Late-Onset Neutropenia After Rituximab Treatment

Case Series and Comprehensive Review of the Literature

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Abstract: Rituximab is a chimeric monoclonal antibody against CD20 that is used mainly for the treatment of CD20-positive lymphoma. Recently, its use has been expanded to include treatment of other nonmalignant diseases such as rheumatologic diseases and autoimmune cytopenia. Correlating with the increased use of rituximab has been an increased number of reports of its late adverse effects. One of these is late-onset neutropenia (LON). Most investigators define LON as grade III–IV neutropenia occurring 3–4 weeks after the last treatment with rituximab, in the absence of an alternative explanation for the neutropenia.

We report 6 cases of LON identified in our institution. Four patients were treated for diffuse large B-cell lymphoma, and 2 patients for follicular lymphoma. Median patient age was 68 years (range, 33–83 yr); LON appeared after a median interval of 77 days (range, 42–153 d) and lasted for a median of 5 days (range, 1–45 d). Five of the 6 patients presented with infectious complications, and 4 patients experienced recurrent episodes of neutropenia. One patient presented with LON and concomitant subacute pulmonary disease that was attributed to rituximab therapy.

In addition to our own case series we present a systematic review of the literature, which we performed to compile data to describe better the syndrome of LON. Systematic studies, case series, and case reports were extracted. Most studies dealing with LON are retrospective by design and are limited by the heterogeneous populations included in the analysis. The incidence of LON is generally reported to be in the range of 3%–27%. Data regarding populations at risk are not consistent, and in some instances are conflicting.

Patients considered at increased risk of LON include patients after autologous stem cell transplantation, patients treated for acquired immunodeficiency syndrome (AIDS)-related lymphoma, and patients treated with purine analogues. Patients who received previous cytotoxic treatment as well as those treated with more intensive chemotherapy or with chemotherapy in combination with radiotherapy are also considered to be at risk of LON. In addition, advanced stages of disease and having received multiple doses of rituximab are risk factors for LON.

The mechanism of LON is poorly understood. Direct toxicity is very unlikely. Some speculate that there may be an infectious etiology involved, as well as an antibody-mediated process, but these ideas have not been substantiated. The concept of a lymphocyte subpopulation imbalance leading to LON has been presented based on the demonstration of T-LGL in peripheral blood and bone marrow of patients with LON. Perturbations in stromal-derived factor-1 and in the BAFF cytokine have also been discussed as potential players in the pathogenesis of LON. A recent study correlated specific polymorphism in the immunoglobulin G Fc receptor FCγRIIIa 158 V/F with increased rates of LON.

The clinical significance of LON is important because it may affect treatment strategies. Of note, infectious complications are not very frequent and not very severe. Pooling data from the major retrospective studies reveals an infection rate of 16.9%. Most infections were mild and resolved promptly. One death occurred from infection during neutropenia. Repeated episodes of LON are not uncommon, but it is so far impossible to identify those patients at risk of these relapsing episodes of LON. Re-treatment with rituximab after LON may result in recurrent episodes, but the implications and risks are uncertain at the present time. The role of growth factors once LON appears is ill defined, and the decision to use them should be made on a case-by-case basis.

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Abbreviations: ACVB = adriamycin, cyclophosphamide, vindesine, and bleomycin, AIDS = acquired immunodeficiency syndrome, ASCT = autologous stem cell transplantation, CT = computed tomography, DLBCL = diffuse large B-cell lymphoma, G-CSF = granulocyte colony-stimulating factor, LON = late-onset neutropenia, NHL = non-Hodgkin lymphoma, R-CHOP = rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone, R-DA-EPOCH = dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, and hydroxydaunorubicin with rituximab, SDF-1 = stromal-derived factor 1, T-LGL = T-cell large granulocyte lymphocyte.

INTRODUCTION

Rituximab is a chimeric monoclonal antibody that specifically depletes B cells by binding to CD20 expressed on the surface of premature and mature B cells. It has been shown to improve the clinical outcome of patients with various forms of aggressive and indolent B cell lymphoma.[9]

Rituximab has been shown to be effective, tolerable, and safe in the treatment of CD20-positive lymphoma.[22] More recently, its use was expanded to other nonmalignant diseases such as rheumatoid arthritis,[45] systemic lupus erythematous,[48] and autoimmune cytopenia.[3] A significant increase in the number of patient exposures to this drug was observed in the past decade. For instance, it is estimated that more than 1 million patients have been treated with Rituxan (Genentech USA/Biogen Idec, South San Francisco, CA) in the past decade (data on file, Genentech USA/Biogen Idec).

Coincident with the increased use stated above, various late adverse effects and complications associated with the use of rituximab were noted. Among these are hematologic, pulmonary, and infectious complications.[22,37] One of the recently recognized side effects of rituximab is late-onset neutropenia (LON).

Rituximab-associated LON is defined by most investigators as a grade III–IV neutropenia (an absolute neutrophil count under 500–1000 cells/μL; according to the National Cancer Institute Common Toxicity Criteria), occurring 3–4 weeks after the last treatment with rituximab and after the absolute neutrophil count

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has recovered to normal or near normal. LON was initially considered a rare phenomenon with a postmarketing rate of less than 0.02% in over 300,000 treated patients.[2] This figure probably underestimates the true incidence due to a lack of routine follow-up of neutrophil counts in the months after rituximab therapy for most patients. Indeed, several prospective and retrospective studies have suggested a much higher incidence of LON.[4–6,12,15,17,24,25,34,47] Since episodes of LON may result in clinically significant disease and may have bearing on clinical decisions, it is important to summarize our current knowledge regarding epidemiologic and clinical aspects of LON.

In the current study, we present a case series of 6 patients identified in our center who developed LON related to rituximab therapy, discuss their characteristics, and review the literature on this evolving subject. Most studies in this systematic review are retrospective and, as such, are not suitable for meta-analysis. We also present an unusual case of concurrent LON and lung injury related to rituximab therapy.

