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"ELASTOVOD PROJECT: Prospective studies to evaluate the preclinical diagnostic potential of hepatic ultrasound-based elastography measurement for predicting the development of Sinusoidal Obstruction Syndrome (SOS/VOD) and hepatic complications in patients undergoing hematopoietic stem cell transplantation (HSCT)"

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ABSTRACT

BACKGROUND

Veno-occlusive-disease (VOD), also known as sinusoidal-obstruction-syndrome (SOS), is one of the main complications of hematopoietic stem cell transplantation and is related to the treatment with pyrrolizidine alkaloids or other toxic agents (chemotherapy for liver-metastasis). Clinical diagnosis using the new criteria from the European Society for Blood and Marrow Transplantation is the reference for SOS/VOD diagnosis. However, increasing evidence suggests the emerging role of several imaging methods that could help the clinician in VOD/SOS assessment. The survival rate is higher when earlier specific therapy is initiated, thus improving early, non-invasive diagnosis of SOS/VOD is strongly needed. We aimed to assess in patients undergoing HSCT, the SOS/VOD diagnostic role of Liver Stiffness Measurement (LSM) evaluated by many elastography techniques.

METHODS

The ElastoVOD project was an interventional study without drugs developed in two sequential phases: the first was monocentric proof of the concept study in Bologna from April 2015 to November 2017; the second was a national multicentre study involving around 47 Italian transplant centres and is still ongoing (clinicaltrial.gov NCT03426358). LSM was performed before HSCT and at day +9/10, +15/17 and +22/24 post-HSCT. EBMT-criteria were used to establish a VOD/SOS diagnosis.

RESULTS

Among the 456 patients undergoing HSCT, 20 patients developed SOS/VOD (4.4%) during the study period. A sudden increase of LSM, compared to the previous assessment and pre-HSCT measurement, was found in all patients who developed SOS/VOD. LSM increases occurred from 1 to more than ten days before clinical SOS/VOD appearance. The SOS/VOD diagnostic performance of increased LSM over pre-HSCT assessment showed Area under the ROC Curve (AUROC) 0.997 (Sens. 75%; Spec. 98.7%). LSM gradually decreased following successful SOS/VOD specific treatment. Interestingly in the adult patient's subgroup, LSM values did not significantly increase in patients experiencing hepato-biliary complications (according to common terminology criteria, CTC) other than SOS/VOD.

CONCLUSION

Elastography techniques measuring liver stiffness (LSM) represent the most recent and promising approach to perform an early, pre-clinical diagnosis and follow-up of SOS/VOD. In our view, a multidisciplinary approach to the SOS/VOD diagnosis should be highly encouraged.

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PART 1 – LITERATURE REVIEW

1. Sinusoidal obstruction Syndrome SOS

Definition and History of the disease

Sinusoidal Obstruction Syndrome (SOS), also known as Hepatic Veno-Occlusive Disease (VOD) was first described by a pathologist from Prague in 1905 as an obliterative endophlebitis in the terminal hepatic veins of the human liver lobule, suggesting syphilis as the only possible etiology [1]. This entity was recognised in South Africa in 1920 as cirrhosis resulting from Senecio poisoning in humans. It was further characterised in the West Indies in the 1950s, based on conspicuous congestion of sinusoids and hemorrhagic necrosis in the centrilobular area while large hepatic veins were patent and there was a non-thrombotic occlusion of central and sublobular hepatic veins by subendothelial oedema and fibrosis[2]. In 1954, terminal vein lesions were described in Jamaican drinkers of bush tea, characterised by eradication of hepatic vein radicals by varying amounts of subendothelial swelling and delicate reticulated tissue[2]. At later stages, a fibrous pericentral scar developed. [3] It was soon recognised that the disease was associated with the consumption of "bush tea". In the early 1960s, studies of the effects of ionising

radiation on mammalian tissues documented that the hepatic vasculature could be damaged by this mechanism [4], in the absence of antecedent vascular thrombosis [5,6]. The most remarkable example of an obliterative venous lesion induced by irradiation was observed in humans with lung tumours treated with radiation; both the lung vasculature and that of the dome of the liver that was included in the radiation field developed vascular obliteration, but not the remainder of the unexposed liver [7]. Shortly after that, 12 patients who were receiving upper abdominal irradiation by the Stanford Linear Accelerator showed evidence of the induction of obliterative venopathy, as a consequence of heavy irradiation of the human liver for metastatic [8]. Thus, by the mid-1960s, the concept of hepatic *veno-occlusive disease* was well-known, caused by either chemical or radiation toxicity, and as a lesion separate from Budd-Chiari syndrome and Banti syndrome [9,10]. In the late 1970s, similar histologic lesions were found in outbreaks in India and Israel, attributed to contamination of wheat and traditional herbal remedies with plant toxins [11,12]. The histological lesions resembled previously described hepatic veno-occlusive lesions observed in rats infected with *Senecio* plant extracts[13] or *Crotolaria*[6]. This type of liver toxicity was ultimately associated with hepatic exposure to plant pyrrolizidine alkaloids, proving these plant toxins as the cause of

veno-occlusive disease in users of herbal teas. A relationship with similar lesions found in cattle exposed to pyrrolizidine alkaloid-containing plants was rapidly established.

Pyrrolizidine alkaloids are many, varying in structure and origin. They are mostly found in plants, of several families, including about 3% of the world's flowering plants. Following ingestion, they are absorbed from the gut and transformed by hepatic cytochrome P450, CYP3A and CYP2B into so-called DHP esters. These metabolites react rapidly with functional groups on DNA, proteins or glutathione to form DHP adducts and rapid spontaneous hydrolysis of DHP esters forms less reactive intermediates that can diffuse outside the liver. DHP adducts may further induce toxic effects. The differences in metabolism among different species are likely to explain part of the species susceptibility to pyrrolizidine alcaloids[14].

In the 1970s, besides the endemic cases related to the consumption of bush teas, epidemic forms of the disease have been described as a result of consuming products made from wheat contaminated with seeds of pyrrolizidine alkaloid-containing plants[15]. These epidemics occurred in a context of war or drought, modifying regular harvesting and allowing for toxic plants to contaminate crops. Attack rates have been up to 30% based on clinical examination and associated with fatalities or complete recovery within the same

epidemics. The findings from endemic cases have been similar to those from histopathological studies, spanning from acute centrilobular congestion with occlusion of central veins to cirrhosis. Such outbreaks of SOS/VOD have still been reported into the 1990s.

Since the mid-1980s, several sporadic cases related to the consumption of herbal remedies have been reported from western countries or China. Recently, attention has been drawn to the possible SOS/VOD occurring as a result of the erroneous substitution of pyrrolizidine alkaloid-containing to non-pyrrolizidine alkaloid in herbal remedy [16]. Epidemiological studies have recently on low-level dietary exposure to pyrrolizidine alkaloids, e.g. consuming honey from plants containing pyrrolizidine alkaloids[17]. There is a lack of evidence for significant toxicity from such low-level dietary exposure, but this is challenging to be verified in humans [14]. They could explain in part some endemic/epidemic toxic liver injury related to co-exposure to DTT, as recently reported for the Hirmi valley disease[18]. Recent efforts have also focused on identifying accurate biomarkers for exposure to pyrrolizidine alkaloids. Serum pyrrole-protein adducts may prove valuable in this regards [16].

The long-used administration of pyrrolizidine alkaloids to animals of various species has allowed a demonstration of direct responsibility

in inducing the liver changes. A reproducible rat model was eventually developed, consisting of gavage with monocrotaline for 1 to 10 days before sacrifice[19]. This model showed early injury to sinusoidal and central vein endothelium, preceding the development of veno-occlusive lesions. The latter could be explained by the contiguity of venous subendothelial areas with the space of Disse, where endothelial denudation allowed accumulation of erythrocytes and cellular or non-cellular debris. These findings lead the new denomination of "SOS" being proposed for the entity, in order to better account for damage to sinusoidal endothelium rather than occlusion of the central vein as a primary event. Coagulative necrosis of hepatocytes was also found to occur later than endothelial injury [19]. This model further provided clues to understand how toxic metabolites, which are produced in the hepatocytes, induce more severe damage to endothelial cells than hepatocytes. Indeed, a decrease in glutathione content was more profound in endothelial cells than in hepatocytes.

Moreover, supply in exogenous GSH through the portal vein protected from SOS/VOD [20]. Further experiments showed that the earliest changes detectable in sinusoidal endothelium, the rounding up of a sinusoidal endothelial cell, was dependent on the production of matrix metalloproteinase nine and two by endothelial

cells[21], which could be induced by a decreased production of nitric oxide [22]. This experimental model also allowed for showing that bone marrow-derived progenitors replace sinusoidal and central venous endothelial cells after injury and that monocrotaline suppresses endothelial cell progenitors in the bone marrow and circulation [23]. Therefore, SOS/VOD appears to be a disease resulting from 2 combined mechanisms:

- toxic injury to sinusoidal/central venous endothelial cells;
- toxic injury to bone marrow progenitors preventing the replacement of the damaged endothelial cells in sinusoids and central veins.

These concepts are highly relevant to hematopoietic stem cell transplantation.

Bone marrow transplantation for humans with leukaemias became a therapeutic option during the 1960s. Initial challenges to this new therapy were the preservation of harvested marrow and achieving successful marrow engraftment[24]. Reports of hepatic veno-occlusive disease in patients undergoing bone marrow transplantation emerged in the 1970's[25],; a number of different studies followed this research and validated the following apparent risk factors: bone marrow transplantation for malignancy, involving intense chemotherapeutic and radiation conditioning regimens;

patient age over 15 years; and in particular, abnormal pretransplant serum levels of liver enzymes[26–28]. Moreover, also the presence of metastatic liver disease, predisposed to veno-occlusive disease [28,29].

In these initial years of veno-occlusive disease as a complication of induction regimen before bone marrow transplantation, the prevalence of veno-occlusive disease varied from 21% to 25% in allogeneic graft recipients [28,30,31], to 5% in recipients of autologous marrow[28,32,33]. In the four decades since routine use of bone marrow transplantation for solid malignancies, lymphomas and leukaemias, induction regimes and therapies have helped to improve, but not to eliminate, the incidence of this condition in the transplant population. Up to date, the incidence of SOS/VOD is primarily in the setting of hematopoietic stem cell transplantation (**Table 01**). The initial definition of VOD suggested the primary involvement of hepatic venules. However, it is now known that the primary sites of toxic injury are sinusoidal endothelial cells due to hepatoxic agents, leading to a loss of sinusoidal wall integrity, endothelium detachment and embolisation towards the centrolobular zone of the hepatic acinus[34]. These events lead to a block in the liver blood outflow, with sinusoidal obstruction and consequent congestion, which can also lead to the development of

(post)sinusoidal portal hypertension (PH)[35]. Other features, such as marked sinusoidal fibrosis, necrosis of pericentral hepatocytes, and the narrowing and fibrosis of central veins, have also been described in liver biopsies[36].

Young children and adults were both affected. Several variants of clinical presentation were described:

- Acute presentation with rapid and massive abdominal swelling and pain associated with haemorrhagic centrilobular necrosis;
- Subacute presentation with recurrent ascites, splenomegaly and hepatomegaly, associated with extensive fibrosis in centrilobular areas;
- Chronic variant indistinguishable at the bedside from cirrhosis of other origins but showing a venocentric type of cirrhosis at histological examination.

A possible full clinical, biochemical and pathological recovery was recorded in half the patients, a rapid death in 20% of patients, and the development of decompensated liver disease in the rest.

Table 01: Main causes of Sinusoidal Obstruction Syndrome (SOS)

Hematopoietic stem cell transplantation (HSCT)
Adjuvant or neoadjuvant chemotherapy with hepatectomy for metastatic liver disease
Radiation-induced liver disease
Chemotherapy for acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL) CD22+
Liver transplantation
Use of herbal remedies
Veno-occlusive disease with immunodeficiency (VODI)

Epidemiology

Although several agents can be the carriers of the syndrome, the most common cause of SOS/VOD in the West today is certainly HSCT[37]. In fact, SOS/VOD affects 14% (5-60%) of patients who received an allogeneic or autologous HSCT[38], with significant differences in its incidence depending on several risk factors[39], the clinical diagnostic criteria chosen and the use of Reduced Intensity Conditioning (RIC) regimens[40].

Pathogenesis

Experimental Investigation in Animals

The first experimental efforts to induce veno occlusive disease in animals concentrated mainly on irradiation. Even though the non-human primate liver is relatively resistant to radiation-induced veno-

occlusive disease, [41], veno-occlusive lesions could be induced in primates [42] and non-primates[43] by exposing them to the chronic irradiation regimes. However, the underlying pathogenesis of this condition was not demonstrated with these early experimental models. Careful ultrastructural examination of human tissues suggested that the initial morphologic change in the hepatic veno-occlusive disease was the obstruction at the level of hepatic sinusoids, followed by obliteration of the terminal hepatic veins [36]. In 1999 a study with a more economical and reproducible rodent model of veno-occlusive disease confirmed this observation [19]. The earliest documented alteration found in rats gavaged with the pyrrolizidine alkaloid monocrotaline and killed between days 1–10 after exposure, was damage to the hepatic sinusoids; fibrosis and obliteration of the terminal hepatic veins occurred as a subsequent lesion. This articulation that toxic injury to the hepatic sinusoids was the primary lesion of the hepatic veno-occlusive disease led to its being renamed sinusoidal obstruction syndrome (SOS)[34].

The rat model of SOS/VOD may be summarised as follows[19,44]. Following a single gavage with monocrotaline, within 24 h–48 h there is ultrastructural evidence of damage to hepatic sinusoidal endothelial cells (SEC), but little clinical or histological evidence of hepatic toxicity. By days 3–5 (early SOS), sinusoidal

obstruction evidence is quite severe, with huge centrilobular necrosis and haemorrhage, damage to endothelial cells of the terminal hepatic veins, subendothelial haemorrhage, and ultrastructural evidence of extensive destruction of the sinusoidal wall. The clinical manifestations are hepatomegaly, ascites, and hyperbilirubinemia. By days 6–7 (late SOS), the characteristic subendothelial and adventitial fibrosis of terminal hepatic veins becomes more evident. There is continued sinusoidal and subendothelial haemorrhage, but the gradual resolution of the ultrastructural evidence of damage to the sinusoidal endothelial cells. By days 8–10, SOS/VOD has entirely resolved in some animals or persisted as a severe pattern of hepatic damage in others.

A detailed study of the first hours after monocrotaline exposure[44] reveal that SECs become swollen, with increased adhesion of leukocytes to the endothelium. Red blood cells dissect beneath the endothelial cells and into the space of Disse, separating the endothelial cells from the underlying hepatocytes and permitting free access of blood to the parenchymal space. In contrast, blood flow in the restricted sinusoidal channel becomes sluggish. The sinusoid is eventually obstructed by an embolism of aggregated sinusoidal lining cells, red blood cells, and adherent monocytes. Kupffer cells are lost along the sinusoidal lining and are replaced with an influx of

circulating phagocytic monocytes which accumulate in the injured centrilobular area. The specific toxicity of monocrotaline is that of its reactive metabolite, monocrotaline pyrrole, which binds covalently to the actin microfilaments of endothelial cells[12]. The F-actin depolymerises causing the disassembly of the actin cytoskeleton and rounding up of the endothelial cells. Increased expression and release of matrix metalloproteinase-9 into the extracellular space causes the breakdown of the extracellular matrix in the space of Disse, allowing further dehiscence of the endothelial cells [44].

These experiments started to suggest that SECs are more susceptible to toxic injury than hepatocytes. SEC glutathione depletion, nitric oxide depletion, increased expression of matrix metalloproteinases and vascular endothelial growth factor (VEGF), and activation of clotting factors are considered some of the most relevant pathogenic factors[45]. In the beginning, drugs leading to SOS/VOD are metabolised exclusively by the hepatocellular cytochrome P450 systems, for which glutathione is an antioxidant recovery mechanism. Hepatic exposure to these drugs causes the depletion of glutathione. Severe deficiency of glutathione levels in SECs renders them susceptible to cell death; prophylactic infusion of glutathione or N-acetyl cysteine prevents the development of SOS/VOD in the monocrotaline-treated rat model[44]; post-hoc administration of

glutathione lowers the degree of sinusoidal injury, but does not avoid it. The central role of glutathione depletion is highlighted by additional evidence including the predisposition to SOS/VOD in humans with a glutathione S-transferase M1 (GSTM1) null genotype who are undergoing bone marrow transplantation[46] or receiving oxaliplatin for metastatic colon cancer [47]. In the second instance, nitric oxide levels in the hepatic vein of rats decrease during induction of SOS/VOD [48]. The induction of SOS/VOD can either be exacerbated by administration of N(G)-nitro-l-arginine methyl ester (l-NAME), an inhibitor of nitric oxide synthase or be mitigated by infusion of V-YRRO/NO, a liver-selective nitric oxide donor prodrug [22]. Vasoconstriction plays different roles as not only provides evidence for nitric oxide depletion in the pathogenesis of SOS/VOD [48] but also shows that adequate nitric oxide levels inhibit vasoconstriction of hepatic stellate cells, which invest the hepatic sinusoidal from within the space of Disse [49].

Matrix metalloproteinase-9 expression increases early in the rat monocrotaline-induced SOS/VOD model; later, the increase in matrix metalloproteinase-2 is of a lower degree. SECs are proved to be a relevant origin of both basal and monocrotaline-induced enzyme expression and release. The development of experimental SOS/VOD is avoided by the Administration of matrix metalloproteinase

inhibitors [21]. This implicates degradation of the extracellular matrix within the space of Disse as contributing to the loss of endothelial cell adhesion. DeLeve and associates utilised the rat model of monocrotaline-induced SOS/VOD to demonstrate that the repair of sinusoidal endothelial cells (SEC) by constitutive bone marrow stem cell is critical to the maintenance of the sinusoidal architecture[23]. Bone marrow suppression by irradiation through impairing SEC repair mechanism may contribute to the pathogenesis of SOS.

The role of VEGF as an angiogenic factor has less clear evidence. Iguchi et al. observed increased serum levels of VEGF during the development of SOS/VOD in human patients[50]. This observation is leaving open the question of whether VEGF-induced acceleration of vasopermeability, neovascularisation, and expression of coagulopathic tissue factors on circulating mononuclear cells may be relevant in SOS/VOD pathogenesis[45]. Nakamura et al. [51] hypothesised that antiangiogenic agents might be a protection against SOS/VOD and decided to utilise sorafenib to test this hypothesis. Sorafenib is a multiple receptor tyrosine kinase inhibitor that is responsible for the inhibition of multiple tyrosine kinases, including the VEGF receptor-2 (VEGFR-2) and -3 (VEGFR-3). Its primary usage is found in the standard treatment for hepatocellular carcinoma

and renal cell carcinoma, but it may also have an antifibrotic effect in the liver and prevent the development of portal hypertension[52,53]. Nakamura treated rats with sorafenib before induction of SOS/VOD by monocrotaline before partial hepatectomy, and showed significant suppression of the morphological features of SOS, with significant improvement in post-hepatectomy survival[51]. While the loss of endothelial cells was not completely blocked, it did suppress the degradation of the extracellular matrix in the space of Disse and uplift of the endothelial cells. Hence, although the link to VEGF is not proven owing to the broad inhibitory action of sorafenib on tyrosine kinases, remodelling of the extracellular matrix in the space of Disse is demonstrated to be a contributing factor to the dehiscence of sinusoidal endothelial cells in the development of SOS. radiation-induced liver disease (RILD) has become the centre of attention due to recent technological advances in radiation therapy [54]. Although whole-liver irradiation has generally been restricted to 30–35 Gy in standard daily fractions of 1.8–2.0 Gy owing to the risk of lethal RILD above these levels, intensity-modulated radiation therapy, 3-dimensional conformal radiation treatment planning, and organ and tumour motion tracking enable treatment of liver cancer with fewer but larger dose fractions. This innovation is called “hypofractionated stereotactic body RT” [55]. Revisiting the original

Cynomolgus monkey model of RILD[42], Yannam et al. [54]recently conducted an escalated dose study in order to evaluate the effect of newer hypofractionated regimes on the liver. The authors demonstrated a higher tolerance for hypofractionated radiation, but that the characteristic histological lesions of SOS/VOD still developed at radiation doses above 40 Gy. However, an additional key finding was that metabolic stress on the liver, such as glucose loading or administration of total parenteral nutrition, produced substantial additional hepatic injury and generated hepatic failure. These findings suggest radiation-induced hepatocellular injury as a critical component of RIDL. Although this injury may be considered as a secondary factor to the inadequate blood supply to perivenous hepatocytes per se, a broader level of injury to the parenchyma may also be operatively generated by the upstream impairment of sinusoidal blood flow.

A separate study developed a new rodent model for oxaliplatin-induced SOS/VOD has been developed [56], owing to the importance of this form of chemotherapy for advanced colorectal cancer. The model consisted of intraperitoneal administration of FOLFOX (folinic acid, 5-fluorouracil, and oxaliplatin) to C57Bl/6 mice, based on the premise that FOLFOX induces endothelial damage and leads to a pro-thrombotic state within the liver. This new model may allow for new

experimental examination of the role of oxidative stress in the pathogenesis of oxaliplatin-induced SOS, and potential amelioration or prevention of SOS/VOD by prophylactic administration of antioxidants. This model may also be necessary for exploring the role of the pro-thrombotic state in SOS/VOD [57], unlike the monocrotaline rodent model of SOS, in which there is no evidence of clotting abnormalities[19].

Recent studies to establish a rodent model of SOS/VOD arising from hematopoietic stem cell transplantation also have recently been successfully met with success, using an allogeneic transplantation model involving male C57BL/6 male mice as donors and female BALB/c mice preconditioned with whole-body irradiation as recipients[58].

Clinical Pathogenesis of Sinusoidal Obstruction Syndrome

Experimental models of SOS/VOD in animals allowed examination of fundamental causes of sinusoidal destruction and vascular compromise. However, there is still a need to perform a clinical investigation of human patients affected by SOS, mainly because the induction pathways vary. Through the 1980s and 1990s, SOS/VOD was a complication of induction regimes for a broad set of patients undergoing allogeneic bone marrow transplantation. The fundamental premise was that, while SOS/VOD developed in the days

and weeks following the actual bone marrow transplantation, it was the marrow ablation regime before transplantation that was the actual cause of the hepatotoxicity. Accordingly, induction regimes were modified to reduce the incidence of SOS.

In the past decade, SOS/VOD has become an issue primarily with patients undergoing HSCT for widely metastatic solid tumours, or intense neoadjuvant chemotherapy for cancer metastatic to the liver. The reported degree of SOS/VOD in these two patient populations remains entirely consistent, therefore requiring a continuing investigation into pathogenesis. An example is the performance of gene expression profiling to examine potential mechanisms for oxaliplatin-induced SOS/VOD in humans (vide infra)[59].

Recently, on 98 patients undergoing preoperative chemotherapy for colorectal cancer metastatic to the liver, followed by hepatic resection[60] SOS/VOD was observed. Of these patients, 80 were treated with preoperative chemotherapy. SOS/VOD manifested in 39 of these patients (39.8%), with the development of SOS/VOD in patients receiving oxaliplatin-based neoadjuvant chemotherapy significantly higher than those receiving non-oxaliplatin-based chemotherapy. Increased CD34 immunohistochemical reactivity in sinusoids was found in histological examination of resected non-tumoral liver tissue, the intensity of which correlated both with

preoperative functional studies of indocyanine green retention, and severity of histological SOS. These studies conclude that sinusoidal capillarization, as evidenced by CD34 expression, should be a component of the hepatic functional deterioration in the setting of SOS.

Histopathological features

SOS/VOD may exhibit acute, subacute, and chronic features, depending on the time of collection of liver material. Unfortunately, as this disease is focal in nature, diagnostic features may not be evident on liver biopsy. During the acute phase of the disease, striking centrilobular congestion is observed, with centrilobular hepatocellular necrosis and accumulation of hemosiderin-laden macrophages (Figure 01).

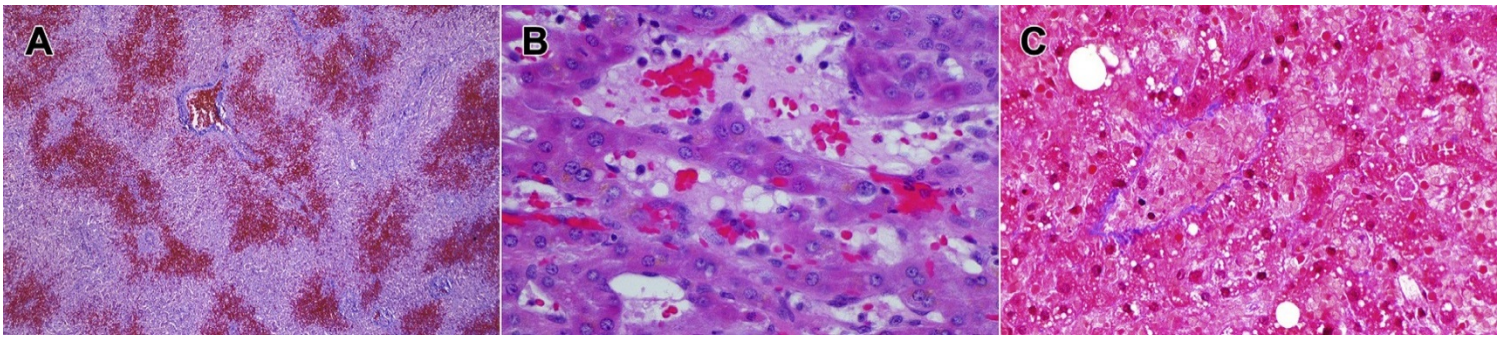

Figure 01 Early histology changes of sinusoidal obstruction syndrome. *(A) Post-mortem liver: low power image showing massive pericentral conges- tion and hemorrhagic necrosis; (B) Liver biopsy: high power image of pericentral region, showing subtotal occlusion of terminal hepatic venule, with entrapped erythrocytes; (C) Liver biopsy: high power image showing dilatation of sinusoids and necrosis of hepatocytes. Terminal hepatic vein is occluded, but collagen deposition has not yet occurred.* (From **Fan, C. Q., & Crawford, J. M. (2014).** Sinusoidal obstruction syndrome (hepatic veno-occlusive disease). *Journ*al of Clinical and Experimental *Hepatology*, *4*(4), 332–346. https://doi.org/10.1016/j.jceh.2014.10.002)

The terminal hepatic venules exhibit intimal oedema, without visible fibrin deposition or thrombosis. As per previous discussions, SOS/VOD originates from toxic injury to the sinusoidal endothelium [61]. The cells round up and slough off the sinusoidal wall, embolizing downstream and obstructing sinusoidal blood flow.

