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Research Article

Predictive Biomarkers in Late Onset Neutropenia Associated with Rituximab

Ali Ogul,[1] Semra Paydas,[2] Abdullah Evren Yetisir,[2] Cem Mirili,[3] Mahmut Büyüksimsek[1]

[1]Department of Medical Oncology, Adana City Training and Research Hospital, Adana, Turkey
[2]Department of Medical Oncology, Cukurova University Faculty of Medicine, Balcali, Adana, Turkey
[3]Department of Medical Oncology, Erzurum Ataturk University Faculty of Medicine, Erzurum, Turkey

Abstract

Objectives: The aim of this study is to determine the biomarkers in LON (Late Onset Neutropenia) associated with rituximab using in lymphoproliferative diseases (LPDs).

Methods: : In this retrospective study 22 cases with LPD treated by rituximab containing regimen and followed at least one year in our center were evaluated for LON.

Results: Grade I, II and IV neutropenia were detected in five, five and one cases, respectively. Lowest neutrophil/lymphocyte ratio (NLR) was 1.2 and 1.7 in cases with and without LON, respectively. Neutropenia developed in 8 of 15 cases with Diffuse large B cell lymphoma (DLBCL), 2 of 2 cases with Follicular Lymphoma (FL) and one case of Small lymphocytic lymphoma (SLL). According to prophylactic Granulocyte colony-stimulating factor (G-CSF) using, neutropenia was detected in all cases given prophylaxis while 6 of 17 cases without prophylaxis. Mean rituximab dose was 4870 mg in cases developing neutropenia while it was 4162 mg in cases without neutropenia.

Conclusion: Lower NLR at the end of treatment cycle, G-CSF using with prophylaxis aim, extranodal involvement and more than 4 gr rituximab using were found as the predictive factors for LON associated with rituximab.

Keywords: Lymphoma, neutropenia, rituximab

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Rituximab is a chimeric monoclonal anti CD-20 antibody (Ab) and is used in CD20 positive lymphomas and various autoimmune diseases including rheumatoid arthritis, wegener granulamatosis etc.[1,2] Rituximab is the first Ab approved in lymphoma treatment with better outcome but in spite of good tolerability profile it shows some side effects including infusion reactions, lymphopenia, infections, asthenia, HBV reactivation, progressive leukoencephalopathy, myocardial dysfunction and interstitial pneumonitis.[2,3] Neutropenia is a rare adverse effect in cases treated by rituximab.[3,4] LON (Late Onset Neutropenia) is defined as the neutrophil count less than 1.5x109/L detected after 4 weeks of the last dose of rituximab and also the exclusion of other causes of neutropenia.[5]

LON is a late onset side effect seen between one month and one year and generally reversible and does not cause life-threatening infections.[6,7] Duration of neutropenia has been reported between five and 77 days and incidence is variable between 3% and 27%.[5,8] The most reliable diagnostic criteria in rituximab related LON is the proliferation of T cell large granular lymphocyte (T-LGL) in peripheral blood and bone marrow.[9] T-LGL proliferation causes CD95 activation and neutrophil apoptosis, LON has been found to be related with increased T-LGL in peripheral blood and

Address for correspondence: Ali Ogul, MD. Adana Sehir Egitim ve Arastirma Hastanesi, Onkoloji Klinigi, Adana, Turkey
Phone: +90 552 254 64 85 **E-mail:** mdaliogul@gmail.com

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bone marrow in various studies and also T-LGLs cause fas-fas ligand secretion, and a new repertoire with the binding to neutrophil surface.[10–13] Some risk factors such as autologous stem cell transplantation, HIV related lymphoma, history of previous chemotherapy, purine analogue using, high dose methotrexate have been defined as the causative factors for LON.[5–8]

Methods

This study is retrospectively performed in the medical oncology clinic of Çukurova University in 2019. Twenty two cases with LPD treated with rituximab containing regimen and followed at least one year were included in this study. Diagnosis of LPD according to the WHO classification, ECOG performance status, Ann Arbor staging, extranodal involvement, complete blood count, bone marrow infiltration, ESR, beta 2 microglobulin, IPI scores in DLBCL, total rituximab used during chemotherapy and chemotherapy regimens, leukocyte/neutrophil nadirs, time to neutropenia and G-CSF prophylaxis using were reviewed. Neutropenia was graded according to the NCICTC version 3.0 (National Cancer Institute Common Toxicity Criteria).

