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Replacement of Calcineurin Inhibitor with Ruxolitinib as GVHD Prophylaxis during Patients with Vascular Endothelial Syndromes after Allogeneic Stem Cell Transplantation

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Abstract

Background: Vascular Endothelial Syndromes (VES) are a range of life-threatening complications that after allogeneic Hematopoietic Stem Cell Transplantation (HSCT), including Transplant-Associated Thrombotic Microangiopathy (TA-TMA), Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome (VOD/SOS), Capillary Leak Syndrome (CLS), Engraftment Syndrome (ES) and Idiopathic Pneumonia Syndrome (IPS)/Diffuse Alveolar Hemorrhage (DAH). Changing immunosuppressive regimen is an important initial step to manage VES because Calcineurin Inhibitors (CNIs) maybe contribute to endothelial cells injury. Our study aimed to determine the therapeutic value of replacing Calcineurin Inhibitors (CNIs) by ruxolitinib during patients with VES.

Methods: 25 patients with hematopoietic malignancies after all o-SCT who developed VES enrolled in this study. All patients received ruxolitinib to replace CNI as GVHD prophylaxis. Ruxolitinib was initiated at 5 mg ~ 10 mg twice a day until 28 days, and then tapered gradually followed by cyclosporine with complete remission of VES therapy.

Results: All 25 patients received CNI with or no MMF as GVHD prophylaxis when diagnosed VES, among them, 12 patients with TA-TMA, 4 patients with CLS, 7 patients with SOS/VOD, and 2 patients diagnosed ES. After ruxolitinib replacement, 18 (72%) patients achieved complete remission of VES. *Cytomegalovirus* (CMV) and Epstein-Barr Virus (EBV) reactivation occurred in 52% (13/25) and 36% (9/25) patients, respectively.

Conclusion: Our data demonstrate that replacement of calcineurin inhibitors with ruxolitinib is a promising treatment option to improve the therapeutic outcome of vascular endothelial syndromes following hematopoietic cell transplantation, but the effect of infection on efficacy should be noted.

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Introduction

Hematopoietic Stem Cell Transplantation (HSCT) is associated with various Vascular Endothelial injury Syndromes (VES), the most recognized systemic diseases include Sinusoidal Obstruction Syndrome/Veno-Occlusive Disease (SOS/VOD), Capillary Leak Syndrome (CLS), Engraftment Syndrome (ES), Transplant-Associated Thrombotic Microangiopathy (TA-TMA), and Idiopathic Pneumonia Syndrome (IPS) [1,2]. The presence of VES risk factors including the toxicity of the conditioning regimen, infections, inflammation (including the allogeneic reaction) and higher Calcineurin Inhibitors (CNIs) or sirolimus trough level [3].

The immune system activation mediating cytokine responses contributing to endothelial injury, hypercoagulability, together with complement activation as a key player of pathophysiological mechanism of VES. Moreover, activation of interferon signature associated with increased STAT1 and STAT2 signaling that resolved after complement blockade [4-6]. Exciting "interferon-complement loop" could perpetuate endothelial injury and promote thrombotic microangiopathy progression [7]. Withdrawal of CNIs/sirolimus may be an effective treatment or lead to reversal of disease, especially in cases of TA-TMA, is a common practice in most transplant centers and is an object of general recommendations [8]. It is traditionally believed that corticosteroids have anti-inflammatory effects that may mitigate endothelial damage and be commonly used; however,

no treatment advantage has been documented [8]. Recent studies have shown that corticosteroid suppress epithelial proliferation in the intestines and exacerbate gastrointestinal damage [9].

Patients with VES who always at high-risk for GVHD and impaired organ function replace CNI with alternative immunosuppressive drug, which should not be associated with endothelium and organ toxic. Ruxolitinib, a selective Janus Kinase (JAK)-1/2 inhibitor has been proved to have a significant efficacy in treating Steroid Refractory (SR)-GVHD, while reserving the GVL effect with mild side effects [10-12]. Especially, ruxolitinib also improve outcomes by reduce inflammatory cytokines in patients with COVID-19 [13]. Recently, the results found that ruxolitinib maybe benefits for complement-mediated endothelial and tissue injuries [13,14]. Thus, despite the lack of data, ruxolitinib seems very promising to replace CNI as a prophylactic agent to manage GVHD in VES therapy.