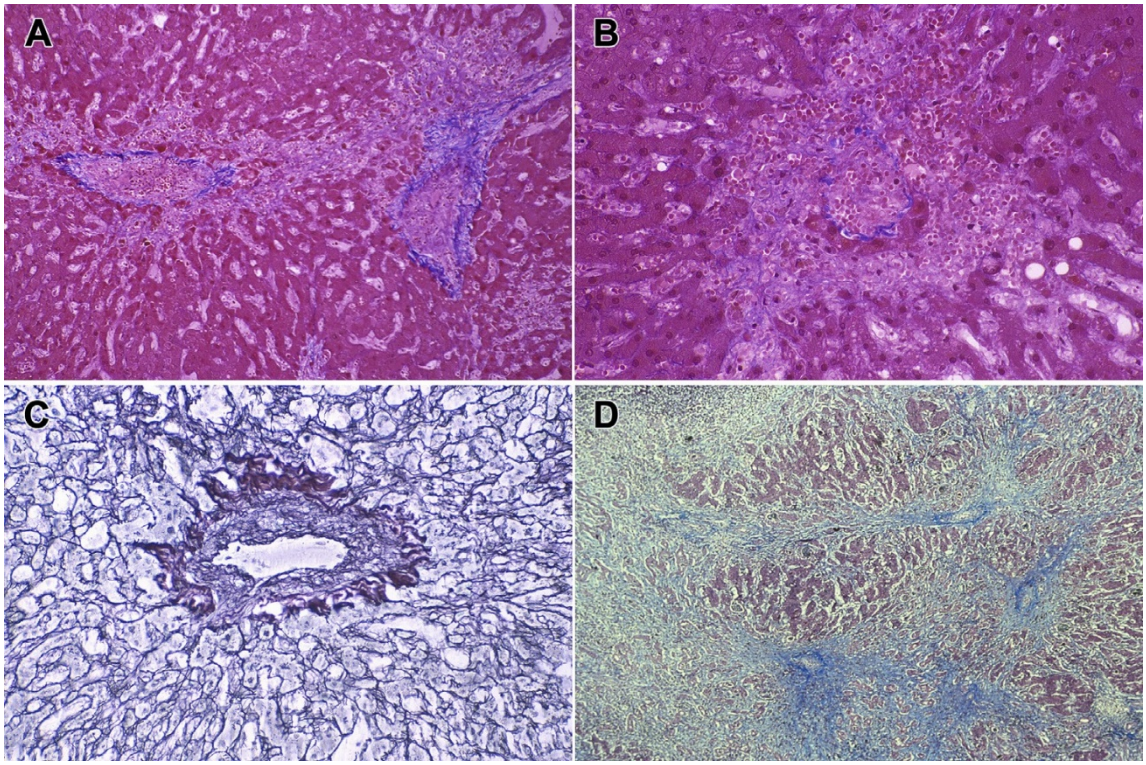

Figure 02 Sinusoidal obstruction syndrome of longer duration, post-mortem livers. *(A) Medium power image showing deposition of loose fibrous tissue in the pericentral region, with early formation of fibrous bridges to adjacent centrilobular areas; (B) Medium power image of the terminal hepatic vein, showing complete loss of pericentral hepatocytes with sharp demarcation zone from viable hepatocytes in the middle of the lobule. The lumen of the terminal hepatic vein is completely occluded, and there is extravasation of erythrocytes in the pericentral space; (C) Higher power image of terminal hepatic vein, showing intraluminal deposition of extracellular matrix, with residual lumen; (D) Low power image of post-mortem liver with SOS/VOD of longer duration, with extensive destruction of the parenchyma and central-to-central fibrous bridging.* *(From* ***Fan, C. Q., & Crawford, J. M. (2014).*** *Sinusoidal obstruction syndrome (hepatic veno-occlusive disease). Journal of Clinical and Experimental Hepatology, 4(4), 332–346. https://doi.org/10.1016/j.jceh.2014.10.002)*

This obstruction is accompanied by dissection of erythrocytes into the space of Disse and downstream accumulation of cellular debris in the terminal hepatic vein. The proliferation of perisinusoidal stellate cells and subendothelial fibroblasts in the terminal hepatic vein follows,

with deposition of the extracellular matrix. However, liver biopsies are rarely obtained during the acute stage.

Over days to weeks (subacute), collagen deposition occurs in and around the affected terminal hepatic venule (Figure 02). This leads to progressive obliteration of the venules, which is easily identified with special stains for either collagen or reticulin[62]. Apparent centrilobular congestion without readily identifiable terminal hepatic veins on haematoxylin and eosin stain should prompt performance of connective tissue stains to exclude SOS. With the persistence of the SOS/VOD lesion into weeks to months (chronic), dense perivenular fibrosis radiating out into the parenchyma develops. The scar tissue contains hemosiderin-laden macrophages, and terminal hepatic vein luminal cannot be identified. Notably, congestion is found in minimal evidence at this stage. There is a possibility of severe destruction of lobular parenchyma, and rarely evolution to cirrhosis.

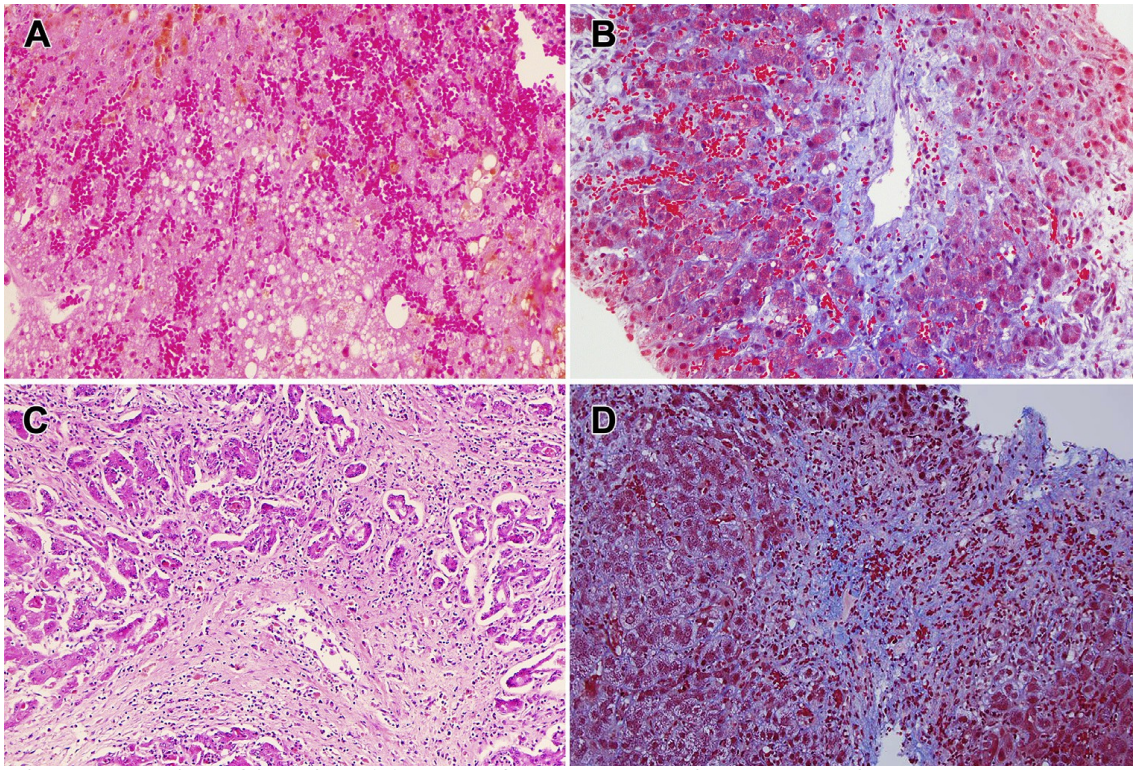

Figure 03 Liver biopsies. *(A) Biopsy from patient with acute SOS, demonstrating extensive dilatation and congestion of sinusoids, and lack of evident terminal hepatic veins; (B) Same liver biopsy, showing terminal hepatic vein with narrowed lumen, intraluminal deposition of extracellular matrix with entrapped erythrocytes; (C) Biopsy from patient with chronic consumption of herbal teas, showing extensive destruction of pericentral parenchyma with deposition of fibrous tissue, inflammation, and partial obliteration of the terminal hepatic vein lumen; (D) Liver biopsy from a different patient, also consuming herbal teas, showing inapparent terminal hepatic vein, extensive extravasation of erythrocytes into pericentral parenchyma, and diffuse pericentral fibrosis.*

*(From **Fan, C. Q., & Crawford, J. M. (2014).** Sinusoidal obstruction syndrome (hepatic veno-occlusive disease). Journal of Clinical and Experimental Hepatology, 4(4), 332–346. https://doi.org/10.1016/j.jceh.2014.10.002)*

When the liver biopsy is performed, either in the acute stage or during a more chronic stage, the histologic findings of SOS/VOD may be strikingly evident (Figure 03). However, the pathologist must be alert to SOS/VOD being the cause of these findings, since histologic findings may be misinterpreted if the centrilobular venopathy is not recognised.

Clinical diagnosis by aetiology

Toxic liver injury related to conditioning for hematopoietic stem cell transplantation

SOS/VOD was first and simultaneously reported by several groups in 1979–1980 as a fatal complication of hematopoietic stem cell transplantation[26]. SOS/VOD developing after allogeneic hematopoietic stem cell transplantation (HSCT) typically occurs within the first month after HSCT, although later onset has been described [63]. Conditioning regimens associated with the onset of SOS/VOD typically consist of cyclophosphamide combined either with total body irradiation or Busulfan[64]. Liver disease occurs in up to 80% of patients following hematopoietic stem cell transplantation (HSCT) for malignancies, ranging from a mild and reversible elevation of serum transaminases to fatal hepatic failure[62]. The prevalence of SOS/VOD is much lower following HSCT for idiopathic aplastic anaemia, reported as 7%, but still carries a high fatality rate (21%) if it occurs[65].

SOS/VOD is part of a broader pattern of toxic liver damage occurring following HSCT[66], in which generalised impairment of liver function in the immediate post-transplantation period, may be further complicated by SOS/VOD in the weeks following

transplantation, or nodular regenerative hyperplasia months later[32]. As a result, the histopathological definition has mostly included the late finding of fibrous obliteration of the central veins, rather than the earlier lesions of central haemorrhagic necrosis, or sinusoidal changes. Still, clinical criteria for diagnosis were identified based on these autopsy data[67].

Despite initial reports of SOS/VOD incidence were in the 60% range[26]; more recently a meta-analysis of 135 studies carried out between 1979 and 2007 set the overall mean incidence of SOS/VOD following HSCT at 13.7% (95% confidence interval = 13.3%–14.1%) [68]. In one study, the cumulative incidence was significantly higher 20 years ago (1985–1996) than in more recent years (1997–2008), attributed to reduced-intensity conditioning regimes for HSCT, and the reduction in the use of unrelated donors for HSCT [38]. Depending on clinical diagnostic criteria and risk factors, the incidence of SOS/VOD has ranged between 0 and 50% among transplantation units, and the fatality rate between 3 and 45%[67]. In an extensive multicentric prospective survey, the incidence was 5.3% [69].

The onset of SOS/VOD after HSCT is characterised by painful hepatomegaly, jaundice, weight gain, and ascites. Severe SOS/VOD is typically associated with multiorgan failure (MOF) and high mortality

rates (>80%)[38,68,70]. The sudden weight gain and the development of hepatomegaly and tenderness are usually observed on day 0 or 1 following HSCT, or 8–10 days after the start of cytoreductive therapy. From the standpoint of clinical parameters, when serum bilirubin values exceed 15 mg/dl and weight gains approach 10–15 kg, mortality exceeds 90% [71]. Despite persistent severe liver dysfunction being a signal of a fatal outcome, liver disease per se is not usually the direct cause of death; most patients die because of to septicaemia, pneumonia, and bleeding and multiorgan failure[68]. Pretransplant hepatitis (elevation of serum transaminases) is a risk factor for liver toxicity after transplantation. Clinical diagnosis of SOS/VOD is based on the occurrence of at least two of the three following symptoms during the first month after bone marrow transplantation: jaundice, development of tender hepatomegaly and right upper quadrant pain, ascites, and unexplained weight gain[72,73]. Serum transaminase levels may also rise substantially, but do not differentiate between potential causes of liver injury.

MODIFIED SEATTLE CRITERIA [14]	BALTIMORE CRITERIA [15]
Presentation before day 20 post HSCT of at least 2 of the following: • Bilirubin >2 mg/dL • Hepatomegaly or right upper quadrant pain • Weight gain (>2%)	Presentation of bilirubin >2 mg/dL before day 21 post HSCT and at least 2 of the following: • Painful hepatomegaly • Ascites • Weight gain (>5%)

EUROPEAN SOCIETY FOR BLOOD AND MARROW TRANSPLANTATION (EBMT) CRITERIA

ADULT POPULATION [9]		PEDIATRIC POPULATION [16]
Classical SOS/VOD (In the first 21 days after HSCT)	Late onset SOS/VOD (>21 Days after HSCT)	No limitation for time of onset of SOS/VOD
Bilirubin ≥ 2 mg/dL and two of the following criteria must be present: • Painful hepatomegaly • Weight gain ≥ 5% • Ascites	(1) Classical VOD/SOS beyond day 21 **OR** (2) Histologically proven SOS/VOD **OR** (3) Two or more of the following criteria must be present: • Bilirubin ≥ 2 mg/dL • Painful hepatomegaly • Weight gain ≥ 5% • Ascites **AND** Hemodynamical or/and ultrasound evidence of SOS/VOD	The presence of two or more of the following[a]: • Unexplained consumptive and transfusion-refractory thrombocytopenia[b] • Otherwise unexplained weight gain on three consecutive days despite the use of diuretics or a weight gain 45% above baseline value • [c]Hepatomegaly (best if confirmed by imaging) above baseline value • [c]Ascites (best if confirmed by imaging) above baseline value • Rising bilirubin from a baseline value on 3 consecutive days or bilirubin ≥2 mg/dL within 72 h [a]with the exclusion of other potential differential diagnoses. [b]≥1 weight-adjusted platelet substitution/day to maintain institutional transfusion guidelines. [c]suggested: imaging (US, CT or MRI) immediately before HSCT to determine baseline value for both hepatomegaly and ascites.

These symptoms/signs should not be attributable to other causes.
Abbreviations: Computed tomography, CT; European Society for Blood and Marrow Transplantation, EBMT; Hematopoietic stem cell transplantation, HSCT; Magnetic Resonance imaging, MRI; Sinusoidal Obstruction Syndrome/Veno-Occlusive Disease, SOS/VOD; Ultrasonography, US.

Table 02: Clinical diagnostical criteria for SOS/VOD (from Ravaioli et al. (2019)
Expert Review of Gastroenterology & Hepatology, 13:5, 463-484)

To date, SOS/VOD diagnosis has been mainly based on clinical and biochemical parameters, which are part of the widely used modified Seattle Criteria or Baltimore Criteria. New clinical criteria have also been proposed by the European Society for Blood and Marrow Transplantation (EBMT), both for paediatric and adult patients[40,74]In addition to the “classical onset” (which usually appears within 21 days after HSCT), EBMT criteria for the first time have proposed the SOS/VOD form, which is a less common “late-onset” (>21 days after HSCT) form [40].

Thy widely used clinical criteria are presented in Table 02. Recommended clinical indices for assessing the severity of SOS/VOD are given in Table 03 [75].

	Mild[a]	*Moderate*[a]	*Severe*	*Very severe - MOD/MOF*[b]
Time since first clinical symptoms of SOS/VOD[c]	>7 Days	5–7 Days	⩽4 Days	Any time
Bilirubin (mg/dL) Bilirubin (μmol/L)	⩾2 and <3 ⩾34 and <51	⩾3 and <5 ⩾51 and <85	⩾5 and <8 ⩾85 and <136	⩾8 ⩾136
Bilirubin kinetics			Doubling within 48 h	
Transaminases	⩽2 × normal	> 2 and ⩽5 × normal	>5 and ⩽8 × normal	>8×Normal
Weight increase	< 5%	⩾5% and <10%	⩾5% and <10%	⩾10%
Renal function	<1.2 × baseline at transplant	⩾1.2 and <1.5 × baseline at transplant	⩾1.5 and <2 × baseline at transplant	⩾2 × baseline at transplant or others signs of MOD/MOF

Abbreviations: EBMT = European society for Blood and Marrow Transplantation; MOD = multi-organ dysfunction; MOF = multi-organ failure; SOS = sinusoidal obstruction syndrome; VOD = veno-occlusive disease. Patients belong to the category that fulfills two or more criteria. If patients fulfill two or more criteria in two different categories, they must be classified in the most severe category. Patients weight increase ⩾5% and <10% is considered by default as a criterion for severe SOS/VOD; however, if patients do not fulfill other criteria for severe SOS/VOD, weight increase ⩾5% and <10% is therefore considered as a criterion for moderate SOS/VOD. [a]In the case of presence of two or more risk factors for SOS/VOD, patients should be in the upper grade. [b]Patients with multi-organ dysfunction must be classified as very severe. [c]Time from the date when the first signs/symptoms of SOS/VOD began to appear (retrospectively determined) and the date when the symptoms fulfilled SOS/VOD diagnostic criteria.

Table 03: EBMT criteria for severity grading of a suspected SOS/VOD (from Mothy et al. (2015)

Seattle and Baltimore's criteria yield differing estimates for the incidence of SOS/VOD [76,77]. These data suggest that the widely used clinical criteria for SOS/VOD point to a syndrome of liver dysfunction where SOS/VOD is relevant together with many other factors, rather than to a discrete clinic-pathological entity. The accuracy of these criteria is limited by the need for a careful exclusion of alternative diagnoses, particularly viral hepatitis, bacterial infections, graft versus host disease, and other drug reactions, all of which are common and frequently combined in the setting of hematopoietic stem cell transplantation. Given the risks of complications, liver biopsy is usually performed in patients in whom the diagnosis of SOS/VOD is unclear, therefore requiring to exclude other diagnoses[72,73]. Due to the potentially dangerous conditions of patients requiring a liver biopsy, the transjugular approach was

recommended to reduce the risks associated with the procedure. Overtime after transplantation, a liver biopsy may become increasingly necessary in order to rule out the diagnostic possibilities in these patients. In these later time frames, coagulation status may be more intact, permitting a percutaneous approach. When the clinical diagnosis was compared to that reached through transjugular liver biopsy, a high rate of a false definite diagnosis of SOS/VOD was found, as well as an underestimation of associated conditions [78,79]. Furthermore, when pre-transplantation and post-transplantation liver biopsy could be compared in the same patient, many features of VOD found after transplantation were already present before, reflecting the importance of taking into account pre-transplant condition and management[80]. A hepatic venous pressure gradient above 9 or 10 mmHg appears to be 85–90% specific for finding SOS/VOD lesions at liver biopsy [64,65].

Major risk factors for the occurrence of this clinical SOS/VOD include[40]:

- the intensity of the conditioning regimen;
- the autologous or allogeneic type of hematopoietic stem cell transplantation;
- exposure to oestroprogestative in women;
- second myeloablative HSCT;

- pre-existent liver disease (as indicated by raised serum transaminase activity).

High-intensity regimens include those using both busulfan and cyclophosphamide, or cyclophosphamide with total body irradiation > 12 Gry., or cyclophosphamide and BCNU and etoposide; or those with an equivalent intensity.

Based on ex vivo studies, cyclophosphamide toxicity to sinusoidal endothelial cells appears to be mediated by metabolism to toxic intermediates in hepatocytes and depletion in glutathione in sinusoidal endothelial cells [81].

In the multicentric European survey mentioned above, SOS/VOD was classified as mild (i.e. self-limited) in 8%; moderate (i.e. with complete resolution but necessitating therapy) in 64%; and severe (i.e. leading to death or not resolving by day 100) in 28% of cases. SOS/VOD related mortality was 1% of the whole series, 18% of SOS/VOD patients, and 67% of severe SOS/VOD [69]. In patients developing SOS/VOD, features of more severe liver disease (increased serum bilirubin or transaminase levels, increased hepatic venous pressure gradient) are associated with a more unfortunate outcome [67].

Prophylaxis with defibrotide has been recommended in children at high risk of SOS/VOD. Its use has also been suggested in such

adults [72,82]. The recommendation is based on a positive randomised controlled trial in 356 children [25]. Twenty-two (12%) patients receiving defibrotide developed clinically defined SOS/VOD by day 30, versus 35 (20%) in the control group ($P = 0.05$). The design was that of an open-label trial. Non-relapse mortality was 9% in both groups. Therefore, the clinical impact of prophylactic defibrotide administration appears to be limited. The drug, however, appears to be well tolerated [26]. Other agents proposed for prophylaxis have included prostaglandin E1, pentoxifylline, heparin and antithrombin, but none of these is recommended or suggested [72]. Ursodeoxycholic acid has been suggested based on randomised trials showing both decreased and unchanged incidence of SOS/VOD but without any impact on mortality [73].

Data on treatment for established SOS/VOD from randomised controlled trials are lacking. Most studies had a retrospective design or, when prospective, used historical controls. Most studies reported apparently improved outcomes. Tolerance appears to be good. As a result, defibrotide has been recommended for treatment of SOS/VOD despite the poor methodological quality of the trials. Tissue plasminogen activator is not recommended as several fatalities related to the use of this agent have been reported. The use of N-

acetyl cysteine proved inefficient in a randomised controlled trial [72,73].

Therefore, the most effective means to control SOS/VOD associated to hematopoietic stem cell transplantation has been to reduce the exposure to chemotherapy and radiotherapy, including conditioning for transplantation and prior therapies for underlying blood disease. Attempts at adjusting the dose of myeloablative agents to individual polymorphism in drug metabolism have thus far not been associated with a decreased incidence of SOS/VOD [83].

Other liver-related diagnoses to be excluded after HSCT include graft-versus-host disease (GVHD); other causes of venous outflow obstruction such as Budd-Chiari syndrome and congestive heart failure; drug reactions, including the toxic effects of hyperalimentation; and infections such as viral hepatitis, fungi, and sepsis.

The pathology of Budd-Chiari syndrome is characterised by centrilobular congestion and potentially parenchymal destruction, but it does not present the occlusion of terminal hepatic veins seen in SOS. Drug reactions show parenchymal damage with hepatocellular apoptosis, cholestasis, and parenchymal inflammation. While severe SOS/VOD may cause similar histology in its more severe stages, identification of the terminal hepatic vein lesions is readily made in

such cases. Importantly, the clinical features of post-transplantation "drug toxicity" and the specific condition of SOS/VOD are similar, and there is no specific management of either condition except supportive. Hence, there is less need to make a specific diagnosis of SOS/VOD by liver biopsy in these unstable patients, as there is a need to exclude other potential causes of hepatic dysfunction. Lastly, patients who undergo stem cell transplantation for non-malignant conditions are less likely to develop liver toxicity, as the doses of cytoreductive therapy are generally lower than those given for malignancy [73].

Due to the concomitant risk of post-transplant GVHD, sirolimus has been used to reduce that risk. However, there is evidence of an increased risk of SOS/VOD after sirolimus-based GVHD prophylaxis[84]. Sirolimus may act as an endothelial toxin and has been associated with another endothelial injury syndrome, thrombotic microangiopathy after transplantation[85]. Cutler et al. have suggested using the biomarkers of endothelial injury (circulating von Willebrand Factor (vWF), thrombomodulin, E-selectin, and soluble intercellular adhesion molecule-1 (sICAM-1)) to predict the development of SOS/VOD following HSCT [86]. Increased serum vascular endothelial growth factor (VEGF) after HSCT also has been correlated with the development of SOS[50].

In summary, SOS/VOD related to hematopoietic stem cell transplantation is a condition decreasing in incidence and severity due to increased attention to reduced exposure to toxic chemotherapeutic agents. Unmet needs consist of accurate biomarkers for early diagnosis and evaluation of patients at risk and well-designed therapeutic trials for the prevention and management of the disease.

Sinusoidal changes after chemotherapy for liver metastasis

The liver is the most common site of metastatic tumour spread, and SOS/VOD may occur in patients with stable metastatic tumours to the liver who are given alkylating agents such as oxaliplatin before surgical resection of the metastatic tumours [87]. In particular, nearly half of patients with colorectal cancer develop liver metastases during their disease[88]. For patients with limited metastatic burden of colorectal cancer in the liver, surgical resection of the hepatic tumour deposits without chemo-inductive therapy offers the best opportunity for cure[89], with a five-year survival rate of 40%–58%[90,91]. However, 60%–70% of patients undergoing initial curative resection suffer recurrent disease, of whom 10%–15% may be considered eligible for repeated resections[92]. The remainder is relegated to chemotherapy. For patients with initially unresectable colorectal metastatic disease, chemotherapeutic regimes can rescue a

reported 12.5% by promoting tumour down-sizing and enabling successful surgical resection of residual disease[93]. Widely utilised regimens for this latter purpose are characterised by a thymidylate synthase inhibitor such as 5-fluorouracil, folinic acid, or capecitabine, combined with either oxaliplatin or irinotecan. More recently, antibody therapy in the form of bevacizumab or cetuximab has been included in these regimes[94]. Thus, there is a potentially large population of patients with advanced colorectal carcinoma who will be subjected to either adjuvant or neoadjuvant chemotherapy. In the paediatric population, chemotherapy accompanying hepatectomy for solid metastatic cancer such as Wilms tumour may also cause hepatic SOS[95,96].

As a consequence of these treatments, an abundance of liver resections specimens has become available for a comprehensive evaluation of the changes in non-tumorous areas. Indeed, specific sinusoidal changes resembling those of SOS/VOD have been described of such patients. Grossly, diffuse liver congestion has a typical nutmeg aspect corresponding on histology to extensive veno-venous dilatation and congestion around small hepatic veins; centrilobular hepatocytes plates are atrophic or disrupted, and a sinusoidal fibrosis is frequently observed without venous obliteration. They consist of congestive sinusoidal dilatation predominating in the

centrilobular area in 30–65% of patients, perisinusoidal fibrosis in 35–40%, centrilobular fibrosis in 30%, atrophy of liver cell plate but few necrotic cells bordering dilated areas, and low-grade nodular regenerative changes in 12–20% [97,98]. According to a widely used classification, the sinusoidal lesions are mild (less than 1/3 of the lobule), moderate (1/3 to 2/3 of the lobule), and severe (extending in the whole lobule) in about 30% of patients each. Only in occasional patients with severe lesions was extravasation of erythrocytes in the space of Disse described [99,100]. Similar lesions were not found in control patients receiving resection without preoperative chemotherapy [90].

Oxaliplatin is a critical agent in such regimes and may cause sinusoidal injury, the onset of SOS, and increased morbidity before or after hepatectomy[99,101,102]. 59% of patients receiving preoperative oxaliplatin treatment before hepatectomy showed histologic evidence of high-grade lesions of SOS/VOD, based on examination of the surrounding resected liver[103]. The presence of such sinusoidal injury had a negative effect both on the intra- and postoperative course of patients undergoing major liver resection. In the first instance, both intraoperative bleeding and transfusion rate were increased in patients with high-grade SOS/VOD lesions, when compared to those with low-grade SOS/VOD lesions. In the second

case, there were more frequent and severe postoperative complications, including the development of a large number of post-operative ascites and liver insufficiency. Ironically, oxaliplatin has also been demonstrated to induce focal hepatic SOS/VOD that can mimic metastatic liver disease.

There is a clear association with oxaliplatin-based chemotherapy, where the incidence of such sinusoidal changes is up to 75%. Still, irinotecan-based chemotherapy is also associated with a 25–40% incidence of similar sinusoidal changes [97,98]. 5- fluorouracile is also was found to be independently associated with the development of sinusoidal changes [104]. Co-administration of bevacizumab has been shown to reduce the incidence and severity of sinusoidal changes [100,104–106]. A murine model of FOLFOX-associated sinusoidal alterations has been established [56]. In this model, however, sinusoidal dilatation and hepatocyte atrophy are marked, but endothelial destruction and hepatocyte necrosis are inconspicuous. There is evidence for activation of VEGF and IL6 pathway mediators both on patients, and in experimental models[56,59,107] There is also evidence that changes induced in the tumour itself by chemotherapeutic agents participate in inducing sinusoidal changes through activation of pro-inflammatory pathways [57]. Therefore, it is still unclear whether the sinusoidal

changes observed in oxaliplatin-based chemotherapy are identical to SOS/VOD observed after hematopoietic stem cell transplantation or pyrrolizidine alkaloid exposure, or are mostly related to pro-inflammatory mechanisms [108].

Clinical manifestations associated with sinusoidal changes in patients with liver metastasis from colorectal carcinoma appear to be mild or absent. However, patients with clinically conspicuous toxicity from chemotherapy could have been denied resection and thus could have been excluded from analyses due to lack of histological data. Reports of clinically significant liver injury in unoperated or operated patients are scarce. They were exclusively observed in patients receiving intrahepatic arterial administration of high doses of oxaliplatin combined with the systemic or hepatic arterial infusion of other agents [109,110]. Therefore, the frequency of pathological findings contrasts with the rarity of the clinical manifestations.

The occurrence of post-hepatectomy SOS/VOD is also linked with early tumour recurrence and decreased long-term survival[111]. The conditioning chemioadiuvants regimens for metastatic colorectal carcinoma (mainly based on. Irinotecan) are not complicated with SOS but by a risk of chemotherapy-associated steatohepatitis[112].

The impact of sinusoidal changes associated with chemotherapy on the outcome of patients has been unclear. Increased postoperative

morbidity after major resection has been reported by some [103,113] but not all [98] investigators. Interestingly, the occurrence of sinusoidal changes has been associated with a decreased tumour response [114,115]. However, there is currently no evidence for increased mortality in patients with sinusoidal changes [114].