CASE SERIES

In the present study we describe 6 patients from a single institution who developed LON after treatment with rituximab (at the Rabin Medical Center, Petah Tikva, Israel, during 2005–2008). LON was defined in accordance with the accepted definition stated above. These patients were defined as having LON because the neutropenia could not be explained by any alternative cause, such as bone marrow involvement, an infectious cause, or an autoimmune process. In addition, their clinical course was typical of LON.

We collected both patient characteristics and LON-related data from medical records (Table 1 and Table 2, respectively). This patient series included 4 patients with diffuse large B-cell lymphoma (DLBCL) and 2 with follicular lymphoma. The median age at the onset of LON was 68 years (range, 33–83 yr); 1 patient had been previously treated with chemotherapy before the index therapy that was associated with LON. One patient developed LON during rituximab maintenance therapy (after previously receiving first-line chemotherapy protocol containing rituximab). All patients except 1 achieved complete remission of the lymphoma, as assessed by positron emission tomography.

LON appeared after rituximab therapy at a median interval of 77 days (range, 42–153 d). Three patients experienced recurrent episodes of LON. These episodes were not precipitated by repeated administration of rituximab, but rather represented a late sequelae of prior rituximab administration (Patients 1, 2, 4). One patient (Patient 5) experienced multiple LON episodes, part of which appeared following re-treatment with rituximab. The first episode of late neutropenia was brief and appeared after treatment with rituximab combined with cytotoxic drugs (fludarabine, cyclophosphamide, and rituximab). This patient received maintenance therapy of 3 additional doses of rituximab that was administered 76 days after the initial episode of LON. After receiving the last dose of maintenance rituximab, he developed 2 additional episodes of LON. The median duration of LON episodes was 5 days (range, 1–45 d). The median total number of doses of rituximab administered before LON was 6 (range, 3–8 doses). Five of the 6 patients presented with infectious complications. Two of these patients presented with recurrent infectious episodes associated with recurrent LON episodes. Febrile neutropenia without an infectious focus or proven bacteremia appeared in 4 patients. One patient presented with *Pseudomonas aeruginosa* bacteremia, while another patient presented with *Pseudomonas aeruginosa*-related ecthyma gangrenosum. One patient presented with pneumonia in the right middle lobe. Growth factors such as granulocyte colony-stimulating factor (G-CSF) were given to 5 of the 6 patients to enhance the recovery of neutrophils. Bone marrow examination was performed in 4 cases. In all cases a left shift in the granulocytic line as well as maturation arrest were noted. In 2 cases, lymphoid aggregates in the bone marrow were also a prominent feature. In 1 case, T-cell aggregates were identified, while in the other case a mix of B- and T-cell populations, with T-cell predominance, was described. A scarcely positive staining for CD20 and CD79A probably represented residual lymphoma involvement in this case.

Patient 6 presented with LON and concurrent subacute interstitial lung disease that presented as fever, hypoxia, and dyspnea. Computed tomography (CT) of the chest revealed ground-glass opacities in the upper lung fields and linear atelectasis in the lung bases. There was no evidence of pulmonary embolism on CT angiography. Pulmonary function tests revealed a restrictive pattern with impaired diffusion capacity and a mild obstructive component. An extensive infectious workup (including bronchoalveolar lavage and transbronchial biopsy) was negative, and the diagnosis of drug-related pulmonary toxicity was established. Potential etiologic candidates included cyclophosphamide, methotrexate, filgrastim, and

TABLE 1. Characteristics of 6 Patients With Late-Onset Neutropenia

Patient	Age*/Sex (yr)	Primary Diagnosis	Ann Arbor Stage at Diagnosis	Therapy Preceding LON	Previous\Additional Therapy	Patient Outcome
1	33/F	Mediastinal DLBCL	IIA	R-VACOP-B	None	CR
2	55/F	Follicular lymphoma	IVA	FCR	COP	CR
3	83/M	DLBCL	IVA	R-CHOP	None	CR
4	75/F	DLBCL	IA	R-CHOP	None	CR
5	65/M	Follicular lymphoma	IVA	Rituximab maintenance	Chlorambucil, FCR	Disease relapsed
6	71/M	DLBCL	IVB	R-CHOP	Concurrent intrathecal methotrexate	CR

Abbreviations: COP = cyclophosphamide, vincristine, and prednisolone; CR = complete remission; FCR = fludarabine, cyclophosphamide, and rituximab; R-VACOP-B = rituximab, vincristine, doxorubicin, cyclophosphamide, etoposide, prednisone, and bleomycin.

*Age at onset of LON.

TABLE 2. LON Characteristics in Study Patients

Patient	LON Appearance (days from last rituximab)*	LON Duration (days)*	No. of LON Episodes	Nadir of Neutrophil Count	Total Rituximab Doses	Infectious Complications	G-CSF Administration	BM Findings
1	77, 126, 153	6, 2, 5	3	100	7	1st episode: Febrile neutropenia with *Pseudomonas aeruginosa*-related ecthyma gangrenosum 3rd episode: Febrile neutropenia	Yes	Maturation arrest in granulocytic cell line
2	79, 117, 134	4, 5, 8	3	70	3	1st episode: Febrile neutropenia with pneumonia 2nd episode: Febrile neutropenia	Yes	Myeloid maturation arrest, T-cell aggregates
3	153	3	1	400	8	*Pseudomonas aeruginosa* bacteremia	Yes	NA
4	47, 145	1, 38	2	200	3	None	Yes	Maturation arrest, left shift
5	77, 115, 168	1, 3, 45	3†	300	8	Febrile neutropenia	Yes	Maturation arrest, lymphoid aggregates, suspected residual involvement of lymphoma
6	42	9	1	600	5	Febrile neutropenia	No	NA

Abbreviations: BM = bone marrow, NA = not available.

*For patients with more than 1 LON episode, data are shown sequentially divided by a comma.

