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The diagnostic value of anti-rituximab Ab in Iraqi patient with chronic lymphocytic leukemia

Iqbal Muaafi Abdullah
Microbiology & Immunology Department, College of Medicine, University of Baghdad, Baghdad, Iraq

Yaseen M. Taher
University of Aliraqia, college of medicine, department of medicine, M.B.Ch.B., C.A.B.M.S., F.I.C.M.S.Hemato

Alaa Fadhel Alwan
Prof. FICMS (int.Med)FICMS (clin.hem) Consultant Hematologist The National Center of Hematology AL-Mustansiriyah University

Mohammed Maaruf Al-Ani
Prof. M.Sc.,FICMS/Path-Clinical Immunology College of Medicine, University of Baghdad, Baghdad, Iraq

***Abstract*---**Patients who receive monoclonal antibodies are at risk of producing anti-drug antibodies (mAbs). Rituximab, a chimeric anti-CD20 mAb, is used to treat CD20-positive B-cell malignancies and autoimmune illnesses such chronic lymphocytic leukemia (CLL). Thirty patients were grouped according to the first diagnosis, the staging of the CLL, follow-up examinations to measure their exposure to Rituximab after 3-6 months of treatment, and to the excluding criteria another Thirty healthy volunteers age and gender-matched were utilized as a control group.The mean age between study and control groups was (61.0 ± 10.7 vs. 59.8 ± 9.7 years); Stage II was the most common type of CLL (53.3%) followed by III (35%) and stage IV (10%) in the control group. The male to female ratio was 2:1 in both groups, with no significant differences between the two.There was no significant difference between control and CLL in ARA levels, (0.050 vs. 0.053, p-value = 0.525) at the begging of the study.Median Rituximab antibodies (ARA) did not change significantly from baseline to the end of the study in CLL patients (0.053 to 0.055, p-value = 0.551).

***Keywords*---**CLL, ARA, ANTI CD20 AB, rituximab.

Introduction

Rituximab is a glycosylated immunoglobulin G1-mAb that contains human kappa and IgG1 constant region sequences as well as murine light- and heavy-chain variable region sequences. Rituximab binds to the B-lymphocyte transmembrane protein CD20, which can be found on both healthy and cancerous B cells (excluding stem cells, pro-B cells, and plasma B cells)[1].Rituximab, which is given intravenously and has been used to treat B-cell malignancies since its licensure 20 years ago, has become a standard treatment for follicular lymphoma, diffuse B-cell lymphoma, chronic lymphocytic leukemia, and mantle cell lymphoma [2].Anti Rituximab Antibody (ARA) and immune response (IRs) have been found in SLE and pediatric nephrotic syndrome cases [3,4].The high-titer ARA results show that ARA may have caused the significant IR following the second rituximab infusion. Because the second IR was stronger than the first, it was supposed that ARA had amplified the second IR;The ARA generation during the first chemoimmunotherapy phase was described in retrospective studies as having a high fever and pale erythema; Because ARA is not routinely tested, its prevalence cannot be precisely measured (Kidoguchi et al., 2020).Rituximab is more likely than other mAbs to elicit IR (77%) [5]. Extreme IRs, on the other hand, could be caused in part to ARA-related toxicity [6]. Despite being one of the most effective and widely utilized therapeutic mAbs to date, Rituximab's efficiency is limited by inter-individual heterogeneity and the development of resistance Rituximab resistance has been defined in a variety of ways, but it is most often defined as a lack of response or usable progression within 6 months of starting a rituximab-containing therapy. According to the results of rituximab monotherapy trials, Rituximab tolerance occurs in 30 to 60% of indolent Non Hodgkins Lymphoma NHL-naive patients [7] However, this is likely to exaggerate the strategy's true cumulative occurrence.