Determinants for the development of sinusoidal changes after chemotherapy for colorectal cancer have not been identified yet. Genetic polymorphism in drug metabolising enzymes, such as glutathione S-transferase, has been incriminated [47]. Preoperative laboratory indices designed to predict liver fibrosis in patients with hepatitis C virus-related liver disease involves such calculations as aspartate aminotransferase (AST) to platelet ratio index (APRI) and fibrosis scoring systems[116,117]. Recently, these clinical parameters have been applied to patients undergoing oxiplatin-based induction chemotherapy for metastatic colorectal carcinoma; the evidence suggests that the APRI score, along with a low preoperative platelet count, may be reliable predictors of SOS/VOD severity following hepatectomy [103]. In the post-hepatectomy time-frame, a clinical marker of risk for SOS/VOD has been found in the measurement of circulating Hyaluronic Acid as a measure of endothelial function [117]. More recently, monitoring of

post-hepatectomy splenic volume by imaging methods has been found to be a useful predictor of risk for the development of SOS[90]. Moreover, polymorphisms in a transmembrane drug transporter gene (ATP7B), have been identified for increasing the susceptibility to SOS/VOD after oxaliplatin-based chemotherapy[57]

Of note, particular focal lesions simulating metastasis can be attributed to sinusoidal changes. A distinctive feature of such focal lesions is their ill-defined margin on hepatobiliary phase of gadoxetic acid-enhanced and diffusion weighed MR imaging [118].

In summary, oxaliplatin-associated sinusoidal lesions is a pathological entity with minimal clinical expression, which is not entirely specific for oxaliplatin in the context of chemotherapy for liver metastasis of colorectal cancer. The impact on patients' outcome is uncertain or minimal. Similarity with pyrrolizidine alkaloid or hematopoietic stem cell transplantation is only partial, probably related to the use of clinically fewer toxic doses in most protocols than those used for preparation to hematopoietic stem cell transplantation.

Radiation-Induced Liver Disease

Radiation therapy (RT) without chemotherapy or hepatectomy still constitutes a central approach for the treatment of cancer metastatic to the liver and host conditioning for bone marrow transplantation.

Patients with RILD typically present with fatigue, weight gain, hepatomegaly, ascites, and an isolated elevation in alkaline phosphatase in months following hepatic RT[8]. Liver biopsy reveals the characteristic features of SOS/VOD affecting terminal and sub lobular hepatic veins[80].

RILD is a severe complication of RT. In patients who do not die of this condition, liver healing happens over several months as the vascular congestion resolves.

Sinusoidal changes related to other drugs or conditions

Table 04 presents the agents, which have been reported to be associated with SOS/VOD. All of these agents share, causing bone marrow suppression in addition to SOS/VOD.

Table 04 Drugs reported being associated with SOS/VOD.

6-mercaptopurine
6-thioguanine
Actinomycin D
Azathioprine
Busulfan
Cytosine arabinoside
Cyclophosphamide
Dacarbazine
Gemtuzumab-ozogamicin
Inotuzumab-ozogamicin
Melphalan
Oxaliplatin
Urethane

The relationship of azathioprine to SOS/VOD is particularly unclear for several reasons: i) there is no animal model; ii) dose relationship is not apparent; iii) similar to oxaliplatin, lesions have been described under various denomination including SOS/VOD but also peliosis, sinusoidal dilatation and nodular regenerative hyperplasia; iv) conditions for which azathioprine was administered have been reported to be associated with sinusoidal changes including Crohn's

disease [108], renal transplantation and liver transplantation [119,120].

A singular example of SOS/VOD occurring in the setting of intensive cytotoxic chemotherapy is the use of Mylotarg® (gemtuzumab ozogamicin, GO) and Besponsa® (inotuzumab ozogamicin, INO) for acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL), respectively [121,122]. GO is composed of a humanised conjugated antibody carrier linked to a potent antitumor antibiotic (calicheamicin). It targets the CD33 antigen expressed on the surface of AML cells, which is relatively absent from non-hematopoietic tissues (also present on sinusoidal endothelial cells) and primitive hematopoietic stem cells. This population of GO-treated patients show a prevalence of SOS/VOD at as 9.1%[123]. Despite the 6-month mortality rate in this population of SOS-affected patients is high (68%), three-quarters of the deaths were due to progression of the AML. The overall risk of death from SOS/VOD and multiorgan failure was 2.7%. Briefly, the histopathological findings in such patients are characterised by dilatation and congestion of sinusoids limited to centrilobular zones around the terminal hepatic vein; endothelial cells of veins and sinusoids are damaged, leading to a substantial hematic deposition in Disse space and hepatocyte necrosis around the central veins.

INO is a humanised monoclonal antibody to the human CD24 cell surface marker which is highly expressed on malignant lymphoblastic leukaemia cells[122]. INO is conjugated to a cytotoxic agent called ozogamicin. When inotuzumab binds to CD24, it is internalised, and the ozogamicin is released by the action of lysosomal enzymes on the linker molecule that joins it to the monoclonal antibody. The released intracellular ozogamicin causes a break in double-stranded DNA that leads to apoptotic cell death. This monoclonal antibody conjugate is effective in inducing remissions in refractory acute lymphoblastic leukaemia and was given accelerated approval for this indication in the United States in 2017. Inotuzumab ozogamicin is available as 0.9 mg of lyophilised powder in single-dose vials under the brand name Besponsa[122]. The recommended dose regimen depends on the following factors: body weight, day of therapy, and whether treating refractory vs relapsed acute lymphoblastic leukaemia. Common side effects include bone marrow suppression, infections, fatigue, fever, nausea, headache, abdominal pain and haemorrhage. Less common but potentially severe side effects include severe myelosuppression, severe infections, clinically significant bleeding and haemorrhage, related infusion reactions, QTc interval prolongation and embryo-fetal toxicity[122].

In prelicensure clinical trials of INO, up to half of the patients had serum ALT or AST elevations during therapy which were above five times the upper limit of normal (ULN) in 2% to 5%. Hyperbilirubinemia is also common during inotuzumab therapy, generally occurring without carrying ALT or AST elevations. More importantly, a variable proportion of patients (ranging from 2% to 35%) showed clinically apparent sinusoidal obstruction syndrome (SOS) after inotuzumab ozogamicin therapy. The incidence was even higher (15% to 25%) in patients who receive inotuzumab and subsequently underwent hematopoietic cell transplantation (HCT). Symptoms of nausea, right upper quadrant pain, weight gain and abdominal distension (from ascites) arose within 5 to 20 days of the infusion and were followed in some patients by progressive rises in bilirubin, serum aminotransferase and alkaline phosphatase levels. Clinically apparent SOS/VOD has a poor prognosis, and the mortality rate is as high as 70%, most patients dying of multiorgan failure. Allogeneic hematopoietic cell transplantation, use of other antineoplastic agents and the presence of preexisting liver disease are recognised as the main risk factors for developing SOS/VOD after inotuzumab therapy [122].

The cause of the serum enzyme elevations during inotuzumab ozogamicin therapy is not known, but they are likely due to direct

toxicity of the conjugate. The propensity of inotuzumab ozogamicin to cause sinusoidal obstruction syndrome may be explained by the fact that hepatic sinusoidal endothelial cells express low levels of CD24 on the cell surface and the antibody conjugate may be taken up by these cells resulting in their damage and release of apoptotic fragments into sinusoids causing obstruction[122].

The mild-to-moderate serum aminotransferase elevations that occur during inotuzumab ozogamicin therapy are generally transient and asymptomatic and do not require dose modification or delay in therapy. More careful monitoring and suspension of further infusions should be carried out when there is evidence of elevations above five times the upper limit of normal, at least until levels return to normal or near-normal levels. Management of SOS/VOD generally relies on careful attention to fluid balance and treatment of complications. There are no proven means of prevention or treatment of SOS/VOD due to inotuzumab ozogamicin, although pretreatment with ursodiol and acute management with defibrotide are often utilised. The adverse events profile and hepatotoxicity of inotuzumab ozogamicin are similar to those caused by gemtuzumab ozogamicin, a similar monoclonal antibody-conjugated directed to CD33 that is used to treat refractory or relapsed acute myeloid leukaemia [122].

In summary, drugs which are simultaneously toxic for hepatic endothelial and hematopoietic cells appear to be at risk of inducing SOS/VOD. However, conditions characterised by a dysregulated immunity, and therefore indications for immunosuppressive agents including azathioprine also appear to be risk factors for sinusoidal changes, which makes the relationship with immunosuppressive agents challenging to establish. It can be suggested that the combination of several factors is relevant to the pathogenesis of these uncommon lesions: an immune-related injury and a drug-induced injury.

Liver Transplantation

SOS/VOD as a cause of liver graft dysfunction after liver transplantation is rare but has a poor prognosis[124]. This needs to distinguish from post-transplant perivenulitis, as part of graft rejection[125]. If present, SOS/VOD is more likely to occur in living-related transplant grafts[126] or patients were receiving azathioprine as part of their immunosuppressive regime[119]. Better optimisation of immunosuppression is suggested as an approach to reduce the incidence of this rare event[119].

Herbal Toxins

Recent studies have focused on the use of hepatotoxic herbal remedies by humans [127]. An estimated 500 herbal products are

distributed worldwide and are represented as harmless healthy products without side effects[127,128]. Over 60% of patients in the U.S. is seen by a physician report use of herbal remedies[129]. It has been estimated that more than 6000 plant species contain pyrrolizidine alkaloids with different chemical structures[130].

Symphytum officinale, commonly known as comfrey, is one of the most famous herbs containing pyrrolizidine alkaloids [131]. This herb has been used as an external remedy for bone fractures, joint inflammation, and wound healing, and internally for gastroduodenal ulcers and gastritis[132]. The comfrey-induced injury involves fibrous obliteration and destruction of hepatic veins, leading to cirrhosis[133,134]. However hepatotoxicity (including SOS), acute or chronic hepatitis, cholestasis, hepatic necrosis or fibrosis, cirrhosis, and outright liver failure, can occur with a much broader array of herbal products[135], depending on what plant components have been used, storage conditions for the plant products, mislabeling or misidentification of the plant, and outright contaminations[18,136]. Even *Echinacea*, despite being widely considered as being completely free of toxicities, is known to contain pyrrolizidine alkaloids and may induce hepatotoxicity[137].

Clinical presentation of hepatotoxicity arising in the setting of herbal product use is non-specific, involving fatigue, loss of appetite, and

potentially jaundice. Hepatomegaly, ascites, and hyperbilirubinemia may be evident. Liver enzymes may be elevated in either hepatitis, cholestatic or combined pattern. Similar to the onset of SOS/VOD in the post HSCT setting, there are no symptoms or laboratory findings that specifically implicate SOS.

The presence of this form of liver injury requires a liver biopsy and attentiveness to this possibility in examining the biopsy tissue. More severe chronic exposure to the hepatotoxins in comfrey can manifest clinically in hepatomegaly and ascites, with SOS/VOD again confirmed by liver biopsy[138,139], even in the very young child given comfrey tea[140]. The presence of perivenular necrosis, endophlebitis and fibrotic alterations of the terminal and sub-lobular hepatic veins are consistent with SOS[141]. However, histological examination of liver tissue may be difficult to perform owing to ascites and coagulopathy. In such cases, diagnosis of SOS/VOD may have to rely on clinical features and a retrospective history of drug or herb exposure. Recently, a useful supplement to conventional diagnostic methods is found in the development of an ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) assay for identification of pyrrole-protein adducts in peripheral blood [142].

With either mild or moderate cases of SOS, removal of the offending agent may offer good prospects for clinical recovery. In the most

severe cases of herbal toxin-induced SOS/VOD in adults, hepatic failure may ensue. Notably, a fatal case of SOS/VOD has been discovered in the preterm infant born of a mother consuming herbal teas[134].

Because botanicals are self-prescribed, self-administered, and widely available, they are difficult to control and difficult to document[119]. Generally, mixed formulations are conventional. The point should be made that severe hepatotoxicity, including SOS, may be quite rare, given the extensive use of herbal products and the relative infrequency of severe clinical presentations[141]. Nevertheless, healthcare providers are recommended to obtain accurate information about their patients' use of herbal remedies and to make sure that they receive a good education about their potential hepatotoxicities.

VOD-SOS/VOD with Immunodeficiency

Mellis and Bale described in 1976, cases of infants from three families who died within seven months by the veno-occlusive disease of the liver[143]. Authors described the infants with the evidence of immune deficiency, including hypogammaglobulinemia, multiple infections (mainly, Pneumocystis jerovici, enteroviral infections, and mucocutaneous candidiasis), and lymphoid tissues devoid of

germinal centres and mature plasma cells. An additional 19 cases of what is now termed autosomal recessive hepatic veno-occlusive disease with immunodeficiency syndrome (VODI) have been identified over the ensuing years. VODI is associated with an 85% mortality if unrecognised and untreated with intravenous immunoglobulin and P. jerovici prophylaxis. The lack of hepatic veno-occlusive disease in other forms of inherited immunodeficiency suggests that this is a primary feature of VODI.

In 2006, Roscioli et al. [144] described mutations in the promyelocytic leukaemia protein (PML) nuclear body protein Sp110 in six children from five families of Lebanese ethnicity, who met the clinical criteria for VODI. The immunodeficiency consisted of a combined T and B cell immunodeficiency. The B cell immunodeficiency showed signs of evolving severe hypogammaglobulinemia, absent memory (CD19+ CD27+ IgD−) B cells and tonsillar lymph nodes and circulating CD19+ B cell numbers and percentages within the normal range. The T cell immunodeficiency was represented by reduced numbers of memory (CD4+ CD45RO+ CD27−) T cells, and low or reduced intracellular T cell cytokine expression after antigen stimulation. SP110 is an immunoregulatory gene expressed in T and B lymphocytes within lymph nodes, spleen and liver. The affected infants in four of the

families had a homozygous single-base deletion, 642delC (P214PfsX15) in exon 5. There were no living affected individuals in the fifth family, but the consanguineous parents and unaffected children of these families exhibited a heterozygous single-base deletion, 40delC (Q14SfsX25) in exon 2 of SP110. The central role of the SP110mutation in VODI, along with identification of novel mutations and critical clinical and immunological features, has been confirmed in an extensive additional study[145]. This and a limited number of other studies have discovered that early disease recognition and management (e.g., immunoglobulin replacement therapy and trimethoprim-sulfamethoxazole prophylaxis) can improve the dismal mortality rates[146]. HSCT, when carried out before disease onset, can be curative. In one publication, the antenatal diagnosis was possible for a family with a known risk of this disease[147].

Interestingly, SP110 is a single-copy gene in humans. However, in all inbred strains of laboratory mice, it is part of a rearranged and the amplified genomic region containing approximately 60–2000 copies of Sp110 and 20 adjacent genes[148]. Therefore it has not been possible to produce a genetic knockout model of Sp110 for the study of the tissue pathobiology of this syndrome. Thus, despite the potential unique opportunity to gain insights from SP110 mutation

into the immunopathogenesis of hepatic veno-occlusive disease (SOS), the evolution of the hepatic vascular lesions in VODI has not been elucidated.

Treatments and preventions

Supportive Measures

Current management of SOS/VOD is characterised mainly by supportive care, with a focus toward fluid management, adequate oxygenation and transfusional support to minimise ischemic liver injury, and avoidance of hepato/nephrotoxins[149,150]. Correctly, patients with mild SOS, as defined by clinical indicators, can be observed[73]. Patients with evidence of moderate SOS/VOD should be treated expectantly with mild diuretics. This helps mitigate against fluid overload while preserving renal blood flow and avoid prerenal azotemia and potential hepatorenal syndrome. If ascites occurs, paracentesis may be required. However, the number of ascites removed should be modest, in the range of 1 litre per day, to not harm renal blood flow[75]. Patients with severe SOS/VOD require treatment.

Defibrotide

The use of Defibrotide (DF) for the treatment of SOS/VOD is supported by a large number of clinical trials showing that DF

improves both complete response and survival. Richardson et al. [150]first reported on 19 patients with SOS/VOD and multiorgan failure who had received Defibrotide for the management of severe SOS/VOD in the United States. The efficacy of Defibrotide (DF) for the treatment showed complete resolution of SOS/VOD in 8 patients (42%), 6 of who survived for longer than 100 days with no significant bleeding observed[151]. Several trials have subsequently confirmed the efficacy of DF in this setting, including a similar European multicenter compassionate-use study, which treated 40 patients and demonstrated a 55% complete response (CR) rate, with 43% patients alive after 100 days[152]. Importantly, the US Food and Drug Administration granted access to DF in the United States by issuing an investigational new drug compassionate-use treatment protocol in December 2007. An interim analysis of 269 patients enrolled between December 2007 and March 2011 at 67 US centres revealed that 32% of patients achieved a CR at day-100 post-HSCT, with overall day-100 survival of 50% [153]. To date, DF has been used in more than 240 transplantation centres across 33 countries as a result of a sizeable compassionate-use program and the ongoing named patient programs.

Based on an overall experience in more than 1800 patients, DF continues to demonstrate remarkable safety and tolerability despite

an acutely ill patient population, with manageable toxicities and low rates of attributable haemorrhage [70]. A study from Spain consisted of a population of 845 patients, who received allogenic HSCT over 24 years. Of 845 patients, a total of 117 developed SOS[154]. Overall mortality from SOS/VOD decreased, from a reported 22% in 1997 to 14% in 2008, despite no change in clinical severity of the SOS[154]. For those patients with severe SOS/VOD and multiorgan failure, only 2 of 8 (25%) of patients receiving defibrotide died, versus 14 of 18 (78%) for those severely ill patients receiving other treatments.

Tissue-Plasminogen Activator (tPA)

Anticoagulation and thrombolytic therapies using tissue-plasminogen activator (t-PA) have been used, but are reported to be ineffective and are associated with significant bleeding complications[70,155]. The use of tissue plasminogen activator with or without heparin has been evaluated in many studies and small case series. However, results have generally been disappointing; although about one-third of patients show improvement in their SOS/VOD with thrombolytic therapy, life-threatening haemorrhages are frequent, and no survival advantage is apparent[149].

For pediatric patients developing SOS/VOD after chemotherapy and hepatectomy, careful supportive measures and anti-fibrinolytic drugs

are the standard treatment. In one report, two patients with life-threatening SOS/VOD who failed to respond to the standard therapeutic approach were successfully treated with non-activated protein C supplementation[156].

Methylprednisolone

High dose methylprednisolone has also been utilised in the treatment of SOS. Some studies reported that response was defined as a reduction in the total serum bilirubin by 50% or more within 10 d of initiation of corticosteroids. Sixty-three per cent of patients responded, and 28/48 (58%) were alive at 100 days from transplant[72]. A recent pediatric retrospective study described by Myers et al., 2013, 9 patients who received methylprednisolone[157]. Eight of these patients had multi-organ failure secondary to SOS. The response was defined as a 50% reduction in bilirubin level by ten days after the commencement of therapy. Six patients responded to treatment. Four patients also received treatment with defibrotide. Overall survival was 78%[157]., methylprednisolone may be taken into consideration for the usage in the treatment of SOS/VOD.

Transjugular intrahepatic portosystemic shunt (TIPS)

TIPS insertion has been used to decompress the portal circulation, and relieve ascites in some patients with hepatic SOS, but in some

others, this procedure has been shown to worsen the process, with no signs of improvement of the outcome [158–160].

Liver Transplantation

Liver transplantation has been reported as a treatment for SOS[161–163]. However, it should only be taken into consideration for patients with severe liver failure who are expected to have a good outcome in the absence of liver disease, and those who have undergone bone marrow transplantation for benign disease. Liver transplantation is usually contra-indicated when malignancy is present because of the high rates of recurrence.

Prevention

Holding to the adage that “an ounce of prevention is worth a pound of cure”, prevention remains the primary tool in the clinician's arsenal for managing SOS/VOD in the HSCT population[75]. The first step is the identification of patients at higher risk for developing SOS, namely those with pre-existing liver injury or global systemic inflammation[164]. These include patients with pre-existing viral hepatitis, alcoholic hepatitis, or steatohepatitis, or other causes of elevated liver enzymes, massive exposure to hepatotoxic agents, a history of infection and long antibiotic exposure, or patients with a reduced diffusing capacity of the lung[75]. Risk factors arising

directly from HSCT include those receiving high doses of total body irradiation (TBI), cyclophosphamide exposure and dosage, oral busulfan use, and exposure to other hepatotoxic medications[69,165–168]. Over the last decade, there is evidence that the reported incidence of SOS/VOD following allogeneic hematopoietic stem cell transplantation has diminished, with an improved outcome [38]. This has been attributed to reduced-intensity conditioning HSCT (RIC-HSCT) for patients myeloablative-HSCT from unrelated donors.

Patients identified as being at higher risk for SOS/VOD should be considered for prophylaxis. Ursodeoxycholic acid (ursodiol) beginning two weeks before transplantation is commonly used. Nevertheless, there is not enough evidence as to whether early prophylactic use of ursodeoxycholic acid (ursodiol) reduces the incidence of SOS/VOD in the HSCT population [169–173]. Institution of low-dose heparin prophylaxis is a more aggressive approach, as it requires an infusion regime, and the evidence for the efficacy of this prophylaxis also is equivocal[174–176]. Evidence shows that glutamine supplementation, use of peripheral blood progenitor cells as opposed to those derived from the bone marrow, and T-cell depletion as prophylaxis against graft-versus-host disease has reduced the prevalence of SOS[177,178]. In the pediatric population undergoing HSCT, it has been discovered that a prophylactic regime

of enoxaparin or ursodeoxycholic acid and vitamin E has proven valuable [179].

Lastly, aggressive fluid management and control of fluid balance during HSCT are essential. Although not proven to prevent SOS, successful fluid management can reduce hepatic congestion and potentially eliminate a source of the hepatic insult. Inputs, outputs, and weight are strictly recorded daily, with aggressive diuresis to maintain euvolemia.

In the population of patients with metastatic colorectal carcinoma, the addition of bevacizumab to oxaliplatin-based chemotherapy can attenuate the incidence and severity of SOS[100,180]. Bevacizumab (Bmab) is a monoclonal humanised antibody directed against VEGF, and is an approved drug for first-line treatment of metastatic colorectal cancer, but only as part of combination chemotherapy[181]. It is reassuring that this drug may also be helpful to avoid one of the life-limiting complications of one of the companion chemotherapeutic agents, oxaliplatin.

2. Role of imaging in SOS/VOD diagnosis

Due to the suboptimal performance of clinical criteria, histological examination of liver tissue (liver biopsy) and hepatic venous pressure gradient (HVPG) measurement may be necessary in order to obtain a conclusive diagnosis[31]. However, since these techniques are invasive and carry a high risk of bleeding and infective complications, particularly in pancytopenic patients, even when the safer trans-jugular approach is used, they are only performed in selected cases[73].

Several radiological techniques have recently been proposed to identify SOS/VOD development, with promising results. Physicians involved in HSCT processes are required to understand the specificities and pitfalls of each method. Head-to-head consultations among transplanters, hepatologists and radiologists to select the best method for a patient-tailored diagnostic approach represent a challenge and an exciting field for future clinical research.

Diagnostic role of Hepatic Venous Pressure Gradient (HVPG) in SOS/VOD assessment

SOS/VOD is considered as a paradigmatic model of sinusoidal PH[77]. HVPG still represents the gold standard for PH measurement[77]. HVPG represents the gradient between the

wedged pressures (WHVP) and the free hepatic venous pressures (FHVP). When blood flow in a hepatic vein is stopped by a wedged catheter (WHVP), the proximal static column of blood transmits the pressure from the other vascular territory (hepatic sinusoids) to the catheter. WHVP thus reflects the hepatic sinus pressure and not the portal pressure itself [77]. In a normally functioning liver, due to the pressure balance through interconnected sinusoids, the WHPV is slightly lower than the portal pressure, although this gradient is clinically insignificant. Based on HVPG values, the patients can be classified into i) normal when the HVPG is less than five mmHg; ii) with portal hypertension when the HVPG ranges from 5 to 9 mmHg and iii) with clinically significant portal hypertension (CSPH) when the HVPG is higher than ten mmHg. When CSPH occurs, clinical manifestations (ascites, hepatomegaly, splenomegaly…) can occur.

Thus, HVPG can be useful to predict and diagnose SOS/VOD through the assessment of the degree of PH[182,183]. In 98% of cases and with adequate sample size, measuring HVPG by the trans-jugular route[184,185] enables a liver biopsy (LB) to be safely carried out also in cytopenic patients[186].

The first case report describing high HVPG values in a patient with SOS/VOD dates back to 1981. Since then, only a few studies have investigated its accuracy in relatively large cohorts. Carreras et al.

[25,26] showed that all 36 out of 217 patients with histological SOS/VOD diagnosis presented an HVPG > five mmHg; and over 80% CSPH[79,187]. Shulman et al [78] described a specificity of 91% (PPV 86%) for HVPG ≥10 mmHg in SOS/VOD diagnosis in 24 out of 60 patients who underwent HSCT. A similar result was also recently reported by Kis et al [185] in a large cohort of 141 patients: HVPG ≥10 mmHg presented a 90.8% specificity, and 77.3% sensitivity, PPV 75.6%, NPV 91.6%.

Besides its diagnostic role, HVPG is also a significant prognostic factor of SOS/VOD, as HVPG ≥20 mmHg is associated with more unsatisfactory outcome and higher mortality. Regarding PH improvement after therapy, few studies have described an HVPG and portosystemic gradient reduction after Transjugular Intrahepatic Portosystemic Shunt (TIPS) placement in severe cases of SOS/VOD[160,188] the role and benefit of this therapeutic option are however still controversial.

Regarding Oxaliplatin-induced SOS/VOD, only one recent study[158] has investigated the role of HVPG before surgical resection of liver metastases. Pelegrina et al. found that 4 out of 9 patients with a histological diagnosis of SOS/VOD presented PH at baseline [189]; none of which had CSPH. Higher values of HVPG were associated with SOS/VOD diagnosis and significant surgical complications.

In conclusion, despite many authors and guidelines considering the HVPG measurement as one of the most accurate imaging techniques for SOS/VOD diagnosis, some aspects still need to be addressed: i) HVPG is an invasive technique and available only in centres with specific expertise; ii) a limited number of studies are available; iii) there is no clear interpretation of values belonging to a "grey area" [between 5 (PH) and 10 mmHg (not CSPH)]; iv) and therefore its diagnostic role in mild-moderate cases of SOS/VOD still needs to be determined.

Therefore, only in cases of suspected and unclear SOS/VOD, primarily determined by discrepancies between the various diagnostic assessments carried out, can HVPG with trans-jugular LB confirm the diagnosis with confidence.

Very probably, given the continuous improvements in non-invasive techniques for SOS/VOD diagnosis, the role of HVPG in the diagnostic work-up could become more marginal soon.

Diagnostic role of ultrasound imaging (US) in SOS/VOD assessment

Of the non-invasive radiological techniques, liver ultrasound (US) is one of the most commonly studied. It enables hepatic venous

circulation changes to be assessed through dynamic scans by Color-Doppler US.

Currently, the role of US in SOS/VOD assessment mostly regards a differential diagnosis, i.e. cholangitis, cholestasis, acute GvHD, fulminant viral hepatitis, mycotic liver infections or Budd-Chiari Syndrome [190–192].

However, the latest EBMT criteria for SOS/VOD stated that US characteristics, in addition to other clinical criteria, should be prioritised and even be mandatory in the context of a diagnosis of late-onset SOS/VOD[73].

The first evidence of the role of US in this syndrome dates back to the 1990s, when various authors described typical signs in a case series of patients who developed SOS/VOD-induced by HSCT or other forms of chemotherapy, in particular, hepatosplenomegaly, ascites, thickening of the gall bladder wall, ill-defined borders of the hepatic vessels, and failure to visualize the significant hepatic veins (HV)[193,194]. Gallbladder wall thickness is due to PH-related congestion and correlates with HVPG[195]. Similarly, portal vein (PV) flow decreases/reverses, or “to-and-fro” flow and triphasic hepatic venous outflow is lost due to the hepatic congestion leading to a loss of liver compliance[196].

From these results, the authors proposed the use of Color-Doppler US as an easy, non-invasive, repeatable and reliable tool for the daily monitoring of the effects of treatment with the recombinant tissue plasminogen activator (tPA), which was the SOS/VOD pharmacological treatment at that time[196]

However, evidence regarding the usefulness of US in SOS/VOD assessment has always been controversial. Around the same time, in fact, two prospective studies[197,198] found no strong association between the above described US signs and SOS/VOD diagnosis, and proposed a mean hepatic arterial resistive index (HARI) of ≥ 0.75 as a new indicator of SOS/VOD development, observing significantly high values in 95% of patients with SOS/VOD [198]. On the other hand, after finding no significant differences between patients with and without SOS/VOD, Teefey et al. [199] questioned the role of HARI and other signs, such as HV and PV flow, in SOS/VOD assessment.

