Treprostinil reduces endothelial damage in murine sinusoidal obstruction syndrome

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Abstract
Sinusoidal obstruction syndrome (SOS) is a major complication after hematopoietic stem cell transplantation and belongs to a group of diseases increasingly identified as transplant-related systemic endothelial disease. Administration of defibrotide affords some protection against SOS, but the effect is modest. Hence, there is unmet medical need justifying the preclinical search for alternative approaches. Prostaglandins exert protective actions on endothelial cells of various vascular beds. Here, we explored the therapeutic potential of the prostacyclin analog treprostinil to prevent SOS. Treprostinil acts via stimulation of IP, EP₂, and EP₄ receptors, which we detected in murine liver sinusoidal endothelial cells (LSECs). Busulfan-induced cell death was reduced when pretreated with treprostinil in vitro. In a murine in vivo model of SOS, concomitantly administered treprostinil caused lower liver weight-to-body weight ratios indicating liver protection. Histopathological changes were scored to assess damage to liver sinusoidal endothelial cells, to hepatocytes, and to the incipient fibrotic reaction. Treprostinil indeed reduced sinusoidal endothelial cell injury, but this did not translate into reduced liver cell necrosis or fibrosis. In summary, our observations provide evidence for a beneficial effect of treprostinil on damage to LSECs but unexpectedly treprostinil was revealed as a double-edged sword in SOS.

Key messages
- Murine liver sinusoidal endothelial cells (LSECs) express prostanoid receptors.
- Treprostinil reduces busulfan-induced cell death in vitro.
- Treprostinil lowers liver weight-to-body weight ratios in mice.
- Treprostinil positively affects LSECs in mice but not hepatic necrosis/fibrosis.

Keywords Prostacyclin · VOD · SOS · HSCT · Defibrotide · Transplant-related toxicity

Abbreviations
SOS Sinusoidal obstruction syndrome
HSCT Hematopoietic stem cell transplantation
ALT Alanine aminotransferase
LSEC Liver sinusoidal endothelial cells
PGI₂ Prostacyclin
PGE₂ Prostaglandin E₂
cAMP Cyclic adenosine monophosphate
IP I prostanoid
EP E prostanoid
Allo-HSCT Allogeneic stem cell transplantation
18S 18S ribosomal RNA
qPCR Quantitative PCR
H&E Hematoxylin-eosin
WBC White blood cell
Materials and methods

Isolation of primary murine cells and experiments with transformed murine LSECs

Primary hepatocytes and LSECs were isolated by liver perfusion as described previously [22]. Isolated primary hepatocytes and LSECs were immediately homogenized for RNA extraction. Vijay H. Shah (Mayo Clinic and Foundation, Rochester, MN, USA) kindly provided transformed murine sinusoidal endothelial cells (TSECs) with stable expression of SV40 large T-antigen [23]. TSECs were cultured in endothelial cell medium containing 5% fetal bovine serum, 1% penicillin/streptomycin, and 1% endothelial cell growth supplement (ECGS) (ScienCell Research Laboratories, San Diego, CA). For cell viability experiments, 24 h after plating (5 × 10^3 cells/24-well plate), 10 μM treprostinil was given to the medium. After 1 h pretreatment with treprostinil, busulfan (Sigma-Aldrich, Vienna, Austria) was added at concentrations ranging from 10 μM to 1 mM. Cell viability was assessed after 48 h. For this purpose, cells were washed twice with PBS, trypsinized, and counted using a hemocytometer and trypan blue. For MTT cell metabolic activity experiments, 24 h after plating (1.5 × 10^6 cells/96-well plate) in phenol-red free medium, 10 μM treprostinil was given to the medium. After 1 h pretreatment with treprostinil, busulfan was added at concentrations ranging from 125 μM to 1 mM. MTT assay was assessed after 48 h according to manufacturer’s instructions and absorbance was measured at 570 nm (Thermo Fisher Scientific, Waltham, MA). For [3H]cAMP accumulation assays, TSECs were incubated for 16 h with medium containing [3H]adenine (1 μCi ml^-1) and samples were processed as described previously [24–26]. Treprostinil (Remodulin®) was kindly provided by SciPharm SàRL (2540 Luxembourg City, Luxembourg).

