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Neutropenia following Rituximab in paediatric non-malignant diseases: case series and review of the literature

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INTRODUCTION

Rituximab (RTX) is a chimeric monoclonal antibody directed against CD20 on the surface of B lymphocytes.[1] It has been used to treat B-cell neoplasms with good response. Its use was subsequently expanded to other non-malignant diseases including renal, rheumatological and other autoimmune conditions.[2-4]

With increased use of RTX, adverse effects associated with the use of RTX, including RTX related neutropenia (RRN), have been well studied. However, the exact mechanism of neutropenia remains undetermined, and is thought to be an idiosyncratic adverse effect. Late onset Neutropenia (LON) is defined as an absolute neutrophil count (ANC) < 1.5 x 10^9/L after treatment with RTX, at least four weeks after the last infusion, up to 12 months after.[5,6] Early onset Neutropenia (EON), occurs within four weeks of RTX treatment and is less commonly reported.[7]

In the midst of emerging data on RRN in the adult population, there is still a paucity of literature in paediatric population. We aim to characterise RRN in paediatric patients with non-malignant diseases through a case series of seven patients in our centre and a thorough review of the literature.

METHODS

Records of all patients who received RTX for non-malignant diseases in the paediatric department, KK Women's and Children's Hospital in Singapore from 1 July 2007 to 31 October 2017 were reviewed. Patients who developed neutropenia, defined by ANC <1.5 x 10^9/L on full blood count, were identified and included in the study. Demographic characteristics, diagnosis, number of RTX courses and doses, time to RRN, duration of RRN and ANC nadir were recorded. We also evaluated the outcomes including presence of infection, use of Granulocyte-Colony Stimulating Factor (GCSF) and recurrence of RRN.

A literature review was performed for manuscripts from inception to 31 December 2019 that described patients with neutropenia after treatment of non-malignant diseases with RTX. The search was performed in MEDLINE, OVID and Cochrane using the following keywords: “neutropenia”, “Rituximab” and excluded “leukemia” and “lymphoma”. We excluded studies which described treatment of other malignant conditions or studies not describing neutropenia after RTX. Our search was limited to literature written in English. All data shown here were extracted from the reports found. We identified fifteen case studies and nine case reports that met our criteria.

This study was approved by SingHealth Centralized Institutional Review Board (CIRB: 2018/2454) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Non-parametric analyses were used to describe data and were shown as median (interquartile range, IQR) for continuous variables and percentages for categorical variables. Chi-squared or Fisher’s exact, Mann Whitney U or Kruskal Wallis tests were applied to compare differences between groups where appropriate.

RESULTS

A total of 29 patients received RTX, a total of 100 RTX doses between July 2007 and October 2017. Twelve patients developed neutropenia, of which five were excluded, as neutropenia episodes were not related to RTX. After exclusion, a total of seven cases of RRN were identified, and the details are shown in Table I. The median age at which they first received RTX was 14 years (IQR 9.0-15.0). There was no significant difference compared with the group who did not develop RRN (median 15 years, IQR 11.8-15.3, p=0.15).

A subgroup of 14 children received RTX for treatment of rheumatological diseases, of which four developed RRN, with a frequency of 28.6%. The median age of those who

developed RRN in these patients was 14 years (IQR 11.0-14.8), which was significantly younger than the group who did not (median age 15.5 years, IQR 15.0-17.3, p=0.03).

There were five cases of EON and two cases of LON. The median time to neutropenia from the last dose of RTX was 16 days (IQR 14.0-30.0), and the median duration of RRN was 9 days (IQR 5.0-16.0 days). Case 5 had an exceptionally long duration of RRN, as the RRN was mild and patient was reviewed as outpatient, with a longer interval between outpatient visits and repeated blood tests compared to other patients.

Infections were reported in four patients requiring intravenous antibiotics. Sources of infection included respiratory, gastrointestinal and urinary tract infections. There was no mortality. RRN resolved in all patients, but three patients required GCSF. Two patients (Case 2 and Case 4) were re-challenged with subsequent doses of RTX, and both did not have recurrence of neutropenia thereafter.

Our literature review identified fifteen case series and nine case reports. The paediatric studies and adult studies are summarized in Table II and Table III respectively.