†Second and third episodes of LON appeared after re-treatment with rituximab.

rituximab, all of which are known to confer potential pulmonary toxicity.[1,26,28,30,52] Despite the difficulty in establishing causality,[27] the timing and the pattern of the pulmonary disease were most consistent with rituximab-related pulmonary toxicity. The pulmonary disease promptly subsided following treatment with corticosteroids, and the patient's pulmonary function tests returned to normal within 1 month of receiving treatment.

LITERATURE REVIEW

Literature Review Methods

We conducted a MEDLINE (National Library of Medicine, Bethesda, MD) search with the subject heading "rituximab" combined with "neutropenia," "late onset neutropenia," or "delayed onset neutropenia" to identify literature pertaining to the subject of LON associated with rituximab treatment. Our search was limited to literature written in English. All data shown here were extracted from the reports found, but occasionally articles lacked relevant clinical or laboratory data.

Literature Review Results

LON emerged as a possible adverse event as soon as rituximab entered prospective systemic evaluation in clinical trials. Maloney et al[31] and McLaughlin et al[33] were among the first to demonstrate this phenomenon in their work assessing 4 weekly doses of rituximab (375 mg/m^2) in patients with relapsed low-grade lymphoma. In the aforementioned trial, 2 of the 37 patients (5.4%) developed isolated neutropenia of unknown etiology, beginning 4 and 10 months after therapy, respectively, and resolving within 1 month. In the later trial evaluating 166 patients, 13 cases of late neutropenia were described (7.8%). Of these, 5 cases were of grade III severity and 1 of grade IV severity.

Davis et al[11] assessed the efficacy and safety of re-treatment with rituximab in 58 patients with relapsed low-grade or follicular lymphoma that were previously treated with this drug. Rituximab was administered in the same frequency and dose as stated above. LON developed in 1 patient 1 month after completion of the last rituximab dose. It is noteworthy that this patient had developed a similar pattern of LON upon his previous exposure to rituximab. During a 1-year follow-up, 2 patients developed LON with neutrophil counts of less than 1000/μL (grade III neutropenia, 3.4%). In these trials, there were no reported infections related to these neutropenic episodes. Two large prospective trials assessed the utility of rituximab maintenance therapy after completing standard chemotherapy with or without rituximab. The first trial by Van Oers et al[50] evaluated patients with relapsed or resistant follicular lymphoma. The second trial by Forstpointner et al[14] dealt with recurring and refractory follicular lymphoma and mantle cell lymphoma. Both trials showed an increase in the incidence of neutropenia during maintenance therapy. In the former study, neutropenia was documented in 10.8% of patients in the rituximab maintenance group as compared with 5.4% in the observation group ($p = 0.07$). The higher infection rate in the maintenance group was attributed to the rate of neutropenia in this group. The latter trial showed a nonsignificant difference in grade III and IV neutropenia (13 cases in the rituximab arm vs. 6 cases in the observation arm).

Since LON was not a predefined clinical endpoint in any of the aforementioned prospective trials, it is difficult to characterize the incidence and clinical features of this syndrome based on these trials.

Several retrospective trials have been reported in recent years that have attempted to define the incidence and natural history of LON. A summary of the major outcomes of these studies is presented in Table 3. Data and outcomes are diverse, as the patient populations were very heterogeneous.

Patients with indolent and aggressive lymphoma, "naïve" and heavily pretreated lymphoma patients, and patients with nonhematologic immune-mediated diseases are all potential candidates for treatment with rituximab. Chaiwatanatorn et al[6] retrospectively investigated LON in a mixed cohort of 53 patients with either follicular lymphoma, mantle cell lymphoma, or DLBCL. One patient with chronic myelogenous leukemia treated for iso-antibody suppression following bone marrow transplantation was also included in the cohort. The results of this group were compared with data obtained from the records of 93 patients with non-Hodgkin lymphoma (NHL) treated with a regiment that did not contain rituximab. Seven patients (13.2%) developed LON in the study. All of them had been previously treated with chemotherapy for their disease. The median time for onset of LON from last rituximab administration was 122 days, and the median duration of neutropenia was 9 days. On 4 occasions the patients received filgrastim (G-CSF) because of the neutropenia. Five of the patients were treated with rituximab as monotherapy. One patient had a recurrent episode of LON upon repeated treatment with rituximab 2 years after the initial treatment with this drug that was complicated with LON. On 5 occasions the diagnosis of LON was made from routine peripheral blood counts. One patient presented with buccal cellulitis, and another with pneumonia. Bone marrow examinations were performed in 6 cases and revealed selective depletion of neutrophil precursors either beyond the myelocyte (2 cases) or the metamyelocyte stage (4 cases). No episodes of LON were noted in the historical cohort of the control group that received solely chemotherapy.

Dunleavy et al[12] retrospectively investigated a mixed population of lymphoma patients treated with an identical chemotherapy. A population including 76 patients with DLBCL and mantle cell lymphoma received a combination of dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide and hydroxydaunorubicin with rituximab (R-DA-EPOCH). The number of treatment cycles ranged from 3 to 8 depending on the protocol. Importantly, lymphoma patients infected with the human immunodeficiency virus (HIV) were also included in the study. In an effort to avoid potential false results, only patients who achieved complete remission were included in the analysis. The study was controlled by a historical cohort of 54 patients treated with a non-rituximab-containing DA-EPOCH protocol. During follow-up of at least 1 year, 6 of 76 patients (8%) in the rituximab-treated group developed LON. LON was much more prevalent among patients with AIDS-related lymphoma. As many as one-quarter of these patients (2 of 8 patients) developed LON. Median time to onset of LON was 175 days, with a median duration of 12 days (among patients in whom quantification of LON duration was feasible). One patient developed buccal cellulitis and was treated with antibiotics and filgrastim. Bone marrow examination performed in 2 patients demonstrated either normal or mildly decreased cellularity with a mild decrease in granulocytes and a left shift. In the control group, who had received only chemotherapy, no episodes of LON were reported.