Statistical Analysis

All analyses were performed using IBM SPSS Statistics Version 20.0 statistical software package. Categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean and standard deviation and as median and minimum-maximum where appropriate. Chi-square test was used to compare categorical variables between the groups. The normality of distribution for continuous variables was confirmed with the Shapiro Wilk test. For comparison of continuous variables between two groups, the Student's t-test or Mann-Whitney U test was used depending on whether the statistical hypotheses were fulfilled or not. The statistical level

Table 1. Characteristics of patients with and without neutropenia

	With neutropenia (n=11)	Without neutropenia (n=11)	p
Age	53.1 (20–76)±18.7	59.7 (47–82)±11.6	0.746
Gender (Female), n(%)	7 (32)	5 (22)	0.342
Diagnosis, n(%)			0.689
DLBCL*	8 (36)	7 (32)	
EMZL†	0	3 (14)	
FL‡	2 (9)	0	
Mantle cell lymphoma	0	1(5)	
SLL§	1(5)	0	
Stage			0.732
I-II	5 (22)	3 (14)	
III-IV	6 (28)	8 (36)	
Bone marrow bX			0.822
Normo-hipercellular	6 (28)	9 (40)	
Hipocellular-infiltrate	5 (22)	2 (9)	
Beta-2 microglobulin	3.66 (1.98–6.96)±1.43	3.79 (1.67–9.35)±2.38	0.840
Sedimentation	30.3 (2–72)±20.4	34.8 (6–78)±25.3	0.766
ECOG-PS‖			0.074
0–1	10 (45)	5(22)	
2–4	1 (5)	6 (28)	
LDH¶	405 (130–1249)±374.7	240.4 (147–385)±78.7	0.072
Use of G-CSF in chemotherapy			0.035
Yes	5 (22%)	0	
No	6 (28%)	11 (50%)	
Neutrophil lymphocyte ratio	1.23 (0.07–2.03)±1,03	1.7 (0.86–4.33)±0.51	0.023
Total rituximab dose (mg)	4870 (2250–8004)±1400	4162.3 (3168–5400)±699.8	0.088
Time to neutropenia (day)	234 (119–465)±107.5	216 (116–319)±62.3	0.898

*DLBCL: Diffuse large B cell lymphoma, †EMZL: Extranodal marginal zone lymphoma, ‡FL: Follicular Lymphoma, §SLL: Small lymphocytic lymphoma, ‖ECOG-PS: Eastern Cooperative Oncology Group Performance Status, ¶LDH: Lactate dehydrogenase.

of significance for all tests was considered to be 0.05. SPSS referance: IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.

Results

Female/male ratio was 12/10, nine cases were older than 60 years, ECOG performance status was 0–1 in 15 cases, 14 cases had stage III-IV disease. Fifteen cases had DLBCL, 3 had extranodal MZL, 2 had FL, one case had MCL an one case had SLL. Bone marrow biopsy was hypercellular or normocellular in 15 cases and hypocellular or infiltrated in 7 cases. Clinical/laboratory characteristics of the patients have been shown in.

Neutropenia was detected in 11 cases (50%). Neutropenia was detected in 8 of 15 cases with DLBCL and 2 of 2 cases with FL and one case with SLL. Neutropenia was not detected in none of the cases not receiving prophylactic G-CSF (11 cases) while was detected in 5 of 5 cases (100%) receiving prophylactic G-CSF (p=0.035). NLR in first four weeks was 1.23 and 1.7 in cases detected neutropenia and not detected neutropenia (p=0.023). Total rituximab used in treatment period was 4870 in neutropenia developing cases while it was 4162 mg in neutropenia not developing cases (p:0,088). Age, sex, ESR, beta 2 microglobulin, and LDH levels were not different in cases with and without neutropenia. Bone marrow involvement was detected in 5 of 11 cases with neutropenia while this was in 1 of 11 in cases without neutropenia (Table 1).

LDH levels were higher than normal limits in 7 cases with neutropenia while 5 cases in without neutropenia. Among cases with LON grade I, II and IV neutropenia were detected in 5, 5 and 1 cases, respectively. Grade II neutropenia was detected on day 256 (between 119–465 days). Grade IV neutropenia was detected on day 212, neutrophil count in this case was 50 cells/µl and fludarabine had been used in this case. Underlying lymphoma subtype developing LON was DLBCL in 5 cases, FL in one case according to the NCICTC version 3.0. G-CSF was found to be necessary in only one case with grade IV neutropenia. Lowest and highest rituximab dose in cases developing LON was 3978 and 8004 mg, respectively (Table 2).