Chemo sensitization and other synergistic processes increase responses, lowering the risk of Rituximab resistance [8].Rituximab, as an immunotherapy medication, is heavily reliant on the host to maximize its cytotoxic effect Inhibition of rituximab activity may promote the retention of pathogenic memory B-cells and trigger disease relapse due to the earlier reconstitution of the B-cell compartment; differentiation between non-neutralizing antibodies that do not inhibit the therapeutic effect of a medicinal product and neutralizing anti-drug antibodies has been identified [9]. As a result, there are a variety of potential sources of resistance, including tumor- or host-related origins. Despite widespread expression of the CD20 antigen, different B cell lymphoproliferative neoplasms respond differently to Rituximab treatment. CD20 expression density varies by case; CLL cells have been discovered to express less CD20 on their surface than other B cell lymphomas, which was originally blamed for CLL's poor response to rituximab monotherapy [10].

Subjects and specimens

A prospective study was carried out on three primary groups, ranging in age from 47 to 81 years old. Prior to participating in this analysis, the scientific ethical committee of Medicine College/Baghdad University was consulted, and consent forms was acquired from each participant. Thirty-three adult patients with

chronic lymphocytic leukemia were included in this investigation (CLL). Patients were subjected to follow-up examinations to measure their exposure to Rituximab after 3-6 months of treatment.In the selection of CLL patients, there was an exclusion criteria:patients who have been diagnosed with other type of malignancies AND patient who was diagnosed with CLL of grade A. All patients was selected according Rai staging chart for fulfill the study criteria.

Rai Stage	High levels of lymphocytes	Enlarged lymph nodes	Enlarged spleen or liver	Anemia	Low levels of platelets
0	Yes	No	No	No	No
I	Yes	Yes	No	No	No
II	Yes	Yes or no	Yes	No	No
III	Yes	Yes or no	Yes or no	Yes	No
IV	Yes	Yes or no	Yes or no	Yes or no	Yes

Chart adapted from the *American Society of Hematology, Kay et. al. 2002, vol. 1:193, Table 8*.

A control group of thirty age and gender matched samples with no history of haematological or chronic disease was used. Blood count indices were acquired and calculated using an automated blood count analyzer at the time of sampling. Between October 2018 and October 2019, samples were collected from patients who had recently been diagnosed with CLL by a specialist and were sent to the Department of Oncology in Baghdad Medical City and the National Center for Research and Hematology. A data form was given to each patient. Each patient's sociodemographic information, such as age, sex, and residence, was collected, as well as their complete medical history. All hematological studies that were registered were documented.

Antibody to Rituximab (Rituxan®, Mabthera®) ELISA(ARA)

The Matriks Biotek Antibody to Rituximab (Rituxan®, Mabthera®) Enzyme-Linked Immuno-Sorbent-Assay (ELISA) Kit is used to measure antibodies to rituximab (Rituxan®, Mabthera®) in serum and plasma. Mabthera® is a registered trademark of Roche, Inc., and Rituxan® is a registered trademark of Biogen Idec, Inc.The Matriks Biotek Antibody to Rituximab (Rituxan®, Mabthera®) ELISA is a double antigen sandwich assay for the determination of antibodies against rituximab in serum and plasma samples. The Antibody to Rituximab (Rituxan®, Mabthera®) ELISA from Matriks Biotek is a double antigen sandwich test for detecting antibodies against rituximab in serum and plasma samples, antibodies to rituximab in patient serum and plasma samples are captured by the medication rituximab coated on the microtiter wells' walls. The results are evaluated by a cut-off value which is estimated by multiplying the mean OD450nm of the negative controls by 3. I.e.; If “Sample OD450/the mean OD450 of Negative Controls” is ≥3, the sample is POSITIVE.

Statistical Analysis

GraphPad Prism software, San Diego, California USA, used to analyse data for this study. P value considered when appropriate to be significant if less than 0.05.Data analysis: Anderson darling test was used to determine whether continuous variables have a normal distribution. U test used to analyze the differences in median of two groups (if they do not follow normal distribution). Scatter plot used to present the regression analysis, r (correlation coefficient or standardized beta) used to assess the relationship between different variables. U test used to analyze the differences in median of two groups (if they do not follow normal distribution).