In this study, we focus on vascular endothelial syndromes including SOS/VOD, CLS, TA-TMA and ES. We conducted a pilot study to evaluate the effectiveness and safety of ruxolitinib replaces CNI to manage GVHD during VES therapy.

Materials and Methods

Patient characteristics

This was a retrospective review of 25 consecutive patients with VES who had undergone HSCT at the Nanfang Hospital of Southern Medical University and Zhongshan Hospital of Sun Yat-sen University between February 2019 and November 2021. A waiver of informed consent and waiver of authorization to use and disclose protected health information was granted since this study did not involve direct patient contact, and therefore, involved no more than minimal risk to the subjects included.

Inclusion criteria

The diagnosis criteria of SOS, CLS, TA-TMA and ES according to Pagliuca et al. [2] publish data. Hematologic response was defined as normalization of LDH, disappearance of schistocytes, and improvement in transfusion requirements at 4 weeks after initiation of VES therapy. An improvement in transfusion requirement would mean the patient requires fewer Red Blood Cell (RBC) and platelet transfusions given 2 weeks after the last day of VES treatment. Complete response was defined as a hematologic response with organ recovery. Even though increased Serum creatinine (Scr) is not part of the TMA criteria, a documented Scr above the range of normal would be documented as acute kidney injury for the purpose of documenting organ injury. Remission was defined as recovery of clinical symptoms, absence of microangiopathic hemolysis and normalization of abnormal biochemistry.

Treatment

At the time of VES diagnosis, all patients withdraw CNI or Sirolimus, and Immunosuppression Manipulation (ISM) with ruxolitinib (initiated at 5 mg ~ 10 mg twice a day until 28 days, and tapered gradually followed by cyclosporine with complete remission of VES therapy), concomitant corticosteroids (0.5 mg/kg to 1 mg/kg per day for 3 days, with subsequent tapering over 1 week), and Mycophenolate Mofetil (MMF) were continued, and/or other supportive care measures. Supportive care measures included transfusion support, treatment of active infections, anti-hypertensives, and renal replacement therapy.

The end points

The primary endpoint was hematologic response with organ recovery. Acute GVHD (aGVHD) was graded according to the Mount Sinai Acute GVHD International Consortium criteria. The secondary endpoints included the incidence of aGVHD, chronic GVHD (cGVHD), safety of treatment, incidences of primary disease relapse, Overall Survival (OS), and incidence of *Cytomegalovirus* (CMV) or Epstein-Barr virus (EBV) reactivation.

CMV and EBV-DNA monitoring

The EBV-DNA and CMV-DNA loads in the blood were measured regularly by real-time quantitative polymerase chain reaction (RQ-PCR). The threshold for EBV-DNA and CMV-DNA copies in the blood provided by the manufacturer (ZJ Bio-Tech Co. Ltd., Shanghai, China) was less than 500 copies/ml. The EBV-DNA and CMV-DNA loads in the blood were monitored weekly for the first 3 months after transplantation, every 2 weeks from the 4th to the 6th month post-transplant and then once per month from the 7th to the 12th month. Once CMV-DNA in the blood was positive, ganciclovir was used until CMV-DNA turned negative consecutively 2 times. Foscarnet was an alternative to ganciclovir in case of cytopenia.

Statistical analysis

Patient, disease, and transplantation-related variables were expressed as median and ranges, or as percentages, as appropriate. Life tables and survival analysis and confident intervals are required for the presentation of outcome. For all other analyses, significant differences between two groups were examined using two-sample Student's t-tests, Chi-square test or Fisher's exact test depending on types of data.

Statistical analysis was performed using GraphPad Prism (GraphPad Software, Version 9.3.1.) and SPSS (Statistical Package of Social Sciences, Version 25.0). A two-sided P value less than 0.05 was considered to indicate a statistically significant difference.

Result

Patient characteristics

From February 2019 to November 2021, a total of 25 patients were diagnosed with VES, including 18 (72%) male and 7 (28%) female (median age, 45 years; range, 15 to 79 years). The most frequent VES overall was TA-TMA (12 patients, 48%), followed by 7 patients with VOD/SOS, 4 patients with CLS, and 2 patients with ES. Baseline characteristics are summarized in Table 1.