The most critical effort in US research on SOS/VOD assessment was carried out by Lassau and colleagues [200]. In a large prospective study, with 100 patients who underwent high risk-HSCT, 25 developed SOS/VOD. The authors performed grey-scale and Doppler US examination before and then weekly after HSCT while patients were still hospitalised. Fourteen US criteria were then developed. The

seven grey-scale morphological US criteria were: hepatomegaly, PV diameter >12 mm, gallbladder wall thickening > 6 mm, HV <3 mm, splenomegaly, ascites, and visualisation of paraumbilical veins. On the other hand, the flow demodulation in the portal vein, a decrease in spectral density, portal flow <10 cm/sec, congestion index < 0.1, HARI > 0.75, monophasic flow in HV and flow in paraumbilical vein were assessed as being the best seven Doppler US criteria.

These US criteria have already been validated as US signs of portal hypertension by several medical ultrasound societies (e.g. European Federation of Societies for Ultrasound in Medicine and Biology, EFSUMB) and routinely used worldwide [201]. From these criteria, three scores have been developed: one for the B-mode US, one for Doppler-US, and one total score. A Doppler-US criteria score of >3 was correlated with CSPH, HVPG ≥10 mmHg [202]. A total score of >6, with 83% sensitivity, 87% specificity, 68% PPV and 94% NPV, were significantly associated with the diagnosis of SOS/VOD in patients who underwent HSCT. These criteria differ significantly between patients with SOS/VOD and those with GvHD of the liver, thus allowing a differential diagnosis between these forms. Finally, a total score of >8, in addition to total serum bilirubin >80 μmol/L, were identified as a negative predictive factor of mortality [200]

The same French research group in 2002 validated the previously described criteria in 71 children who developed SOS/VOD after intensive myeloablative therapy with Busulfan, in order to also establish their prognostic role in the pediatric population[202].

When a clinical diagnosis of SOS/VOD was provided, US examination (grey and Doppler-US) was performed. In the univariate analysis, three B-mode US criteria (i.e. splenomegaly, ascites and visualization of the paraumbilical vein), and three Doppler-US criteria (i.e. reversed flow or less than 10 cm/sec in the portal vein, decrease in spectral density of the portal flow and flow in the paraumbilical vein) were correlated with the severity of SOS/VOD. Of the above criteria, only ascites, splenomegaly and the visualisation of the paraumbilical vein were independently associated with the SOS/VOD severity degree in multivariate analyses. Finally, a total score of ≥9 was chosen as a predictive cut-off for grade 3 SOS/VOD severity. In conclusion, the authors suggested that an early Doppler-US could predict the SOS/VOD grade at the time of diagnosis, also with a higher sensitivity than bilirubin levels and HVPG.

In contrast, McCarville et al [203] evaluated eight US parameters in 202 prospective examinations of 48 pediatric patients who underwent HSCT: direction and velocity of PV flow, thickness of the gallbladder wall, HARI, Doppler flow pattern in the HVs and inferior

vena cava, echotexture of the liver, diameter of the common bile duct, and presence of ascites. No differences were identified between patients who developed SOS/VOD (n=29) and those who did not. Only the Doppler flow pattern in the HV seemed to be inversely associated with SOS/VOD diagnosis; however, significant variations in range values were observed. Flow demodulation in PV (inversion) was also associated with SOS/VOD diagnosis but was only present in a few cases. These authors concluded that the US parameters evaluated could not reliably predict or diagnose SOS/VOD in their pediatric group[204].

Since then, little new evidence in the US imaging field has been established[205,206]. An interesting paper by a group in Turkey was recently published, which focused on the diagnostic value of hepatic arterial maximal systolic velocity (V_{max}) assessed by Doppler-US in pediatric patients[204]. In 18 out of 36 patients who developed SOS/VOD, the V_{max} of the hepatic artery was significantly associated (p-value<0.001) with clinical SOS/VOD diagnosis, based on modified Seattle criteria. No other Doppler-US parameters were found to be associated with SOS/VOD by these authors.

The proof of concept study on the use of contrast-enhanced US (CEUS) in the early diagnosis of SOS/VOD[206] is also worth mentioning. The contrast enhancement pattern was equal to the

patchy liver enhancement described in other radiological examinations such as multi-detector row CT and MRI (see below), reflecting the hepatic hemodynamic changes.

A group in Japan has recently established a novel scoring system (HokUS-10) based on 10 US variables, such as gallbladder wall thickening, ascites, and blood flow signal in the paraumbilical vein, which predicted the SOS/VOD diagnosis with a sensitivity of 100% and specificity of 95.8% in 10 patients[207]. HokUS-10 is a more effective and simplified scoring system than the one proposed by Lassau et al.[200], and if adequately validated, could prove to be an excellent diagnostic tool for SOS/VOD diagnosis.

Despite much evidence regarding the utility of US imaging as a diagnostic tool, its role is still controversial due to the non-uniformity of results and various limitations of the method[192]. These examinations require an expert sonographer in Doppler-US. Furthermore, Color Doppler-US signs, which mirror severe portal hypertension, usually arise when an advanced stage of SOS/VOD has already developed; thus, its application may be minimal for the early diagnosis of SOS/VOD.

Role of Ultrasound Elastography techniques in SOS/VOD assessment

Ultrasound elastography techniques are a group of new and non-invasive methods, which evaluate the stiffness of parenchymatous organs through the detection of the distortion velocity of the tissue induced by shear waves[208]. The liver stiffness measurement (LSM) thus detects changes in the mechanical properties of the liver which occur whenever the architecture of its parenchyma is modified by the disease. Stiffness is increased, and elasticity is diminished due to fibrosis, inflammation, congestion, central venous pressure, and cholestasis [209–214].

In over ten years, many studies and meta-analyses have shown the close relationship between LSM and HVPG in advanced chronic liver disease (ACLD) patients, confirming LSM as an accurate surrogate of PH[[215–217]. Of the ultrasound elastographic techniques, only transient elastography (TE) and point-shear wave elastography (p-SWEs) by acoustic radiation force impulse (ARFI) imaging have been investigated in SOS/VOD assessment.

The first impression of changes in LSM in patients that developed SOS/VOD was reported by Fontanilla et al. in 2011[206]. The authors showed that the shear wave velocity, assessed by ARFI, increased in the two patients who developed SOS/VOD (2.75 and 2.59 m/sec,

respectively). More recently, Auberger et al. found that a pre-transplant LSM cut-off of 8 kPa could predict and differentiate patients that developed liver toxicity after HSCT[218]

A critical study on the role of ultrasound elastography in post-HSCT complications was performed by Karlas and colleagues, who prospectively enrolled 59 patients[219]. Major complications (grade 4 Common Terminology Criteria CTC for adverse events) [220] occurred in seven patients: four with acute GvHD and severe liver toxicity, two with SOS/VOD development and one case of transplant rejection. The authors showed that baseline liver and spleen size, liver perfusion, TE and right lobe LSM (R-ARFI) did not differ significantly between patients with and without severe complications. The only baseline left lobe LSM (L-ARFI) values were significantly increased in the group with complications. In general, these authors found a slight increase in LSM values in almost all patients throughout the post-HSCT period, probably due to oedema and hepatic inflammation caused by the high number of drugs administered in this context. On the other hand, in five patients who developed severe hepatic complications, LSM by TE was significantly higher (6.2±1.5 kPavs4.7±1.7 kPa, p=0.043) in comparison with the whole population cohort. However, the authors were unable to differentiate among the different post-HSCT complications.

Several research groups have recently focused on ultrasound elastography techniques in SOS/VOD assessment. A proof of concept of elastography usefulness in SOS/VOD assessment has now been achieved by Park's group, who evaluated the role of ARFI in a murine model with histologically diagnosed SOS/VOD[221].

The authors induced SOS/VOD with different severity stages in rat models, by monocrotaline gavage (n=40) or by intraperitoneal injection of 5-fluorouracil, leucovorin and oxaliplatin (FOLFOX) (n=16), in order to obtain acute and chronic SOS/VOD development respectively. Liver Shear Wave Velocity (SWV), assessed by ARFI imaging in the median lobe, was higher in the SOS/VOD-rat models than the matched control group (1.29–2.24 m/s vs 1.01–1.09; $p \leq 0.09$). Subsequently, two liver pathologists evaluated rat liver samples by assessing the presence of SOS/VOD features (defining an "SOS-score"). Liver SWV was significantly related to the histological SOS/VOD score ($r = .858$, $p < .001$) and lobular inflammation ($r = 0.819$, $p < .001$). Moreover, in acute monocrotaline-induced SOS/VOD, the liver SWV gradually and significantly decreased in the post-treatment period, while in FOLFOX-induced SOS/VOD, the stiffness values decreased after two weeks of FOLFOX treatment interruption. These behaviours seem to describe different sinusoidal injuries due to heterogenic pathogenetic patterns.

Thus, these results in the murine model may better explain the role of liver stiffness variations in SOS/VOD diagnosis.

A recent study by our group[222]investigated the predictive role of LSM changes, assessed by TE, in SOS/VOD development after HSCT in paediatric patients. Of the 22 patients enrolled, five patients developed SOS/VOD after HSCT. LSMs were carried out at baseline (before HSCT), and subsequently at the bedside at days 7-10, 17-20 and 27-30 after HSCT. No significant differences were observed at baseline LSM between patients that developed SOS/VOD and those that did not. In fact, in patients that developed SOS/VOD, LSM values increased markedly compared to the previous measurement, reaching values from 10.3 to 59.3 kPa. LSM increases were observed from 3 to 6 days before clinical SOS/VOD diagnosis. Based on these preliminary results, a national multicentre prospective study in Italy ("ElastoVOD Study" ClinicalTrial.gov: NCT03426358) was set up, aimed at confirming the prognostic role of LSM. All our above results are better and extensive, shown in part two of the present thesis dissertation. Similarly, a recent study by Reddivalla et al. [223] evaluated SWV by 2D-SWE in 25 paediatric patients at baseline and days +5 and + 14 after HSCT. An SOS/VOD diagnosis was made in five patients, observing no differences in pre-conditioning SWV between SOS/VOD and the control group (1.24 ± 0.09 m/s vs 1.41 ±

0.18 m/s, p=0.06). Interestingly, a significant increase in SWV velocity was observed in patients that developed SOS/VOD (at day +5: by 0.25 ± 0.21 m/s vs. 0.02 ± 0.18 in the control group; at day +14: by 0.91 m/s ± 1.14 m/s vs. 0.03 m/s ± 0.23 m/s). These authors concluded that an increase in SWV generally preceded a clinical and US-based SOS/VOD diagnosis by 9 and 11 days, respectively.

The first monocentric study on an adult population of patients undergoing HSCT was recently published by our group [224]. We confirmed that LSM increases occurred from +2 to +12 days before clinical SOS/VOD appearance and gradually decreased following successful SOS/VOD specific treatment also in the population of adult patients. Moreover, for the first time, we showed that LSM values did not significantly increase in patients experiencing hepato-biliary complications (according to common terminology criteria, CTC) other than SOS/VOD [224]. Again, all our above results are better and extensive, shown in part two of the present thesis dissertation.

These results need to be validated by extensive prospective studies in order to define the most suitable application of LSM in clinical practice [192]. However, many experts agree that liver elastography is a promising method since it is a non-invasive, bedside method for the early diagnosis and follow-up of SOS/VOD. Besides, it is well tolerated by patients, reproducible, and provides an objective

quantitative value, preventing radiations, intravenous contrast agents, and risks of bleeding or infection.

Diagnostic role of Computed Tomography (CT) in SOS/VOD assessment

CT is routinely used in patients undergoing HSCT to evaluate transplant complications, particularly gastrointestinal Graft-versus-Host-Disease (GvHD)[225]. However, its role in SOS/VOD diagnosis in HSCT patients has only been investigated in the last few years. Most studies with CT derive from other contexts, such as SOS/VOD after liver transplantation or induced by other chemotherapy drugs (carboplatin, cisplatin and oxaliplatin) and hepatotoxic agents (pyrrolizidine alkaloids which are found in plants and shrubs).

To our knowledge, Erturk et al. were the first to retrospectively evaluate the CT scans of 18 patients after HSCT, with histological diagnosis of SOS/VOD, GvHD or both. Ascites, periportal oedema and right hepatic vein narrowing, were found to be the most specific findings for SOS/VOD, while small-bowel wall thickening seemed exclusive to GvHD[226].

Other studies have focused on the diagnostic role of CT in small cohorts of patients that developed SOS/VOD induced by pyrrolizidine alkaloids (PA). In 2012, Wu et al. observed some recurrent findings

by multi-detector row CT, Angio-CT and multiplanar reconstruction (MPR) in 10 patients with SOS/VOD-induced by "*Gynura-Rhizome*": all patients presented ascites, hepatomegaly (>18 cm), pleural effusion and gallbladder wall thickening[227]. In most patients, periportal oedema was also visible. After administration of the contrast agent, during the portal phase, a diffuse heterogeneity in hepatic enhancement was observed. Angiographic reconstruction was not able to identify hepatic veins in any of the patients. A larger group of 39 patients with SOS/VOD caused by another plant, "*Sedum aizoon*", was studied by Shao et al. with CT assessment[228]. The findings of this study agreed with the results described above. Besides, the authors highlighted the presence of splenomegaly, CT heterogeneous density of liver parenchyma, with widespread hypodense areas and again non-uniform contrast-enhancement.

A more exhaustive study was carried out by Kan et al. [229] in 2016, in order to establish the diagnostic value of CT radiological findings. The authors compared the CT-scans of 71 patients with SOS/VOD-induced by "*Gynura Segetum*" ingestion with 222 patients with hepatic cirrhosis or Budd-Chiari syndrome. In the SOS/VOD cases, typical findings were ascites (100%), hepatomegaly (78.9%), gallbladder wall thickening (87%) and pleural effusion (70.4%). After contrast administration, the entire cohort of SOS/VOD patients

showed heterogeneous hypoattenuation, and 93% presented patchy liver enhancement. These CT characterizations were present in a small subgroup of Budd-Chiari patients (19.3% hypo attenuated areas, and 28.1% irregular contrast-enhancement). In addition, hepatic vein narrowing was present in 87.3% of SOS/VOD patients, while other signs of portal hypertension such as collateral circulation and splenomegaly were more common in the cohort of control patients (cirrhosis and Budd-Chiari syndrome) [229]. CT-patchy liver enhancement showed a sensitivity of 93%, specificity of 92.8%, a positive predictive value (PPV) of 80.5%, a negative predictive value (NPV) of 97.6%, and an overall accuracy of 91.8% in SOS/VOD diagnosis. On the other hand, the CT-heterogeneous hypo attenuation showed a sensitivity of 100%, a specificity of 95.1%, PPV of 86.6%, NPV of 100%, and overall accuracy of 96.3%. The authors thus concluded that these radiological signs were the most specific features of SOS/VOD development by CT examination. In fact, all of the above radiological findings resulted in a superior SOS/VOD diagnostic performance than merely using clinical signs such as ascites, jaundice and hepatomegaly. Accordingly, the diagnostic performance of contrast CT was better than clinical Seattle criteria in PAs-induced SOS/VOD.

In 2017, further confirmation came from a meta-analysis carried out by Yang[230], who considered 11 studies involving a total number of 326 patients, in order to better establish CT feature incidence in SOS/VOD diagnosis. The authors analysed the presence of the above described CT findings, confirming that both heterogeneous hypoattenuation and patchy enhancement, with or without narrowing or an invisible hepatic vein were the essential features for SOS/VOD diagnosis.

The diagnostic role of the CT scan was also evaluated in patients undergoing Oxaliplatin-based chemotherapy. In the last few decades, the use of preoperative chemotherapy has facilitated radical surgical resection in patients with previously unresectable liver metastasis[231]. Unfortunately, the Oxaliplatin-based regimen has also been related to essential liver complications, such as SOS/VOD development, which could considerably influence the outcome of patients requiring liver resection surgery[102,232].

CT-scans could be very useful in this context, as they are the main radiological techniques used for cancer staging and surveillance in these patients[233]. According to Bethke and colleagues, sinusoidal congestion in SOS/VOD causes a delay in the inflow of radiocontrast agent in contrast-enhanced CT, and thus it could arise in the portal venous phase with heterogeneous attenuation of hepatic

parenchyma[233]. The pattern of "Post Oxaliplatin Heterogeneity of Liver parenchyma" (POHL) appears to be related to SOS/VOD development. In fact, in 2015, Han and colleagues [234], also confirmed the association of POHL (61.9%) with SOS/VOD development in 270 patients treated with oxaliplatin-based chemotherapy using serial contrast-enhanced CT. POHL was predominantly located in the peripheral area (67.1%) and right hepatic lobe (62.4%). A multivariate analysis POHL was independently associated with SOS/VOD clinical factors. Furthermore, more severe forms of POHL appear to be related to increasing spleen size and decreased attenuation of the hepatic parenchyma[234].

Similar results have been recently found by Cayet et al.[235] who evaluated preoperative CT-scans in 67 patients undergoing hepatic surgical resection after neoadjuvant chemotherapy for colorectal cancer metastasis. The authors identified not only a heterogeneity pattern but also other SOS/VOD-related CT features in 29 patients who presented a histological SOS/VOD diagnosis. A clover-like sign, increase in spleen volume of >30% and peripheral distribution of heterogeneity were identified as independent predictors of SOS/VOD. Regarding Oxaliplatin-based chemotherapy, a new SOS/VOD presentation has been increasingly reported. Some case reports[236–

238] have described unusual radiological CT-scans after Oxaliplatin chemotherapy with “focal SOS/VOD lesions”, as a new form of "chemotherapy-induced sinusoidal injuries". A CT-scan of focal SOS/VOD can often be misinterpreted as hepatic metastasis, leading to unnecessary biopsies and hepatectomies. Mass-forming sinusoidal injuries, mimicking metastatic liver tumours, usually appear at contrast-enhanced CT as hypodense, unenhanced lesions, which are often categorised as indeterminate lesions by radiologists[239]. More information is still needed to recognise pseudo metastasis at CT-scan. Despite the above shreds of evidence, CT examination for SOS/VOD suspicion is not routinely used in clinical practice, especially in HSCT patients, and further studies are needed to identify the role of CT in the early and differential diagnosis of SOS/VOD[192].

Diagnostic role of Magnetic Resonance Imaging (MRI) in SOS/VOD assessment

Although evidence is still limited to clinical case series, the interest in MRI as a new radiological technique for SOS/VOD diagnosis has increased.

The first observations on MRI features were reported by van den Bosch et al. in 2000 [240] who observed hepatomegaly, hepatic vein

narrowing, gallbladder wall thickening, periportal cuffing, ascites and pleural effusion in two patients who developed SOS/VOD after HSCT. These findings were explained by the authors as a result of increased resistance to lymphatic and venous inflow. They concluded that MRI could be a useful radiological tool to be performed after the Doppler-US examination. In fact, in addition to Doppler-U S, MRI enables slow portal venous flow to be differentiated from hepatic veins thrombosis and to define better various US findings that could be nonspecific (e.g. gallbladder wall thickening in SOS/VOD is associated with high signal intensity on T2-weighted images).

Two years later, another case [241] of SOS/VOD-induced by the recreational drug “popper” was studied with an MRI and compared with Budd-Chiari syndrome MRI-signs. Unlike Budd-Chiari syndrome, MRI features in SOS/VOD included signs of liver congestion spread throughout the parenchyma, with highly heterogeneous signal intensity and multiple contrast enhancement points (patchy liver enhancement), consistent with collateral vessels. In Budd-Chiari syndrome, on the other hand, hepatic congestion changes are typically observed in peripheral areas of the liver and are determined by venous shunts which provide hepatic venous drainage from central areas and the caudate lobe of the liver. Accordingly, hypotrophy of peripheral zones and hypertrophy of central non-

congested zones were observed only in Budd-Chiari syndrome patients.

Despite these slight differences, the authors concluded that the most reliable MRI-signs capable of differentiating between the two syndromes are the observation of hepatic veins or inferior cava vein obstruction.

Although supported by a few studies [242], patchy liver enhancement in Contrast-enhanced MRI seems to be associated with SOS/VOD development.

In 2014, a Korean research group directed by Zhou[243]compared the CT and MRI findings of 16 patients who had developed SOS/VOD induced by “Gynura Segetum”. Of these, eight patients had a CT evaluation, three patients had an MRI assessment, and five patients had both techniques. CT and MRI features were in agreement. In the MRI, all patients showed ascites, main right hepatic vein narrowing and “patchy enhancement” after gadolinium infusion. Furthermore, in 87.5%, 50% and 75% of patients, hepatomegaly and gallbladder wall thickening, periportal hyper-intensity and periportal cuffing in T2-weighted were described, respectively.

Zhou and collegues[243]described for the first time, both in CT and MRI examination, the so-called “clover sign” in 25% of all patients,

which consisted in an area of liver parenchyma with regular enhancement in venous phases, surrounding the main hepatic vein. Finally, the authors established a significant association between the extent of abnormal enhancement areas and severity of SOS/VOD.

In 2017, a Chinese group reported the largest retrospectively study on MRI evaluation in patients with pyrrolidine alkaloid-related SOS/VOD[244]. Of the 90 patients who developed PA-SOS/VOD, 39 underwent MRI. The distribution and severity of hypointense heterogeneity were evaluated according to 4-point scales. The most common distribution of heterogeneous hypointensity was diffuse (76.92%), and right lobar predominance (17.95%) and hypointensity (92.31%) was scored as severe (grade 4).

In 74.36% of cases, non-homogeneous enhancement around the hepatic veins, defined as "claw-shaped enhancement", was described and explained as an expression of differential perfusion in PA-induced SOS/VOD.

It is also worth highlighting various observations on MRI with hepato-specific contrast agents in patients with SOS/VOD diagnosis. In patients with colorectal liver metastases undergoing hepatic surgery resection after neoadjuvant Oxaliplatin-based chemotherapy, SPIO-MRI (Super Paramagnetic Iron Oxide contrast-MRI) is a preoperative exam, which is highly sensitive in detecting liver

metastases. Performing preoperative SPIO-MRI in 60 patients, Ward et al. [245] observed a reticular hyperintensity in T2-weighted sequences, in 24 patients who developed SOS/VOD (confirmed by histological examination) with a sensitivity, specificity, PPV and NPV of 87%, 89%, 83% and 92%, respectively. In the study, two observers were involved whose task was to identify and classify linear and reticular hyper-intensity, in a 4-grade severity scale. Notably, the correlation between SPIO-MRI and histological examination was better for severe forms of SOS/VOD.

A similar study was performed in 42 patients by Shin et al. [246] in 2012, assessing preoperative MRI with another hepato-specific contrast, Gadolinium EthOxyBenzyl DieThylenetriamine Pentacetic Acid (Gd-EOB-DTPA), also known as Primovist. In 16 out of 42 patients who developed SOS/VOD after chemotherapy for hepatic metastasis, a reticular hypo-intensity in areas with hepatocellular dysfunction and injury was observed in hepato-biliary phases. The authors concluded that Gd-EOB-DTPA-MRI, compared with histological examination, achieved a sensitivity of 75% and a specificity of 96-100%, with lower accuracy in mild SOS/VOD identification. A further recent report described Gd-EOB-DTPA-MRI findings in a case of SOS/VOD occurring after thiopurine

administration in a patient with Crohn's disease, confirming the previous typical MRI signs also in this scenario[247].

In patients undergoing Oxaliplatin-based chemotherapy, unlike with CT-scans in which focal SOS/VOD mimics liver metastasis, some authors have suggested the role of MRI in differentiating "chemotherapy-induced sinusoidal injury" from real metastasis.

As described by Han et colleagues in 2014[118] a lesion with ill-defined margins, non-spherical shape and intermingled signal intensity pattern is suggestive of focal SOS/VOD, while metastatic nodules usually presented a well-defined margin, spherical shape and homogeneous signal intensity in the gadoxetate-enhanced hepatobiliary phase (HBP-MRI). Also, 69% of focal SOS/VOD in the hepatobiliary phase presented a reticular heterogeneity of background liver tissue. Above all, the enhancement pattern of the focal SOS/VOD lesions at HBP-MRI and diffusion-weighted (DW-MRI) was different from the typical metastasis pattern.

Notwithstanding these intriguing data on the diagnostic role of MRI examination, in particular with hepato-specific contrast, further studies with a larger cohort of patients who develop SOS/VOD, are needed in order to identify the role of MRI in the early diagnosis of SOS/VOD and in differential diagnoses with other liver vascular lesions[192].

Diagnostic role of Nuclear Medicine in SOS/VOD assessment

Experience with nuclear medicine in SOS/VOD evaluation is minimal, although the first evidence suggesting the application of these techniques in this context originates from as early as 1975. The first cases [248] assessing scintigraphy with ^{99}mTc-pertechnetate in patients with a confirmed histological diagnosis of SOS/VOD, showed hepatomegaly and multiple widespread areas of hypo-captation of radionuclides. The authors underlined that the above findings, although not highly specificity for SOS/VOD, have a very different scintigraphy pattern from Budd-Chiari syndrome (right, a large and single area of hypo-captation).

A Japanese research group [249] recently used Rectal Portal Scintigraphy to investigate the development of SOS/VOD in a patient undergoing an unrelated cord blood transplant. This technique is based on nuclear medicine and is proposed as a new, non-invasive and potentially safe method for detecting patients who developed SOS/VOD after HSCT. Rectal portal scintigraphy with ^{99}mTc-pertechnetate is a method to evaluate of portosystemic shunting using the portal shunt index (SI), calculated from radioactivity curves of the liver and the heart. A radioactive tracer absorbed from the rectum passes through the inferior mesenteric vein into the portal vein, the

liver and then the right heart. In the case of reports, 60% of patients developed SOS/VOD, SI indicating severe PH (normal values <10%). Despite its potential, only prospective studies with large cohorts will be able to investigate and possibly confirm these results.

Other authors have proposed 18-FluoroDeoxyGlucose Positron Emission Tomography Computed Tomography (18-FDG-PET/TC) to detect patients who developed SOS/VOD.

After various case reports[250] in 2016 Kim and colleagues [251] showed that hepatic FDG uptake on PET/CT significantly intensified after the onset of SOS/VOD in 35 patients who underwent chemotherapy for colorectal liver metastases. Since FDG-PET/CT is frequently used to evaluate chemotherapy response in tumoral lesions, Kim underlined that an SOS/VOD-increased uptake could be responsible for errors in oncological evaluations [250]. A possible explanation for altered hepatic FGD uptake may lie in microvascular SOS/VOD physiopatogenetic disturbances, resulting in sinusoidal congestion state and endothelial cell dysfunction [251]. It has been reported that FGD uptake distribution could coincide with widespread hypodense areas seen at CT, compatible with the alterations previously described [97].

Based on the inadequate current evidence of this technique in the assessment of SOS/VOD, it is not possible to draw definitive

conclusions regarding the role of nuclear medicine as a useful tool when deciding on how to treat this disease [192].