Discussion

LON related with rituximab is relatively rare but important late onset complication and its incidence is variable. Grade II and higher neutropenia was detected in 7% and 27% of the cases reported by Tesfa[14] and Cattaneo[15] in 113 and 72 cases respectively. Grade III and IV neutropenia have been reported by Lai et al in 13% of the cases with DLBCL.[16] In our small group LON was detected in 4% of the cases with grade III-IV neutropenia while it was 22% if grade II was used as cut off for LON. LON in NHL has been evaluated in two studies and lower clearence in patients with stage III-IV disease and especially in female patients as compared with satge I-II disease in but not in other study.[17–18] Rituximab clearance has been found to be lower in female cases with older than 70 and this has been attributed to tendency to neutropenia in these cases.[18] Our single female case of FL with grade IV LON had stage IV- extranodal disease without bone marrow involvement and had been treated by fludarabine containing regimen which has been found to be predisposing factor in rituximab associated LON (5.8).

Table 2. Characteristics of patients with Late Onset Neutropenia

Patient no	1	2	3	4	5	6
Age/Sex	60/F	66/M	33/M	69/F	20/F	62/F
Diagnosis	DLBCL *	DLBCL	DLBCL	DLBCL	DLBCL	FL
Stage	2	4	2	4	4	3
Received treatment	CHOP-R †	Benda-R‡	CHOP-R	CHOP-R	CHOP-R	FCR
Previous treatment	None	§CVP-R	CHOP-R	None	None	None
Response	CR‖	PR¶	PR	PR	CR	CR
Duration of neutropenia (day)	310	465	137	251	119	212
Grade of neutropenia	Grade 2	Grade 2	Grade 2	Grade 2	Grade 2	Grade 4
Neutrophil count (cells/µl)	1180	1450	1460	1200	1240	50
Need of G-CSF prophylaxi in chemotherapy	Yes	No	No	No	Yes	No
Need of G-CSF in R-LON	No	No	No	No	No	Yes
Bone marrow status (before treatment)	Normocellular	Infiltrate	Normocellular	Infiltrate	Infiltrate	Normocellular
Total rituximab dose (mg)	3978	8004	4500	4500	4459	4200

*DLBCL: Diffuse large B cell lymphoma; †CHOP-R: Cyclophosphamide, doxorubicin, vincristine, prednisone, rituximab; ‡Benda-R: Bendamustine, rituximab; §CVP-R: Cyclophosphamide, vincristine, prednisolone, rituximab; ‖CR: Complet response; ¶PR: Partial response.

Complete response has been found in in our three of four cases with LON and this suggests the higher rituximab plasma level due to lower rituximab clearance in females. It will be interesting to compare the neutrophil levels in females and males and different age groups.

LON development in cases with lower NLR within posttreatment four weeks and the need for G-CSF prophylaxis in all five cases with LON suggest that these two parameters may be important predictive factors for LON. In our analysis higher rituximab using was found in cases with neutropenia (4870 mg vs 4162 mg). LON developed in 465 days in our one case receiving 8004 mg rituximab. However LON is rare after one year of rituximab using Although O'Brien et al.[19] did not find an increased toxicity in CLL cases treated with high doses of rituximab (up to 2250 mg/m^2) cumulative rituximab dose may be related with LON.

LON has been found to be related with age, sex and diagnostic risk factors But these factors have not not been to be predictive.[17,18] In our small study lowest NLR ratio in first four weeks after cytotoxic chemotherapy has been found to be predictive for the development of neutropenia. In addition ≥4 gr total dose of rituximab may be an important predictive for neutropenia.

Limitations

Major limitation of our study is the low number of the cases and restropective nature. Although there were more cases in this period in our unit, we evaluated only the patients followed regularly.

Conclusion

Low NLR ratio in the first four weeks of the treatment of rituximab containing regimen, G-CSF prophylaxis, DLBCL diagnosis, extranodal involvement, purine analog and rituximab using more than 4 gr have been found to be predictive for LON in cases treated by rituximab.

Disclosures

Ethics Committee Approval: This article was approved by the Adana City Education and Research Hospital Clinical Research Ethics Committee dated 18.12.2019 with the ID of 650. Ethical approval was not required based on the law and the national ethical guidelines of our country and written informed consent was not required for individual patient because of retrosprective nature. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained for the image material used. Informed consent forms were obtained from all subjects.