Analysis of gene expression by quantitative PCR

Total RNA was isolated with TRI® Reagent (Sigma-Aldrich, Vienna, Austria) according to the manufacturer’s protocol. Total RNA was reverse-transcribed into complementary DNA (cDNA) using High Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA). The levels of murine transcripts encoding prostanoid I and prostaglandin receptors (Ep1, Ep2, Ep3, Ep4, and Ip) were assessed by quantitative PCR (qPCR). Murine 18S ribosomal RNA (18s) was used as a reference gene for qPCR. Each reaction condition was performed in triplicates. Relative abundance was calculated using the 2^ΔΔCt method (gene-specific expression level relative to that of the reference gene). The specific primer sequences listed in Table 1 were purchased from Microsynth AG (Balgach, Switzerland).
Mice

Male BALB/c and C57BL/6J mice were either purchased from the Jackson Laboratory (Bar Harbor, ME) through Charles River Germany (Sulzfeld, Germany) or were bred in-house (C57BL/6J). Mice were between 8 and 10 weeks old, and their body weight was in the range of 20–25 g.

Animal housing and husbandry were in accordance with the recommendations and requirements defined by the Federation of Laboratory Animal Science Associations (FELASA) in Europe. Mice were kept on a 12-h light-dark cycle in isolated ventilated cages with ≤5 mice/cage (Smart Flow and Easy Flow; Tecniplast, Buguggiate, Italy) at 21 ± 3 °C. Mice were fed autoclaved standard laboratory chow (commercial control diet for mice; Ssniff R/M-H, Soest, Germany) and water ad libitum. Animal technicians monitored animal welfare and health status daily under the supervision of a veterinarian. The experimental protocol was reviewed by the animal ethics committee of the Veterinary University of Vienna, approved by the Austrian Ministry of Science and Research under licenses BMWFW-68.205/0103-WF/V/3b/2015 and BMWFW-68.205/0047-V/3b/2018 and conducted according to the guidelines of FELASA and ARRIVE. Predefined humane end points included emaciation (i.e., weight loss > 25%), loss of activity (reduced mobility, prolonged crouching), loss of grooming/ruffled fur, or labored breathing. Mice meeting these criteria were killed by cervical dislocation.

Experimental animal model

The murine model of SOS described by Zeng et al. [27, 28] is based on an allogeneic hematopoietic stem cell transplantation (allo-HSCT), where preconditioning is achieved by whole body irradiation. In that model, the maximum of SOS is observed 15 days after allogeneic hematopoietic stem cell transplantation. After adaptation for 7 days, BALB/c mice were randomly divided into three groups: healthy control, allo-HSCT, and allo-HSCT + treprostinil. Mice assigned to allo-HSCT + treprostinil were pretreated 1 day before HSCT with treprostinil subcutaneously (0.15 mg kg\(^{-1}\) 8 h\(^{-1}\)) as described previously [24]. On the same day, recipient mice, i.e., the groups allo-HSCT and allo-HSCT + treprostinil, were exposed to whole body irradiation (7.5 Gy, split doses, 2 Gy min\(^{-1}\); Siemens Primus, 6MV, Siemens Austria); mice were placed in single chambers of an irradiation pie with 15 mice per pie. The radiation dose delivered was verified with a dosimeter. On the next day, recipient mice received 5 × 10\(^6\) bone marrow mononuclear cells containing 1.14 ± 0.26% hematopoietic stem cells via tail vein injection. Bone marrow mononuclear cells were obtained from donor C57BL/6 mice. Mice belonging to the group allo-HSCT + treprostinil were daily treated with subcutaneous injections of treprostinil for 15 days. Thereafter, all mice (healthy control, allo-HSCT, allo-HSCT + treprostinil) were euthanized.

Histology and immunohistochemistry

On day 15 after HSCT, mice were weighed and livers were immediately removed after euthanasia, weighed, fixed in 4% formalin, and embedded in paraffin for subsequent slicing. Liver weight was normalized for body weight by calculating the liver weight/body weight ratio. Sections prepared from formalin-fixed, paraffin-embedded organ specimens were stained with hematoxylin-eosin (H&E) or with Masson-Goldner trichrome using standard protocols. Light microscopic images were captured with a PixeLINK camera and the corresponding acquisition software on a Zeiss Imager Z.1.

