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Original Article

FcgRIIIa 158 V/F polymorphism predicts rituximab-induced late onset neutropenia in newly-diagnosed CD20-positive B-cell lymphoma but is not correlated with long-term survival: A prospective study at a single institution with long-term follow-up

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ABSTRACT

Objective: Rituximab is a commonly used treatment for CD20-positive B-cell lymphoma. Late onset neutropenia (LON) has been identified as a complication associated with rituximab. FcgRIIIa 158 V/F polymorphism has been correlated with LON. We want to explore the relation between FcgRIIIa 158 V/F polymorphism and survival outcome.
Materials and Methods: We examined a cohort of patients with newly diagnosed CD20-positive B-cell lymphoma who were treated with rituximab-based therapy. We identified patients with LON and analyzed their characteristics and survival outcomes. Furthermore, we used a multiplex polymerase chain reaction to detect FcgRIIIa 158 V/F polymorphism and correlated this with LON. After a 10-year follow-up, we analyzed the survival outcomes between groups with different FcgRIIIa polymorphisms.
Results: Seventy-two consecutive patients with CD20-positive B-cell lymphoma patients were examined. Eleven (15.2%) of these patients developed LON. The V/V and V/F polymorphisms were significantly associated with the occurrence of LON ($p = 0.031$), yielding an odds ratio for the development of LON of 1.47 (95% confidence interval, 1.21–1.78). The log-rank test showed no overall survival difference both in the occurrence of LON and different polymorphisms.
Conclusion: The FcgRIIIa polymorphism was significantly associated with development of LON. Furthermore, neither FcgRIIIa polymorphism nor LON predicted a patient's survival outcome according to the results of a long-term follow-up study.

1. Introduction

Rituximab is a genetically engineered chimeric murine/human monoclonal antibody that is directed to the CD20 antigen, which is found on the surface of premature and mature B cells. Rituximab has proven efficacy in the treatment of CD20-positive B cell lymphoma. It works in the indolent and aggressive histological types, and also in untreated or relapsing populations [1]. Therefore, rituximab has become widely used. Currently, inclusion of rituximab is the standard treatment for CD20-positive B cell lymphoma.

Late onset neutropenia (LON) is a well-recognized late complication of rituximab-based chemotherapy. There is currently no uniform definition of LON [2], but it is generally defined as the unexplained occurrence of neutropenia at least 4 weeks after the completion of rituximab administration and full hemogram recovery [3,4]. Rituximab-related LON has been reported as a rare late-onset complication. As awareness of this adverse effect increases, the incidence of LON has been reported to range from 5.6%

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to 27.3% [3–9] based on different treatment regimens and patient populations.

The mechanism underlying LON is still unknown. Several suspicious etiologies of LON have been discussed, including superimposed infections, the bystander effect, and lymphocyte subpopulation imbalance [10]. The known risk factors for LON include intensified treatment and advanced disease status.

FcγRs have been shown to mediate phagocytosis, antibody-dependent cell-mediated cytotoxicity, and other inflammatory mediators central to the protective and injurious properties of antibodies [11]. Immunoglobulin G (IgG) Fc receptor (FcR) polymorphisms that influence the binding of IgG have been described [12–14]. A polymorphism of FcγRIIIa has been identified at position 158 with a V or F residue, and it was reported that the FcγRIIIa 158 V allele is associated with rituximab-induced LON after autologous hematopoietic stem cell transplantation [15].

We previously reported an 11.3% incidence in LON following frontline rituximab-based treatment in a large cohort of patients with newly diagnosed CD20-positive B cell lymphoma. The FcγRIIIa 158 V/V and V/F polymorphisms were significantly associated with the development of rituximab-related LON [16]; however, the data addressing the correlations between long-term survival and occurrence of LON and FcgRIIIa 158 V/F polymorphism have not been discussed.

According to the extended data, we determined whether the FcγRIIIa 158V/F polymorphism or LON is correlated with clinical outcomes in patients diagnosed with CD20-positive B cell lymphoma who received rituximab-based chemotherapy.

