

Rotavirus as a significant cause of prolonged diarrhoeal illness and morbidity following allogeneic bone marrow transplantation

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Summary:

Infective diarrhoea is common among allogeneic stem cell transplant (SCT) recipients, frequently caused by viruses and may be difficult to differentiate from acute graft-versus-host disease (GVHD). Viral pathogens may directly or indirectly impact upon transplant-related mortality. Rotavirus is one of the most common causes of diarrhoea worldwide, but one of the least studied causes of diarrhoea post SCT. In this retrospective study we describe 21 cases of confirmed rotavirus infection in allogeneic SCT recipients. Most of these cases may occur in clusters during the winter and spring period. Symptoms of rotaviral infection were diarrhoea (95%), vomiting (62%), abdominal pain (38%), weight loss and loss of appetite in 38 and 29% of the cases, respectively. Possible extraintestinal manifestations of rotavirus infection were observed. The duration of the symptoms in this series ranged from 4 days to 4 months with median of 15 days. Patients with rotavirus infection were invariably lymphopenic and/or on immunosuppression for GVHD. Of the patients diagnosed with rotavirus, 86% required hospitalisation. In 57% of the cases, other viral pathogens were isolated near to the rotavirus infection period. Rotavirus infection is an important cause of prolonged diarrhoea post SCT, causing significant morbidity and frequently requiring hospitalisation.

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treatment.¹ After conditioning regimen-associated mucositis has abated the most common causes of diarrhoea in bone marrow transplant recipients are acute graft-versus-host disease (GVHD) of the gut, intestinal infection and diarrhoea caused by medications.^{2–6} The incidence of infection-related post transplant diarrhoea is normally around 13%, but has been reported to be up to 40%, with viruses being the most common pathogens.^{5,7} Transplant related mortality (TRM) is higher in patients with viral infections, either by direct effects on infected vital organs or indirectly by causing GVHD or poor immune recovery.

Rotavirus is a virus with a segmented double-stranded RNA genome and is one of the most common causes of gastroenteritis worldwide. It is an important cause of severe diarrhoea in otherwise healthy children.⁸ It is uncommon in adults except those in close contact with children, hospitalised patients and the elderly. The incidence of rotavirus detected from stool samples of patients who have received a bone marrow transplant has been reported to be as high as 10% and appears to be the same in allogeneic and autologous transplants.^{7,9} Viral strains involved are occasionally antigenically distinct from those causing enteritis in infants.¹⁰ Rotaviral infection in immunocompetent hosts is recognised as a self-limiting, superficial enteritis of mature differentiated enterocytes^{11,12} with an average symptom duration of 3–9 days.

We have seen a number of particularly problematic cases of rotavirus diarrhoea in the transplant population in Bristol and decided to analyse our experience of this to see whether certain characteristics are associated with the infection and possibly an unfavourable outcome.

Diarrhoea is a common complication of allogeneic bone marrow transplant. Diarrhoea early post transplant is mainly due to intestinal damage caused by cytoreductive

Patients and methods

Patients

We describe 21 patients with rotavirus associated diarrhoea post allogeneic bone marrow transplantation. Our patients were aged from 7 months to 32 years (median age 6.5 years) and had received peripheral blood stem cell or bone marrow transplants between April 1995 and May 2003 at the Bone Marrow Transplant Unit, Royal Hospital for Children, United Bristol Healthcare NHS Trust. Stool

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samples were collected from all patients with diarrhoea and underwent systematic screening for the likely pathogens, which partly depended on the clinical scenario, including the timing of diarrhoea. The patients were found to be rotavirus positive by electron microscopy of stool. The electron microscopy at our disposal is of very high quality, and the results were reproducible and confirmed by culture results. Data regarding these patients were collected retrospectively using hospital case notes.

Patients were considered to have proven rotavirus infection if they had gastrointestinal symptoms associated with the virus detected in faeces by electron microscopy at least once.