Results

There was no significant difference between control and CLL in ARA levels (0.050 vs. 0.053, p-value = 0.525)

Table 2
Assessment of ARA marker data at baseline

	Control	CLL	p-value
Number	30	30	-
ARA AU/ml *	0.050 (0.044-0.063)	0.053 (0.045-0.065)	0.525
<0.15 pg/dl	30 (100%)	30 (100%)	-
Data presented as median (interquartile range; 25% - 75% percentile)			
ARA:Anti Rituximab Ab			

*Arbitrary Unit

As this figure showed the relationship between ARA and CLL

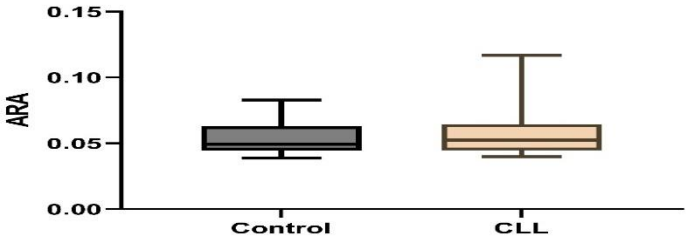


Figure 3. Boxplot of ARA with CLL for patient and control group at baseline

Assessment of change of ARA from baseline to the end of the study in CLL patients

Median Anituximab antibodies (ARA) was not change significantly from baseline to the end of the study in CLL patients (0.053 to 0.055, p-value = 0.551)

Table 3
Assessment of ARA marker for CLL patients

	Baseline	End of the study	p-value
Number	30	30	-
AR	0.053 (0.045-0.065)	0.055 (0.048 – 0.064)	0.551
<0.15 pg/dl	30 (100%)	28 (93.3%)	
≥0.15 pg/dl	0 (0%)	2 (6.7%)	

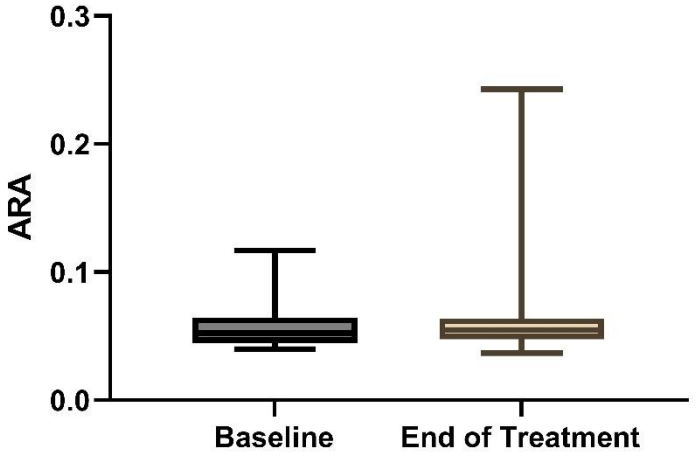


Figure 3. Boxplot of ARA change from baseline to the end of therapy for CLL patients

Assessment of clinical and hematological variables for CLL patients

The most common presentation was spleen involvement (63.3%), followed by lymph node (56.7%) and liver (33.3%); the rest of the hematological parameters are illustrated in table 3.4.

Table 4
Assessment of clinical and hematological variables for CLL patients

	CLL	Normal rang
Number	30	
WBC	89.7 ± 61.7	4.5-11.0 x 10^9 cell/l
ALC	76.7 ± 55.6	10^9 cells/liter
Hemoglobin	11.5 ± 2.4	gr/l
Platelet	172.0 ± 76.4	150 to 400 × 10^3/L
Presentation		
Spleen enlargement	19 (63.3%)	
Liver enlargement	10 (33.3%)	

Lymph node enlargement	17 (56.7%)	

Table 5
Assessment of the relationship between ARA at baseline and the other variables in CLL patients

	ARA at baseline	
	r	p-value
Age	-0.289	0.109
Stage	-0.185	0.327
WBC	0.009	0.962
ALC	0.020	0.914
Hb	0.221	0.240
Platelet	-0.180	0.342

Assessment of change in markers according to disease progression

A total of 9 (30%) patients had relapsed after chemotherapy from 30 CLL patients.

Assessment of change in markers according to disease progression at the end of therapy

There was no significant difference between relapsed patients and those in remission in median levels of ARA, as illustrated in table 6.