Techniques	Common findings	Advantages	Disadvantages
Hepatic Venous Pressure Gradient (HVPG)	Increase in Hepatic Venous Pressure Gradient (> 10 mmHg predicts CSPH and VOD/SOS diagnosis with good accuracy/a specificity over 90%)	Good accuracy in CSPH assessment Concurrent liver biopsy (LB) can be performed, leading to a conclusive VOD/SOS diagnosis Trans-jugular approach safer than others Prognostic Value	Invasive High bleeding or infectious risk (pancytopenic patients) Contrast medium and radiation exposure risks Unclear evaluation in 'grey area' (5–10 mmHg) Available in few centers High expertise required
Grey Scale Ultrasound (US)	Hepatomegaly, splenomegaly, ascites, gallbladder wall thickening, dilatation of portal vein (diameter > 12mm), narrowing of hepatic veins, paraumbilical vein recanalization	Non-invasive No contrast medium or radiation exposure risk Bedside feasible Available in most centers First-line technique for differential diagnosis	Operator-dependent No VOD/SOS specific signs Non-uniformity of results No quantitative values for assessing PH degree
Doppler Ultrasound (Doppler-US)	Reversal or reduction (mean velocity <10 cm/sec) of portal vein flow, Congestion Index < 0.1, hepatic artery Resistive Index (HARI) > 0.75, monophasic flow in hepatic veins, detected flow in paraumbilical vein, decrease in hepatic arterial maximal systolic velocity (Vmax)	Non-invasive No contrast medium or radiation exposure risk Bedside feasible Good accuracy in PH assessment Useful in differential diagnosis	Strongly operator-dependent High expertise required PH signs only appear in advanced stages of VOD/SOS High non-uniformity of results
Contrast-enhanced Ultrasound (CEUS)	Patchy liver enhancement, similarly to other contrast-enhanced imaging techniques	Non-invasive No radiation exposure risk No nephrotoxic iodine-contrast exposure risk Bedside feasible	Very limited evidence Strongly operator-dependent High expertise required Available in few centres Contrast medium exposure risk Not feasible with severe cardiological disease
Ultrasound Elastography Techniques: Transient Elastography by FibroScan® (TE) Point-Shear Wave Elastography (p-SWEs) by Acoustic Radiation Force Impulse imaging (ARFI)	Increase in Liver Stiffness Measurement (LSM) assessed by Transient Elastography by FibroScan® (TE) and Point-Shear Wave Elastography (p-SWEs) by Acoustic Radiation Force Impulse imaging (ARFI), from 1 to 9 days before clinical diagnosis; normalization of values, in accordance with VOD/SOS resolution	Non-invasive No contrast medium or radiation exposure risk Bedside feasible Easy to perform and high reproducibility (inter-operator) Provides objective and quantitative value Related to congestion and PH grades Increased stiffness values indicate need for clinical diagnosis, useful in early assessment	Evidence still limited Performance in differential diagnosis needs to be assessed Not feasible if ascites, severe obesity, pacemaker and implantable device
*Computed tomography (CT)**	Most relevant features: heterogeneous hypoattenuation of liver parenchyma, patchy liver enhancement at Contrast-enhanced scans. Other signs: Ascites, hepatomegaly, periportal edema, gallbladder wall thickening, pleural effusion, narrowing or invisible hepatic veins. In patients underwent oxaliplatin-based chemotherapy for liver metastasis: heterogeneous attenuation of hepatic parenchyma in portal venous phase (so called 'Post Oxaliplatin Heterogeneity of Liver parenchyma' – POHL), predominantly located at the peripheral area and right hepatic lobe. In few cases: focal SOS/VOD mimicking liver metastasis	Non-Invasive Available in most centres Useful in differential diagnosis (e.g. GvHD.) Largely used in patients with liver metastasis, in post-oxaliplatin preoperative evaluation Good accuracy for SOS/VOD diagnosis, specific signs especially in contrast- enhanced scans	Contrast medium and radiation exposure risks Not bedside feasible Expert radiologist is required Cost No quantitative values related to PH Limited evidences, further studies are needed (HSCT context)
*Magnetic Resonance imaging (MRI)**	Most relevant features: highly heterogeneous signal intensity, patchy liver enhancement in gadolinium-enhanced sequences. Other signs: Ascites, hepatomegaly, periportal cuffing, gallbladder wall thickening, pleural effusion, narrowing of hepatic veins. In patients underwent oxaliplatin-based chemotherapy for liver metastasis: utility of hepato-specific contrast-enhanced MRI, with reticular hypointensity or hyperintensity (according to the different contrast agent used).	Non-Invasive Available in most centres No radiation exposure risk Good accuracy for SOS/VOD diagnosis, specific signs especially in contrast- enhanced scans Used in patients with liver metastasis, for post-oxaliplatin preoperative evaluation Good differentiation between focal-SOS/VOD and liver metastasis Usefulness of hepato-specific contrast medium	Gadolinium contrast related risk Not bedside feasible Expert radiologist is required High cost No quantitative values related to PH Limited evidence in HSCT context (cases series) Not feasible in patients with pacemakers, defibrillators, other implanted electronic devices, metallic clips or implants
*Positron Emission Tomography (PET)**	Significantly intensified 18-FluoroDeoxyGlucose (FDG) uptake liver areas; the distribution coincides with widespread hypodense areas at CT	Non-Invasive Available in most centres	Very limited evidence Exposure to radiation Expert radiologist is required Not bedside feasible High cost No quantitative values related to PH

*Very limited evidence in HSCT context

Table 05: The most significant findings and pros- and cons- of various imaging techniques in SOS/VOS diagnosis (from Ravaioli et al. (2019) *Expert Review of Gastroenterology & Hepatology, 13:5, 463-484)*

Integrative imaging approach to SOS/VOD diagnosis

Only in the HSCT context is there an established and reference standard for SOS/VOD diagnosis which is represented by clinical criteria [74] however with the new evidence, imaging may be useful

for oncohematologists and hepatologists to detect early or mild development of VOD (Table 05). We recently underlined the important potential significance of the use of liver elastography as an accurate, easy-to-use, non-invasive, bedside technique, which is repeatable and capable of early and preclinical diagnosis of VOD / SOS. A prompt diagnosis of VOD / SOS/VOD would increase the probability of response to drug therapies and a decrease in SOS/VOD-related mortality [192].

There is no doubt that most clinical and radiological research on SOS/VOD diagnosis has focused on the context of HSCT due to the crucial clinical implications[192]. Consequently, we proposed that in the next five years, the contribution of radiological techniques to SOS/VOD diagnosis in the HSCT-context could radically change from a solely clinical to an integrated clinical-radiological diagnosis.

We believe that the clinical criteria and non-invasive imaging techniques should be integrated into multidisciplinary diagnostic algorithms aimed at guiding the clinical decision-making of SOS/VOD diagnosis after HSCT where imaging could play an important role (Figure 04). We also believe that a multidisciplinary team would be highly recommended for a prompt diagnosis and tailored management of patients with SOS/VOD [192].

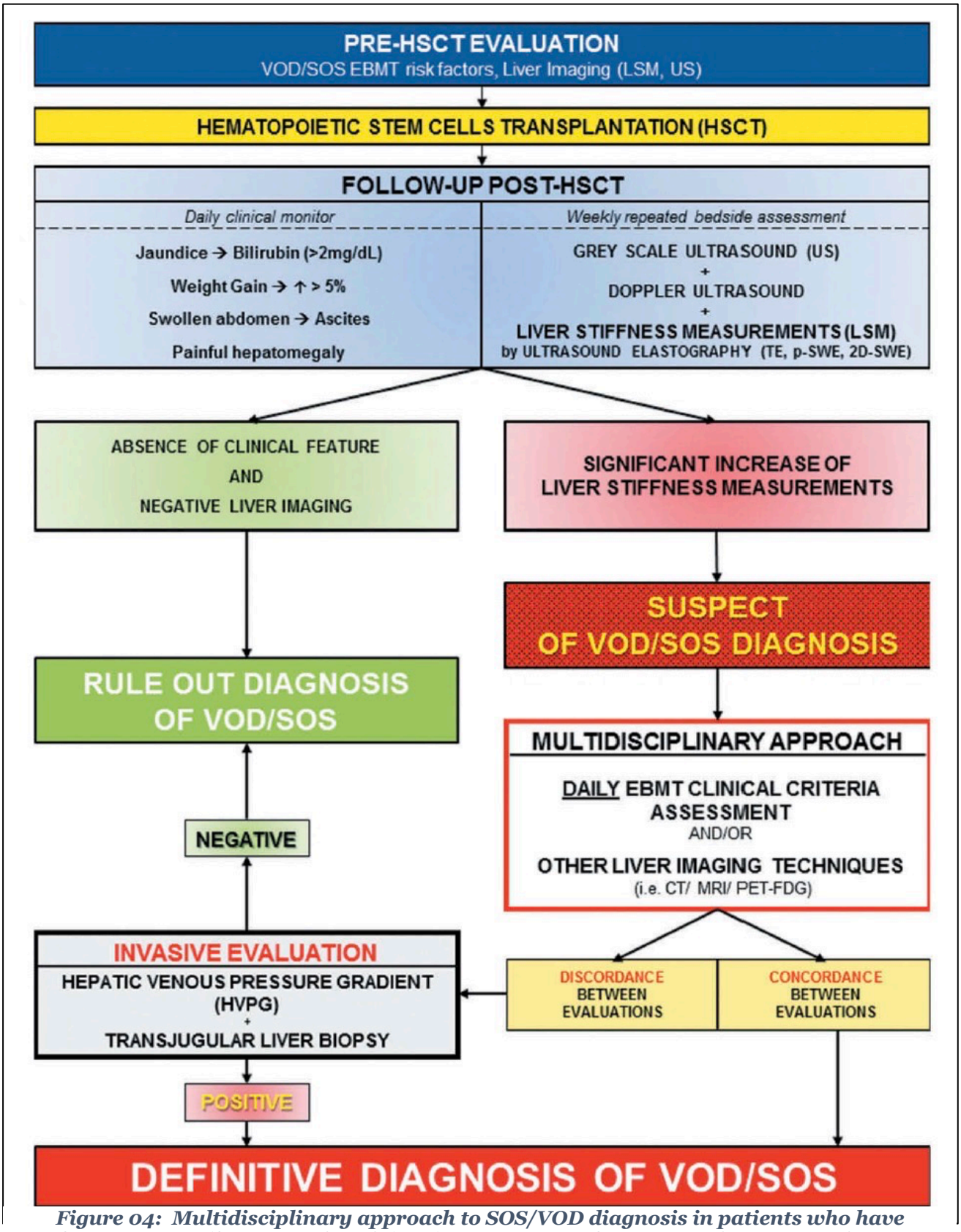

Figure 04: Multidisciplinary approach to SOS/VOD diagnosis in patients who have undergone HSCT *(from Ravaioli et al. (2019) Expert Review of Gastroenterology & Hepatology, 13:5, 463-484)*

PART TWO
– RESEARCH STUDIES –

PART 2 – RESEARCH STUDIES

INTRODUCTION

Veno-Occlusive Disease (VOD), also known as Sinusoidal Obstruction syndrome (SOS), is a relatively rare but potentially life-threatening complication which might affect patients after Hemopoietic Stem Cell Transplantation (HSCT)[73]. It occurs roughly in 14% (5-60%) of patients after HSCT[38] depending on several known factors[35,252–254]. From a pathophysiology point of view, the conditioning regimens lead to a toxic injury in sinusoidal endothelial cells with consequent damage of hepatic *acini,* with a blood outflow block causing sinusoidal portal hypertension (PH)[36,255,256]. The clinical signs such as ascites, weight gain, painful hepatomegaly and jaundice, which are the clinical diagnostic criteria for SOS/VOD[30,31,40,74] are directly connected to the PH. Despite these clinical criteria being crucial to establishing SOS/VOD diagnosis, several conditions can mimic or overlap SOS/VOD, (e.g. cholangitis, bacterial sepsis, acute GvHD and biliary sludge…) and often histological confirmation is needed to discriminate accurately among multiple differential diagnoses[72].

Moreover, severe forms of SOS/VOD lead up more frequently to severe multiorgan dysfunction/failure (MOD/MOF)[256] with a

mortality rate that can reach, in the historical cohort, up to 80%[154]. However, it has been demonstrated that an early and prompt diagnosis, leading to earlier specific treatment initiation[257], significantly improved at day +100 survival both in a subset of patients with MOF and in those without MOD[258,259].

For this reason, the recognition of SOS/VOD in its milder forms or at earlier stages[260] assumes a high grade of relevance. New ultrasonography scores and technological ultrasound improvement may help the physician to confirm the diagnosis in the suspect of SOS/VOD [191,200,207]; however, early recognition of SOS/VOD remains a challenge. Invasive methods, such as the measurement of Hepatic Vein Pressure Gradient (HVPG) and trans-jugular liver biopsy approach, can lead to a conclusive SOS/VOD diagnosis with high specificity although their use may be limited in selected cases and well-trained centres[72].

Recently, Ultrasound Elastography techniques, assessing the Liver Stiffness Measurements (LSM), have been investigated as a possible surrogate of PH degree and its complications[215,217,261,262]. Indeed, many studies and meta-analyses confirmed the close relationship between LSM and Hepatic Vein Portal Gradient (HVPG), the gold standard method to evaluate PH, in patients with advanced chronic liver disease (ACLD)[214,263,264].

A recent paper has shown that LS measurement using Point-Shear Wave Elastography (p-SWEs) by Acoustic Radiation Force Impulse (ARFI) and Transient Elastography (TE) by Fibroscan® (Echosens, Paris, France) could represent an additional diagnostic parameter for adult patients with suspected post-transplant hepatic injury; in fact, LS pretransplant values using ARFI < 1.25 m/sec were correlated with the absence of severe complications after allo-HSCT[219].

Here, I would report the results from our extensive evidence and results collected so far, from the ELASTOVOD project evaluating the role of LSM assessed in SOS/VOD development in patients undergoing allogeneic HSCT for haematological malignancies.

METHODS

Study design

The project was an interventional study without drugs developed in two sequential phases: the first was a monocentric proof of the concept study in Bologna from April 2015 to November 2017; the second was a national multicentre study involving 47 Italian transplant centres and is still ongoing (clinicaltrial.gov NCT03426358). The results of the first phase have already published in peer-reviewed international journals[222,224,265]

Aims:

The primary aim of the project was assessed in a large, mixed (adult and paediatric) population undergoing HSCT, the role of Liver Stiffness Measurement (LSM), assessed by different elastography methods, in SOS/VOD diagnosis.

Moreover, in these patients undergoing HSCT, we secondly aimed to:

- identify the actual incidence of SOS/VOD in the adult/ paediatric Italian population;
- evaluate the role of biochemical, ultrasound scores systems and clinical variables as possible predictors for SOS/VOD;
- define the role of LSM in the differential diagnosis among other liver-related HSCT complications;

- compare the application of LSM and ultrasound (US) in SOS/VOD assessment;
- to evaluate the role of LSM in SOS/VOD follow-up management.

Patients population

The project population was all consecutive adult or paediatrics patients undergoing allogeneic HSCT at the Institute of Hematology "L. and A. Seràgnoli" and Hemato-Oncologic Pediatric Unit, Sant'Orsola-Malpighi University Hospital, Bologna, Italy (first and second phases) or from the 47 participating HSCT-transplant centres in the multicentre phase (**Figure 05)**

Inclusion/exclusion criteria

Figure 05: Italian maps of the centers involved in the ElastoVOD

The inclusion criteria were allogeneic HSCT transplant from any donor and hematopoietic stem cell source, with either myeloablative and reduced-intensity conditioning, haematological indication for allogeneic transplant, age between 3 and 70 years.

The exclusion criteria were patients with pathological obesity (BMI over 40 kg/m^2), with ascites and presence of advanced chronic liver disease (ACLD) at pre-HSCT assessment, in order to avoid possible technical LSM bias.

Study schedule

The study protocol, in both phases, foresaw a clinical evaluation, biochemical assays, a grey-scale and the colour-doppler US, and LSM evaluation of all patients included at enrolment (within a month before HSCT). Then the LSM subsequent evaluations were done by means of a dense schedule of LSM assessment on days +9/10 (T1), +15/17 (T2) and +22/24 (T3) after HSCT, three times a week assessment of biochemical laboratory tests and a daily clinical assessment of presence of SOS/VOD criteria from the start of preparing regimen until 30 days after transplant. In presence of clinical suspicion of SOS/VOD, according to the EBMT criteria[9], during the time interval between two subsequent scheduled time points (T0-T1, T1-T2, T2-T3 or >T3), in addition to the above schedule of the study protocol, a Colour-Doppler US, LSM and an

intense clinical monitoring were carried out as required by the severity of the clinical feature. In case of LSM increasing value was found at any scheduled time points, Colour-Doppler US, biochemical tests and intensive clinical monitoring were further assessed to confirm/exclude the diagnosis, according to the EBMT recommendations when SOS/VOD was diagnosed, weekly LSM evaluations until clinical SOS/VOD resolution were performed.

VOD/SOD diagnosis

SOS/VOD of the liver was defined by EBMT clinical diagnostic criteria for adults and pediatric patients (Table 02). Accordingly, painful hepatomegaly, weight gain, and bilirubin ≥ 2.0 mg/dL with or without ultrasound signs (only in late-VOD) were considered as typical clinical signs for SOS/VOD diagnosis; in doubtful cases to confirm clinical diagnosis further invasive diagnostic methods (liver biopsies and Hepatic Venous Portal Gradient, HVPG) have been performed. SOS/VOD grading was defined by EBMT criteria for severity grading [9 (). VOD with MOF was defined as the presence of renal or pulmonary dysfunction in addition to VOD. Furthermore, the classical modified Seattle or Baltimore criteria[40] were also recorded.

Other hepatobiliary complications

The occurrence of other hepato-biliary complications after HSCT such as hepatic GvHD, infective hepatobiliary diseases and other Drug-Induced Liver Injury (DILI) following clinical definitions[266–269] were also evaluated. Moreover, patients with liver biochemical alterations were stratified based on the common terminology criteria for adverse events (CTC AE) version 4 [220].

Liver Stiffness Measurements (LSM)

In the monocentric phase, LSM values were assessed bedside of patients by Transient Elastography (TE), using the FibroScan® apparatus with "M" probe (Echosens, Paris, France) after overnight fasting and after a complete abdominal ultrasound (US) examination. In the multicentre phase, the LSM values were evaluated based on the local experience of elastometric techniques, including in addition to TE, also pSWE and 2D-SWE techniques (Figure 06).

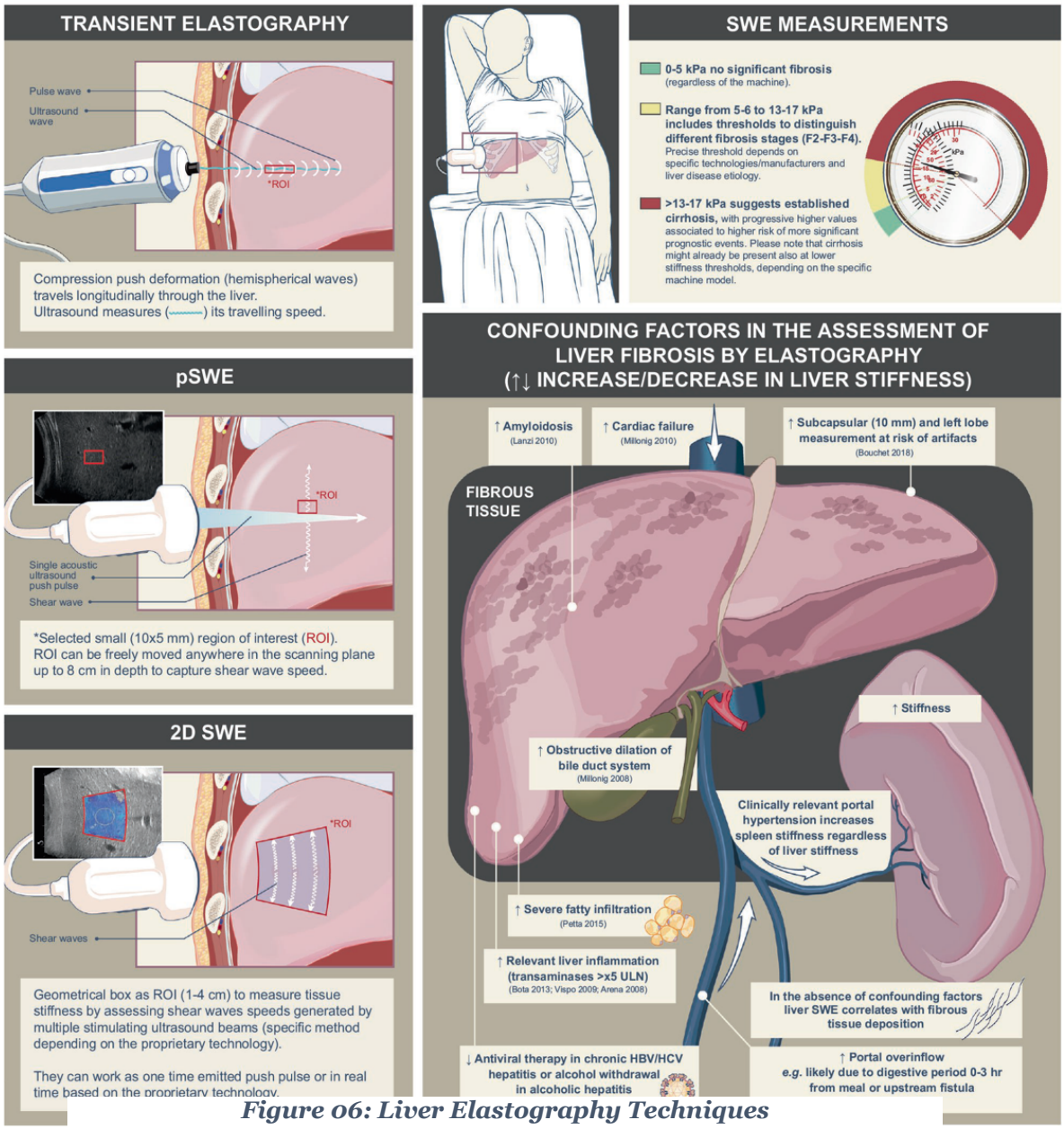

Figure 06: Liver Elastography Techniques

(Figure reproduced from Mulazzani et al. J Hepatol. 2019;70(3):545-547)

LSM values were obtained as previously reported [214] and the reliability criteria considered in accordance with the last EFSUMB Guidelines and Recommendations on the Clinical Use of Ultrasound Elastography[208].

Abdominal Ultrasound (US)

All patients underwent baseline gray-scale and colour-doppler US examinations before HSCT when LSM increased and in case of the presence of one among the SOS/VOD clinical signs criteria (Table 02) in the time interval between scheduled time points. US findings, according to Lassau criteria[200], were evaluated. US examination was performed at the bed-side throughout hospitalisation with 3 MHz convex probe.

Supportive care

Patients were allocated in single, air-positive pressure rooms with HEPA-filtered air. Anti-infectious prophylaxis was given, according to the local practice, with levofloxacin and anti-mould drugs (fluconazole for HLA identical sibling transplant and voriconazole in all the other transplants) during the neutropenic phase. All patients received acyclovir and cotrimoxazole in the standard schedule until nine months from transplant or as long as immunosuppression lasted.

CMV and EBV monitoring was performed once-twice a week, according to the risk of the pairs during the first 100 days from transplant and once a month after that. All patients received filtered and irradiated blood products.

Ethics:

This project was conducted following the Helsinki Declaration and approved by the local institutional review committee [Institutional Ethics Committee of the Sant'Orsola-Malpighi University Hospital (Bologna, Italy)]; protocol number 125/2015/O/ Sper. (monocentric phase) and 95/2017/U/Sper. (multicentric phase). Subsequently, the protocol was approved for confirmation by all the local institutional review committees of the participating centres involved. Written informed consent was obtained from each patient before inclusion in the study.

Data management: REDCap Sofware

Data were collected and managed using REDCap electronic data capture tools hosted at the Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Italy [270,271].

Research Electronic Data Capture (REDCap) is a free web application developed by a Vanderbilt University IT team to acquire data for clinical research and to create databases and projects. It is compliant with the Health Insurance Portability and Accountability Act (HIPAA), US legislation that provides privacy and data security provisions to safeguard medical information and is highly secure and intuitive to use.

The REDCap project was developed to provide scientific research teams with intuitive and reusable tools for collecting, archiving and disseminating clinical research data specific to a given project.

Some key features can be identified to support the use of REDCap for project implementation and data acquisition [132]:

- Collaborative access to data between departments and academic institutions;
- User authentication and role-specific security;
- Intuitive electronic data collection (eCRF) cards;
- Validation of data in real-time, verification of completeness of the information and other mechanisms that guarantee the quality of the data collected;
- Ability to assign and control data;
- Data storage and sharing;
- Centralised data storage and backup;
- Data export function in statistical programs;
- Batch import function of data from other systems;
- Possibility to host multiple projects simultaneously;
- A tool can meet the different data collection needs of projects related to a wide range of scientific disciplines.

To access REDCap, you must be aware of an institutional agreement with Vanderbilt University. REDCap is a flexible tool that can be used

on multiple operating systems such as Windows and Mac, and a Web server is required. Usually, there is an electronic archive administrator who performs the installations to start updates and support users, who need a device connected to the Internet and a REDCap account to use this online platform.
It is also useful for users to have a data analysis tool, such as Microsoft Excel, SAS, SPSS, to manage and evaluate data collected by REDCap projects.

A fundamental prerequisite when collecting data for a study is the revision and approval of the project by the Ethics Committee.
It is also essential to define the data to be collected before starting to build a project, since having clear the objective for which these data are collected is an important step that determines how to organise data collection on REDCap.

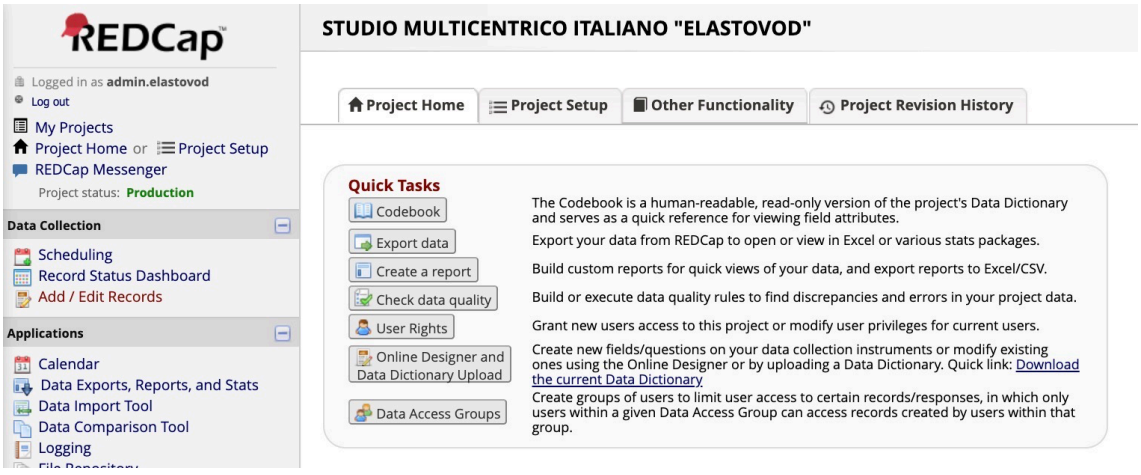

Figure 07.01: Web-based application REDCap home page for ElastoVOD project

To start a new project, users can use the settings on REDCap to guide them in creating it.

The steps are:

- Determine the main tools of the project and the various fields necessary for data collection, and these can be inserted according to a ramification logic and actions;
- Create event sheets to the planning system that allows you to manage any visits or checks to which the patient must submit; it is also possible to carry out questionnaires that can be sent directly to the patient by e-mail, which is useful for collecting data for the project;
- Set user rights and permissions: the rights of each user who is granted access to the project must be defined individually, depending on the user's profile.

The REDCap platform can be accessed through any personal computer or device connected to the internet, using the URL address of reference. For our department, the REDCap software is hosted at https://redcap.unibo.it/gastroenterologia/

Once present on the electronic archive portal, each authorised investigator is asked to enter their credentials:

In the case of multi-centre projects, personal credentials lead the

investigator back to their centre of membership, and this will only have access to the data of the enrolled patients belonging to their centre. Only the administrator has access to the data of all the centres involved in the project (**Figure 07**).

Access control is entirely configurable: the project owner can authorise access to study staff and users can be marked and removed from data exports based on the conditions set by the study protocol.

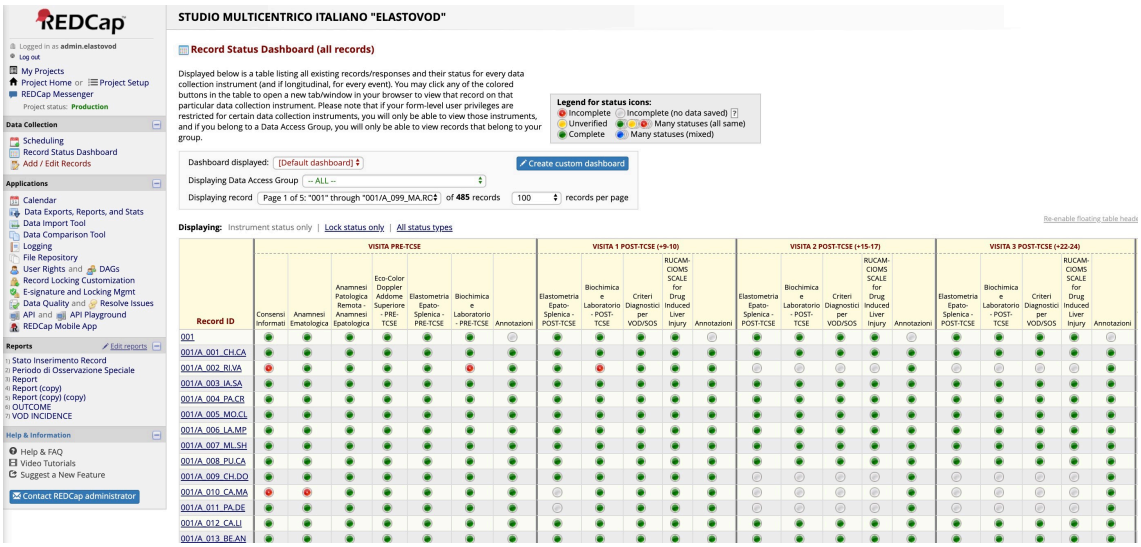

Figure 07.02: Web-based application REDCap Record status dashboard page for ElastoVOD project

Every operation performed on this platform is monitored and can be quickly recovered; this ensures a certain degree of security with regards to the management of the data entered within this electronic archive. Furthermore, all the tools and data registered on REDCap are hosted on secure servers, and a backup can be performed at regular intervals, to ensure that no information is lost. Users are not required to have any basic knowledge to use REDCap,

but for each module and function, video tutorials and help texts are available on the site that allows a steep learning curve. Once the personal credentials have been entered in the login page, the main page of the eCRF will be accessed, and it will be possible, by clicking on 'Add / Edit Records', to access the screen for entering the new patient.