There were four paediatrics case series and one paediatric case report of RRN and in all of them RTX was used to treat refractory paediatric nephrotic syndrome.[8-11] The frequency of RRN ranged from 5% to 33%, with the highest frequency reported in patients treated for cyclosporin and steroid resistant nephrotic syndrome. Fujinaga et al reported that all patients with SRNS and RRN were less than ten years old. Similarly, Kamei et al also reported that the median age of patients who developed neutropenia (6.4 years old) was significantly younger than the patients who did not (12.5 years old). In both case series, neutropenia occurred at a later onset, with a median of 89 and 66 days, respectively. Both Fujinaga et al and Kamei et al reported use of GCSF in majority of the neutropenic instances (2 out of 3 and 10 out of 11, respectively), with an overall median duration of neutropenia of 3 days in both case series.

Fujinaga et al reported no serious infection in patients with RRN, while Kamei et al reported neutropenic infections in nine out of the eleven patients. There was no reported mortality.

Among the adult case series, RTX was used for treatment of autoimmune or rheumatic disease in 11 of them.[7,12-28] In these case series, the frequency of RRN ranged from 1.5% to 15.0%. Majority of the cases were LON, with the median time to LON more than four weeks in all except two case series (both of which reported 2 cases each). The use of GCSF in adult studies ranged from 0% to 54%. The frequency of infection also varied widely. Out of 104 patients who developed RRN cumulatively, 35 patients developed infections, with no associated mortality reported. A total of 49 patients were re-challenged with RTX after RRN, and majority (74%) did not have recurrence of neutropenia.

DISCUSSION

In our monocentric case series, we describe seven paediatric patients who developed RRN. This is the first case series that included paediatric patients who received RTX for rheumatological conditions, with majority of them diagnosed with Systemic Lupus Erythematosus (SLE). The overall frequency in our case series was 24%, and in the subgroup of rheumatologic disease, 30%. This is significantly higher than the frequency of RRN in the adult case series and most of the paediatric case series. It is important to acknowledge that all our patients who developed RRN were receiving other immunosuppressive agents at the time of neutropenia, and the possibility that these agents may have an association cannot be completely ruled out. However, none of our patients developed neutropenia before RTX despite use of similar immunosuppressive medications.

The median age of children receiving RTX in our case series was older than those reported by Fujinaga et al and Kamei et al, understandably so given the typical age of presentation of pediatric rheumatological disease like SLE and juvenile dermatomyositis

(JDM). Despite this difference, our patients with rheumatological diseases who developed RRN were significantly younger than those who did not develop RRN (p = 0.03). This association between younger age and development of RRN is consistent with the findings by Fujinaga et al and Kamei et al.

Interestingly, most of our patients developed EON instead of LON, with a median time to RRN of 16 days. This is different from both paediatrics and adult case series reported thus far, even with adult patients who received RTX for rheumatological conditions. The exact mechanism of RRN is not well understood, with multiple mechanisms proposed, from autoantibodies binding to neutrophil surfaces,[28] to associations with BAFF (B cell activating factor) and SDF-1 (Stromal-derived factor-1) impairing neutrophil egress from the bone marrow,[29] to T-LGL (T cells with phenotype consistent with large granulocyte lymphocyte) proliferation triggering neutrophil apoptosis.[30] However, they have not been consistently found or proven. Nevertheless, most proposed mechanisms attempt to explain the development of LON, which is more commonly seen. It is not clear if EON occurs through different mechanisms.

Our data supports current literatures on paediatric case series that RRN is usually not associated with serious infections that lead to significant adverse outcomes. Most reported infections in the paediatric population are self-limiting, including uncomplicated respiratory tract and urinary tract infections. However, adults with RRN develop more neutropenic fever and complicated infections including abscess and bacteraemia.[22] This is contrary to the usual belief that paediatric patients have lower immunity and hence greater infection risk, especially when neutropenic. Nonetheless, it is still important to monitor paediatric patients with RRN for infection and administer early treatment.

Our data suggests that GCSF therapy may not be necessary as the only difference GCSF makes is in the time to recovery of ANC; patients treated with GCSF have normalisation of

their ANC after a median of 5 days compared those who did not receive GCSF with a median of 13 days, and this is not statistically significant. There are also no differences in infection rates and the overall prognosis of RRN has been shown to be good even in patients who did not receive GCSF, consistent with previous experience.

In conclusion, while the mechanisms of RRN are not well understood, it is a well described adverse effect. The characteristics of RRN in the treatment of paediatric rheumatological diseases seen in our case series are unique compared to adult population and paediatric patients with nephrotic syndrome, and the incidence is higher, as well. Most of the patients described in this case series developed EON instead of LON. Patients need to be monitored routinely post administration of RTX for development of neutropenia, and patients and parents should be appropriately counselled. The overall prognosis of RRN is good, and is usually not associated with serious infections.