Fukuno et al[15] described LON in 3 of 54 (5.6%) NHL patients. LON appeared 76 days after exposure and lasted for a median of 6 days. Of the 3 patients in whom LON was detected, 2 were patients with follicular lymphoma who presented with recurrent episodes of LON, 1 of which presented with recurrent grade IV neutropenic fever. Bone marrow demonstrated hypocellularity with neutrophil maturation arrest accompanied by dysplasia of the myeloid series, or selective depletion of the myeloid series, or a normocellular bone marrow

TABLE 3. Retrospective Trials Assessing LON After Rituximab Therapy: Major Outcomes

Study First Author (ref.)	No. of Patients	Patient Characteristics	Rate of LON % (patients)	Median Time From Last RTX to LON (days) (range)	Recurrent Episode of LON (No./No. of Patients)	Median Duration of LON Episode (days) (range)	Median No. of RTX Infusions (range)
Chaiwatanatorn[6]*	53	Mixed population	13% (7)	122 (32–168)	1/7	9 (4–148)	4 (2–8)
Dunleavy[12]*	76	Mixed population	8% (6)	175 (77–204)	0/6	12 (1–16)	6 (3–6)
Fukuno[15]*	54	Mixed population	5.6% (3)	73 (34–111)	2/3	6 (5–16)	4
Cattaneo[5]†	72	Mixed population	27.3%¶ (21)	70 (21–161)	NA	77 (7–161+)	4 (2–8)
Nitta[34]‡	107	Mixed population	21.5%** (23)	124 (46–384)	3/23	28 (5–84)	NA
Tesfa[47]†	113	Mixed population	7%†† (8)	88 (30–295)	1/8	54 (10–120)	8 (5–9)
Cairoli[4]§	10	ASCT patients	70% (7)	38 (14–84)	2/7	12 (7–145)	2§§
Lemieux[25]§	39	ASCT patients	15% (6)	158 (84–193)	NA	9 (8–93)	NA
Hirayama[17]*	14	ASCT patients	42% (6)	NA	NA	NA	NA
Lai[24]‡	121	DLBCL	13.2%‡‡ (16)	129 (39–277)	NA	68 (3–349)	6¶¶ (1–8)
Rios-Fernandez[39]‡	23	AI diseases	4.3% (1)	119	NA	5	4
Jones[19]*	65	ANCA Vasculitis	3% (2)	120 (90–150)	NA	NA	NA

Abbreviations: See previous tables. AI = autoimmune, ANC = absolute neutrophil count, ANCA = antineutrophil cytoplasmic antibody, RTX = rituximab.

Each of the cited studies defines LON differently:

*Denotes studies that defined LON as grade IV neutropenia. Time from treatment to neutropenia varied between studies: Chaiwatanatorn et al and Fukuno et al required the neutropenia to occur >4 wk from drug exposure; Hirayama et al required >4 wk to elapse from ASCT to neutropenia; Dunleavy et al required >60 d to neutropenia onset in order to define LON.

†Denotes studies that defined LON as grade II neutropenia. Time from treatment to neutropenia varied between studies: Cattaneo et al required the cytopenia to occur 21–180 d after drug exposure; Tesfa et al required >4 wk to elapse from drug exposure to neutropenia. They also required at least 2 d of neutropenia.

‡Denotes studies that defined LON as grade III neutropenia. Time from treatment to neutropenia varied between studies: Lai et al followed the general definition of neutropenia occurring 4 wk to 6 mo after completion of treatment (but followed patients for >2 yr); Rios-Fernandez et al required the neutropenia to occur >30 d from drug exposure; Nitta et al did not set a specific time frame.

§Denotes studies in which clear definitions of LON are not available.

¶Grade IV neutropenia appeared in 11.1% of cases (9 patients). Rate calculations are for the sum of rituximab courses, which totaled 77 as some patients were treated with more than 1 course of rituximab.

**Grade IV neutropenia appeared in 9.3% of the cohort (10 patients).

††Grade IV neutropenia appeared in 5.3% of the cohort (6 patients).

‡‡Grade IV neutropenia appeared in 3% of the cohort (4 patients).

§§Two doses of rituximab given at a median of 52 and 66 d post-ASCT.

¶¶Number of rituximab doses in this study reported for the whole cohort.

in 2 patients with follicular lymphoma and 1 with DLBCL, respectively.

Cattaneo et al[5] examined a very heterogeneous population of 72 NHL patients comprised of "naïve" and relapsed NHL patients. They were treated with 77 courses of rituximab either as monotherapy or in combination with anthracycline-based and/or fludarabine-based combination chemotherapy. Twenty-five percent of the cohort received rituximab as part of the high-dose chemotherapy before autologous stem cell transplantation (ASCT) or as part of in vivo purging preceding ASCT. In this study, a broader definition of LON was used including grade II–IV neutropenia. Using this definition, the authors identified 21 cases of LON (27.3%). The calculated rate of LON was 19.4% for grade III–IV neutropenia and 11.1% for grade IV neutropenia. Eight cases of neutropenia were associated with other cytopenias. Median onset of LON occurred 70 days from the last rituximab dose and lasted a median of 77 days. Four cases of major infection occurred in LON patients (19%). One patient died of *Pneumocystis jirovecii* pneumonia and cytomegalovirus reactivation. Other patients suffered from cytomegalovirus pneumonitis, mycobacterial pneumonia, and bacterial pneumonia. In comparison, only 2 of the 56 patients who did not develop LON presented with an infectious event (3.6%; $p = 0.043$). It is noteworthy that no difference in LON rate was observed between patients treated with rituximab as monotherapy and patients receiving rituximab-containing chemotherapy. In this study, 7 patients were treated with filgrastim while suffering from LON.