VES was diagnosed at a median of 46 days post-HSCT (range, 7 to 402 days). Treatment for VES in started at a median of 1.5 days (range, 0 to 3 days) following VES diagnosis, and first remission at a median time of 11 days (range, 2 to 43 days). First-line therapy for VES was CNI discontinuation, and ISM with ruxolitinib (10 mg ~ 20 mg daily), corticosteroids, and Mycophenolate Mofetil (MMF) were continued. All patients were treated with supportive care. One patient was treated with rituximab. No patients were treated with defibrotide.

Treatment response

23 of the 25 patients obtained a hematologic or a complete response at 4 weeks after initiation of VES treatment according to our clinical response criteria. Besides, a hematologic response at 4 weeks after treatment was noted in 18 patients achieving a complete response with organ recovery. A greater number of patients had disappearance of reticulocyte, normalization of LDH, and improvement in HB

Table 1: Baseline demographic and clinical characteristics.

	VES patients (N=25)
Median age (years)	45 (15-79)
Gender	
Male	18 (72%)
Female	7 (28%)
Diagnosis	
MDS/MPN	9 (36%)
Leukemia	15 (60%)
Lymphoma	1 (4%)
Transplant modality	
HLA-haploidentical	17 (68%)
HLA-identical	7 (28%)
Other	1 (4%)
Donor source	
PBSC	11 (44%)
PBSC+BM	11 (44%)
PBSC+UCB	3 (12%)
Conditioning regimen	
BUCY	23 (92%)
TBI-CY	2 (8%)
Injury type	
CLS	4 (16%)
VOD/SOS	7 (28%)
TA-TMA	12 (48%)
ES	2 (8%)

Table 2: Comparison of hematological indices for patients receiving ruxolitinib.

	Before Ruxolitinib	After Ruxolitinib	P value
HB (g/L)	64 (31,123)	79 (35,123)	P=0.065[a]
PLT (× 10^9/L)	15 (4,74)	34 (4,179)	P=0.000[b]
LDH (U/L)	324 (102,3376)	232 (102,1188)	P=0.007[b]
CR (μmol/L)	84 (38,720)	69 (38,288)	P=0.04[b]

[a]Paired samples test; [b]Wilcoxon signed ranks test

and platelets transfusions. Hematological indices at onset and after treatment were observed in patients, and platelets improved most significantly after treatment, with median value of 34 (range 4 to 179, P<0.05) (Table 2).

Risk factors of development VES

On multivariable analysis, the risk factors that were associated with development of VES included acute GVHD, infections, CNI and other unknown factors. A common origin shared between VES and acute GVHD had already been proposed several years ago [15]. Our results show that 16 out of 25 patients (64%) had acute GVHD at VES diagnosis. In our study, 9 (36%) patients had liver injury, 2 (8%) patients had kidney injury, 12 (48%) patients had gastrointestinal involvement, 6 (24%) patients had bladder involvement, 5 (20%) patients presented with systemic edema, and 5 (20%) patients had ≥ 3 organs involved (Table 3).

Infection-related events were frequent in the present cohort. Patients were divided into two groups according to the presence or absence of infection before VES diagnosed. We observed that patients without co-infection were more likely to achieve a response compared with those with coinfection. The level of HB rose significantly in the non-coinfected group when patients was in remission [84 (52~123) *vs.* 56 (35~100) P<0.05], and PLT level were pullback to some extent [49 (15~161) *vs.* 20.5 (7~179) P<0.05]. Also, LDH level were decreased [196 (102~338) *vs.* 324 (168~1188) P<0.05]. There are no significant differences related to response in age, sex and hematological indices changes in different groups at start of ruxolitinib treatment were found (Table 4).

Patient outcomes

Contrasting the effect of ruxolitinib before and after, we found that HB, LDH, PLT and CR levels were obviously improved in the non-coinfected group (P<0.05). In contrast, there were no statistically significant differences in most indices in coinfected group (P>0.05) (Table 5 and Figure 1). These results indicated that patients without other infections before the use of ruxolitinib had better outcomes and treatment effect.

During the ruxolitinib treatment periods, 8 patients experienced disease progression, including 6 patients with TMA, 1 patient with VOD, 1 patient with CLS. Five of them had comorbid other infections before the use of ruxolitinib, and eventually died from infection and disease progression.