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Table I. Characteristics of patients with RRN at our institution

Case	Gender	Age (yr)	Diagnosis	Time to RRN from last RTX (days)	No. of RTX Courses	Concomitant medications	RTX dose (mg/m2)	ANC Nadir (10^9/L)	Infection	Duration of RRN (days)	GCSF
1	M	9	PV	15	2	MMF, Pred	757	0.14	UTI	6	Yes
2	F	16	SLE	17	7	MMF, Pred, HCQ	613	0.01	RTI	4	Yes
3	F	14	SLE	42	2	Aza, Pred	729	0.76	None	16	No
4	F	14	SLE	30	2	MMF, CYP, HCQ,	581	0	GI	5	Yes
5	M	10	JDM	14	1	MMF, HCQ	813	1.19	None	145	No
6	M	1	Enceph	10	3	None	355	0.98	UTI & RTI	9	No
7	F	15	SRNS	16	4	MMF, CSA	393	0.08	None	13	No

PV Pemphigus Vulgaris, *SLE* Systemic Lupus Erythematosus, *JDM* Juvenile Dermatomyositis, *Enceph* Encephalitis, *SRNS* Steroid Resistant Nephrotic Syndrome, *MMF* Mycophenolate Mofetil, *Pred* Prednisolone, *HCQ* Hydroxychloroquine, *Aza* Azathioprine, *CYP* Cyclophosphamide, *CSA* Cyclosporine, *UTI* Urinary Tract Infection, *RTI* Respiratory Tract Infection, *GI* Gastrointestinal Infection, *GCSF* Required Granulocyte-colony Stimulating Factor, *Spont* Spontaneous recovery of neutropenia without treatment.

Table II. Case Series - Characteristics of Pediatric Patients with RRN from Literature Review

Author	Ref	Age (years)	Frequency of RRN (%) (RRN no. / RTX no.)	Median time to RRN (days) (interquartile)	No. of pts with infection	Mortality (n=)	Median duration of RRN (days)(range)	Required GCSF (n=)	Recurred / Re-challenge	Diagnosis
Bonanni et al (2018)[8]	[8]	3-13	1.4% (2/130)	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	SRNS and SDNS
Fujinaga et al (2019)	[9]	2-12	33% (2/6)	N.A.	0	0	N.A.	N.A.	N.A.	CSA SRNS
Fujinaga et al (2016)	[10]	2-16	5% (3/60)	89 (69-175)	0	0	3 (3-28)	2	0/2	SDNS
Kamei et al (2015)	[11]	4-10	9.6% (11/114)	66 (56-130)	9 - FUO (4), Tonsillitis (2), Sinusitis (2), URTI (1)	0	3 (2-7)	10	0/5	CSA SRNS & SDNS

Time to RRN time till onset of Neutropenia (days) from last treatment with RTX, *GCSF* Granulocyte colony-stimulating factor, *Recurred / Re-challenge* number of patients that had recurrence of RRN after re-challenge with RTX / number of patients that were re-challenged with RTX after resolution of neutropenia, *N.A.* information Not Available, *SRNS* Steroid Resistant Nephrotic Syndrome, *SDNS* steroid dependent nephrotic syndrome, *CSA SRNS* cyclosporin and steroid resistant nephrotic syndrome

Table III. Case Series and Reports - Characteristics of Adult Patients with RRN from Literature Review