2. Materials and methods

A total of 100 newly diagnosed patients with CD20-positive B cell lymphoma who received rituximab-based chemotherapy at a single institution between April 2003 and January 2009 were included in this prospective cohort study. The diagnosis of B cell lymphoma, including the aggressive and indolent histology types, was based on the pathologist's determination and expression of CD20 on the lymphoma cell. After giving informed consent, patients were enrolled in the observative cohort study. Blood sampling for genetic study was optional. The definition of LON in our study included the following criteria: (1) an absolute neutrophil count <1500/μL; (2) neutropenia occurring >4 weeks after the last rituximab-based treatment; and (3) exclusion of an alternative etiology of neutropenia. Exclusion criteria were as follows: (1) cirrhosis (hepatitis B- or C-related); (2) neutropenia (<1500/μL) before the first cycle of chemotherapy; and (3) concurrent use of medications that may cause neutropenia. After completion of frontline therapy, all patients had complete blood counts every 2–3 weeks for 6 months. Furthermore, patients who developed LON continued to receive close follow-up in the outpatient clinic (1 visit every 1–2 weeks) with a complete blood count every 2 weeks until full neutrophil recovery. The study was approved by the Tzu Chi Dalin General Hospital institutional review board.

Heparinized blood samples were collected from all patients who gave informed consent. Genomic DNA was extracted from peripheral blood mononuclear cells using a DNA extraction kit. A polymerase chain reaction-based method to determine FcγRIIIa genotypes was used, as previously described [17].

The mean values and standard deviations were calculated to describe the data population. The Pearson chi-square test was used to evaluate the distribution of the two groups (dividing the three genotypes into VV/VF vs. FF) between each group. Statistical analyses were performed using the Fisher exact test or two-tailed *t*-test. Multiple clinical factors (gender, age, history of hepatitis C virus infection, history of hepatitis B virus infection, chemotherapy regimen, and lymphoma histology) were evaluated using univariate analyses with Cox proportional hazards regression for indicators suggesting the development of LON. Survival and follow-up times were calculated from the day of pathologic diagnosis to the day of last follow-up or death. We used commercial statistical software (SPSS version 17.0; SPSS Inc., Chicago, IL, USA) to perform statistical analyses. Different groups were compared with respect to baseline characteristics using a *t*-test for continuous variables and the chi-square test for categorical variables. The Kaplan–Meier method was used for survival analysis. A p value <0.05 was considered statistically significant.

Table 1
FCγRIIIa polymorphism versus late onset neutropenia (LON).

Variable	V/V	V/F	F/F	Total
	Number	Number	Number	
LON	2	9	0	11
No LON	5	37	19	61
LON/(LON + No LON)	28.6%	19.6%	0%	
Total	7	46	19	72

3. Results

A cohort of 100 newly diagnosed patients with CD20-positive B cell lymphoma were consecutively treated with rituximab-containing chemotherapy without autologous stem cell transplantation. Because blood sampling was not enforced, only 72 patients with FCγRIIIa 158 V allele polymorphism were available after completion of informed consent. We performed a subgroup analysis of these 72 patients to interpret the association between FCγRIIIa 158 V allele polymorphism, LON, and survival outcome.

Among the patients, 11 (15.2%) who received rituximab-containing chemotherapy developed LON. In the LON group, two

Table 2
Demographic characteristic of patients included in the study ($n = 72$).

Variable	VF/VV	FF	p
	Number (%)	Number (%)	
Age (y), mean ± SD	61.29 ± 15.38	62.26 ± 13.99	0.809
Gender			
Male	25 (47)	10 (53)	0.683
Female	28 (53)	9 (47)	
Hypertension			
Yes	6 (11)	7 (37)	0.013
No	47 (89)	12 (63)	
Diabetes mellitus			
Yes	9 (17)	4 (21)	0.692
No	44 (83)	15 (79)	
Hepatitis B carrier			
Yes	11 (21)	4 (21)	0.978
No	42 (79)	15 (79)	
Hepatitis C			
Yes	8 (15)	1 (5)	0.266
No	45 (85)	15 (95)	
IPI score			
0–1	18 (34)	7 (37)	0.821
2–4	35 (66)	12 (63)	
Histology type			
Aggressive	45 (85)	16 (84)	0.942
Indolent	8 (15)	3 (16)	
Stage			
I–II	27 (51)	7 (37)	0.291
III–IV	26 (49)	12 (63)	
Bone marrow involvement			
Yes	9 (17)	5 (26)	0.378
No	44 (83)	14 (74)	

patients had the V/V polymorphism, nine patients had the V/F polymorphism, and none had the FcgRIIIa 158 F/F polymorphism. In the non-LON group, five patients had the V/V polymorphism, 37 patients had the V/F polymorphism, and 19 patients had the FcgRIIIa 158 F/F polymorphism (Table 1). The prevalence rate of LON was 28.6% among patients in the FcgRIIIa 158 V/V group, 19.6% in the FcgRIIIa 158 V/F group, and 0% in the FcgRIIIa 158 F/F group.