A key code must identify each entered record. The REDCap platform, to protect the identity of the persons involved in the study, generates a unique identification code associated with its data collection cards for each subject, which allows each centre to maintain the association with the patient's data.

If several centres are involved in the study, it is a good idea to assign to each centre an identification code to be inserted in the ID-record to distinguish the records based on the centre to which it belongs.

Once the ID record has been created, the "Record Home Page" is accessed. The investigator will have to complete the tools on this page during the study period. By clicking on the "buttons", the different tools to be completed will open, entering the necessary data.

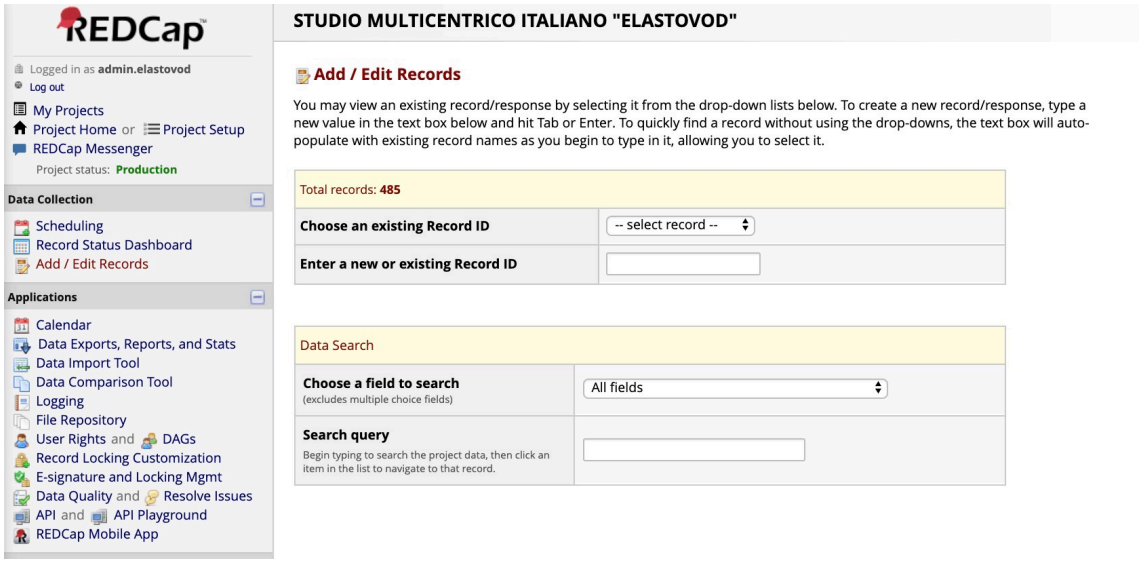

Figure 07.03: Web-based application REDCap ID records management page for ElastoVOD project

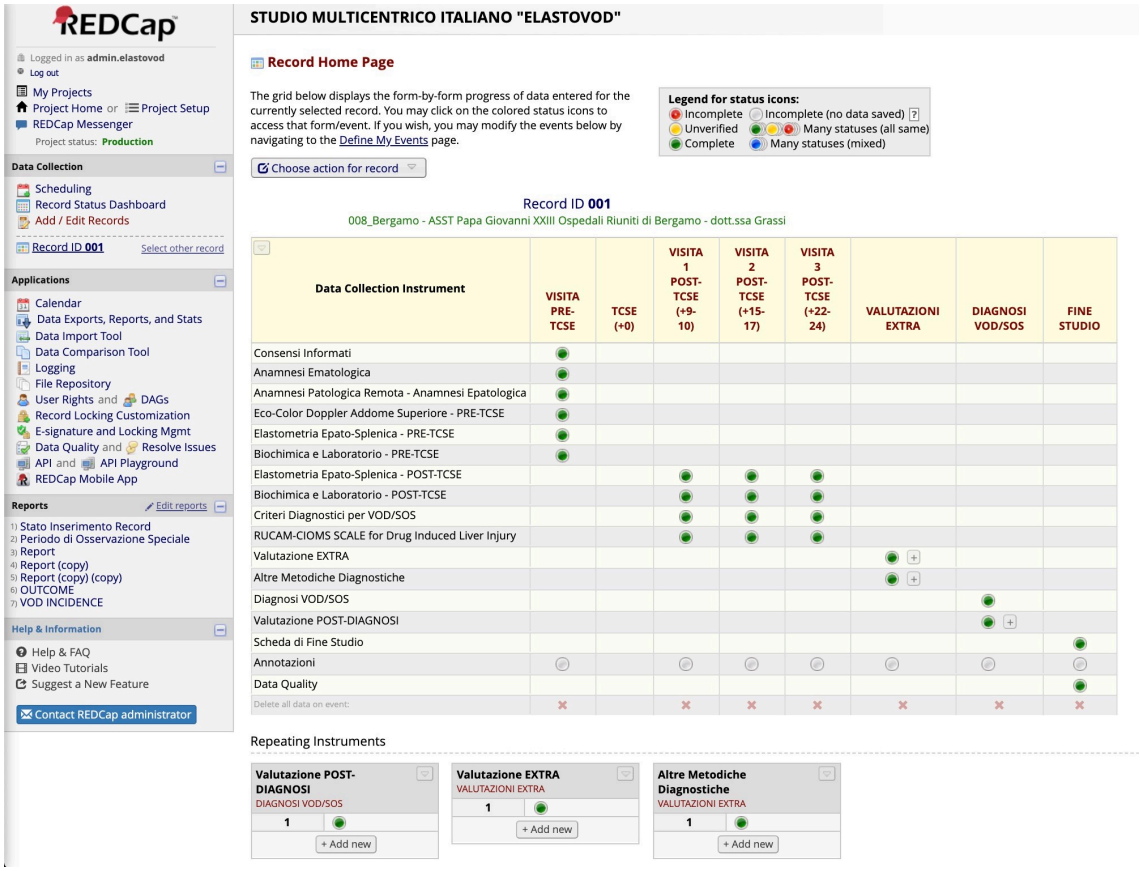

Figure 07.04: Web-based application REDCap Record home page with all instrument for ElastoVOD project

On the REDCap platform, there is also a Scheduling function (programming), through which appointments for each Record are automatically generated, and one of Calendar (calendaring) synchronised; this facilitates the organisation of visits or events planned within the project.

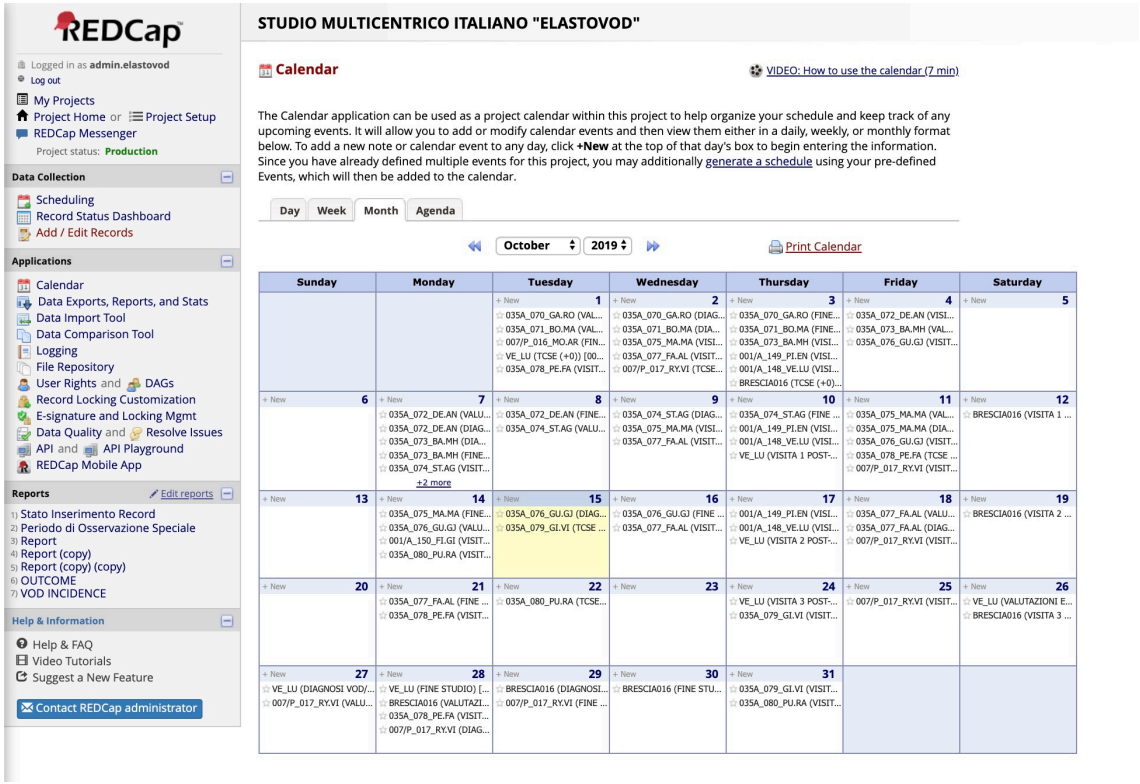

Figure 07.05: Web-based application REDCap Calendar and scheduling page for ElastoVOD project

All data entered is subject to a revision system that must be implemented from the beginning of data collection to detect any data entry errors or update data entry instructions, if necessary. Several tools allow data review:

a) Automatic controls during data entry or import: REDCap automatically checks if the information is entered with correct formatting and if there are missing values in the mandatory entry fields;
b) Monitoring of data review: each data entry form has a status that can be used to track data entry and data review progress; different colours are presently based on the data entry status
c) Report generator: allows users to create, save, export and print customised data reports. Once the report has been saved, it can be viewed by all users who have been granted access to the project. For example, it is possible to create reports to check the chronological order of the dates present in the various events.
d) PDF: the data of one or all records can be downloaded in a single PDF file that contains the data entry page and the data of all or a single instrument. It can be used if the data needs to be shared in a short time;
e) Graphical display and statistics: you can view any data report in aggregate graph format and as descriptive statistics.
f) Data export: data can be exported to Excel, SAS, SPSS, R or STATA;
g) Data quality module: the purpose of this module is to help you quickly find discrepancies and errors in the project. This

module contains rules that can be customised and the same can be used at any time during the study. Users can also exclude any results that they do not wish to see in future analyses.

When the data review is complete, the project can be moved to the inactive mode, and the data can be viewed, but it cannot be changed or deleted. The database is thus ready for statistics.

Spread and Implementation of Research Findings

An internet site (www.elastovod.it) has been developed to increase the dissemination to the scientific community of the results and the project network. The multicentric study protocol has been published on ClinicalTrial.gov (https://clinicaltrials.gov/NCT03426358).

Sample size calculation

No sample size was calculated for the first phase of the project (monocentric) Then, for the multicentric part of the project, we determined the sample size by estimating the proportion (Confidence Intervals for One Proportion Technique, PASS Sample Size Software)[272,273]. A sample size of 595 produces a two-sided 95% confidence interval [0.078 – 0.128] with a width equal to 0,050

when the sample proportion is 0,100. Considering an dropout rate of 20%, to preserve statistical significance, the total number of subjects who must be enrolled in the multicenter study is 744 patients.

Statistical analysis

Statistical analyses were performed using Stata/SE (Version 15.1; Stata Corp, Texas, United States of America) for Windows; continuous variables are expressed as median and values of 25% and 75% percentiles; categorical data are expressed as numbers (percentages). For group comparisons of categorical and continuous variables, Chi-square test or Mann-Whitney test and McNemar's test were used, as appropriate. Multiple linear regression analysis was performed to establish a regression equation able to predict the CCI preoperatively after HR. Univariate logistic regression analyses were performed to predict the risk factors of SOS/VOD development after HSCT. After evaluation of the multicollinearity, multivariable logistic regression analyses were carried out on variables which reached $p<0.1$ at univariate analysis. The final multivariate regression model was built from the set of candidate variables by removing the predictors based on p values, in a stepwise manner. For multivariable logistic regression, the model discrimination and calibration were reported together with AIC (Akaike information criterion) and BIC (Bayesian information criterion) measures for comparing maximum

likelihood models. Model discrimination was assessed calculating the Area under the Receiver Operator Characteristic (AUROC) curve (or c-statistic), whereas model calibration was determined by Hosmer-Lemeshow (H-L) technique and AIC assessed the goodness of fit of the models. Basing on the multivariable logistic regression model, the novel nomogram was built and internally validated using bootstrap resampling. We made an a priori decision to consider only models with AUROC c-statistic ≥ 0.70 adequate and to test the equality of the area under the curves, DeLong test was performed [274]. All p values referred to two-tailed tests of significance. $P<0.05$ was considered significant.

RESULTS

Monocentric paediatric population

During the study period, 22 (16 males, six females, mean age: 140 months, min 54, max 245) out of 30 patients who had undergone HSCT were enrolled according to the previously defined inclusion criteria. The indication for HSCT and the baseline clinical and demographic characteristics of the patients enrolled are shown in **Table 06.**

Subject number	*Sex*	*Age (year)*	*Diagnosis*	*Conditioning regimen*	*Type of SCT*	*VOD*	*Day after HSCT*	*VOD treatment*
1	M	5.7	AML	BU-MEL-CPM-ATG	MUD	No		
2	F	12.2	ALL	BU-THIO-CPM-ATG	MUD	Yes	22	Defibrotide
3	M	5	ALL	FLUDA-TREO-MEL-ATG	MUD	No		
4	F	20.4	ALL	BU-THIO-CPM	Sibling	Yes	25	Paracentesis
5	M	8.4	Ewing sarcoma	BU-MEL	Autologous	Yes	24	Diuretics+UDCA
6	M	4.5	ALL	BU-THIO-CPM+ ATG	PMUD	Liver toxicity		Diuretics+UDCA
7	M	9.4	Beta-thalassemia	THIO-FLUDA-TREO-ATG	Sibling	No		
8	F	14.5	Severe aplastic anemia	FLUDA-CPM-ATG	PMUD	Liver toxicity	11	Diuretics+UDCA
9	M	15.8	Severe aplastic anaemia	THIO-FLUDA-TREO-ATG	Sibling	No		
10	M	6	Beta-thalassemia	BU-THIO-FLUDA-ATG	PMUD	No		
11	M	19	AML	TREO-THIO-FLUDA	Sibling	No		
12	M	15.8	AML	TREO-THIO-FLUDA+ATG	PMUD	No		
13	M	13.2	AML	TREO-THIO-FLUDA	Haploidentical	No		
14	F	11.4	ALL	TREO-THIO-CPM	MFD	No		
15	M	16.5	LH REC	FLUDA+ MEL	Sibling	No		
16	F	9.3	Beta-thalassemia	BU+FLUDA+THIO	MUD	No		
17	M	10.6	AML	FLUDA+TREO+THIO	MUD	Yes	19	Defibrotide+ Diuretics
18	F	16.9	Severe aplastic anaemia	FLUDA-CPM-ATG	MUD	No		
19	M	5.4	ALL (relapse)	TREO-THIO-FLUDA	Haploidentical	GvHD		Defibrotide
20	M	8.7	Ewing sarcoma	BU-MEL	Autologous	No		
21	M	14.8	ALL (relapse)	BU-THIO-FLUDA-ATG	Haploidentical	No		
22	M	13.6	Ewing sarcoma	BU-MEL	Autologous	No		

Abbreviations: ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; ATG = anti thymocyte globulin; BU = busulphan; CPM = cyclophosphamide; CPMpost = CPM after HSCT; F = female; FLUDA = fludarabine; GvHD = graft-vs-host disease; M = male; MEL = melphalan; MFD = matched family donor; MUD = HLA matched unrelated donor; PMUD = HLA partially unrelated matched; THIO = thiotepa; TREO = treosulfan; UDCA = Ursodeoxycoholic acid.

Table 06. Demographic and clinical characteristics of patients enrolled in the monocentric population

Except for patients 5, 20 and 22 who had Ewing's sarcoma and had undergone autologous transplantation, all patients underwent allogeneic transplantation. Table 06 also reports the pre-HSCT conditioning regimens. Patients 2, 4, 5 and 17 developed VOD; according to the diagnostic criteria, our patients were classified as moderate VOD (all necessary treatment, no one died from VOD). Patients 6 and 8 developed a clinical picture suggestive of liver toxicity; patient 19 developed GvHD.

The TE measurement was successful in all patients; none had ascites during TE assessment, as documented by the preliminary ultrasonography examination. The LS value for each assessment and the VOD diagnosis are shown in Figure 08; no evident differences were observed at baseline LS values between patients who developed VOD and those who did not. Of the patients who developed VOD, patient 2 had an increase in LS 18 days after HSCT, developing VOD 4 days later; patient 4 had an increase in LS 17 days after HSCT, developing VOD 5 days later; patient 5 had an increase in LS 18 days after HSCT, developing VOD 6 days later and patient 17 had an increase in LS 16 days after HSCT, developing VOD 3 days later. The LS values increased in the measurement carried out before VOD diagnosis, and these values were different from the baseline and the previous values.

In the two patients who developed liver toxicity, there was a gradual and early increase in LS, starting from the fifth day after HSCT. Of these patients, the first one had spontaneous resolution of the liver toxicity, and the second one developed multi-organ failure and died on day 44 after HSCT.

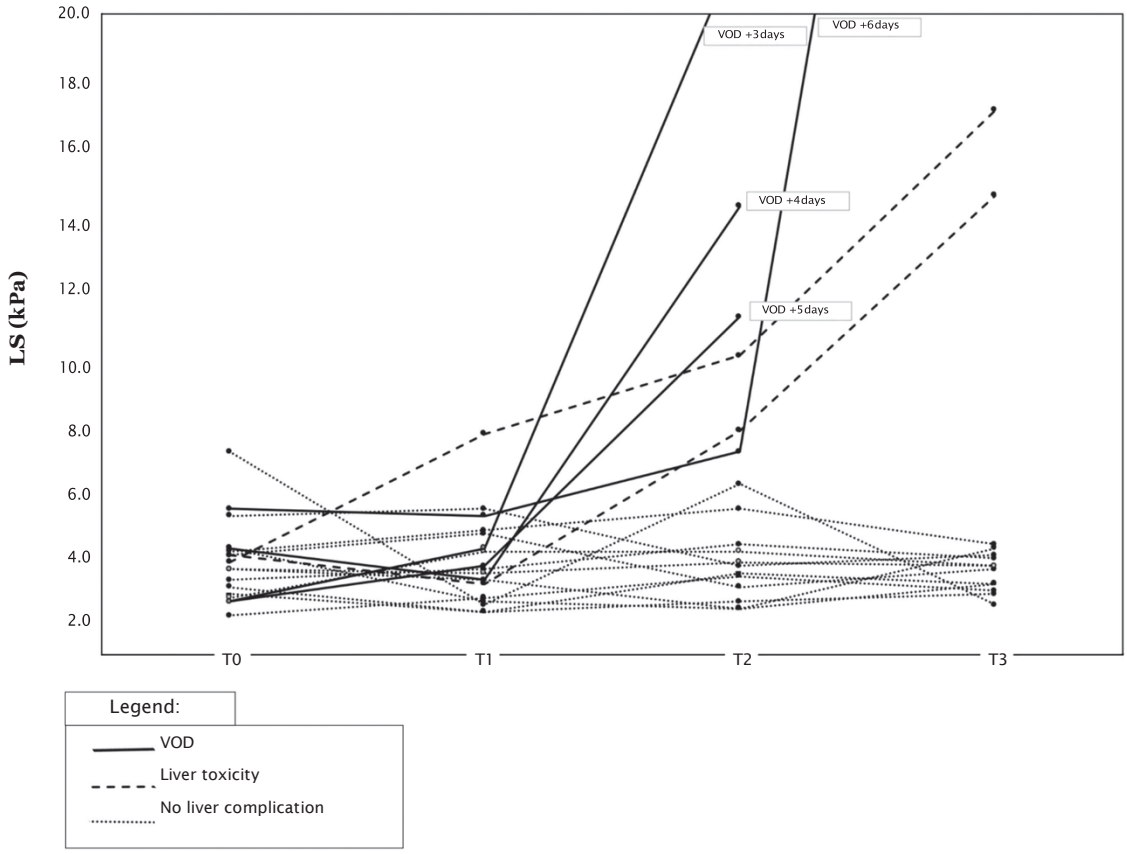

Figure 08. Variation of LS values at each determination for all patients

At T2 in patient 2, LS suddenly increased four days before VOD; in patient 4, LS suddenly increased five days before VOD; in patient 5, LS suddenly increased six days before VOD and in patient 17, LS suddenly increased three days before VOD (continuous lines). The early LS values which progressively increased in patients who developed liver toxicity (dashed lines). The LS values in patients without liver complications (dotted lines).

In a subgroup of patients, we also assessed the changes in LS values after SOS/VOD diagnosis and treatment. The patient's characteristic was reported in Table 07.

Characteristics	Patient 1	Patient 2	Patient 3
Age (y)	7	14	8
Sex	F	F	F
Diagnosis	ALL B Ph +	ALL B	ALL T
Stem Cell Source	BM	BM	BM
Cells received	12.0×10^8/kg	5.5×10^8/kg	6.3×10^8/kg
Type of STC	MUD	PMUD	MUD
Conditioning Regimen	BU-THIO-CPM-ATG	BU-THIO-CPM	BU-THIO-CPM-ATG
GvHD prophylaxis	Ciclosporin—MTX	Ciclosporin—MTX	Ciclosporin—MTX
GvHD	Yes (skin, grade 1)	No	No
Day of VOD diagnosis	25	18	19

Table 07: Demographic and clinical characteristics of the paediatrics patients followed after VOD diagnosis

Abbreviation: ALL, acute lymphoblastic leukaemia; ATG, anti-thymocyte globulin; BM, bone marrow; BU, busulphan; CPM, cyclophosphamide; GvHD, graft-versus-host disease; MTX, methotrexate; MUD, matched unrelated donor; PMUD partially matched unrelated donor; STC, stem cell transplant; THIO, thiotepa.

Case I

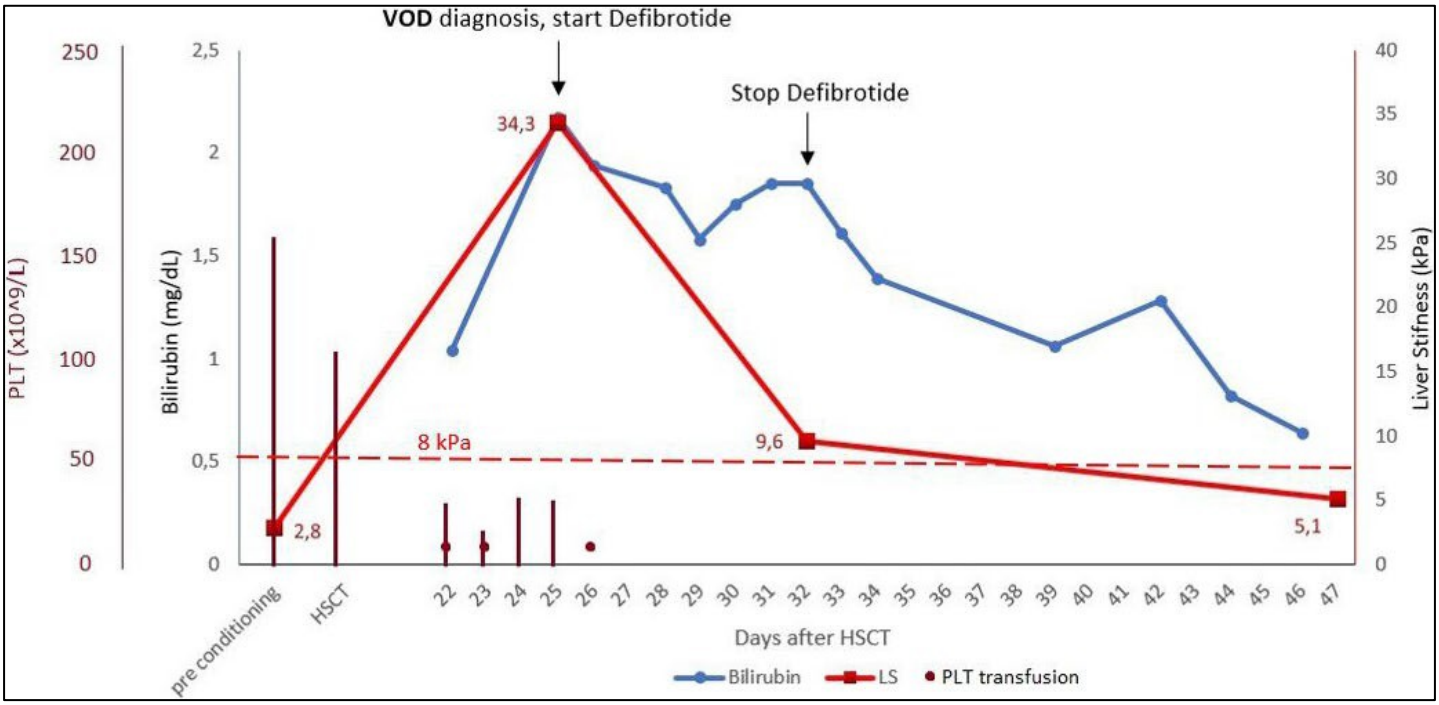

Figure 09.01: Clinical course of patient CASE I

The first patient (Table 07, Figure 09.01) underwent HSCT for ALL Ph+. Conditioning regimen included busulphan (0.8 mg/kg from −8 to −5), thiotepa (10 mg/kg on −4), cyclophosphamide (60 mg/kg −3

and −2), and thymoglobulin (rabbit anti-thymocyte globulin (ATG) 2 mg/kg from −4 to 0.2); for GvHD prophylaxis, ciclosporin (3 mg/kg iv from −1 to +35, than orally) and short-term methotrexate (respectively 12/8/8/8 mg/m^2 on days +1/+3/+6/+11) were used. Preconditioning regimen liver stiffness and the first two valuations after SCT were below the cut-off of 8 kPa. Neutrophil and platelet engraftment was achieved on day +13 and day +48, respectively.

On day +25, for the presence of hyperbilirubinemia (2.17 mg/dL), weight gain >5% above baseline value, painful hepatomegaly, and transaminase elevation (AST 273 U/I, ALT 132 U/I), the diagnosis of SOS/VOD were made. On the same day, liver stiffness measurement was performed, revealing a pathological value (34.3 kPa), and supportive therapy (albumin, diuretics, antithrombin III) and treatment with defibrotide (25 mg/kg) were started. The clinical condition of the patient improved rapidly together with the normalisation of transaminase and bilirubin in the following days; there was no trans- fusion-refractory thrombocytopenia. On day +33, defibrotide was stopped, and another measurement of liver stiffness was performed, showing a decreased value of 9.6 kPa. The last liver stiffness on day +47 was normal: 5.1 kPa. The patient was later dismissed on day +53.