Author	Ref	Frequency of RRN (%) (RRN no./ RTX no.)	Time to RRN (days) median/mean (interquartile)	No. of pts with infection (details of infection and proportion (%))	Mortality (n=)	Median duration of RRN (range)	Resolution /Required GCSF (n=)	Recurred/ Re-challenge	Diagnosis
Abdulkader et al. (2014)	[5]	4.6% (5/108)	151/142 (103-176)	2 – Pneumonia (100%)	0	14 (7-15)	3 / 2	0/3	5 RA
Adler et al (2019)	[7]	N.A. (1)	18	1 - RTI	0	27	0/1	0/0	Bullous pemphigoid
Akram et al. (2016)	[12]	N.A. (1)	30	0	0	7	1/0	N.A.	IM & SS
Arroyo-Avila et al. (2015)	[13]	N.A. (1)	19	0	0	20	1/0	0/1	SLE
Besada et al. (2012)	[14]	5.8% (8/N.A.)	165/189 (122-276)	4 – Pneumonia (25%), Pharyngitis (25%), FWOC (25%), C diff diarrhea (25%)	0	6.5 (3-270)	8/0	0/5	5 GPA, 1 RA, 1 cryo, 1 JIA
Breuer et al. (2014)	[15]	N.A. (11/N.A.)	138/152 (105-151)	3 – Cellulitis (33%), Febrile neut w/o source (67%)	0	N.A.	5/6	1/3	10 RA, 1 SLE, 1 MCTD
Enriquez et al. (2007)	[16]	N.A. (1)	5	1 - Febrile neut w/o source	0	7	0/1	0/0	SLE with lupus nephritis
Gottenberg et al (2005)	[17]	15% (2/13)	12/12 (10-15)	1- Febrile neut w/o source	0	N.A.	1/0	N.A.	SLE
Jones et al. (2009)	[18]	3% (2/65)	120 /- (-)	N.A.	N.A.	N.A.	N.A.	N.A.	ANCA vasculitis
Knight et al. (2016)	[19]	11.9% (7/59)	86 / 99 (62-131)	5- UTI (40%), RTI (40%), Abdo pain, fever (20%)	0	N.A. (1-45)	3/4	3/3	5 GPA, 2 MPA
Marotte et al. (2008)	[20]	N.A. (1)	N.A.	0	0	N.A.	0/1	0/0	RA

Mealy et al (2015)	[21]	N.A. (2/N.A.)	17/17 (7-28)	1 - Sinus infection (100%)	0	1.5 (1-2)	0/2	0/1	NMO
Monaco et al. (2016)	[22]	6.5% (11/168)	74/ 90 (53-102)	4 – HAP (25%), BS (25%), Cellulitis (25%), Perianal abscess (25%)	0	4 (1-10)	8/ 3	0/8	9 RA 2 ANCA vasculitis
Ogawa et al (2017)	[23]	36.4% (4/11)	72.6/N.A. (43-122)	0	0	N.A.	3/8	N.A.	AITH
Plate et al. (2014)	[24]	N.A. (1)	90	0	0	3	1/0	1/1*	NMO
Rios-Fernandez et al. (2007)	[25]	4.3% (1/23)	119	1- Febrile neut w/o source	0	5	0/1	0/0	Pemphigus Vulgaris
Rissanen et al. (2017)	[26]	N.A. (1)	90	1 – Dental infection	0	14	0/1	N.A.	Relapsing MS
Salmon et al. (2015)	[27]	1.5% (40/2624)	RA – 135/- (-) AID – 150 /- (-)	5 – UTI (40%), RTI (20%), Febrile neut w/o source (40%)	0	N.A.	35/5	3/19	25 RA, 7 SLE 7 ANCA-V, 1 myositis
Tesfa et al. (2011)	[6]	5.2% (11/209)	102/134 (67-195)	7 – Bacteremia (86%), Febrile neut w/o source (14%)	0	9 (4-20)	5/6	1/5	GPA 3, SLE 3, RA 5
Yamazaki et al (2019)	[28]	N.A. (1)	188	0	0	17	1/0	0/0	MCNS

HAP hospital acquired pneumonia, *BS* biliary sepsis, *UTI* Urinary Tract Infection, *RTI* Respiratory Tract infection, F*ebrile neut w/o source* = febrile neutropenia without source, *FWOC* fever with oral candidiasis, *C diff diarrhea* C difficile positive diarrhea, *N.A.* information not available, *AITH* autoimmune thrombotic and homeostatic disorders, *RA* Rheumatoid Arthritis, *ANCA* anti-neutrophil cytoplasmic antibody, *GPA* Granulomatosis with polyangitis, *MPA* microscopic polyangitis, *SLE* Systemic Lupus Erythematosus, *NMO* neuromyelitis optica, *MCTD* mixed connective tissue disease, *Cryo* cryoglobulinemia, *MCNS* Minimal change Nephrotic Syndrome, *MS* multiple sclerosis, *IM & SS* Inflammatory myopathy & systemic sclerosis overlap. Of note, one patient was reported to have recurrence of neutropenia without re-challenge 42 days after first episode of LON (Breuer 2014) and 21 days after resolution of first episode of LON (Abdulkader 2014). The recurrence was excluded in the recurrence column for both these studies as there was no repeat dose of RTX.