The FCγRIIIa 158 allele polymorphism was divided into two groups (V/V, V/F vs. F/F). Because the sample size was too small for comparison, we combined the VV/VF group and compared the VV/VF group with the F/F group. The patient baseline characteristics are presented in Table 2. The characteristics of the treatment modality and treatment responses are listed in Table 3. There were no differences in baseline data, comorbidities, histologic types, clinical stages, prognostic indices, or rituximab-based chemotherapy regimen, although there were slightly more patients with hypertension (Table 2, hypertension, $p = 0.013$) in the FF group than the VV/VF group. In contrast, the occurrence of late onset neutropenia ($p = 0.031$) and the complete response rate were higher in the VV/VF group. Patients with VV/VF had a significantly increased rate of LON compared with the F/F group ($p = 0.031$). The odds ratio for VV/VF patients to develop LON was 1.263 (95% confidence interval, 1.098–1.449).

We next estimated the overall survival of the FcγRIIIa polymorphism groups. There were no significant differences between patients with the FcγRIIIa F/F polymorphism and patients with the V/F or V/V genotype ($p = 0.649$; Fig. 1). We further examined the survival of patients with or without LON. Similarly, there was no difference in survival between patients who did and did not have LON ($p = 0.563$; Fig. 2). There were also no differences in the progression-free survival between FcγRIIIa polymorphism groups, or between patients with and without LON (Figs. 3 and 4).

Analysis of the major prognostic factors based on univariate Cox regression analysis showed that age and initial treatment response were correlated with better survival outcomes, rather than the international prognostic index score, histology type, gender, LON, or FcgRIIIa polymorphism (Table 4).

4. Discussion

This is the first prospective cohort study to interpret the correlation between survival outcome and occurrence of LON in patients with newly diagnosed CD20-positive B cell lymphoma, who were treated with rituximab-based chemotherapy without salvage high-dose chemotherapy plus autologous stem cell rescue. The follow-up period was up to 10 years in length.

We previously showed that rituximab-induced LON was correlated with FcγRIIIa genotypes in patients with CD20-positive B cell lymphoma. The objective of this clinical study was to lay a foundation for the clinical results. Again, the occurrence of LON was significantly associated with FCγRIIIa 158 V allele polymorphism. Neither LON nor FCγRIIIa 158 V allele polymorphism showed a survival effect.

The incidence of LON has been reported to range widely between 5.6% and 27.3% [3,5,8,9,18–20] in separate series, probably because of various definitions of rituximab-associated LON and different patient populations. The incidence of LON in the current extended study involving patients with untreated CD20-positive B cell lymphoma was 15.2% (11 of 72 patients). The prevalence was

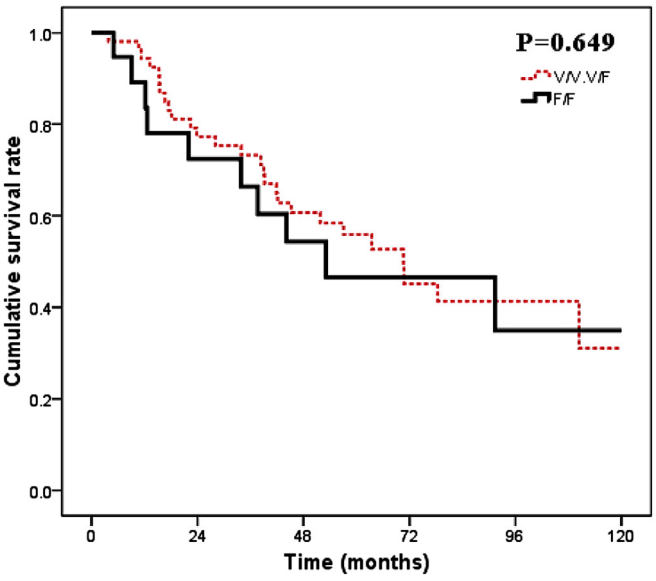

Fig. 1. Overall survival curves for lymphoma cancer according to VF type.