Case II

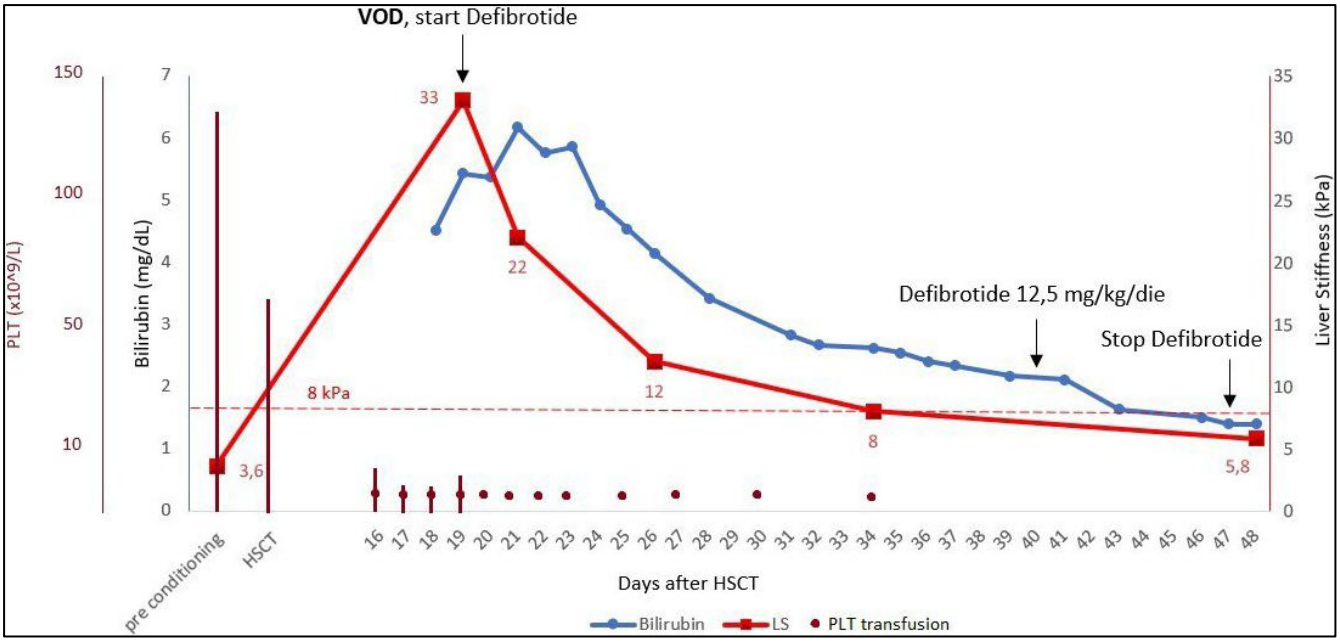

Figure 09.02: Clinical course of patient CASE II

The second patient (Table 07, Figure 09.02) underwent HSCT for a relapsed B-lineage ALL. Conditioning regimen was based on busulphan (0.8 mg/kg from −8 to −5), thiotepa (10 mg/kg on −4), cyclophosphamide (60 mg/kg −3 and −2); GVHD prophylaxis consisted of ciclosporin (3 mg/kg iv from −1 to +33, then orally) and short-term methotrexate (respectively 12/8/8/8 mg/m^2 on days +1/+3/+6/+11). The basal value of liver stiffness at conditioning regimen start was 3.6 kPa.

Neutrophil and platelet engraftment was achieved on day +19 and day +39, respectively. On day +18, the patient displayed a significant gain of weight concomitantly with hyperbilirubinemia (4.52 mg/dL),

painful hepatomegaly, and transfusion-refractory thrombocytopenia: thus, VOD/ SOS/VOD was diagnosed, supportive therapy (albumin, diuretics, anti-thrombin III) and treatment with defibrotide (25 mg/kg) started.

The next-day valuation of the liver stiffness was performed, showing a pathological value of 33 kPa. The measurement was carried out multiple times in the following weeks as we noticed a progressive decreasing trend, alongside the normalisation of bilirubinaemia and the improvement of the patient's clinical condition (22 kPa on +21, 12 kPa on +26, eight kPa on +34, and 5.8 kPa on 48). Defibrotide was weaned on day +40 (12.5 mg/kg) and stopped on day +46. The patient was discharged on day +50.

Case III

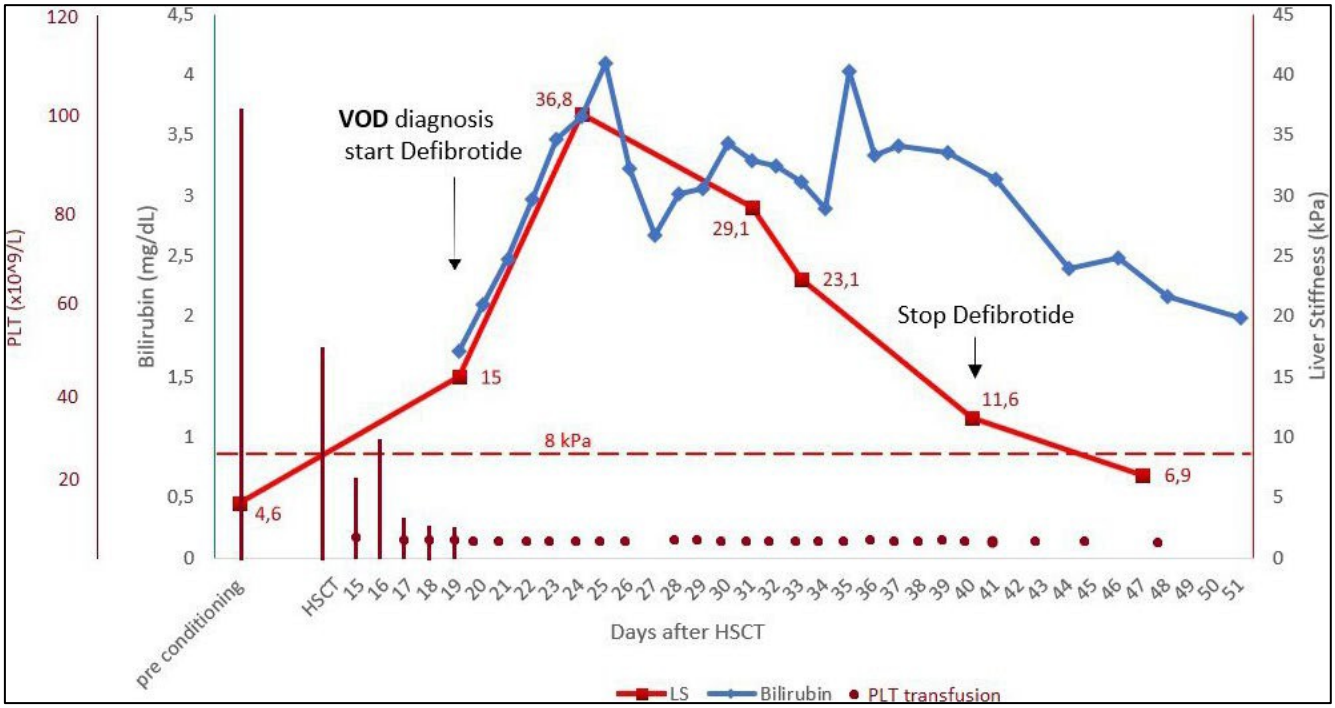

Figure 09.03: Clinical course of patient CASE III

The third patient (Table 07, Figure 09.03) underwent HSCT for high-risk T-lineage ALL. Conditioning regimen included busulphan (0.8 mg/ kg from −8 to −5), thiotepa (10 mg/kg on −4), cyclophosphamide (60 mg/kg −3 and −2), and thymoglobulin (rabbit ATG 5 mg/kg from −4 and −2); for GVHD prophylaxis, ciclosporin (3 mg/kg iv from −1 to +20, than orally) and short-term methotrexate (respectively 15/10/10 mg/m^2 on days +1/+3/+6) were used. Preconditioning regimen liver stiffness and the first two valuations after SCT were lower than eight kPa. Neutrophil engraftment was achieved on day +12. On day +19, the patient presented painful hepatomegaly and unexplained gain of weight; a measurement of liver

stiffness was performed, showing a pathological value of 15 kPa. Diagnosis of severe SOS/VOD was made, and therapy with defibrotide (25 mg/kg) started.

In the following days, there was a further progressive gain of weight alongside rising bilirubinaemia (max value 4.1 mg/dL on +25), so another LS measurement was performed that resulted dramatically increased since the last one (36.8 kPa on +24). A bladder catheter was placed, and furosemide infusion began, for the worsening of renal function. During the next two weeks, four more evaluations with TE were carried out: These resulted progressively decreased (29.1 kPa on day +31, 23.1 kPa on day +34, 11.6 kPa on day +40) and became, at last, standard (6.9 kPa on day +47). Treatment with defibrotide was stopped on day +41. After the diagnosis of SOS/VOD, the patient needed daily platelet transfusion and, even at the moment of discharge (day +53), full platelet engraftment was not obtained.

Monocentric adult population

Over the study period, of the 89 patients referred to the transplant centre, two were excluded because of BMI>40 and ACLD at enrolment; 9 patients did not perform HSCT (unavailable donor or progression of the disease) after being initially enrolled. As shown by **Figure 10**, 78 patients fulfilled all the inclusion criteria and underwent HSCT. During the study period, two patients were transferred to the Intensive Care Unit (ICU), and four patients died for not-liver related causes. One patient died because of severe SOS/VOD.

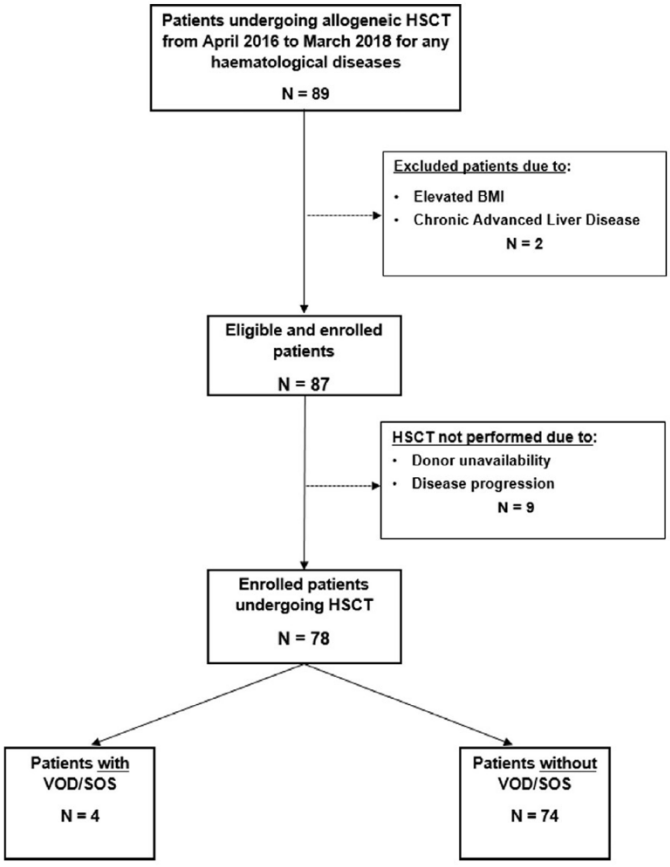

Figure 10: Study flow-chart of the monocentric adult cohort

The clinical and transplant characteristics of the patients enrolled are shown in **Table 08**, respectively. Prior to HSCT (from the diagnosis of haematological disease to 10 days before transplantation), almost all patients (92.3%) underwent blood transfusions (median ferritin level 738 ng/mL (68 – 1333) and 32 (41.1%) have received more than 20 red cells blood transfusions; the baseline median LSM was 4.2 kPa (3.8-5.3).

During the study period, 4 out of 78 patients (5.1%) met the clinical diagnostic criteria of SOS/VOD. The median day of clinical SOS/VOD diagnosis was +17 (+4 - +28). The features of patients who developed SOS/VOD are reported in **Table 09.** Most of the SOS/VOD were severe/very severe (3/4), and one of them led to MOF and death a few days later (pts.3). These patients received a higher number of blood transfusion (>20) before HSCT and MAC regimen based on Busulfan or Cyclophosphamide (4/4) than the patients who did not develop SOS/VOD (**Table 10**).

Accordingly, with SOS/VOD Risk Factors by EBMT, **Table 11** shows that among pre-HSCT risk factors, the patients underwent previous therapy with Inotuzumab ozogamicin (INO) (3/4) or others hepatoxic drugs (1/4) and presented pre-HCST iron overload (2/4).

With regards to the US findings at SOS/VOD diagnosis **(Table 12)**, according to Lassau criteria [200] (increased diagnostic likelihood when fulfilled ≥three criteria): three patients (3/4) presented ≥3 Grey-scale US morphologic criteria, and none presented ≥3 US colour doppler criteria. Only portal vein diameter >12 mm was presented in all patients with SOS/VOD diagnosis.

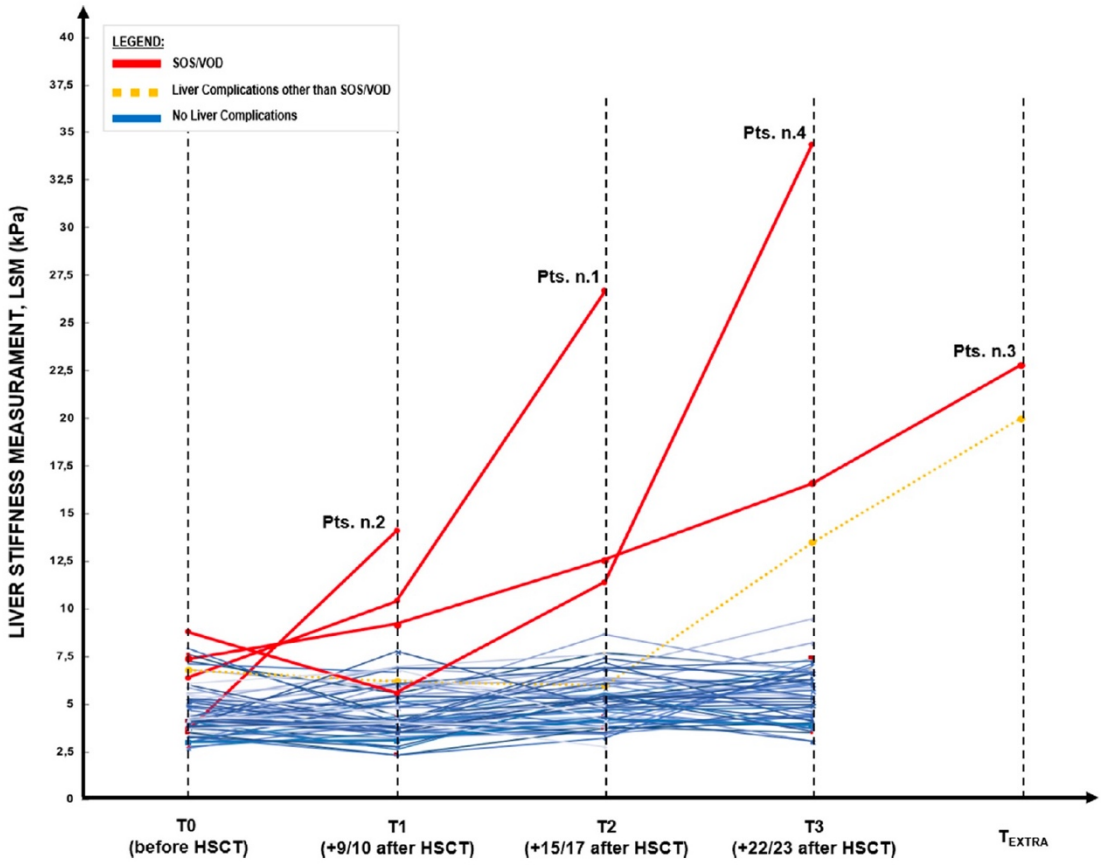

Figure 11: Variation of LSM values at each determination for all patients. The solid red line represents patients who developed SOS/VOD after HSCT; solid blue line, patients who did not develop SOS/VOD after HSCT; yellow dotted line, patients who developed liver complications other than SOS/VOD

LSM values for each time points were reported in **Figure 11**; LSM showed increased values in all patients who developed SOS/VOD (continuous red lines). The pre-HSCT (To) LSM did not differ

between patients with or without (6.9 kPa vs 4.2 kPa; p-value 0.079) SOS/VOD diagnosis. The LSM values increased in the assessment carried out before SOS/VOD clinical diagnosis, and these values were significantly different from the T0 and the previous assessments. In general, the LSM values showed a sudden increase some days (from 2 to 12) before the clinical SOS/VOD diagnosis (**Table 09**).

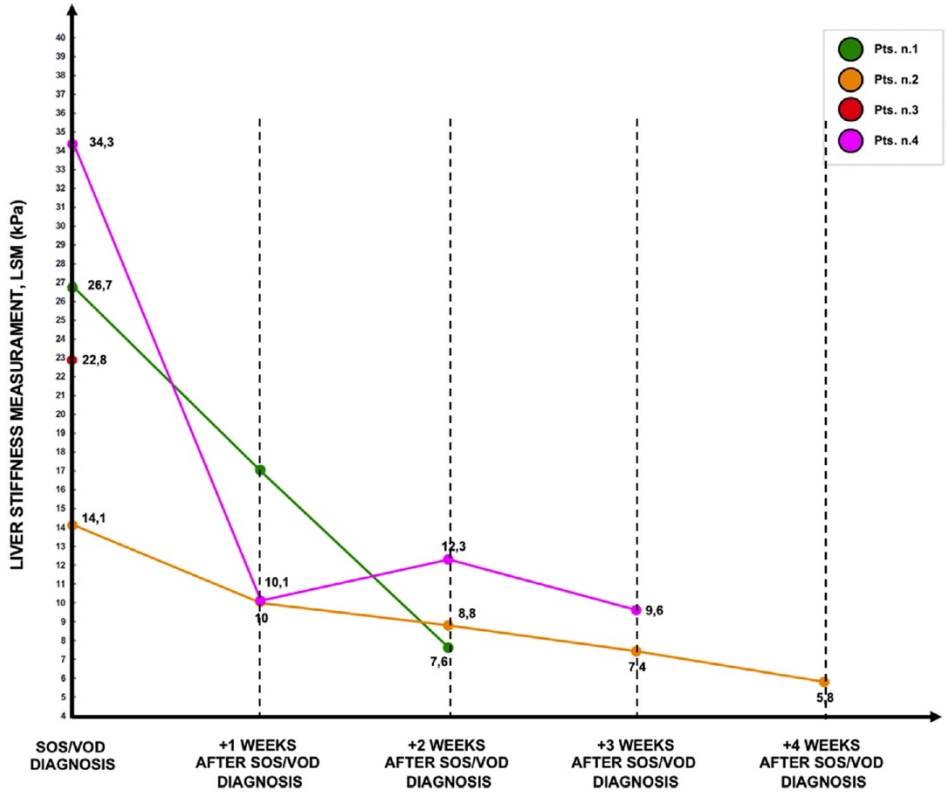

Figure 12: Variation in LSM values after SOS/VOD-specific treatment of patients with SOS/VOD diagnosis

After starting SOS/VOD specific treatment (Defibrotide and diuretics) LSM values consensually decreased, reaching pre-transplant LS values within 2-4 weeks after SOS/VOD diagnosis; one

patient, who died 20 days after severe SOS/VOD diagnosis, did not show the LSM decrease (**Figure 12**).

Logistic regression analysis demonstrated that an LSM increase after HSCT over pre-HSCT assessment, was significantly associated with SOS/VOD diagnosis (OR: 1.837; 95%CI [1.1107- 3.0384] p-value<0.001); furthermore, ROC analysis of LSM increase over pre-HSCT assessment resulted in an AUC of 0.997 with a sensitivity of 75% and specificity of 98.7%. For instance, an increase of ≥+10 kPa over pre-LSM-HSCT assessment have shown a sensitivity of 100% and specificity of 98.7% for SOS/VOD diagnosis.

During the study period, among patients who did not develop SOS/VOD (n=74), 62 patients had shown no or mild-to-moderate therapy-associated complications without severe involvement of the liver after HSCT (CTC grades 0-3). In 12 cases, severe liver complications (CTC 4-5) occurred: n=2 acute cholecystitis, n=1 cholangitis, n=2 DILI related to antimycotic drugs, n=1 hepatic GvHD, n=5 isolated liver biochemical alterations and n=1 died after a fulminant EBV-related hepatitis reactivation.

The median LSM after HSCT maximum values reached in group CTC 0-3 (6.1 kPa; 5kPa – 6.8kPa), were not significantly different (p-values 0.724) compared to patients in group CTC 4-5 (6.6 kPa; 4.8kPa –8.6kPa). Median LSM after HSCT maximum values reached

significantly different ones (p-value<0.001) when we compared patients with CTC 4-5 to patients with SOS/VOD development (**Figure 13).**

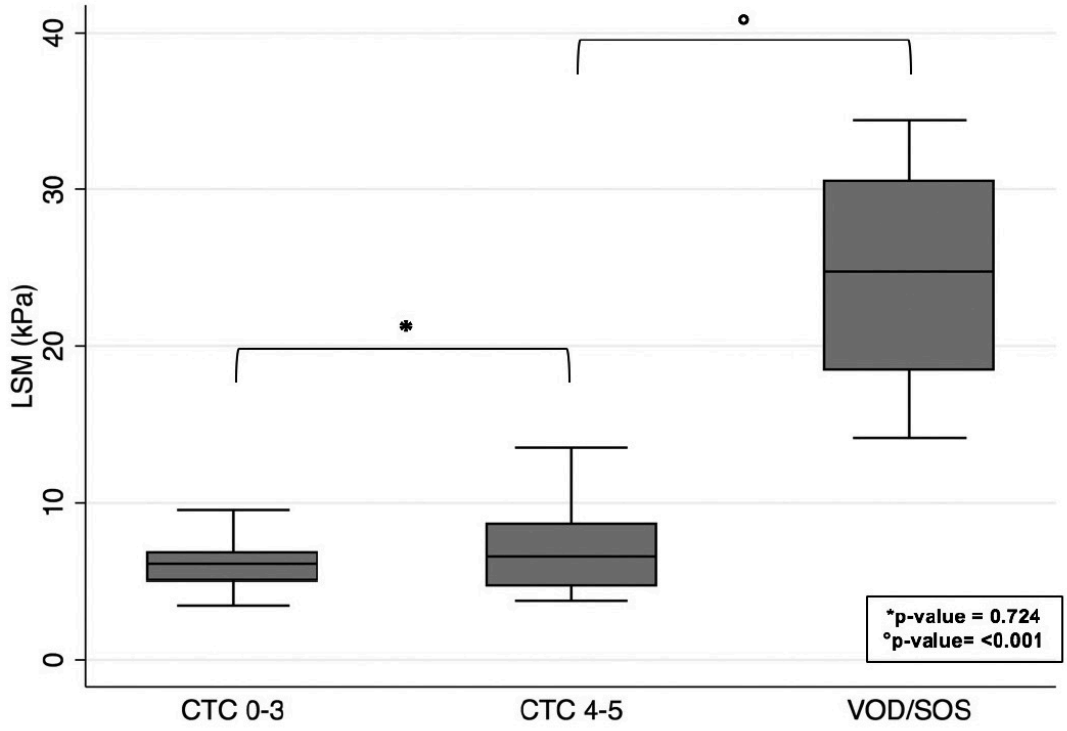

Figure 13: Boxplot of maximum LSM values after HSCT in patients with SOS/VOD diagnosis by CTC degree

Regardless of this, only in the case of a 67-year-old patient with Philadelphia positive acute lymphoblastic leukemia, as shown in **Figure 11**, LSM showed a sudden increase (13.5 kPa) in the absence of any clinical signs of VOD / SOS; in the following month the patient presented mildly painful hepatomegaly, and developed a tender abdomen with ascites. For SOS/VOD suspicion a trans-jugular liver biopsy was performed (HVPG=21 mmHg) which described a

widespread dilatation of hepatic sinusoids, with a massive presence of atypical elements, as B Lymphoblastic Leukaemia infiltration; at the same day, LSM values was 20 kPa.

We believe that the case described above is an uncommon clinical situation in which the infiltration of liver sinusoids by leukaemia mimics the presence of sinusoidal obstruction typical of the pathogenesis of VOD / SOS/VOD after HSCT.

Characteristic	Study population (N = 78)	Without VOD/SOS (n = 74; 94.9%)	With VOD/SOS (n = 4; 5.1%)	*P* Value*
Male sex, n (%)	39 (50)	37 (50)	2 (50)	1
Age, yr, median (IQR)	54 (40-60)	54 (41-60)	46 (31-60)	.696
BMI, kg/m^2, median (IQR)	25 (22.6-27.5)	25.1 (23-27.5)	21.5 (21.1- 23.8)	.358
Ethnicity				.106
Caucasian	73 (93.6)	70 (94.5)	3 (75)	
African/African American	3 (3.8)	3 (4.1)	0 (0)	
Asiatic	2 (2.6)	1 (1.4)	1 (25)	
Diagnosis, n (%)				.133
Acute myelogenous leukemia	35 (44.9)	34 (45.9)	1 (25)	
Acute lymphoblastic leukemia	14 (17.9)	12 (16.2)	2 (50)	
Myelodysplastic syndrome	11 (14.1)	11 (14.9)	0 (0)	
Hodgkin lymphoma	5 (6.4)	5 (6.8)	0 (0)	
Non-Hodgkin lymphoma	4 (5.1)	4 (5.4)	0 (0)	
Multiple myeloma	2 (2.6)	2 (2.7)	0 (0)	
Myelofibrosis	3 (3.8)	3 (4.1)	0 (0)	
Chronic myelogenous leukemia	3 (3.8)	3 (4.1)	0 (0)	
Severe aplastic anemia	1 (1.3)	0 (0)	1 (25)	
Blood transfusion history, n (%)				**.029**
≤20 transfusions	46 (58.9)	46 (62.2)	0 (0)	
>20 transfusions	32 (41.1)	28 (37.8)	4(100)	
HCT-CI Sorror total score, median (IQR)	0 (0-2)	0 (0-1.5)	2 (.75-3)	.247
LSM at baseline, median (IQR)	4.2 (3.8- 5.3)	4.2 (3.8-5.2)	6.9 (5.7-7.8)	.079

Significant *P* values are in bold type.
HCT-CI indicates hematopoietic cell transplantation comorbidity index.
* Wilcoxon rank-sum test/Fisher exact test.

Table 08: Baseline Characteristics of Monocentric Study Population and by SOS/VOD Diagnosis

Patient	Sex, Age, yr	Disease	Baseline LSM, kPa	Number of Risk Factors*	Conditioning Regimen	Clinical VOD/SOS Diagnosis*	Serum Bilirubin >2 mg/d	Hepatomegaly	Weight Increase >5%	Ascites on ultrasound	Decreased Mean PV Flow Velocity	Increased LSM, kPa	VOD/SOS Severity Grading*	Management
1	Male, 26	Aplasia	6.4	6	MAC	+7	Yes, +7	Yes, +7	Yes, +7	Yes, +7	No	10.4 at +5; 26.3 at +7	Severe	Defibrotide, diuretics
2	Female, 60	AML	3.7	7	MAC	+1	Yes, +1	Yes, -1	Yes, -1	Yes, +3	Yes, +7	14.4 at -1	Severe	Defibrotide, diuretics
3	Male, 32	ALL	7.4	6	MAC	+27†	Yes, +7	Yes, +27	Yes, +27	Yes, +27	Yes, +46	9.2 at +7; 12.6 at +15; 16.6 at +21; 22.8 at +46	Very severe (MOF)	Defibrotide, diuretics
4	Female, 60	ALL	8.8	6	MAC	+29	Yes, +29	Yes, +29	Yes, +27	No	Yes, +27	11.4 at +18; 34.3 at +26	Moderate	Diuretics

PV indicates paraumbilical vein; MAC, myeloablative conditioning; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia.
* According to Mothy et al [8].
† Diagnosis also made with HVPG assessment (+21 mmHg).