Mixed lymphoma populations were also retrospectively evaluated by Nitta et al[34] and Tesfa and colleagues.[47] Nitta et al evaluated 107 patients with various forms of indolent and aggressive lymphoma that were treated with a wide range of rituximab-containing chemotherapy protocols. Follicular lymphoma and DLBCL were the most prevalent diagnoses, and rituximab added to cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) with or without radiotherapy was the dominant treatment protocol. With a median follow-up of over a year, 23 patients (21.5%) developed LON, defined in this study as grade III–IV neutropenia. The rate of grade IV neutropenia was 9.3%. LON appeared 124 days after the last dose of rituximab, and its median duration was 28 days. Filgrastim was administered in only 1 case (with a prompt neutrophil

response). Only 1 patient developed a mild infectious complication (mild tonsillitis), and 3 patients had a second episode of LON following recovery from the first episode. Bone marrow was assessed in 2 patients and demonstrated either maturation arrest of the myeloid line or almost normal findings. No LON episodes were reported in the control group that consisted of 52 patients who had been treated with non-rituximab-containing regimens.

Tesfa et al[47] described LON as grade II–IV neutropenia and demonstrated a rate of 7% (8 patients) in a cohort of 113 patients with various types of lymphoma. Grade IV LON occurred in 6 patients (5.3%). LON occurred at a median of 88 days from administration of the last rituximab dose, and its duration was a median of 54 days. One patient had a recurrent episode of LON. Filgrastim was given to 2 patients. Fever complicated the course of neutropenia in 3 patients.

ASCT patients were specifically investigated with regard to LON in a few retrospective and prospective trials. Higher rates of LON were noted in this population, although some of these trials either lacked a clear definition of LON or included in the analysis earlier events of neutropenia than generally accepted[20,42,53] (data of these trials are not presented). Cairoli et al[4] studied 14 follicular lymphoma and mantle cell lymphoma patients who were treated with a rituximab-containing conditioning chemotherapy. Ten patients also received 2 additional rituximab doses after ASCT. Seven of 10 patients (70%) developed LON after a median of 38 days. It persisted for a median of 12 days. Two patients experienced multiple LON episodes. Of note, no infectious complications occurred in this group. Bone marrow analysis showed hypocellular marrow with normal trilineage hematopoiesis consistent with recovery from transplant.

Lemieux et al[25] studied LON in DLBCL patients treated with ACVB (adriamycin, cyclophosphamide, vindesine, and bleomycin) followed by ASCT. Rituximab was added to this protocol and in some cases was administered in proximity to the transplantation. LON occurred in 6 of the 39 patients (15%), at a median of 158 days from the last rituximab dose and for a median of 9 days. A high rate of infectious complications was noted, with 3 patients presenting with febrile neutropenia and 1 with cellulitis. One other patient was hospitalized with diarrhea and hypotension. Bone marrow examinations were carried out in 5 patients with LON. Four showed maturation arrest with accumulation of promyelocytes and myelocytes. One sample showed a hypoplastic bone marrow with no signs of granulopoiesis impairment. Three of 6 patients were treated with filgrastim.

Hirayama et al[17] investigated LON in a group of 14 patients with either follicular lymphoma or DLBCL who underwent ASCT and achieved complete remission following transplantation. LON developed in 6 patients (42%), between 2 and 6 months after transplantation. No episodes of LON were described in the control group that consisted of 18 patients treated with a non-rituximab-containing regimen.

Tsai et al[49] retrospectively evaluated a small series of 7 patients with progressive intermediate-grade NHL who had been treated with rituximab following ASCT. Rituximab was administered a median of 148 days after transplantation. Two patients developed grade III–IV LON. Of these, only 1 patient developed grade IV neutropenia. It appeared 74 days after initial treatment, presented as febrile neutropenia, and resolved after 4 days of G-CSF therapy. Bone marrow examination in this patient showed a normocellular appearance with myeloid hyperplasia and a left shift in maturation consistent with a recovering bone marrow. Two prospective trials reported LON in patients treated with rituximab as an in vivo purging agent as well as a posttransplantation adjuvant in ASCT patients.

Flinn et al[13] enrolled 25 patients with low- or intermediate-grade NHL in a trial that included the administration of 1 dose of rituximab in addition to a standard stem cell mobilization protocol followed by a standard high-dose preparative regimen and transplantation. An additional posttransplantation rituximab dose was added 7 days after platelet independence was achieved (rituximab was administered at the standard 375 mg/m^2 dose). Six cases of LON (24%) were reported, starting at a median of 99.5 days posttransplantation. Four of these patients presented with a febrile illness, reportedly consistent with a viral infection. In all patients LON resolved spontaneously or after administration of filgrastim.

Horwitz et al[18] assessed the role of rituximab as an adjuvant to high-dose therapy, and ASCT, in a group of 35 patients with recurrent or refractory NHL, most of them with DLBCL. Rituximab was administered in 2 separate 4 weekly standard dose infusions, at 42 days and 6 months posttransplantation. Twenty-one patients developed grade II–IV neutropenia (60%). Grade III–IV neutropenia was observed in 19 patients (54%). We note that 13 patients had recurrent episodes of LON, consisting of a total of 46 episodes. Of these, 21 episodes of grade IV severity (46%) were noted. All episodes of LON resolved within a week, either spontaneously (one-third of episodes) or with G-CSF support (all others). One episode of fever but no serious infection resulted from the neutropenia.

Lai et al[24] retrospectively studied a relatively homogenous population of DLBCL patients treated with R-CHOP protocol. All patients were treated with this protocol as first-line therapy and achieved complete remission. One hundred twenty-one patients were studied and followed for 2 years. LON was defined as grade III–IV neutropenia. It appeared in 16 patients (13.2%) at a median of 129 days, and lasted for a median of 69 days. Grade IV LON appeared in 4 patients (3%). About half of LON patients received filgrastim. One patient developed a non-life-threatening urinary tract infection and pulmonary tuberculosis.