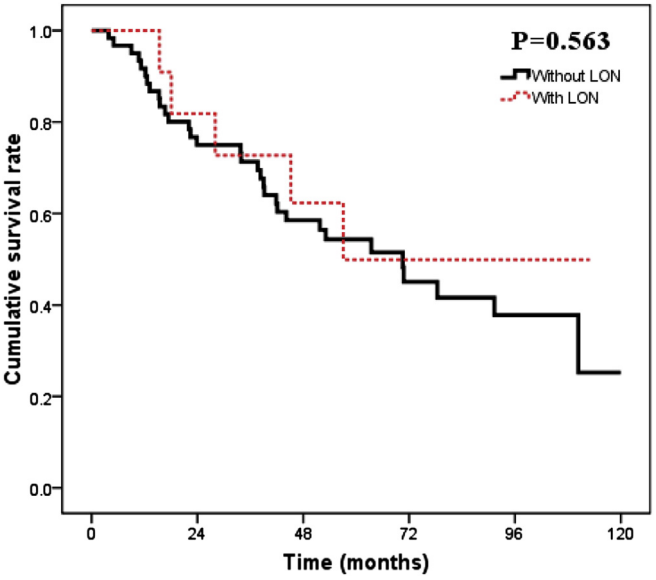

Fig. 2. Survival curves for lymphoma cancer according to LON status., LON = late onset neutropenia.

Table 3
Therapeutic regimen and outcome ($n = 72$).

Variable	VF/VV	FF	p
	Number (%)	Number (%)	
CHOP-like regimen			
Yes	46 (87)	16 (84)	0.780
No	7 (13)	3 (16)	
ESHAP/EPOCH			
Yes	20 (38)	11 (58)	0.128
No	33 (62)	8 (42)	
Other regimen			
Yes	15 (28)	8 (42)	0.268
No	38 (72)	11 (58)	
Late onset neutropenia			
Yes	11 (21)	0 (0)	0.031
No	42 (79)	19 (100)	
Initial treatment response			
CR	42 (79)	13 (68)	0.341
Non-CR	11 (21)	6 (32)	
Final disease status			
CR	29 (55)	5 (26)	0.033
Non-CR	24 (45)	14 (74)	

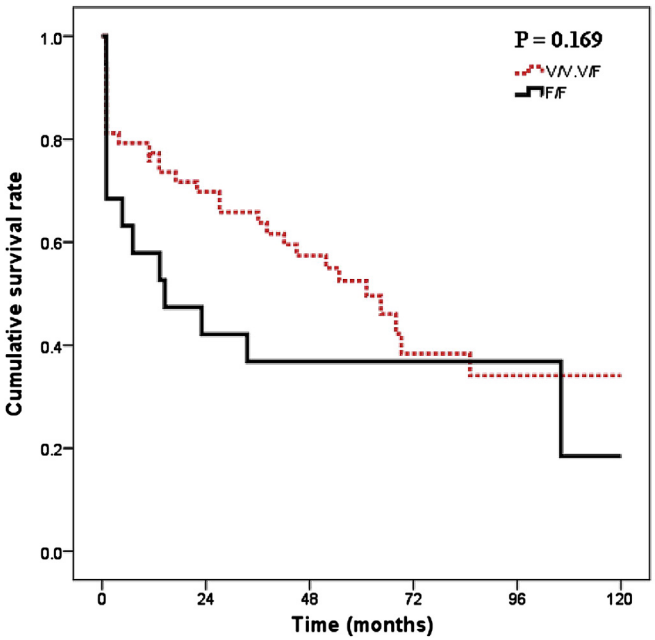

Fig. 3. Progression-free survival curves for lymphoma cancer according to VF type.

higher compared with previously reported data (11.3%; 9 of 80 patients) [16]. The difference in the current study was attributed to not excluding patients with bone marrow involvement and analysis of FCγRIIIa 158 V allele polymorphism. The above prevalence rates of LON were based on different definitions, which were similar and within the range of previously published data [3,5,8,19].