We further performed a survival analysis to calculate the 100-day survival rate, and the maximum observation time was 150 days (Figure 2). The analysis revealed that in the non-coinfected group, the cumulative proportion surviving was 100% on the 100th day (after VES diagnosis), while only 46% in coinfected group. The median survival time for the coinfected patients was 34.18 days. Also, as of the upper limit of observation time, all patients in the non-coinfected group survived. Moreover, Survival declined particularly rapidly during the first 40 days of treatment, that is, during this period patients were prone to death and survival was poor. To sum up, patients in the coinfected group had, as expected, inferior survival outcomes, and the results were statistically significant (P<0.05).

Adverse events of ruxolitinib

In our study, ruxolitinib side effects consisting of cytopenia and virus reactivation. Regarding drug-related toxicities, ruxolitinib was by and large well-tolerated, and only two patients (8%) developed severe hematuria and high CMV-DNA loads that necessitated discontinuation or reduction of the dose of ruxolitinib. Twelve patients (48%) had grade ≥ III cytopenia during ruxolitinib therapy, of whom only 3 (12%) had grade ≥ III neutropenia. Since a remission could be achieved after receiving supportive care (such as RBC/platelet infusion, and G-CSF et al.), most patients remained on active ruxolitinib therapy for VES (Table 6).

Also, ruxolitinib might increase the incidence of infections, which remained a major concern. Reactivation of the *Cytomegalovirus* (CMV) or Epstein-Barr virus (EBV) reactivation in 13 of all patients (13/25, 52%). More concretely, 13 patients had CMV reactivation, 9 patients had EBV reactivation and 9 patients had both viruses' reactivation. With ganciclovir or foscarnet treatment for CMV reactivation patients and rituximab preemptive therapy for EBV reactivation patients, none of the patients developed CMV disease or EBV post-transplantation lymphoproliferative disorder even though ruxolitinib therapy was continued. Sixteen patients (64%) were complicated with pulmonary infection, including pulmonary fungal diseases in two patients, sputum culture positive in *Flavobacterium*

Table 3: Specifics of acute GVHD at the time of VES diagnosis.

Patient	aGVHD	Organs involved						
		Liver	Kidney	GI	Bladder	Systemic edema	Skin	≥ 3 organs involved
1	YES			YES GI=4	YES	YES	YES Skin=4	YES
2								
3		YES			YES	YES		YES
4	YES			YES				
5	YES	YES		YES GI=4	YES			YES
6								
7								
8	YES	YES liver=4						
9	YES	YES				YES	YES	
10	YES			YES			YES	
11	YES		YES	YES				
12	YES	YES	YES		YES			YES
13	YES					YES		
14								
15								
16								
17	YES	YES		YES				
18		YES		YES			YES	
19	YES			YES			YES	
20	YES			YES			YES Skin=4	
21	YES			YES	YES	YES		YES
22	YES	YES		YES				
23	YES			YES	YES			
24	YES	YES						
25								

Table 4: Clinical characteristics in coinfected group and non-coinfected group.

	Coinfected group	Non-coinfected group	P value
Number	10	15	
Gender (Male/Female)	6/4	12/3	P=0.261[a]
Age	43.5 (26,63)	46.0 (15,79)	P=0.523[b]
Clinical data at diagnosis			
HB (g/L)	56.0 (31,110)	71 (55.123)	P=0.08[c]
PLT (× 10^9/L)	10.5 (4,74)	24 (5,59)	P=0.09[b]
LDH (U/L)	23 (181,3376)	324 (102,1268)	P=0.346[b]
CR (μmol/L)	377.5 (46,278)	94 (38,720)	P=0.579[b]
Clinical data after ruxolitinib			
HB (g/L)	56 (35,100)	84 (52,123)	P=0.002[c]
PLT (× 10^9/L)	20.5 (7,179)	49 (15,161)	P=0.025[b]
LDH (U/L)	324 (168,1188)	196 (102,338)	P=0.021[b]
CR (μmol/L)	74 (43,288)	69 (38,99)	P=0.657[b]

[a]Fisher's exact test; [b]Wilcoxon rank sum test; [c]T-test

meningitis in one patient, *Staphylococcus aureus* infection in one patient and mixed infection (Fungal/*Klebsiella pneumoniae)* in one patient. Of the entire cohort, two patients developed sepsis caused by *Pseudomonas aeruginosa* or *Escherichia* (Table 6).