Other infective agents and gut GVHD were excluded as primary causes of the diarrhoeal episodes in this group of patients. The presence of other viral pathogens and *Clostridium difficile* 6 weeks prior to or after the isolation of rotavirus was also recorded.

Methods for pathogen detection

Rotavirus was detected in the faeces of symptomatic patients by negative staining transmission electron microscopy (TEM) using standard procedures¹³ in the Department of Virology, Health Protection Agency Regional Laboratory, Bristol. Diarrhoeal stool samples were also examined for *C. difficile* toxin by neutralisation of cytotoxicity in Vero cells, and by standard virus isolation techniques in MRC5, PLC and Hep2 cell lines.

Rotavirus antigen in stool of children <7 years was detected using IDEIA™ Rotavirus (DakoCytomation) and performed according to the manufacturer's recommendations, and confirmed by TEM.

Results

Patient population, viral isolation and clinical features

The bone marrow transplant unit in Bristol Royal Hospital for Children treats approximately 60% children (patients <16 years) and 40% adults. We retrospectively studied the clinical characteristics of rotavirus positive cases in this unit. Between April 1995 and May 2003, 21 cases of rotavirus-induced diarrhoea were confirmed, with virus detection mainly by electron microscopy from stool in all cases and ELISA™ in 10% of the cases. In this period, the unit performed over 500 allografts. Rotavirus could be detected on one or more occasions depending on the duration of the illness. Patient characteristics are described in Table 1. The median age of this patient group was 6.5 years, ranging from 7 months to 32 years. The majority of the patients with rotavirus diarrhoea were children (17 patients). There was also a higher incidence of rotaviral diarrhoea in males (57%). No obvious relationship between rotavirus induced diarrhoea and the type of conditioning regimen could be observed, although the number of patients receiving each regimen was small.

All transplants were T-cell depleted. The majority of rotavirus related diarrhoea occurred before day 100 post transplant (62% of cases), ranging from day 0 to 23 months

Table 1 Patient characteristics

Number of patients	21
Median age	6.5 years (7 months–32 years) 17 (81%) children, 4 (19%) adults
Males/females	12 (57%)/9 (43%)
<i>Diagnosis</i>	
AML	5 (24%)
ALL	12 (57%)
Other	4 (19%)
<i>Conditioning</i>	
Cy/TBI	9 (43%)
Bu/Cy	2 (9%)
Others	10 (48%)
<i>Type of transplant</i>	
Sib.Allo	5 (24%)
MUD	11 (76%)
MiUD	4 (19%)
Haplo	1 (5%)
T-cell depletion	21
Engraftment $N > 1.0$ (median)	16 (11–24 days)
CMV pos	10 (48%)
Lymphocyte count (median)	0.1 (0–1.8 × 10 ⁹ /lt)
<i>Immunosuppression on diagnosis</i>	
CSA	20 (95%)
Tacrolimus	1 (5%)
Steroids	12 (57%)
<i>Isolation of other viral pathogens</i>	
Resp.Viruses	12 (57%) (7 single)
Adenovirus	6 (29%)
CMV	5 (24%)
Astrovirus	2 (10%)
	1 (5%)
<i>C. difficile</i>	3 (14%)
<i>GVHD</i>	13 (62%)

Isolation of other viral pathogens: Most of these patients had two viral pathogens isolated. Five patients had more than two viral pathogens isolated during the same period. Patients with multiple viral pathogens secrete the rotavirus for a longer period of time (median 3 months) and appear to have longer duration of symptoms due to rotavirus (median 3 months).

post transplant (median 16 days post transplant) and in 62% was associated with acute or chronic GVHD.

Six patients were already hospitalised upon isolation of rotavirus. Four of these patients were receiving their transplant and two were admitted for post transplant complications.