Keane et al [21] reported a prospective study with a 5-year follow-up period, and determined outcomes in 115 patients with newly diagnosed diffuse large B cell lymphoma who received uniform rituximab-cyclophosphamide/hydroxydaunorubicin/oncovin/predni sone. Keane et al [21] reported that FCγRIIIa 158 V/V was highly

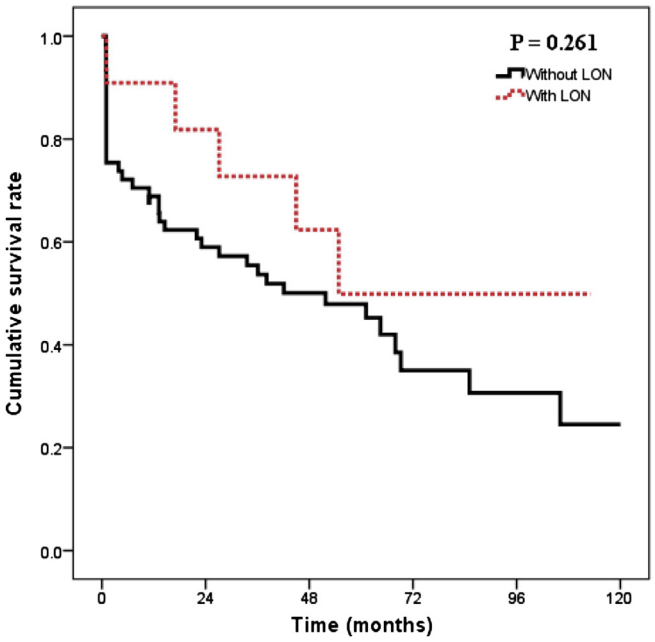

Fig. 4. Progression-free survival curves for lymphoma cancer according to LON status. LON = late onset neutropenia.

Table 4
Univariate analysis.

Variable	Death event (%)	HR	95% CI	*p*
VF type				
VF/VV	27/53 (51)	1		0.649
FF	10/19 (53)	1.18	(0.57–2.45)	
Age (y)		1.04	(1.01–1.06)	0.006
Gender				
Male	19/35 (49)	1.34	(0.70–2.57)	0.376
Female	18/37 (54)	1		
IPI score				
0–1	11/25 (44)	1		0.661
2–4	26/47 (55)	1.17	(0.57–2.40)	
Histology type				
Aggressive	33/61 (54)	1		0.138
Indolent	4/11 (36)	0.45	(0.16–1.29)	
Initial treatment response				
CR	22/55 (40)	0.18	(0.09–0.35)	<0.001
No CR	15/17 (88)	1		
LON				
Yes	5/11 (14)	0.76	(0.29–1.95)	0.565
No	32/61 (86)	1		

correlated with the prevalence of LON. LON and FCγRIIIa polymorphism did not predict overall survival and progression-free survival. Although there were several minor differences in enrolled populations and interventions, the results were not in conflict with the results reported in this study.

Hincks et al [22] concluded that the occurrence of LON predicted the better response to rituximab-based chemotherapy. This argument was based on the observation that only one patient failed to achieve a complete response among 92 patients with LON from several retrospective studies. Similarly, in the current study, only one of 11 patients with LON did not achieve a complete response. However, up to five of 10 patients who achieved complete remission had recurrence of disease during the follow-up period. Furthermore, only one patient with recurrent lymphoma achieved complete remission again. According to the above description, we hypothesize that rituximab might enhance the response of rituximab in patients with LON but not against the natural resistance of underlying malignancy.

There were several limitations in the current research. First, our study included patients with heterogenous pathological diagnoses, which included aggressive and indolent lymphoma with totally different disease characteristics and nature course. It is not feasible to interpret the clinical impact of FCγRIIIa 158 V allele polymorphism in specific lymphoma entities. Second, there were confounding factors from heterogenous treatment regimens. Third, lack of cases with salvage high-dose chemotherapy plus autologous stem cell rescue does not fully match current clinical practice. The role of LON in patients with autologous stem cell transplantation should be further determined.

5. Conclusion

Our study demonstrated that the presence of the FCγRIIIa 158 V allele predicted the occurrence of LON. However, there was no prognostic relevance between FCγRIIIa polymorphism and newly diagnosed CD20-positive B cell lymphoma in patients who received rituximab-based chemotherapy. Larger studies are needed for a definite confirmation of the clinical applicability and prognostic significance of FCγRIIIa polymorphisms.

References

[1] Coiffier B. Rituximab therapy in malignant lymphoma. Oncogene 2007;26: 3603–13.