Discussion

Hematopoietic Stem Cell Transplantation (HSCT) is associated with various Vascular Endothelial injury Syndromes (VES), including a series of complications represented by TA-TMA. There

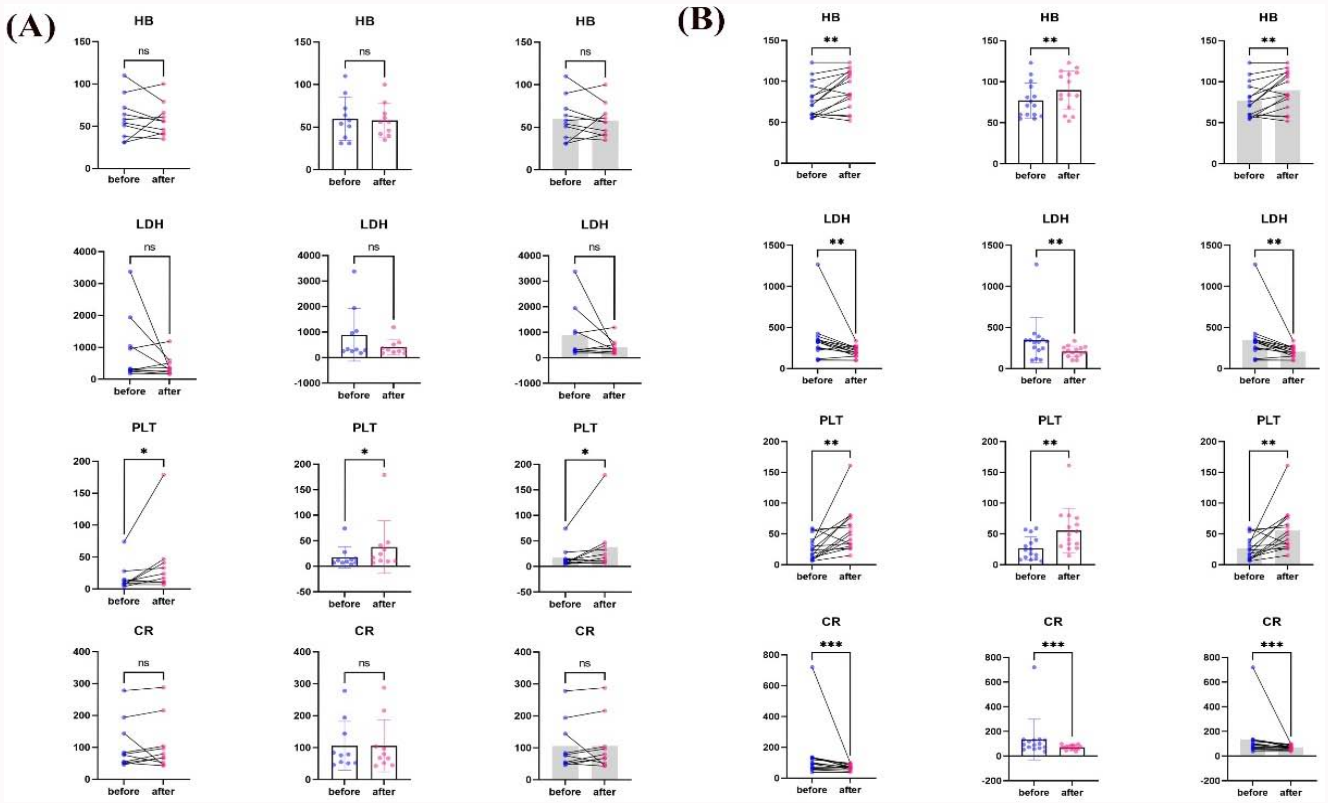

Figure 1: Wilcoxon signed ranks test compared the HB, LDH, PLT and CR levels in coinfected group (A) and non-coinfected group (B).

