2 (Negative)	**44 (74.6%)**	**1 (20%)**	**0.010***
≥ 1. 2 (Positive)	**15 (25.4%)**	**4 (80%)**	
Statistically, significant differences (P ≤ 0.05) are indicated with an asterisk (*). NHL: Non-Hodgkin's Lymphoma; N: Number; ANA: Antinuclear Antibodies; LCA: Leukocyte Common Antigen			

Table 5: Association between LCA and ANA in NHL.



VAriable	Optimal Cutoff	AUC	P value	Sensitivity	Specificity	PPV	NPV	Accuracy
ANA	>0.8	**0.876**	**< 0.001****	77.27	95.24	89.5	88.9	89.1

Figure 1: Diagnostic performances of ANA for discriminating NHL patients with relapse (A) ROC curve obtained by plot at different cutoffs for ANA in NHL patients with relapse. (B) The most discriminative cutoff concentration of serum ANA was >0.8 with an AUC value of 0.876. The sensitivity and specificity were 77.27 and 95.24 %, respectively. Highly statistically significant differences (P ≤ 0.001) are indicated with asterisks (**).

patients with NHL were positive for ANA, the cause for that is many patients had diffuse large B-cell lymphoma (DLBCL) at presentation. Altintas et al. [12] evaluated the prevalence of ANA antibodies in patients with NHL, 13.4% of these patients were positive for ANA antibodies. They did not evaluate serial ANA levels in terms of relapse.

ANA positivity was absent in newly diagnosed patients with NHL. This may be explained by antigen binding of overall available antibodies [13]. Complete remission and partial remission occurred less in ANA antibody-positive patients in comparison to ANA antibody-negative patients that may be due to suppression of antibody production by treatment.

In this study, it was demonstrated that positive ANA was associated with relapse in NHL patients. ANA positive patients had the advanced disease in comparison with negative patients, thus ANA may support its use as a poor prognostic marker for monitoring relapse in NHL. One report suggested that median survival was longer in patients who did not demonstrate autoimmune markers than in those who did [14]. Although the reason for ANA-positivity in relapse remains unknown. It is possible that the antibodies are produced by the immune system, in response to a release of tumor-associated antigens. Immunoregulatory disturbances of the immune system, chronic stimulation of lymphocytes and genetic susceptibility increase the likelihood of initiating mutations [15].

In this study, clinical and laboratory data have been analyzed for 19 patients with ANA. There was a lack of significant correlation between ANA positivity and these features, demonstrating the lack of strict correlation between ANA and autoimmune symptoms. Another study reported a prevalence of ANA without clinical manifestation [16], there was a significant association between ANA and LCA as the most cases with negative LCA are negative ANA and most cases +ve LCA, are +ve ANA. High expression of LCA was previously observed in metastasis [17]. This finding confirms our results with the presence of ANA in relapse. According to the data, the serum level of ANA could be a valuable biomarker for diagnosing relapse in NHL. The AUC of ANA for distinguishing NHL with a relapse from all controls was 0.876 and was higher than that of complete remission and partial remission (0.670, 0.659 respectively) with a cutoff value for ANA >0.8. High levels of anti-nuclear antibodies have been found in many human disorders of the immune system, such as systemic lupus erythematosus (SLE) [18]. As hypomethylation at the T and B-lymphocyte-associated gene, loci is a general feature in SLE patients [19,20], loss of DNA methylation at certain loci might strongly associate with these human disorders. Therefore, it is important to study the links between the alteration of epigenetic markers, such as DNA methylation and initiation/occurrence of NHL. In addition, as DNA methyltransferases (DNMTs) and some histone methyltransferases (HMTs) function as the main enzymes that required for the establishment and maintenance of DNA methylation [21-23], it is also essential to investigate the correlation between the mutation/instability of these enzymes and NHL in patients. Follow-up studies of ANA in NHL will help to identify its role in the mechanisms of relapse in NHL.

There is a limitation of the present study. The number of the patients evaluated was relatively small. Some of the insignificant differences in might become significant with a larger number of patients.

Conclusion

Antinuclear antibodies are associated with lymphomas. The finding of a positive ANA in NHL patients strongly suggests the presence of relapse. According to our data, ANA could be used as a marker to discriminate NHL with relapse.