[2] Wolach O, Bairey O, Lahav M. Late-onset neutropenia after rituximab treatment. Medicine 2010;89:308–18.
[3] Chaiwatanatorn K, Lee N, Grigg A, Filshie R, Firkin F. Delayed-onset neutropenia associated with rituximab therapy. Br J Haematol 2003;121:913–8.
[4] Voog E, Morschhauser F, Solal-Celigny P. Neutropenia in patients treated with rituximab. N Engl J Med 2003;348:2691–4.
[5] Nitta E, Izutsu K, Sato T, Ota Y, Takeuchi K, Kamijo A, et al. A high incidence of late-onset neutropenia following rituximab-containing chemotherapy as a primary treatment of CD20-positive B-cell lymphoma: a single-institution study. Ann Oncol 2007;18:364–9.
[6] Dunleavy K, Tay K, Wilson WH. Rituximab-associated neutropenia. Semin Hematol 2010;47:180–6.
[7] McLaughlin P. Late-onset neutropenia following rituximab. Leuk Lymphoma 2006;47:965–6.
[8] Fukuno K, Tsurumi H, Ando N, Kanemura N, Goto H, Tanabashi S, et al. Late-onset neutropenia in patients treated with rituximab for non-Hodgkin's lymphoma. Int J Hematol 2006;84:242–7.
[9] Lemieux B, Tartas S, Traulle C, Espinouse D, Thieblemont C, Bouafia F, et al. Rituximab-related late-onset neutropenia after autologous stem cell transplantation for aggressive non-Hodgkin's lymphoma. Bone Marrow Transplant 2004;33:921–3.
[10] Stamatopoulos K, Papadaki T, Pontikoglou C, Athanasiadou I, Stavroyianni N, Bux J, et al. Lymphocyte subpopulation imbalances, bone marrow hematopoiesis and histopathology in rituximab-treated lymphoma patients with late-onset neutropenia. Leukemia 2008;22:1446–9.
[11] Ravetch JV, Clynes RA. Divergent roles for Fc receptors and complement in vivo. Annu Rev Immunol 1998;16:421–32.
[12] Osborne JM, Chacko GW, Brandt JT, Anderson CL. Ethnic variation in frequency of an allelic polymorphism of human Fc gamma RIIA determined with allele specific oligonucleotide probes. J Immunol Methods 1994;173:207–17.
[13] Satoh T, Kobayashi M, Kaneda M, Tanihiro M, Okada L, Ueda K. Genotypical classification of neutrophil Fc gamma receptor III by polymerase chain reaction-single-strand conformation polymorphism. Blood 1994;83:3312–5.
[14] Reilly AF, Norris CF, Surrey S, Bruchak FJ, Rappaport EF, Schwartz E, et al. Genetic diversity in human Fc receptor II for immunoglobulin G: Fc gamma receptor IIA ligand-binding polymorphism. Clin Diagn Lab Immunol 1994;1:640–4.
[15] Weng WK, Negrin RS, Lavori P, Horning SJ. Immunoglobulin G Fc receptor Fc_RIIIa 158 V/F polymorphism correlates with rituximab-induced neutropenia after autologous transplantation in patients with non-Hodgkin's lymphoma. J Clin Oncol 2010;28:279–84.
[16] Li SC, Chen YC, Evens AM, Lee CC, Liao HF, Yu CC, et al. Rituximab-induced late-onset neutropenia in newly diagnosed B-cell lymphoma correlates with Fc receptor FcgammaRIIIa158[V/F] polymorphism. Am J Hematol 2010;85:810–2.
[17] Leppers-van de Straat FG, van der Pol WL, Jansen MD, Sugita N, Yoshie H, Kobayashi T, et al. A novel PCR-based method for direct Fc gamma receptor IIIa (CD16) allotyping. J Immunol Methods 2000;242:127–32.
[18] 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.
[19] 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.
[20] 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.
[21] Keane C, Nourse JP, Crooks P, Nguyen-Van D, Mutsando H, Mollee P, et al. Homozygous FCGR3A-158V alleles predispose to late onset neutropenia after CHOP-R for diffuse large B-cell lymphoma. Int Med J 2011;42:1113–9.
[22] Hincks I, Woodcock BE, Thachil J. Is rituximab-induced late-onset neutropenia a good prognostic indicator in lymphoproliferatve disorders? Br J Haematol 2011;153:411–3.