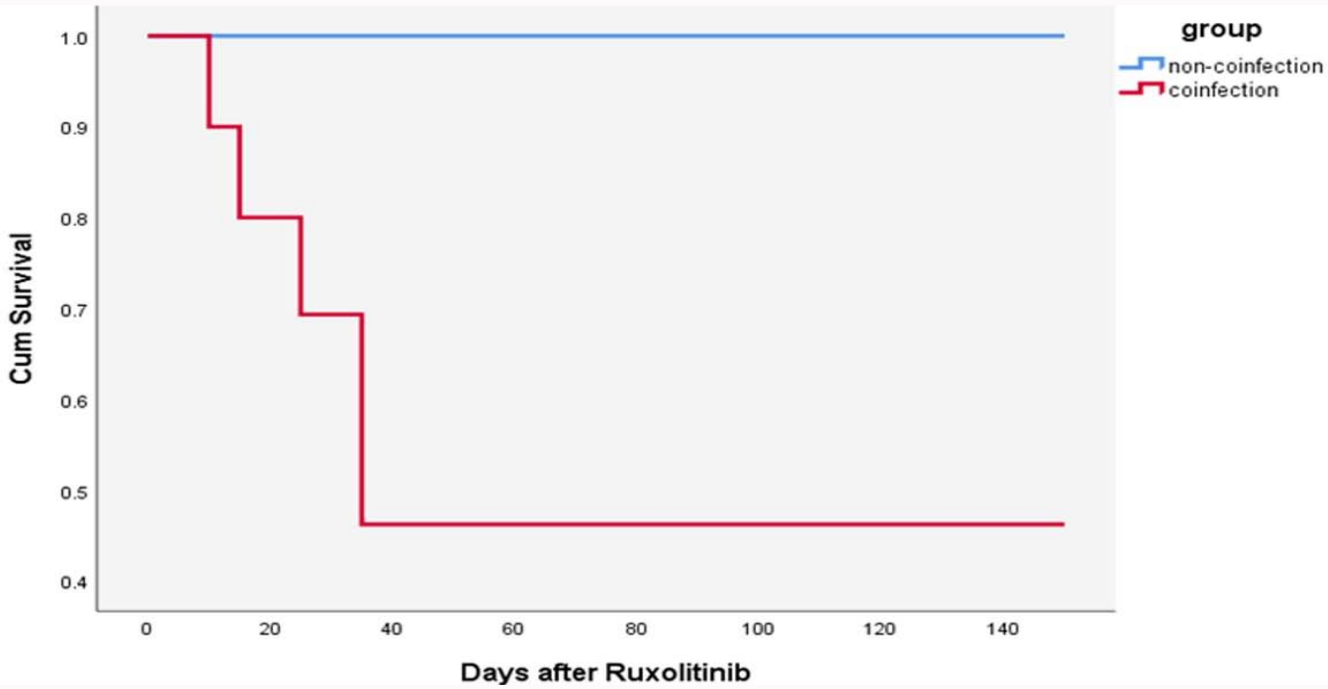

Figure 2: Cumulative survival on day 100. Efficacy of ruxolitinib to replace CNI in this small cohort was associated with infection, and patients in the coinfected group had lower cumulative survival rate on day 100. ($p<0.05$).

is growing evidence of the role of complement dysregulation in its pathophysiology [2,16,17]. Endothelial injury is fundamental to the pathogenesis of VES. Initiation of endothelial injury can be portrayed as a 'two-hit' process consisting of generation of risk factors [18-20]. In this context, ruxolitinib appear particularly promising as VES treatment, which in favor of alleviating microvascular endothelial injury by inhibiting the release of inflammatory cytokines.

The mainstay of initial management of VES is withdrawal or

Table 5: Comparison of hematological indices within groups (before and after treatment) in different groups (coinfected and non-coinfected group).

	Non-coinfected Group	Coinfected Group
Before_ HB - After_ HB	P=0.007[a]	P=0.758[a]
Before_ PLT - After_ PLT	P=0.003[b]	P=0.038[b]
Before_ LDH - After_ LDH	P=0.004[b]	P=0.260[b]
Before_ CR - After_ CR	P=0.001[b]	P=0.386[b]

[a]Paired samples t-test; [b]Wilcoxon signed ranks test

Table 6: Cytopenias, infections, dose reductions/modifications and supportive treatment for patients receiving ruxolitinib.