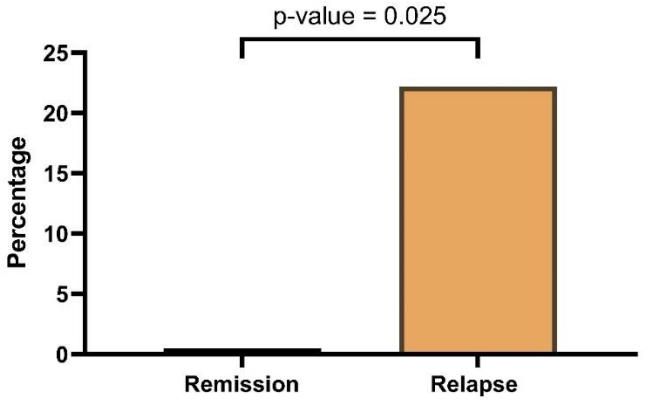
Assessment of biomarkers at the end of therapy in CLL patients according to the outcome of treatment

	Remission	Relapse	p-value
	21	9	-
ARA	0.055 (0.049 – 0.063)	0.055 (0.047 – 0.151)	0.570

Anti-rituximab antibodies (ARA) were significantly higher in relapsed patients compared to those in remission.

Assessment of biomarkers levels at the end of therapy in CLL patients according to the outcome of treatment

	Remission	Relapse	p-value
	21	9	-
ARA	0	2 (22.2%)	0.025



Discussion

Patients who are given Rituximab may develop an anti-rituximab antibody, known as a "human antichimeric antibody." When these patients are treated with different antibodies, they are more likely to develop adverse responses. Immune responses to rituximab have been substantially more common in rheumatoid arthritis patients than in people with other disorders so far. Up to 2% of patients with B-cell malignancies generated human anti-chimeric antibodies during clinical trials [(11)]. In the current study all patients had negative Anti-rituximab antibodies (ARA) at baseline (before the initiation of chemotherapy cycles), and after receiving rituximab only 2 out of 30 CLL patients develop positive ARA (6.7%). Additionally, there was significant association between the development of ARA and risk of relapse (p-value = 0.025).

Several studies examined the development of ARA in many diseases, ARA occurred in 23% of patients treated with rituximab for Membranous Nephropathy and was linked to the need for at least two rounds of rituximab for resistant MN or relapses [(12)]. While in Crohn's disease, it developed in 61% of the patients who were treated with an average of 3.9 infusions (range, 1 to 17) per patient over a 10-month period [(13)]. After two intravenous infusions of infliximab at 3 mg/kg, it developed in 13% of individuals with rheumatoid arthritis; with successive infusions, the incidence of antibody positivity grew to 30% and 44%, respectively (at 3 months and 6 months, respectively)[(14)]. According to other research, roughly 11% of patients examined generated anti-drug antibodies, which is consistent with prior findings [(15)].It had been reported that the incidence of ARA in lymphomas 1 – 2% [(16)].

In Moskalets study of 36 patients with B-chronic lymphocytic leukemia, the incidence of ARA was nil before starting medication and was shown to be positive in 8 (33%) of those who had previously received rituximab [(17)]. Our finding was lower compared to Vladimirovna study 2020, in which they examined the incidence of antibodies to rituximab in patients with CLL, in which 8 patients (33%) had positive antibodies at the end of 4 – 6 cycles RCF protocol, of these cases (6 cases were resistance/ relapsed CLL) [(18)] . The development of innovative,

next-generation anti-CD20 mAbs with alternative modes of action is another major line of study to combat rituximab resistance. Obinutuzumab, a second-generation type II anti-CD20 mAb that was recently approved in combination with bendamustine followed by single-agent maintenance therapy for the treatment of patients with Follicular lymphoma (FL) who do not respond to or whose disease progresses after treatment with rituximab or a rituximab-containing regimen, is an example of this approach. (19).

Conclusion

Anti-rituximab antibodies (ARA) was considerably greater in relapsed patients than those in remission in this investigation.

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