Data regarding LON in the presence of nonhematologic diseases are relatively scarce. Goh et al[16] conducted a small open label prospective study assessing the efficacy of rituximab (given in 4 weekly standard dose infusions) as an adjuvant therapy for the treatment of pemphigus vulgaris. Of 5 patients assessed, 1 developed grade III LON that appeared 19 weeks after therapy and was complicated by community-acquired pneumonia.

Subsequently, Rios-Fernandez et al[39] retrospectively reviewed 23 patients, with various autoimmune diseases, who had rituximab added to their immunosuppressive regimen. Of these, only 1 patient with pemphigus vulgaris developed LON of grade IV severity on day 191 after rituximab treatment. This patient presented with febrile neutropenia that resolved following empirical antibiotic therapy and filgrastim administration.

In a recent study, Jones et al[19] retrospectively analyzed 65 patients treated with rituximab for refractory antineutrophil cytoplasmic antibody-associated vasculitis. Grade IV LON was exhibited in 2 patients (3%) 3 and 5 months, respectively, after the second rituximab course.

DISCUSSION

In recent years rituximab has become a fundamental component of therapy for B-cell malignancies and is administered in various clinical settings: as monotherapy for treatment and maintenance therapy of low-grade lymphoma, as part of standard chemotherapy protocols, and as an adjuvant to high-dose therapy or as part of in vivo purging protocols in patients treated with

ASCT. Its role in nonhematologic immune-mediated diseases is becoming better defined as data continue to accumulate. The ever-growing array of indications for rituximab therapy resulted in a steep increase in exposure of patients to rituximab over the past years and, consequently, in the recognition of late adverse effects. LON, once believed to be a negligible adverse event, has been increasingly studied in an effort to define its risk factors, natural history, and clinical consequences.

As previously stated, 1 of the difficulties in characterizing LON is that very diverse and heterogeneous populations have been assessed in most studies. Populations differ in disease histology, stage, past exposure to treatment, and therapeutic protocols. Moreover, the majority of trials assessing LON are retrospective and are limited by this methodology in accurately defining LON characteristics.

Incidence

The incidence of LON, as calculated from the various studies, is in the range of 3%–27%, with the exception of 2 small retrospective series dealing with ASCT patients in which LON appeared in 42% and 70% of patients.[4,17] The diverse incidence data are in part due to the different definitions of LON used in the various studies (Table 3). Based on these studies, LON appears after a median of 38–175 days from last rituximab dose, and its median duration is 5–77 days.

Populations at Risk and Risk Factors

Identifying populations at risk for LON was attempted in some studies. Higher rates of LON were observed in studies dealing with ASCT patients.[4,13,17,18,25] In another study, patients with AIDS-related lymphoma had a higher incidence of LON.[12] Two studies conducted on a heterogeneous lymphoma population were able to pinpoint statistically significant risk factors for the development of LON. In 1 trial the number of rituximab doses (>4 vs. <4) and previous chemotherapy were significantly associated with LON, while hypogammaglobulinemia and ASCT were not.[5] In a second trial, disease stage (stage I and II vs. stage III and IV), chemotherapy regimen (R-CHOP or rituximab with cyclophosphamide, vincristine and prednisone [R-CVP] vs. more intensive regimens), ASCT, and radiotherapy in primary treatment were significantly associated with LON while age, sex, disease histology, bone marrow involvement, and abbreviated chemotherapy were not.[34] In a later trial that was carried out in a relatively homogenous population of patients with DLBCL treated with R-CHOP protocol, no significant predictive factor was identified upon univariate analysis. This included lack of significance for age, stage, lactate dehydrogenase, Eastern Cooperative Oncology Group (ECOG) performance status, bone marrow involvement, international prognostic index (IPI), blood counts, and albumin level.[24] It is noteworthy that in 1 study, no difference in late cytopenia rate was observed in patients treated with rituximab alone as compared to patients treated with rituximab in combination with chemotherapy. The same study also suggested that the previous use of purine analogues was associated with post-rituximab cytopenia.[5]

In a recent study, specific polymorphism in the immunoglobulin G Fc receptor FCγRIIIa 158 V/F was correlated with higher rates of LON in patients with NHL after ASCT.[53]

Pathogenesis

Despite the interesting work of several groups, the mechanism of LON is not completely understood. Direct toxicity of rituximab is unlikely to be the direct cause of LON for several reasons. First, CD20 is not expressed on granulocytes or on stem or progenitor cells. Although direct cytotoxicity (such as antibody-dependent cellular cytotoxicity) via the neutrophil Fc receptor FCγRI (CD64) remains a theoretical option, there are currently no data to support this mechanism. In this context, it is interesting to point out that LON seems to be unique to rituximab among monoclonal antibodies. Furthermore, the late onset of neutropenia is not correlated with rituximab pharmacokinetics and pharmacodynamics. An infectious etiology has been hypothesized by some investigators,[32] and a few cases of LON associated with parvovirus B19 infection have been published so far.[7,23]

Voog et al[51] suggested that the mechanism may be related to immune-mediated neutropenia. They described a series of 8 patients with LON and demonstrated by direct immunofluorescence IgG type antibodies bound to the surface of neutrophils in 2 of these patients. However, this finding has not been consistently demonstrated in further studies.

Stamatopoulos et al and Papadaki et al have argued in favor of an immune-mediated mechanism associated with lymphocyte subpopulation imbalance leading to LON. This claim was initially based on the observation that 2 patients with LON displayed (in the peripheral blood and bone marrow) the absence of B cells and predominance of T cells with a phenotype consistent with large granulocyte lymphocyte (T-LGL).[36] T-LGL phenotype has been shown to be associated with neutropenia either through neutrophil apoptosis triggered by the FAS\FAS ligand pathway,[29] or through a Fas\Fas ligand independent cytokine\chemokine-related myelosuppression.[8] Subsequently, this group described 34 rituximab-treated patients. Of these, 11 patients presented with LON and T-LGL in peripheral blood. Highlighting the complexity of eliciting the exact mechanism of LON were the additional 4 LON patients lacking T-LGL and the 2 patients without LON, exhibiting T-LGL phenotype in peripheral blood in this group.[35]

Further support for the immune theory of LON came from a case report by Rose et al[40] describing a 46-year-old DLBCL patient who developed LON following ASCT. Neutropenia was refractory to G-CSF and eventually responded to cyclosporine treatment. Saikia et al[41] described a 54-year-old man with follicular lymphoma who developed LON and hypogammaglobulinemia after rituximab maintenance therapy, who presented with esophageal ulcers and febrile episodes. Following treatment with intravenous immunoglobulins the LON resolved within 1 month.