Patient	Cytopenia			Infection			
	After ruxlitinib	Dose reduction	Support therapy	Virus reaction	Others	Treatment	Outcome of infection
1	IV anemia/IV thrombocytopenia	Discontinuation	RBC/platelet/fresh frozen plasma infusion	CMV/ EBV	Pulmonary bacterial infection	GCV, caspofungin	Died from severe post-transplant infection
2	II anemia/II thrombocytopenia	NO	Fresh frozen plasma infusion	NO	Pulmonary infection	Caspofungin	Cured
3	III anemia/IV thrombocytopenia	NO	RBC/platelet infusion, EPAG	NO	Pulmonary infection	Moxifloxacin, cidofovir	Remission
4	III anemia/III thrombocytopenia	NO	RBC/platelet infusion	NO	Pulmonary bacterial infection	Tigecycline, caspofungin	Cured
5	III anemia/IV thrombocytopenia/IV neutropenia	NO	RBC/platelet infusion, G-CSF	CMV/ EBV	Pulmonary infection (flavobacterium meningitidis)	IVIG, cidofovir, meropenem	Progression
6	III thrombocytopenia	NO	Platelet infusion	NO	Sepsis(escherichia)	Imipenem	Cured
7	III thrombocytopenia	NO		NO			
8	III anemia/IV thrombocytopenia/IV neutropenia	NO	RBC/platelet infusion	NO			
9	III anemia/II thrombocytopenia	NO	RBC/platelet infusion	NO	Pulmonary infection	Imipenem	Cured
10	III anemia/IV thrombocytopenia	NO	RBC/platelet infusion	CMV/ EBV	Pulmonary infection	Moxifloxacin, azithromycin, caspofungin, GCV	Died from infection and TMA progression
11	III anemia	NO	RBC/platelet/fresh frozen plasma infusion	CMV/ EBV	Pulmonary infection, sepsis(aeruginosa)	Meropenem, FOS, GCV, IVIG, CMV/EBV-CTL	CMV/EBV (-), pulmonary infection remission
12	III anemia/II thrombocytopenia	NO	RBC/platelet infusion	CMV	Pulmonary infection	cephalosporin, caspofungin,cidofovir, IVIG	Cured, CMV (-)
13	III anemia/IV thrombocytopenia	NO	RBC/platelet infusion	CMV	Pulmonary infection (staphylococcus aureus)	cephalosporin, levofloxacin	Body temperature returned to normal, CMV(+)
14	II thrombocytopenia	NO		NO	Pulmonary bacterial infection		Cured
15	II anemia/III thrombocytopenia	NO	RBC/platelet infusion	NO	Urinary tract infection, pulmonary fungal infection	cephalosporin, caspofungin	Cured
16	II anemia	NO		NO			
17	III anemia/IV thrombocytopenia	10 mg bid >5 mg bid	RBC/platelet infusion, G-CSF	CMV/ EBV	Pulmonary infection	Cephalosporin, caspofungin, IVIG, GCV, FOS, voriconazole	Cured, CMV/EBV (-)
18	II thrombocytopenia/II neutropenia	NO		CMV/ EBV		Valacyclovir	CMV/EBV (-)
19	II thrombocytopenia	NO		CMV/ EBV		GCV, caspofungin	CMV/EBV (-)
20	III anemia/IV thrombocytopenia/III neutropenia	NO	RBC/platelet infusion, G-CSF	NO	Intra-abdominal infections, sepsis (candida glabrata)	IVIG, caspofungin,mer openem,cephalosporin, vancomycin	Sepsis remission
21	III anemia/III thrombocytopenia/II neutropenia	NO	RBC/platelet infusion, G-CSF, EPAG	CMV/ EBV	Pulmonary infection (fungal/KP)	levofloxacin, voriconazole	Cured, CMV/EBV (-)
22	II anemia/IV thrombocytopenia/II neutropenia	NO	RBC/platelet infusion, EPAG	CMV	Pulmonary infection	FOS, moxifloxacin	CMV (-)
23	III anemia/IV thrombocytopenia	NO	RBC/platelet infusion	CMV/ EBV	Pulmonary infection	Cidofovir, cephalosporin	EBV (-)
24	III anemia/III thrombocytopenia	NO	RBC/platelet infusion, EPAG	CMV		GCV, cidofovir, IVIG	CMV (-)
25	III thrombocytopenia	NO	RBC/platelet/fresh frozen plasma infusion	NO	Intra-abdominal infections	caspofungin, tigecycline	Cured