References

1. Maxwell SA, Mousavi-Fard S (2013) Non-Hodgkin's B-cell lymphoma: advances in molecular strategies targeting drug resistance. Exp Biol Med (Maywood) 238: 971-990.
2. Armitage JO, Berg AR, Purtilo DT (1993) Adult non-Hodgkin's lymphoma. In: Bick RL, ed. Hematology: Clinical and Laboratory Practice. St. Louis, MO: Mosby-Year Book, Inc: 875-893.
3. Jaffe ES, Harris NL, Stein H, Vardiman JW (2001) World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues (3rd edn) Lyon: IARC.
4. Li QZ, Karp DR, Quan J, Branch VK, Zhou J, et al. (2011) Risk factors for ANA positivity in healthy persons. Arthritis Res Ther 13: R38.
5. Ma XY, Zhang H, Qu YJ (2010) Autoantibodies and the correlation of malignant tumour research. Ji Lin Yi Xue 31: 4801-4802.
6. Swissa M, Cohen Y, Shoenfeld Y (1992) Autoantibodies in the sera of patients with lymphoma. Leuk Lymphoma 7: 117-122.
7. Timurağaoğlu A, Duman A, Ongüt G, Saka O, Karadoğan I (2000) The significance of autoantibodies in non-Hodgkin's lymphoma. Leuk Lymphoma 40: 119-122.
8. Emlen W, BD, O Neill L (1979) Clinical significance of antinuclear antibodies: comparison of detection with immunofluorescence and enzyme-linked immunosorbent assay. Arthritis Rheum 40: 1612-1618.
9. Zou HY, Gu X, Yu WZ, Wang Z, Jiao M (2015) Detection of serum antinuclear antibodies in lymphoma patients. Genet Mol Res 14: 16546-16552.
10. Bilici A, Yapici HS, Ercan S, Seker M, Ustaalioglu BB, et al. (2012) The prevalence and significance of autoantibodies in patients with non-Hodgkin's lymphoma: are they correlated with clinicopathological features? J BUON 17: 502-507.
11. Uskudar Teke H, Gulbas Z, Bal C (2014) Serum levels of cytokines and prevalence of autoantibodies in lymphoma patients and their prognostic value. J BUON 19: 191-197.
12. Altintas A, Cil T, Pasa S, Danis R, Kilinc I, et al. (2008) Clinical significance of elevated antinuclear antibody test in patients with Hodgkin's and Non-Hodgkin's lymphoma: a single center experience. Minerva Med 99: 7-14.
13. Blomjous FJ, Feltkamp-Vroom TM (1971) Hidden antinuclear antibodies in seronegative systemic lupus erythematosus patients and in NZB and (NZB x NZW) Fl mice. EurJ3 Immunol 1 :396-8.
14. Bairey O, Blickstein D, Monselise Y, Lahav J, Stark P, et al. (2006) Antiphospholipid antibodies may be a new prognostic parameter in aggressive non-Hodgkin's lymphoma. Eur J Haematol 76: 384-391.
15. Ehrenfeld M, Abu-Shakra M, Buskila D, Shoenfeld Y (2001) The dual association between lymphoma and autoimmunity. Blood Cells Mol Dis 27: 750-756.
16. Jardin F, Lévesque H, Tilly H (2005) Auto-immune manifestations in Non-Hodgkin's lymphoma. Rev Med Interne 26: 557-571.
17. Nandedkar MA, Palazzo J, Abbondanzo SL, Lasota J, Miettinen M (1998) CD45 (leukocyte common antigen) immunoreactivity in metastatic undifferentiated and neuroendocrine carcinoma: a potential diagnostic pitfall. Mod Pathol 11: 1204-1210.
18. Hedrich CM, Tsokos GC (2011) Epigenetic mechanisms in systemic lupus erythematosus and other autoimmune diseases. Trends Mol Med 17: 714-724.
19. Renaudineau Y, Youinou P (2011) Epigenetics and autoimmunity, with special emphasis on methylation. Keio J Med 60: 10-16.
20. Zouali M (2011) Epigenetics in lupus. Ann N Y Acad Sci 1217: 154-165.
21. Okano M, Bell DW, Haber DA, Li E (1999) DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell 99: 247-257.
22. Li E, Bestor TH, Jaenisch R (1992) Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. Cell 69: 915-926.
23. Zhang T, Termanis A, Ozkan B, Bao XX, Culley J, et al. (2016) G9a/GLP Complex Maintains Imprinted DNA Methylation in Embryonic Stem Cells. Cell Rep 15: 77-85.