The median lymphocyte count of the patients at the onset of rotavirus diarrhoea was 0.1 × 10⁶/l. Only four patients had a lymphocyte count >1.0 × 10⁶/l (three of whom had undergone a recent donor lymphocyte infusion). In total, 57% of the patients were receiving steroids, mainly for GVHD, at the time of rotavirus isolation. In our patient population, 24% of the cases experienced late onset of rotavirus infection (>3 months from the day of the transplant). The majority of these patients with late onset (>6 months post transplant) of rotavirus diarrhoea ($N = 6$) had developed GVHD post donor lymphocyte infusions

($N=4$) and were on steroid treatment for this. One adult patient had a child with symptomatic diarrhoea that may have been due to rotavirus. All the patients with late onset rotavirus diarrhoea were lymphopenic (lymphocyte count <0.4) apart from three patients post DLIs.

The presence of other viral pathogens and *C. difficile* was recorded in 12 cases. Most of these cases had two viral pathogens isolated. Five patients had more than two viral pathogens isolated during the same period. Patients with multiple viral pathogens secreted the rotavirus for a longer period of time (median 3 months) and appeared to have longer duration of symptoms due to this (median 3 months).

Diarrhoea and vomiting were the most common symptoms seen, followed by abdominal pain, weight loss and loss of appetite (Table 2). Three of the patients developed a high temperature, two abnormal LFTs (not due to GVHD) and one developed pneumatosis coli, a very rare but previously described complication of rotaviral infection.^{14,15} Possible extraintestinal manifestations of rotaviral infection were recorded in four cases, with abnormal LFTs (two patients) skin rash (one patient) and encephalitis (one patient) but none of these was proven. The duration of symptoms varied between 4 days and 4 months (median duration of symptoms 15 days), with a majority of the patients being symptomatic for about 2 weeks.

Although no therapy has been shown to be effective in patients with rotavirus infections, eight patients from our group received intravenous immunoglobulin alone or in combination with antibiotics. Four received intravenous antibiotics mainly for concomitant fever and one patient received steroids. Six patients with a short-lived self-limiting illness did not receive any treatment.

The majority of this group of patients required hospitalisation (86%) and nutritional supplements (81%) via NG tube or TPN. We observed that a majority of the cases occurred in clusters (16 of the 21 cases or 76%) within 6 weeks of each other: four cases between April and May 1997, four cases between February and May 1998, three cases between January and February 1999, and three cases between April and May 2000. In addition, there appeared

to be a higher incidence of rotavirus illness during the winter and spring (January–May) (Figure 1).

Discussion

Up to 40% of BMT recipients experience infective diarrhoea.^{5,7} Viruses are the most common cause identified. Of the viral pathogens, herpes viruses and adenovirus are better studied due to the frequency of their occurrence and isolation. Rotavirus is the most common cause of viral diarrhoea worldwide causing superficial self-limited enteritis in immunocompetent children with average duration of symptoms between 3 and 9 days. We report a study of the characteristics of rotaviral infection obtained by retrospective analysis of the experience in a single combined paediatric and adult BMT unit. In total, 21 patients had symptomatic proven rotaviral illness. The symptoms were variable, commonly involving the gastrointestinal tract

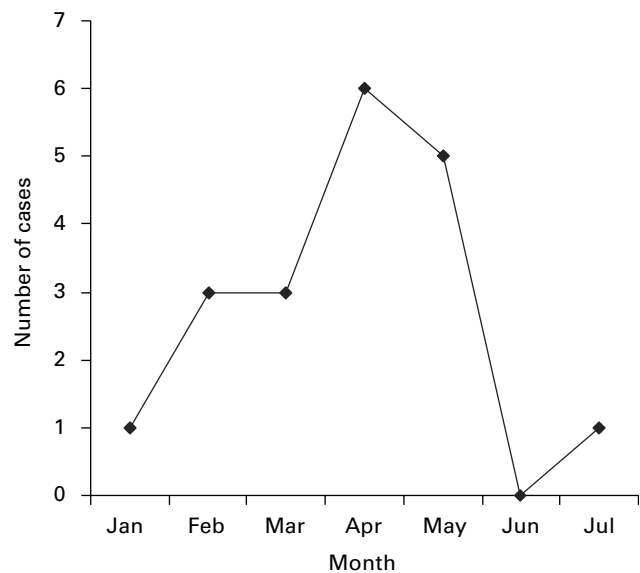


Figure 1 Temporal clustering of cases.