GCV: Gancyclovir; IVIG: Intravenous Immunoglobulin; KP: Klebsiella Pneumoniae; EPAG: Eltrombopag; FOS: foscarnet

minimization of CNI to reduce endothelial damage after HSCT. Carmona et al. [21] demonstrated that calcineurin inhibitors have a proinflammatory effect, which increases thromboxane A2 synthesis and decrease prostacyclin production, promoting procoagulant changes in vascular endothelial tissue, and leading to vascular endothelial cell injury. Traditionally ISM involves stopping the CNI or and mTOR inhibitors, and starting Mycophenolate Mofetil (MMF) and/or corticosteroids due to the risk of GVHD. However, along with our institutional experience, has led us to abandon the strategy of converting to MMF in this period where HSCT patients are particularly vulnerable to GVHD. Corticosteroids are also not an appropriate long-term option for GVHD prophylaxis and can leave patients susceptible to infections and numerous other side effects. Also, recent studies have shown that corticosteroid treatment impairs epithelial regeneration, limiting intestinal recovery in experimental GVHD [9]. A phase III clinical trial showed that the addition of MMF to corticosteroids as initial therapy for acute GVHD did not improve GVHD-free survival [22]. Some studies demonstrated that increased the risk of VES with the greatest risk in steroid-refractory GVHD patients [23,24]. Wall, SA et al. [5] demonstrated that endothelial injury and complement activation also in GVHD histologic evidence, particularly in steroid-refractory GVHD, and ruxolitinib has been approved for its treatments [25]. Therefore, there may be some benefit to switching CNIs or mTOR inhibitors to ruxolitinib to provide improved GVHD control.

In our study, 18 of 25 patients (72%) who were switched to ruxolitinib achieved hematologic resolution. Furthermore, acute GVHD as a common complication at VES diagnosis, and our results show that 16 out of 25 patients (64%) had acute GVHD at VES diagnosis. Immune etiology and inflammation may be a common contributing factor for VES risks, ruxolitinib may thus act as an immunomodulatory agent to protect endotheliocyte and reduce GVHD responsiveness by inhibiting inflammation. Also, researches of Zhao et al. [26] has provided the growing body of evidence to support the use of JAK inhibitors in GVHD [27].

Interestingly, we observed that coinfected patients ended to have poor treatment outcomes and survival outcomes. It should be noted that infection is closely related to the therapeutic effect. Ruxolitinib could reduce infectious tolerance and increase the incidence of CMV reactivation and various infection-related complications [28]. In this study, *Cytomegalovirus* reactivation was observed in 13 of 25 recipient, and CMV infection was controlled by antiviral therapy in all patients even though ruxolitinib therapy was continued, indicating that ruxolitinib treatment does not alleviate CMV treatment response, which is consistent with Zeiser et al. [29] reported.

In fact, the relationship between GVHD, infection, and VES is complex. In addition to diagnosis of GVHD, fungal and viral infections were associated with a greater risk of developing VES [5]. In our study, patients in the non-coinfected group had a more pronounced improvement in the therapeutic effect of replacement of CNI with ruxolitinib than patients in the coinfected group, with better hematologic remission and higher cumulative survival rates. We speculate that patients with VES caused by GVHD or CNI were had a good treatment effect of ruxolitinib, whereas VES caused by infection, treatment with ruxolitinib does not work well. Patients with well-controlled infection might be most likely to benefit from this alternative VES treatment strategy.

We acknowledge that our study is limited by its retrospective nature, and future studies seeking to disentangle the complex relationships between VES, GVHD, infection, and toxicities of the drugs used to treat and prevent these conditions are needed. These results should be interpreted with caution owing to the very small sample size, and the need for careful monitoring of infection remains crucial for patients with VES receiving ruxolitinib.

In summary, replacement of calcineurin inhibitors with ruxolitinib in patients with vascular endothelial syndromes following hematopoietic cell transplantation is an acceptable alternative, but the effect of infection on efficacy should be noted. Our findings may set the stage for future prospective testing of this approach in larger prospective clinical trials.

Acknowledgment

Hanyin Liang and Zhiping Fan contributed equally to this work and should be considered as co-first authors.

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