Scoring of histopathology

Histological sections (two sections/animal) stained with H&E and Masson-Goldner trichrome were evaluated by a board-certified pathologist (JH), who was blinded to the nature of the treatment. The scoring system was based on the system described by Qiao and Zeng et al. [27, 28]. Histological changes were reviewed and scored (0 to 3 points/item) for the following seven pathological features: (i) endothelium injury in liver sinusoid or small hepatic veins, (ii) subendothelial hemorrhage, (iii) internal hemorrhage in the hepatic sinusoid, (iv) liver cell necrosis, (v)
fibrosis of the central veins, (vi) hepatic sinusoidal fibrosis, and (vii) inflammation in the central veins.

**Blood sampling and evaluation of plasma parameters**

Blood of euthanized mice was collected by heart puncture for analysis of blood cell count and biochemical parameters: white blood cell (WBC), red blood cell (RBC), and platelet (PLT) counts were determined using the Vet animal blood counter (scil animal care, Viernheim, Germany). Plasma levels of bilirubin and alanine aminotransferase (ALT) were assessed using the test strip-based Reflotron Plus analyzer (Roche, Basel, Switzerland).

**Statistical analysis**

The primary outcome parameter was hepatomegaly, and the number of animals was based on the following assumptions: the liver-to-body weight ratio (liver weight as % of body weight) was to increase from a mean of 5.5% with a standard deviation of 0.5% to 7.5 ± 0.7% on day 15 [27]. This increase was to be halved by treprostinil. Based on this assumption, we calculated that 18 animals/group were required to detect a statistically significant difference of \( p < 0.05 \) with a 90% probability. We included one and two additional mice in the treprostinil-treated and in the control recipient group, respectively, to account for possible dropouts due to bone marrow transplant failure; treprostinil enhances bone marrow
transplantation [24], hence we assumed that the dropout rate would be smaller. Secondary outcomes included ALT, bilirubin, and pathohistological scores.

Results

Target receptors of treprostinil are expressed by murine LSECs

Treprostinil, a stable analog of PGI2, stimulates IP, EP2, and EP4 receptors [17]. Our working hypothesis posits that treprostinil protects against SOS by acting on LSECs, the cell type thought to be responsible for the initiation of SOS. We isolated mRNA from primary murine LSECs and hepatocytes and evaluated the expression of E prostanoid receptors by qPCR, i.e., Ep1, Ep2, Ep3, Ep4, and Ip. Amplicons of receptors were detected in both primary murine hepatocytes and LSECs (Fig. 1a). The expression of treprostinil-target receptors Ep2, Ep4, and Ip was robust in LSECs with exceeding expression levels of the Ep4 receptor. To reduce animal numbers and circumvent technical difficulties (quantity; maintenance) with primary LSECs, we utilized an immortalized cell line derived from murine LSECs [23]. Similar to primary murine LSECs, these transformed sinusoidal endothelial
cells (TSECs) displayed robust expression of all target receptors of treprostinil, with $\text{EP}_4$ receptor expression being the highest (Fig. 1b). It is difficult to estimate how transcript levels and protein levels correspond, because there are neither good antibodies directed against EP and IP receptors nor suitable commercially available antagonist radioligands. Accordingly, we restored to measuring the cAMP response. Hematopoietic stem and progenitor cells have a comparable expression profile of Gs-coupled $\text{E}$ and I prostanoid receptors ($\text{EP}_2$, $\text{EP}_4$, and IP). At a concentration of 10 μM, treprostinil produced a robust near maximum stimulation of cAMP accumulation in hematopoietic stem and progenitor cells and its action was potentiated by forskolin [22]. Accordingly, we measured the cAMP response of TSECs in the presence of 10 μM treprostinil, 1 μM forskolin, and the combination thereof. The adenine nucleotide pool of TSECs was metabolically labeled with $[^3\text{H}]$adenine and their response to treprostinil...
Treprostinil attenuates busulfan-induced cell death in TSECs

In clinical conditioning regimes based on high-dosed busulfan, the increased risk of SOS is related to the toxicity of busulfan [29]. To test whether the toxicity of busulfan could per se induce cell death in TSECs, the compound was added at increasing concentrations and cell viability was assessed by trypan blue exclusion after 48 h. Indeed, busulfan induced cell death in a concentration-dependent manner (Fig. 2a). For this reason, we next investigated whether pretreatment with treprostinil and its continuous presence reduced busulfan-induced cell death. Therefore, treprostinil was added to TSECs 1 h before the addition of busulfan. Treprostinil attenuated busulfan-induced toxicity, which was assessed by cell viability, metabolic activity, and cell morphology after 48 h (Fig. 2b–d). Given the positive outcome observed in the in vitro studies, we further elucidated the effect of treprostinil in a living organism.