Table 2 Characteristics of rotavirus infection

Time of virus isolation (in relation to day 0)	Day < 100	13 (62%)		
Median 16 days	Day > 100	5 (24%)		
Method of virus detection in stool	EM	21 (100%)		
	ELISA	2 (10%)		
Symptoms	Diarrhoea	20 (95%)	Loss of appet.	6 (29%)
	Vomiting	13 (62%)	Temp > 38°C	3 (14%)
	Abdo. pain	8 (38%)	Abn.LFTs	2 (10%)
	Weight loss	8 (38%)	PR blood	1 (5%)
			Skin rash	1 (5%)
Duration of symptoms (mean)	37 days	Up to 1 week	9	Pneumatosis coli
		Up to 2 weeks	3	1 (5%)
Need for hospitalisation/support	18 (86%)	> 2 weeks	9	Encephalitis
	17 (81%)			1 (5%)
Need for TPN or NG tube	17 (81%)			Deaths
Treatment given	IVIg	8 (38%)	IV antibiotics	4
Cases presented in clusters	16 (76%)		Steroids	1

(GIT) although some patients may have experienced extraintestinal manifestations of rotaviral infection. Rotavirus was more common in children. No correlation between rotavirus infection and the conditioning regimen was observed. The infection tends to occur more frequently (62%) before day 100 post transplant. Lymphopenia immunosuppression and coexistence of acute or chronic GVHD were commonly associated.

There is a significant increase in the duration of rotavirus infection in the immunocompromised host. We observed some particularly persistent diarrhoeal illnesses with symptoms ranging from 4 days to 4 months (median 15 days). Diarrhoea post allogeneic BM transplant is often associated with the conditioning regimen, medications, GVHD and commonly with viral and bacterial pathogens. Here, we have demonstrated that rotavirus can be a significant cause of prolonged diarrhoeal illness and morbidity in the post transplant period. Cases were identified by stool electron microscopy. Temporal clustering during winter and spring was seen. There were associations with lymphopenia, immunosuppression and GVHD. Coexistence of other viral pathogens leads to prolongation of symptomatic period and secretion of the virus. The majority of patients required prolonged hospitalisation and nutritional supplementation. Although no therapy has been shown to be effective early identification reduction in immunosuppression, intravenous or oral¹⁶ immunoglobulins and supportive care might help to reduce the prolonged persistent course of diarrhoeal illness and prolonged in hospital admissions.

Robust infection control precautions need to be in place when dealing with patients with rotavirus infection,¹⁷ and this is especially true in the BMT setting where prolonged excretion of virus may occur. Some rotavirus infections may be acquired nosocomially. Unit policy excludes visitors and staff with diarrhoea (and we do not readmit patients with possible infective diarrhoea), but spread from infected patient to uninfected patient is possible even with the best precautions. Excellent techniques of dealing with infected stool, rigorous handwashing and the nursing of patients in 'teams' may reduce spread. The way to detect in hospital spread is by looking for temporal clustering, but also by molecular methods that detect the same virus in consecutive patients. In cases presenting as clusters or outbreaks, cross contamination should be suspected and excluded.¹⁸

There are significant limitations to this retrospective study. We cannot be certain that all patients with diarrhoea were treated identically. As in all infection studies, there may be under-ascertainment. Viral particles need to be at a certain concentration in stools before they are detectable. Paradoxically, severe diarrhoea may reduce this concentration of particles. Similarly, there have been various approaches to therapy over the 8-year period, thus potentially affecting the natural history of the rotavirus infections we reported.

Further studies in BMT patients should be undertaken using molecular diagnostic methods to enhance sensitivity compared with EM.¹⁹ This would provide firmer information on the incidence of this condition and would allow definitive strain characterisation²⁰ in this group.

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