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References

1. Randall KL. Rituximab in autoimmune diseases. Australian prescriber 2016;39:131–34.
2. Moore DC. Drug-Induced Neutropenia: A Focus on Rituximab-Induced Late-Onset Neutropenia. P T 2016;41:765–8.
3. Grillo-López AJ. Rituximab: an insider's historical perspective. Semin Oncol 2000;27:9–16.
4. Vose JM, Link BK, Grossbard ML, Czuczman M, Grillo-Lopez A, Gilman P, et al. Phase II study of rituximab in combination with chop chemotherapy in patients with previously untreated, aggressive non-Hodgkin's lymphoma. J Clin Oncol 2001;19:389–97.
5. Tesfa D, Palmblad J. Late-onset neutropenia following rituximab therapy: incidence, clinical features and possible mechanisms. Expert Rev Hematol 2011;4:619–25.
6. Saikia TK, Menon H, Advani SH. Prolonged neutropenia following anti CD20 therapy in a patient with relapsed follicular non-Hodgkin's lymphoma and corrected with IVIG. Ann Oncol 2001;12:1493–4.
7. Buch MH, Smolen JS, Betteridge N, Breedveld FC, Burmester G, Dörner T, et al; Rituximab Consensus Expert Committee. Updated consensus statement on the use of rituximab in patients with rheumatoid arthritis. Ann Rheum Dis 2011;70:909–20.
8. Wolach O, Bairey O, Lahav M. Late-onset neutropenia after rituximab treatment: case series and comprehensive review of the literature. Medicine (Baltimore) 2010;89:308–18.
9. Semenzato G, Zambello R, Starkebaum G, Oshimi K, Loughran TP Jr. The lymphoproliferative disease of granular lymphocytes: updated criteria for diagnosis. Blood 1997;89:256–60.
10. Liu JH, Wei S, Lamy T, Epling-Burnette PK, Starkebaum G, Djeu JY, et al. Chronic neutropenia mediated by fas ligand. Blood 2000;95:3219–22.
11. Papadaki T, Stamatopoulos K, Stavroyianni N, Paterakis G, Phisphis M, Stefanoudaki-Sofianatou K. Evidence for T-large granular lymphocyte-mediated neutropenia in Rituximab-treated lymphoma patients: report of two cases. Leuk Res 2002;26:597–600.
12. Chaiwatanatorn K, Lee N, Grigg A, Filshie R, Firkin F. Delayed-onset neutropenia associated with rituximab therapy. Br J Haematol 2003;121:913–8.
13. Voog E, Morschhauser F, Solal-Céligny P. Neutropenia in patients treated with rituximab. N Engl J Med 2003;348:2691–4.

14. Tesfa D, Gelius T, Sander B, Kimby E, Fadeel B, Palmblad J, et al. Late-onset neutropenia associated with rituximab therapy: evidence for a maturation arrest at the (pro)myelocyte stage of granulopoiesis. Med Oncol 2008;25:374–9.
15. Cattaneo C, Spedini P, Casari S, Re A, Tucci A, Borlenghi E, et al. Delayed-onset peripheral blood cytopenia after rituximab: frequency and risk factor assessment in a consecutive series of 77 treatments. Leuk Lymphoma 2006;47:1013–7.
16. Lai GG, Lim ST, Tao M, Chan A, Li H, Quek R. Late-onset neutropenia following RCHOP chemotherapy in diffuse large B-cell lymphoma. Am J Hematol 2009;84:414–7.
17. Jäger U, Fridrik M, Zeitlinger M, Heintel D, Hopfinger G, Burgstaller S, et al; Arbeitsgemeinschaft Medikamentöse Tumortherapie (AGMT) Investigators. Rituximab serum concentrations during immuno-chemotherapy of follicular lymphoma correlate with patient gender, bone marrow infiltration and clinical response. Haematologica 2012;97:1431–8.
18. Pfreundschuh M, Müller C, Zeynalova S, Kuhnt E, Wiesen MH, Held G, et al. Suboptimal dosing of rituximab in male and female patients with DLBCL. Blood 2014;123:640–6.
19. O'Brien SM, Kantarjian H, Thomas DA, Giles FJ, Freireich EJ, Cortes J, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol 2001;19:2165–70.