Pretreatment with treprostinil reduces hepatomegaly

We examined the action of treprostinil in a murine model of SOS, which relied on allo-HSCT in mice preconditioned by whole body irradiation [27]. Our approach was designed to detect a protective effect on the early insult to the sinusoidal endothelium. Accordingly, irradiated recipient BALB/c mice were subcutaneously treated with treprostinil 1 day prior to and the subsequent 15 days after the allo-HSCT (group: allo-HSCT + treprostinil; 0.15 mg kg\(^{-1}\) 8 h\(^{-1}\)). We selected this time interval based on the report of Zeng et al. (2013), where the signs of SOS reached a maximum on day 15. As the idea here was to facilitate HSCT and prevent SOS within the same treatment regime, we used in vivo the dose of 0.15 mg kg\(^{-1}\) 8 h\(^{-1}\) treprostinil, which in previous work had been shown to be optimal to improve HSCT [24]. Consistent with previous observations [27], we observed an increase in liver-to-body weight ratio from about 5.5% in the untreated control group to about 7.5% in recipient mice, which had undergone allogeneic hematopoietic stem cell transplantation (Fig. 3a). Administration of treprostinil protected against hepatomegaly: there was a statistically significant difference between untreated recipient mice and those treated with treprostinil (Fig. 3a). This difference in liver-to-body weight ratio was not accounted for by a distinct change in body weight: both untreated and treprostinil-treated recipient mice lost weight over a similar time course, the nadir was reached between days 6 and 9 (Fig. 3b). Similarly, we also determined the levels of circulating ALT (Fig. 3c) and bilirubin (Fig. 3d): the enzymatic marker of liver cell damage was elevated in recipient mice, when compared to control animals (Fig. 3b), but there was no statistically significant difference between untreated recipient mice and those treated with treprostinil recipient mice (Fig. 3b). In contrast, bilirubin levels were comparable in all three groups (Fig. 3c). In the treprostinil-treated group, one animal had to be euthanized for bone marrow failure, but the overall survival curves did not differ in a statistically significant way (Fig. 3e). In the 38 animals, which had survived until day 15, blood cell counts (i.e., for erythrocytes, leukocytes, and platelets) were comparable and within the normal range (Fig. 3f–h).

Histopathological consequences of treprostinil treatment

The data summarized in Fig. 3 indicated that administration of treprostinil mitigated the development of one of the cardinal symptoms of SOS, i.e., hepatomegaly. However, treprostinil did not have a clear-cut effect on liver damage assessed by plasma levels of ALT. Histological sections of the liver were surveyed to explore the reason for this discrepancy. The changes were scored (0 to 3 points/item) along seven dimensions [27]: the resulting quantification is shown in Fig. 4. Representative micrographs are displayed in Fig. 5. As evident from this analysis that the administration of treprostinil...
reduced damage to the liver sinusoids, because endothelium injury (Figs. 4a and 5a), subendothelial hemorrhage (Figs. 4b and 5b), and internal hemorrhage in hepatic sinusoids (Figs. 4c and 5c) occurred more frequently in animals that had undergone allogeneic hematopoietic stem cell transplantation than in in treprostinil-treated recipient mice. However,
liver cell necrosis was not prevented but rather aggravated in treprostinil-treated recipient mice (Fig. 4d). Consistent with the increased vulnerability of zone 3, the liver cell necrosis was most pronounced at the center of the liver acini but also was associated with inflammation and hemorrhage (Fig. 5d). Similarly, treatment with treprostinil enhanced the inflammatory response surrounding the central veins (Figs. 4g and 5g). Finally, the fibrosis detected by Masson-Goldner trichrome staining was most frequently seen in treprostinil-treated recipient mice in both the hepatic sinusoids (Figs. 4f and 5f) and the area surrounding the hepatic central veins (Figs. 4e and 5e). In our blinded assessment, we also detected cell necrosis of the liver as well as signs of inflammation and fibrosis in healthy BALB/C mice not subjected to any treatment (Fig. 4d–g). The reason for this preexisting pathology is not clear. However, we stress that we verified that the animals did not carry any specific pathogens; in particular, they were free of mouse hepatitis virus.