Recently, Stamatopoulos et al[44] presented an additional study dealing with lymphocyte subpopulations, cytokine expression profiles, and the bone marrow properties of LON patients. Twelve LON patients were studied and compared with a group of 25 healthy donors and 38 rituximab-treated NHL patients without LON. Significant differences in the studied populations included the following: inverted CD4\CD8 ratios in both peripheral blood and bone marrow in 10/12 LON patients vs. 11/38 patients in the control group ($p < 0.01$), a rise in CD8+ count above $1.0 \times 10^9 l^{-1}$ in 8/12 LON patients vs. 8/38 patients in the control group ($p < 0.01$), T-LGL proliferation in 7/12 LON patients vs. 11/38 patients in the control group ($p = 0.064$), an increased proportion of Fas-expressing cells within the CD34+/CD33+ cell compartment and increased Fas ligand RNA expression in the CD3+ cell population of LON patients as compared to healthy donors ($p < 0.0001$). It is noteworthy that all patients and none of the healthy donors had mRNA expression of Fas ligand and interferon-γ. Significantly higher levels of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in LON patients as compared to healthy donors further highlighted the possible role of inflammatory mediators in the development of LON. Bone marrow and peripheral blood flow cytometric studies also revealed a low

granulocytic and erythroid progenitor cell reserve with preserved megakaryocytic progenitor cells as compared to healthy controls. These data correlate with findings described in T-LGL leukemia.

Finally, the bone marrow samples of 10/12 of patients with LON and 27 rituximab-treated patients without LON were inspected. While 7/10 patients demonstrated a significant decrease in the granulocytic series, all LON bone marrow specimens demonstrated a moderate-to-pronounced shift to the left, with abnormal localization of immature precursors (ALIP)-like features extending to maturation arrest in 6/10 cases. In contrast, the majority of non-LON patients demonstrated granulocytic hyperplasia (21/27 patients). It is noteworthy that myelodysplastic changes were observed in the bone marrow samples of all LON patients and in most of the non-LON rituximab-treated NHL controls. This finding has been previously reported[40] and was also seen in patients treated with rituximab as monotherapy. Myelodysplastic syndrome-like changes disappeared upon follow-up bone marrow examination carried out in about half of LON patients and in the majority of the NHL control group.[44]

Possible perturbations in granulocyte homeostasis were the subject of further investigation. Dunleavy et al[12] studied the relation between B-cell recovery and granulocyte counts in 24 patients with mantle cell lymphoma treated with a rituximab-containing protocol (R-DA-EPOCH). The changes in B-cell and granulocyte count at 3-9 months after rituximab therapy were investigated. An inverse correlation between these counts was demonstrated, for example, as B-cell counts rose, granulocyte counts fell ($p = 0.04$). Stromal-derived factor 1 (SDF-1) dynamics were investigated next.

SDF-1 was the focus of further study because of its central regulatory role in neutrophil egression and B-cell lymphopoiesis. SDF-1 levels were measured in the peripheral blood of 17 patients with mantle cell lymphoma at different time points. An elevated SDF-1 level was demonstrated posttreatment. This posttreatment SDF-1 increase was not observed in the non-rituximab-treated DLBCL control group. Furthermore, a decreased SDF-1 level in the period between 3 and 9 months after treatment correlated with an increase in the B-cell number at 9 months ($p = 0.013$). No correlation was found between SDF-1 levels at different time points and changes in granulocyte counts, probably reflecting the complex interactions by which SDF-1 regulates granulocyte egression from bone marrow. Further support for the hypothesis of granulocyte homeostasis perturbations as a contributing mechanism for LON was given by Terrier and colleagues.[46] They presented a case report of a 55-year-old woman with Waldenstrom macroglobulinemia, treated with 4 courses of fludarabine, cyclophosphamide, and rituximab. Subsequently, LON developed and the patient was evaluated for perturbations in a wide array of cytokines. In this report, an elevated level of BAFF, a cytokine involved in B-cell survival, expansion, and development was found. The levels of other key chemokines and cytokines including SDF-1 and TNF-α were within the normal ranges. Rather than an etiologic agent being involved in LON, elevated BAFF levels, which probably express peripheral B-cell depletion, may serve as a marker for elevated risk for LON.

Maturation arrest in the bone marrow of some LON patients, a finding seen in patients with Kostman disease, has led Tesfa et al[47] to test 4 LON patients for HAX1 mutations. All patients exhibited a homozygous state for wildtype HAX1.

In a recent study, Weng et al[53] investigated the relation between specific polymorphism in the immunoglobulin G Fc receptor FCγRIIIa 158 V/F and neutropenia after rituximab therapy. This hypothesis was based on previous observations that 2 immunoglobulin G Fc receptor genotypes, FCγRIIIa 158 V/V and FCγRIIIa 158 H/H, predict response to single agent rituximab therapy in patients with follicular lymphoma. Thirty-three patients with recurrent or refractory NHL, the majority suffering from DLBCL, underwent ASCT. They received 4 weekly courses of rituximab at day 42 posttransplantation. Most of the patients received a second course at 6 months posttransplantation. Fifty-two percent of the patients experienced neutropenia following 1 of the posttransplantation rituximab courses. The median time to neutropenia was 40 days from the last rituximab dose, although it should be stressed that neutropenia at any time post-rituximab was included in the analysis (earliest at 7 d posttreatment).