**Discussion**

SOS is a challenging disease from the perspective of both basic research and clinical management. Toxic injury to liver sinusoidal cells is thought to be the central pathogenic event that occurs in the early phases after HSCT. We employed a murine SOS model, which is known to peak on day 15 and to subsequently resolve spontaneously [27]. Accordingly, we selected day 15 to assess the extent of liver damage. Our observations recapitulated the original findings; this is most readily evident by considering the extent of hepatomegaly and the elevations of ALT levels: our values are in excellent agreement with those reported by Zeng et al. [27]. Similarly and consistent with the observations, we also found that changes in bilirubin levels were less useful to monitor hepatic damage. This is not unexpected because ablation of the bone marrow and the subsequent reconstitution of the bone marrow by hematopoietic stem cell transplantation are likely to affect red blood cell dynamics and thus the production of bilirubin. Our study was powered to detect a beneficial effect of treprostinil provided that it resulted in a reduction in liver weight. Based on hepatomegaly as primary outcome parameter, the treatment of recipient mice with treprostinil was indeed effective in mitigating SOS.

Our working hypothesis posited that treprostinil acted via Gs-coupled prostanoid receptors on the LSECs. We verified the presence of transcripts of Ep2, Ep4, and Ip receptors in LSECs. Similarly, the histological analysis suggested that treprostinil reduced injury to liver sinusoidal endothelial cells. The effect, which we detected in the histopathology, was modest, presumably because our analysis was limited to a snapshot on day 15, i.e., long after the initial injury. More importantly, the histological analysis revealed a damaging effect of treprostinil on the hepatocytes. This is surprising, because prostaglandins are thought to be beneficial in liver injury and their involvement in regeneration is complex: PGE2 and to a lesser extent PGI2 stimulate hepatocyte proliferation [30]; the effect is relevant under in vivo conditions, because genetic ablation of the prostaglandin degrading enzyme 15-hydroxyprostaglandin dehydrogenase (15-PGDH) or its inhibition by a small molecule enhances liver regeneration after partial hepatectomy [31]. Conversely, liver regeneration after surgical removal of liver tissue is blunted upon inhibition of cyclooxygenase-2 and to a lesser extent of cyclooxygenase-1, the enzymes that catalyze the conversion of arachidonic acid to prostaglandin H2, the precursor of PGE2, PGI2, and other prostaglandins [32]. These findings suggest a role of endogenous PGE2 in promoting hepatocyte regeneration. It is less clear which prostanoid receptors mediate the beneficial actions of PGE2: originally, Gs-coupled E prostanoid receptors were shown to be more important for stimulating the proliferation of hepatocytes than Gi-coupled receptors [30], but in ischemia-reperfusion injury, the protective effect was conveyed by stimulation of the EP4 receptor [33]. However, more recently, the beneficial effects of PGE2 and in particular of the EP4 receptor have been questioned [34]; in fact, inhibition of PGE2 synthesis—by deletion of the inducible microsomal PGE synthase-1 or by its inhibition with a small molecule—mitigated liver cell necrosis resulting from ischemia and reperfusion. Similarly, blockage of the EP4 receptor—not of the EP1 or of the EP2 receptor—protected against hepatocyte damage and reduced necrotic areas in mice, in which the EP4 receptor was absent due to genetic deletion [34]. Thus, the available evidence shows that the activation of Gs-coupled prostaglandin receptors results in both enhanced and reduced hepatocyte necrosis depending on the nature of the injury and the time of the snapshot. In fact, the conflicting results of Kuzumoto et al. [33] and of Nishizawa et al. [34] can be rationalized by considering that the time points sampled differed: Kuzumoto et al. [33] examined early events (i.e., 2 h after reperfusion), while the observation period of Nishizawa et al. [34] focused on changes occurring days after ischemia and reperfusion. The pathophysiology underlying damage caused by SOS is presumably more akin to that of ischemia and reperfusion than that of partial hepatectomy. Furthermore, our analysis focused on late events, which are presumably more relevant for the clinical treatment of SOS. Taken together, the previously published findings and our current observations indicate that treprostinil is a double-edged sword. While its action on the LSECs and on platelets may be beneficial, its action on macrophages/Kupffer cells and/or hepatocytes limits the usefulness in the treatment of SOS. This is presumably also true for other agonists, which target Gs-coupled prostanoid receptors.

Defibrotide is the only drug which is currently available for the treatment of SOS [4, 5]. The approval of defibrotide is
The beneficial effect would have outweighed the detrimental action. However, we consider it unlikely that this can be detected with an ethically justifiable number of experimental animals because of the large inherent variability in the course of SOS. Currently, prostaglandins such as PGE$_1$ are not recommended for the treatment of SOS [35], because an encouraging initial trial [15] was not replicated [16]. At the very least, our observations provide an explanation for this failure: in our murine SOS model, stimulation of G$_c$-coupled was protective for the endothelium of the liver sinusoids, but we also detected a damaging effect of treprostinil on hepatocytes. This detrimental action is likely to limit or cancel out any benefit arising from the administration of prostaglandins in human SOS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval The experimental protocol was reviewed by the animal ethics committee of the Veterinary University of Vienna, approved by the Austrian Ministry of Science and Research under licenses BMWF-68.205/0103-WF/V/3b/2015 and BMWF-68.205/0047-V/3b/2018 and conducted according to the guidelines of FELASA and ARRIVE.

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References

1. Cooke KR, Jannin A, Ho V (2008) The contribution of endothelial activation and injury to end-organ toxicity following allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 14(1 Suppl 1):23–32
2. Fan CQ, Crawford JM (2014) Sinusoidal obstruction syndrome (hepatic veno-occlusive disease). J Clin Exp Hepatol 4(4):332–346
3. Mohty M, Malard F, Abecassins M, Aerts E, Alaskar AS, Aljurf M, Arat M, Bader P, Baron F, Bazarbachi A, Blaise D, Ciceri F, Corbicagiou S, Dalle JH, Duarte RF, Fukuda T, Huynh A, Masszi T, Michallet M, Nagler A, NiChonghaile M, Pagluica T, Peters C, Petersen FB, Richardson PG, Ruutu T, Savani BN, Wallhult E,
Martino L, Djuric Z, Milne GL, Chance M, Sanabria J, Dealwis C, Mikkola D, Naidoo J, Wei S, Tai HH, Gerson SL, Ready JM, Posner B, Willson JK, Markowitz SD (2015) TISSUE REGENERATION. Inhibition of the prostaglandin-degrading enzyme 15-PGDH potentiates tissue regeneration. Science 348(6240):aaa2340

32. Rudnick DA, Perlmutte DH, Muglia LJ (2001) Prostaglandins are required for CREB activation and cellular proliferation during liver regeneration. Proc Natl Acad Sci U S A 98(15):8885–8890

33. Kuzumoto Y, Sho M, Ikeda N, Hamada K, Mizuno T, Akashi S, Tsurui Y, Kashizuka H, Nomi T, Kubo A, Kanehiro H, Nakajima Y (2005) Significance and therapeutic potential of prostaglandin E2 receptor in hepatic ischemia/reperfusion injury in mice. Hepatology 42(3):608–617

34. Nishizawa N, Ito Y, Eshima K, Ohkubo H, Kojo K, Inoue T, Raouf J, Jakobsson PJ, Uematsu S, Akira S, Narumiya S, Watanabe M, Majima M (2018) Inhibition of microsomal prostaglandin E synthase-1 facilitates liver repair after hepatic injury in mice. J Hepatol 69:110–120

35. Dignan FL, Wynn RF, Hadzic N, Karani J, Quaglia A, Pagliuca A, Veys P, Potter MN (2013) BCSH/BSBMT guideline: diagnosis and management of veno-occlusive disease (sinusoidal obstruction syndrome) following haematopoietic stem cell transplantation. Br J Haematol 163(4):444–457