Patients were then stratified by FCγRIIIa genotype. It was demonstrated that each additional V allele was associated with a 3-fold increase in the odds of neutropenia (robust $z = 2.08$, $p = 0.038$). There was a trend toward earlier neutropenia with each V allele but this did not achieve statistical significance. A few possible explanations were proposed by the authors.

Among these is, enhanced antibody-dependent cellular cytotoxicity related to the high affinity FCγR, which results in more killing of malignant and normal B cells. This may result in influx of granzyme and lysozyme which, in turn, may kill neutrophils by a bystander effect. Another hypothesis is that the more extensive B-cell lymphopenia observed in specific FCγRIIIa polymorphism may affect the tendency for neutropenia via the complex relations linking granulopoiesis and lymphopoiesis (extensively discussed above).

Clinical Significance

The clinical significance of LON is of paramount importance. Clinical issues such as the risk of infection or the risk of re-challenge with rituximab after an episode of LON may affect treatment strategy and patient outcome.

Generally speaking, infectious complications are not very frequent and not very severe. The pooled number of patients identified with LON in the various cited retrospective studies (note the variations in LON definitions among the studies as described in Table 3) is 106 of 747 assessed patients (14.1%). Of these, 18 infectious events were described (16.9% of LON patients). Individual rates of infection in the studies are in the range of 0–20%. Eight patients were identified with episodes of neutropenic fever, 3 cases of cellulitis and 2 cases of bacterial pneumonia. One patient was described with mild tonsillitis and 1 with cytomegalovirus infection. Two cases of pulmonary tuberculosis were involved, 1 of which had a concurrent culture-positive urinary tract infection. One patient died of *Pneumocystis jirovecii* pneumonia with cytomegalovirus re-activation.

The high rate of infectious complications in our series probably reflects a selection bias. The relatively low infection rate and mild severity of infections in LON patients merits special consideration. Risk of infection in neutropenic patients is thought to be closely related to the depth of the neutropenia and to the length of the neutropenic episode.[10] Both of these parameters are pronounced in some LON patients.

Further increasing the theoretical risk of infection is the hypogammaglobulinemia associated with rituximab therapy. Hypogammaglobulinemia is a well-described sequela of rituximab therapy that can be documented up to 2 years post-exposure.[38,43] Hypogammaglobulinemia was also documented in some of the retrospective studies dealing with LON.[5,17] Although 1 of these studies found no association between hypogammaglobulinemia and post-rituximab cytopenia,[5] the data describing the interplay between hypogammaglobulinemia and LON are not sufficient to draw conclusions about the clinical significance.

The relevance of filgrastim has been described to varying degrees in the different studies and is reported in a descriptive manner. It seems to only minimally affect the rather benign course of most LON patients. However, sufficient data are not available to draw a conclusion regarding its impact on the natural history of LON or on patient outcome.

An interesting insight into the clinical significance of LON was presented by Kato et al.[21] This group retrospectively studied a mixed population of lymphoma patients after ASCT and defined the rate of delayed-onset neutropenia (defined as grade III–IV neutropenia starting 30 d posttransplantation without an apparent cause) and of infectious events independent of posttransplantation rituximab administration. They then examined risk factors and the clinical impact of delayed neutropenia. One hundred eight patients were analyzed. Of these, 56 patients (52%) received rituximab as part of their pretransplantation induction therapy. The cumulative risk of delayed neutropenia after transplantation was 66% and 33% at 1 year in the rituximab-treated and the non-rituximab-treated group, respectively; 117 infectious events were documented during observation. Of these only 24 events occurred during delayed neutropenia (21%). All infectious event were grade 1–3 and were not lethal. In the analysis rituximab usage and female sex were identified as risk factors for delayed neutropenia (odds ratio [OR], 4.5; $p = 0.020$ and OR, 4.7; $p < 0.001$, respectively). In a further analysis delayed neutropenia and age were significant risk factors for total infectious events ($p = 0.001$ and $p = 0.072$, respectively).

One of the noteworthy phenomena observed in the context of LON concerns those who experienced relapsing episodes of LON. These episodes were reported in the LON population in some trials[4,15,34,47] and generally affect only a minority of patients. No distinctive identifiable characteristics or risk factors for recurrence could be obtained from the available data. In our series, over half of the patients experienced multiple episodes of LON.

The dilemma of re-challenge with rituximab is fundamental since this drug is an important component in the treatment of CD20-positive lymphoma and has bearing on clinical endpoints such as survival and freedom from disease progression. Withdrawal of rituximab from further therapeutic combinations may confer adverse prognostic outcome in patients with lymphoma. Since the reason for LON is poorly understood, it is difficult to predict the consequence of re-challenging a patient with rituximab after the first episode of LON. Because host factors probably play some role in LON and because patients tend to be consistent over time in their clinical response, it is conceivable to expect recurrent LON episodes upon re-challenge with rituximab. The published data are scarce, and are probably biased by positive selection.[6,11] In our series, the only patient who was re-challenged with rituximab after an episode of LON presented with additional episodes of LON following re-treatment.

CONCLUSION

In summary, although rituximab is a fairly safe and tolerable drug, LON is a significant late adverse event that merits our attention. Its natural history, etiology, and clinical significance are not yet completely understood despite ongoing research. Prospective trials that include LON as a predefined endpoint are needed to shed light on this evolving subject. Issues that are central to clinical decision making in the setting of LON include the risk of infection and the risk of re-challenge with rituximab after a first episode of LON. Current data are insufficient to establish firm recommendations on these matters. It seems reasonable to recommend a complete blood count in a patient presenting with fever and a past history of exposure to rituximab, since this will alter management of the patient. The role of growth factors (G-CSF) once LON appears is ill defined, and decisions on their use should be made on an individual basis. Routine surveillance of neutrophil counts following rituximab therapy cannot be recommended based on existing data.

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