Table 09: Characteristics of Patients with SOS/VOD

Characteristic	Study Population (N = 78)	Without VOD/SOS (n = 74; 94.9%)	With VOD/SOS (n = 4; 5.1%)	*P* Value*
Conditioning regimen, n (%)				.190
Myeloablative conditioning	52 (66.7)	48 (92.3)	4 (7.7)	
TBI-Cy	2 (3.8)	2 (4.2)	0 (0.0)	.899
TBF	47 (90.5)	45 (93.7)	2 (50)	.523
Bu- Flu	2 (3.8)	1 (2.1)	1 (25)	**.004**
Cy200	1 (1.9)	0 (0.0)	1 (25)	**.05**
Reduced-intensity conditioning	26 (33.3)	26 (100)	0 (0)	.190
TBF	13 (50)	13 (50)	0 (0.0)	.475
TCF	13 (50)	13 (50)	0 (0.0)	.475
GVHD prophylaxis, n (%)				
ATLG-CNI-MTX	73 (93.6)	69 (93.2)	4 (100)	.537
CNI-MTX	2 (2.6)	2 (2.7)	0 (0)	.899
CNI-MMF-PT-Cy	3 (3.8)	3 (4.1)	0 (0)	.852
Stem cell donor, n (%)				
Matched related donor	12 (15.4)	11 (14.9)	1 (25)	.495
Unrelated donor†	53 (67.9)	50 (67.6)	3 (75)	.616
Haploidentical donor	3 (3.8)	3 (4.1)	0 (0.0)	.852
Cord blood	5 (6.4)	5 (6.7)	0 (0.0)	.721
Stem cell source, n (%)				.676
Peripheral blood	58 (74.4)	54 (73)	4 (100)	
Bone marrow	15 (19.2)	15 (20.3)	0 (0.0)	
Umbilical cord blood	5 (6.4)	5 (6.8)	0 (0.0)	

Significant *P* values are in bold type.
TBI indicates total body irradiation; Cy, cyclophosphamide; TBF, thiotepa-busulfan-fludarabine; Bu-Flu, busulfan-fludarabine; TCF, thiotepa-cyclophosphamide-fludarabine; ATLG, anti-T lymphocyte globulin; CNI, calcineurin inhibitor; MTX, methotrexate; MMF, mofetil mycophenolate; PT-Cy, post-transplantation cyclophosphamide.
* Fisher exact test.
† Thirty-four patients (64.1%) were 10/10 HLA-matched, and 19 (35.9%) were <10/10 HLA-matched.

Table 10: Transplantation Characteristics According to SOS/VOD Occurrence

VOD/SOS Risk Factors	Study Population (n = 78)	Without VOD/SOS (n = 74; 94.9%)	With VOD/SOS (n = 4; 5.1%)	*P* Value*
Transplantation-related factors				
Unrelated donor	63 (80.8)	60 (81.1)	3 (75)	.582
HLA-mismatched donor	32 (41)	31 (41.9)	1 (25)	.640
Non-T cell-depleted transplant	78 (100)	74 (100)	4 (100)	1
Myeloablative conditioning regimen	49 (62.8)	47 (63.5)	2 (50)	.625
Oral or high-dose busulfan-based regimen	62 (79.5)	59 (79.7)	3 (75)	.609
High-dose TBI-based regimen	3 (3.8)	3 (4.1)	0 (0)	.852
Second HSCT	7 (9)	6 (8.1)	1 (25)	.319
Patient and disease-related factors				
Older age	21 (26.9)	21 (28.4)	0 (0)	.569
Karnofsky Performance Status score <90%	0 (0)	0 (0)	0 (0)	—
Metabolic syndrome	10 (12.8)	9 (12.2)	1 (25)	.429
Female receiving norethisterone	3 (3.8)	3 (4.1)	0 (0)	.852
Advanced disease (beyond CR2 or relapsed/ refractory)	23 (29.5)	21 (28.4)	2 (50)	.577
Thalassemia	0 (0)	0 (0)	0 (0)	—
Genetic factors (eg, GSTM1 polymorphism, C282Y allele, MTHFR 677CC/1298CC haplotype)	1 (1.3)	1 (1.4)	0 (0)	.949
Hepatic-related factors				
Transaminases 2.5× ULN	0 (0)	0 (0)	0 (0)	—
Serum bilirubin 1.5× ULN	1 (1.3)	1 (1.4)	0 (0)	.949
Active viral hepatitis	3 (3.8)	3 (4.1)	0 (0)	.852
Cirrhosis	0 (0)	0 (0)	0 (0)	—
Abdominal or hepatic irradiation	1 (1.3)	1 (1.4)	0 (0)	.949
Previous use of inotuzumab ozogamicin	4 (5.1)	1 (1.4)	3 (75)	**<.00001**
Other hepatotoxic drugs	2 (2.6)	1 (1.4)	1 (25)	**.004**
Iron overload	3 (3.8)	1 (1.4)	2 (50)	**.006**

Significant *P* values are in bold type.
ULN indicates upper limit of normal.
* Wilcoxon rank-sum test/Fisher exact test.

Table 11: SOS/VOD Risk Factors According to EBMT and by SOS/VOD Diagnosis

Criteria	Patient 1	Patient 2	Patient 3	Patient 4
Clinical VOD/SOS diagnosis	+7	+1	+27	+29
Ultrasound and color Doppler criteria	4	3	8	3
Gray-scale ultrasound morphological criteria, n	4	3	6	2
Hepatomegaly	Yes, +7	Yes, +3	Yes, +27	Yes, +26
Splenomegaly	None	None	Yes, +21	None
Gallbladder wall thickening >6 mm	Yes, +7	None	Yes, +27	None
Portal vein diameter >12 mm	Yes, +7	Yes, +7	Yes, +12	Yes, +26
Hepatic vein diameter <3 mm	None	None	None	None
Ascites	Yes, +7	Yes, +3	Yes, +27	None
Visualization of paraumbilical vein	None	None	Yes, +27	None
Ultrasound color Doppler criteria, n	0	0	2	1
Mean portal flow velocity <10 cm/s or hepatofugal flow	None	None	None	None
Flow recorded in paraumbilical vein	None	None	Yes, +27	Yes +26
Monophasic flow or flow recorded in hepatic veins	None	None	None	None
Hepatic Artery Resistive Index >.75	—	—	Yes, +27	None

Table 12: US Criteria According to Lassau et al. in patients with SOS/VOD

In the following, we reported the interim analysis carried out on the patients' cohort of the Italian multicentre study ElastoVOD enrolled before September 2019. Among the 502 patients who underwent HSCT and enrolled in the study, 46 left and were excluded from the intermediate analyses (**Figure 14**). Most of these were adult patients (83%). During the study period, 20 patients developed SOS / VOD (4.4%). The prevalence of SOS / VOD in the paediatric and adult fields was significantly higher (p-value =0.003) and was 12% and 3% respectively.

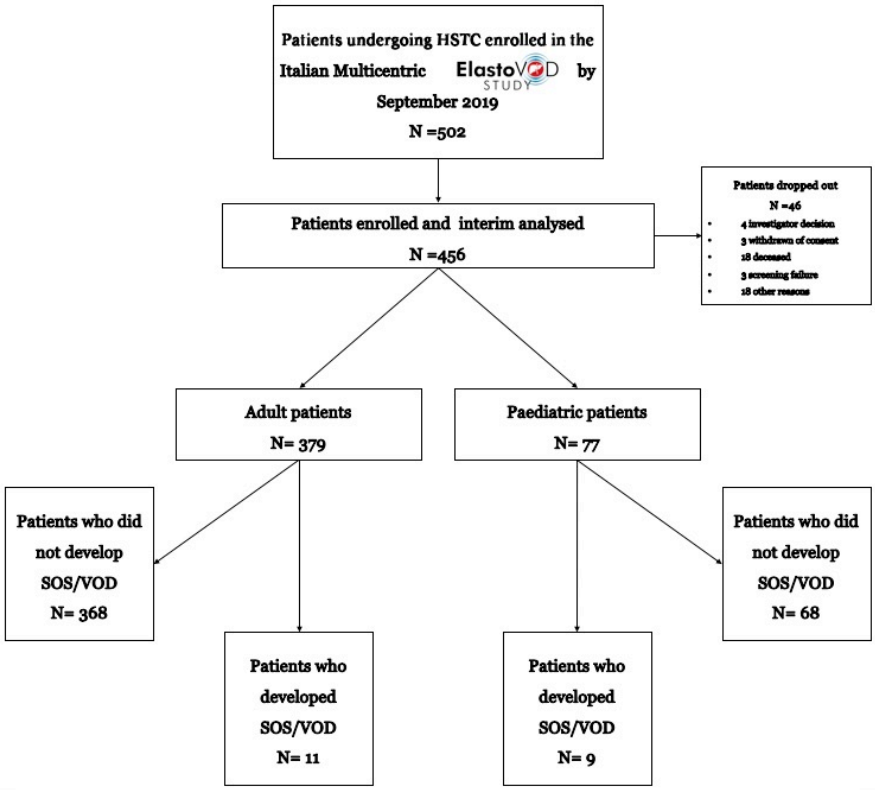

Figure 14: Study flow-chart of the population of the Multicentrer ElastoVOD study since September 2019

The clinical and transplant characteristics of the patients enrolled were summarised in **Table 13**. In these interim analyses, in a large cohort of patients, sex, age, history of blood transfusions, BMI, type of haematological diagnosis, were associated with the SOS / VOD diagnosis. Furthermore, although not wholly statistically significant (p-value =0.081), in the group of patients manifested SOS/VOD, the baseline value of liver stiffness was higher than those that had not. That result was in line with what we observed in the adult cohort as well.

Table 13. *Baseline features of the ElastoVOD study population and by VOD/SOS diagnosis*

	Study population (September 2019) n = 456	**Without VOD/SOS n = 436 (93.6%)**	**With VOD/SOS n = 20 (4.4 %)**	**p-value***
Gender (Male)	287 (63%)	278 (63%)	9 (45%)	**0.053**
Age, median (25th-75th)	49 (26 – 60)	51 (28 – 60)	25 (9 – 40)	**0.0014**
				0.003
Adults	379 (83%)	368 (84%)	11 (55%)	
Paediatric	77 (17%)	68 (16%)	9 (45%)	
BMI (kg/m²), median (25th-75th)	24.1 (20.9 – 27)	24.1 (20.6 – 26.7)	21.3 (18.4 – 22.9)	**0.0127**
Ethnicity				0.264
Caucasian	421 (92.3%)	404 (92.6%)	17 (85%)	
African/African American	11 (2.3%)	11 (2.5%)	0 (0.0%)	
Latin American	10 (2.3%)	9 (2.1%)	1 (5%)	
Asiatic	14 (3.1%)	12 (2.8%)	2 (10%)	
Diagnosis				**0.027**
Acute Myeloid Leukemia	36.5%	40.7%	31.6%	
Acute Lymphoblastic Leukemia	19.2%	14.2%	36.8%	
Myelodysplastic Syndrome	7.7%	8.3%	0%	
Hodgkin Lymphoma	5.8%	4.3%	0%	
Non-Hodgkin Lymphoma	5.8%	7.1%	5.3%	
Multiple Myeloma	3.5%	5.9%	0%	
Myelofibrosis	4.6%	3.2%	0%	
Chronic Myeloid Leukemia	3.1%	2.4%	5.3%	
Thalassemia	2.4%	1.6%	5.3%	
Ewing's sarcoma	1.9%	1.2%	5.3%	
Medullary aplasia	1.5%	1.2%	0%	
Combined immunodeficiencies	1.2%	1.6%	0%	
Sickle cell disease	0.4%	0.4%	0%	
Hemophagocytic lymphohistiocytosis	0.4%	0.4%	0%	
Paroxysmal Nocturnal Haemoglobinurias	0.4%	0.4%	0%	

Severe Aplastic Anaemia	1.9%	1.6%	1.7%	
Others	6.2%	7.1%	0%	
Blood Transfusion History				**0.0304**
≤20 transfusions	221 (48.5%)	218 (50%)	3 (13.3%)	
>20 transfusions	235 (51.5%)	218 (50%)	17 (86.7%)	
HCT-CI Sorror total score, median (25th-75th)	0.97 (0 – 9)	0 (0 – 2)	0.5 (0 – 2.25)	0.3763
Liver Stiffness Measurement at Baseline, median (25th-75th)	**4.40 (3.6 – 5.5)**	**4.2 (3.6 – 5.3)**	**4.9 (4.3 – 6.4)**	**0.0808**

Values are indicated as n (%) unless otherwise defined; *Wilcoxon rank-sum test/ Fisher Exact Test. Abbreviations: BMI Body Mass Index; HCT-CI Hematopoietic Cell Transplantation – Comorbidity Index

In the 47 centres involved, the techniques of transient elastography (FibroScan®) were the most represented (87%). Among the point and 2D Shear Wave techniques, the Philips devices, SuperSonic Images and Siemens were the most used by the centres.

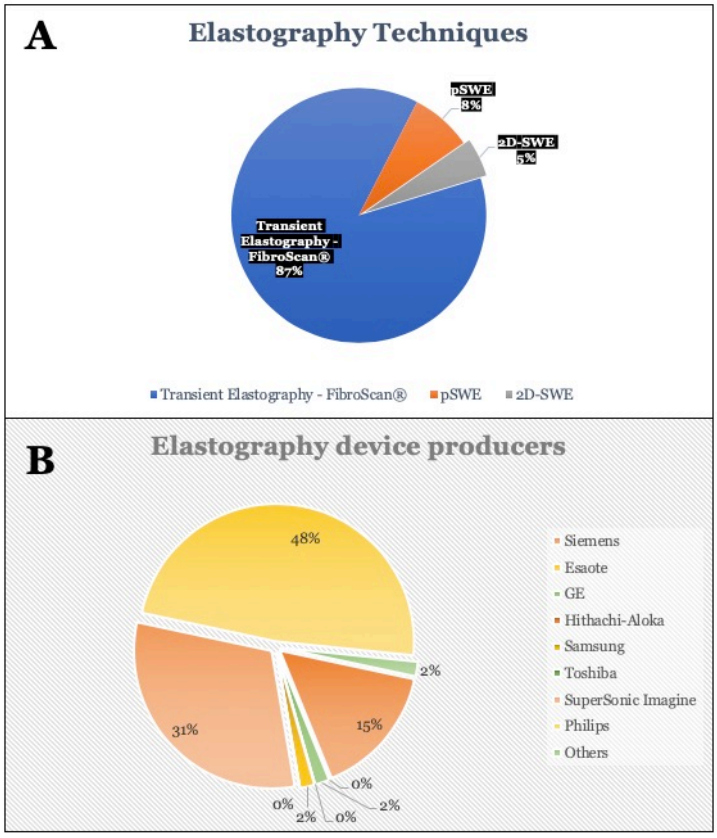

Figure 15: pie chart of the distribution of elastography techniques in the centres involved in the ElastoVOD Multicenter study.
A) Chart of elastography techniques according to the applied physical principle; B) Chart of pSWE / 2D-SWE elastography techniques according to the manufacturer

When we revaluated the SOS/VOD Risk Factors by EBMT in this population cohort, **Table 11** confirmed the pre-HSCT risk factors highlighted in the monocentric adult cohort (previous therapy with Inotuzumab ozogamicin (INO) (4/20) or others hepatoxic drugs (5/20) and presented pre-HCST iron overload (7/20). Overall, the number of pre-HSCT risk factors was significantly higher in those who had developed SOS/VOD after HSCT.

Confirming our previous results in the monocentric cohorts, LSM significantly increased in all patients who developed SOS/VOD anticipating the clinical diagnosis. These suddenly increased values preceded the clinical diagnosis from +1 to more than ten days (**Figure 16).**

Furthermore, the logistic regression analysis confirmed the previous results. In fact, an increase in LSM after HSCT compared to the pre-HSCT evaluation was significantly associated with the SOS / VOD diagnosis (OR: 1.24466; 95% CI [1.1773 - 1.32201] p-value <0.001); furthermore, the increase in LSM compared to the pre-HSCT assessment determined a sensitivity of 13.8% and a specificity of 98.8%. Noteworthy, the negative and positive predictive value of the LSM increase compared to the pre-HSCT evaluation for the SOS / VOD diagnosis was 75% and 96%

respectively. The rise in LSM had correctly classified over 95.46% of patients who developed SOS / VOD.

Table 14. *VOD/SOS Risk Factors according to EBMT in the study population and by VOD/SOS diagnosis*

VOD/SOS RISK FACTORS	Study population (September 2019) n = 456	Without VOD/SOS n = 436 (93.6%)	With VOD/SOS n = 20. (4.4 %)	p-value*
Transplant-related factors				
Unrelated donor	63.5%	62%	79%	0.150
HLA-mismatched donor	44%	42%	32%	0.283
Non-T-cell-depleted transplant	79%	79	90%	0.240
Myeloablative-conditioning regimen	67%	68	74%	0.521
Oral or high-dose Busulfan-based regimen	51%	43	63%	0.274
High-dose TBI-based regimen	4%	3.2	11%	0.136
Second HSCT	9.9%	10.7	5.3%	0.490
Patient and disease-related factors				
Older age	26%	24.5	10%	0.249
Karnofsky score below 90%	8.2%	7.7	0%	0.338
Metabolic syndrome	7%	5.6	10%	0.660
Female receiving norethisterone	1%	1.4	0%	0.747
Advanced disease (beyond second CR or relapse/refractory)	38.6%	37	60%	0.158
Thalassemia	0%	0%	0%	0.794
Genetic factors (GSTM1 polymorphism, C282Y allele, MTHFR 677CC/1298CC haplotype)	5.3%	5.8	0%	0.446
Hepatic-related factors				
Transaminases x 2.5 ULN	2.2%	2.8%	0%	0.506
Serum bilirubin x 1.5 ULN	2.4%	1.6%	0%	0.480
Active viral hepatitis	7%	7.9%	0%	0.760
Cirrhosis	0%	0%	0%	0.816
Abdominal or hepatic irradiation	2.2%	2%	5.3%	0.338
Previous use of Inotuzumab ozogamicin	2.7%	0%	15.8%	**<0.00001**
Other hepatotoxic drugs	12.7%	14%	26.3%	**0.068**
Iron overload	18.2%	16%	37%	**0.030**
N. of risk factors (median; 25th – 75th percentiles)	3.5 (1.5 – 4.5)	3.5 (3 – 4.5)	4.5 (3.5 – 5.75)	**0.01225**

Values are indicated as n (%) unless otherwise defined; TBI Total Body Irradiation; CR Complete Remission; ULN Upper Limit of Normal; *Wilcoxon rank-sum test/ Fisher Exact Test

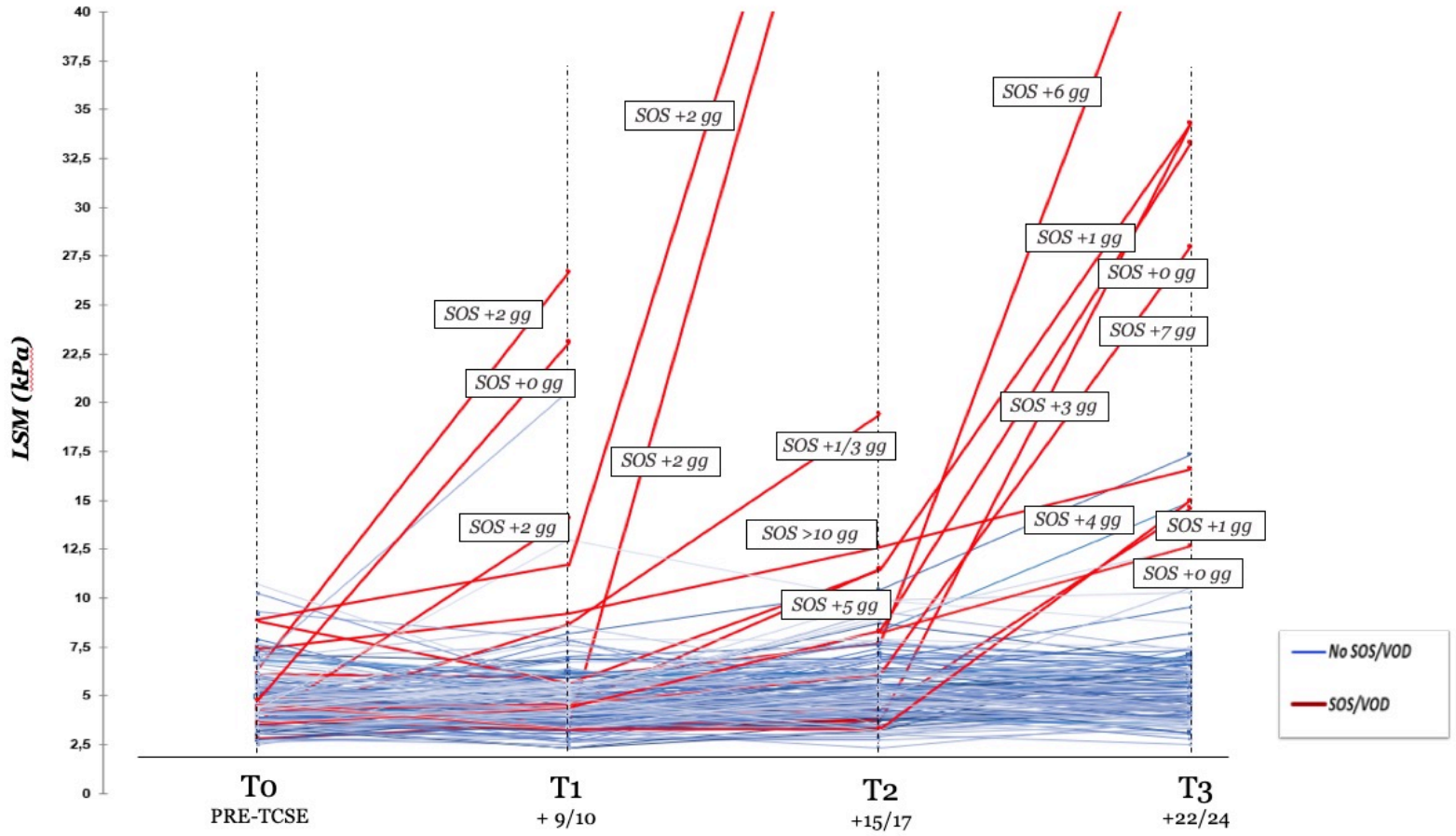

Figure 16: Variation of LSM values in the interim analyses of ElastoVOD study.

The solid red line represents patients who developed SOS/VOD after HSCT; solid blue line, patients who did not develop SOS/VOD after HSCT.

CONCLUSION

The aim of the ElastoVOD project is to evaluate the clinical role of the liver stiffness measurement on SOS/VOD development in a prospective cohort of patients, both adults and paediatrics, undergoing HSCT.

The rationale of using LSM as an early detector of SOS/VOD is related to its pathogenesis: congestion (blood outflow block) and inflammation (loss of sinusoidal wall integrity by toxic injury) lead to sinusoidal PH. All of these conditions have already been demonstrated as determinants of LSM increase in PH[275].

Our data are derived from studies of a single centre on adult, and paediatric patients published and confirmed by the intermediate analysis of the Italian multicentre cohort ElastoVOD on over 450 patients. These results showed that LSM values increased significantly compared to previous and previous pre-HSCT measurements in patients who developed SOS / VOD, allowing for preclinical diagnosis by anticipating the standard EBMT clinical diagnosis from 1 to more than ten days (**Figures 08/11/16**).

The study population showed a mean overall SOS/VOD incidence of 8.6%, with 15% and 4.2% in the paediatric and the adult cohorts respectively. That rates are in line with the current evidence [73,276].

As expected, SOS/VOD occurred mostly in patients with a higher number of SOS/VOD risk factors accordingly with a new classification from EBMT[40,74]. Indeed, patients who developed SOS/VOD have significantly received a more intensive busulfan-based chemotherapy (MAC regimen), more than 20 blood transfusion pre-HSCT (leading to a liver iron overload) and hepatotoxic drugs (mainly Inotuzumab ozogamicin) (**Tables 11/14**). This last result also confirmed in the interim analyses of the multicentre study, supports previous evidence and the recent expert panel review[277,278].

Since the end of the '90s, it has been known the importance of having, besides the clinical SOS/VOD criteria, a radiological technique able to better identify and differentiate SOS/VOD from other clinical HSCT complications. Thus, our protocol schedule contemplated a gray-scale and colour-doppler US examinations when LSM increased or when SOS/VOD diagnosis was established. Indeed, we evaluated the presence of Lassau US criteria[200], confirming that the majority of patients who developed SOS/VOD showed a Lassau score of ≥3 criteria **(Table 12)**. Our results strengthen the suggestions of EBMT and BCSH/BSBMT guidelines[72] stating that US examination could have a pivotal role as first bedside approach in order to assess differential diagnosis between several abdominal post-HSCT complications and to confirm the SOS/VOD diagnosis in uncertain

cases. However, the positivity of US signs is consistent with the clinical occurrence of SOS/VOD criteria, often precluding its use for diagnosis anticipation.

The main findings of this studies are that, for the first time with a prospective design, both in adult and patients undergoing HSCT, the increase of LSM after HSCT, assessed by several elastography techniques (**Figures 08/11/16)**, has been demonstrated to be an accurate predictor of SOS/VOD development. Noteworthy, an LSM increase after HSCT over the pre-HSCT assessment precedes the clinical SOS/VOD diagnosis from 1 to over ten days. These increases in LSM values compared with the patient's previous measures were significantly associated with the SOS/VOD diagnosis. Our results are consistent with the recent study by Reddivalla et al.[223], using a different US elastography method (ARFI), again in a small pediatric population. Besides, in patients who developed SOS/VOD, both in adults (**Figure 12**), and paediatric patients (**Figure 09**), and specially treated, LSM values reduced within 2-4 weeks after diagnosis, reaching pre-transplant values. One could argue that, if further confirmed, this finding could drive the treatment duration, an issue which has not yet been answered by means of previous clinical trials. Furthermore, we could speculate that some patients who developed drug-treated SOS / VOD do not reverse LSM values in the

normal range. That population may be at risk of developing long-term portal hypertension-related complications (esophageal varices, hepatic decompensation, etc.) and therefore deserve extended follow-up analogous to ACLD patients[279].

Finally, we demonstrated in the adult cohort that LSM was able to discriminate between SOS/VOD and other post-HSCT complications, enabling a definitive differential diagnosis. We here showed that the median LSM values of adults patients with SOS/VOD were significantly higher than those of patients with other therapy-associated liver complications (acute cholecystitis, cholangitis, DILI related to antimycotic drugs, hepatic GvHD, isolated liver biochemical alterations and fulminant EBV-related hepatitis reactivation) regardless of the CTC degree **(Figure 13)**. This result is apparently in contrast with Karlas and colleagues[280] who demonstrated that in 5 out 59 transplanted patients who developed severe hepatic involvement after HSCT (CTC grade 4-5), LSM values by TE were significantly higher (6.2±1.5 kPa vs 4.7±1.7 kPa) in comparison to those who did not. One possible explanation to solve this discrepancy could rely on the fact that SOS/VOD was not separately analysed, but it was included in the severe complication group, without any specifications. However, the predictive role and meaning of pre-HSCT LSM values in liver complications is still

unclear. Even if, the initial observation by Auberger et al.[218] who showed that pre-HSCT LSM cut-off of 8 kPa, measured by TE, was able to identify patients developing liver toxicity (defined as bilirubin >2mg/dL) after HSCT; our data and results by Reddivalla et al.[223] did not support the above findings. We did not observe significant differences in pre-HSCT LSM values between patients who developed SOS / VOD and those who did not; although a slight tendency in both the adult cohort and the intermediate analyses of the ElastoVOD multicenter study were observed.

The analyses of the present dissertation have the following limitations: i) a relatively low number of patients with SOS/VOD, even though in line with the incidence of this disease in adults according to the current literature and will be increased by the end of the multicenter Italian study; ii) the LSM assessments were arbitrarily scheduled in three consecutive visits (+9/10, +15/17 and +22/24). Evidence for a standard LSM assessment schedule are not yet available, and our schedule was arbitrarily applied due to the experimental phase of LSM role in this setting; however a daily LSM assessment, hardly practicable in the real clinical setting, could identify the real diagnostic advance of LSM by TE; iii) this study included LSM assessed mainly (>80%) by TE; consequently it is difficult to compare values obtained with other US-based

elastography techniques at this stage, as recently demonstrated[281]; on the other side the interim analyses of the multicenter study confirmed the dynamics of LSM increases in SOS/VOD patients despite the elastography techniques choice. Nevertheless, even if a dedicated device is needed, TE is still considered the best validated and most available elastography technique, and most centres use TE as the standard technique to assess LSM even in the consolidated hepatological indications[282,283]; iv) we excluded patients with pathological (BMI >40 kg/m2) obesity due to a technical limitation of "M" probe of TE device and are conditions which could impair the performance of LSM; for the multicentric study aiming to assess the diagnostic accuracy of LSM for VOD, an intention to diagnose approach (ITD) would be more informative. This approach, also known as the worst-case scenario[283,284], allowing to take into account the feasibility of the index test, will be considered in the final analyses of the ElastoVOD study.

In conclusion, the strength of the ElastoVOD prospective project, resulted of this PhD dissertation, on quite a large number of patients resembles a real-life HSCT practice and suggests that LSM by all the elastography techniques available could be considered a promising method to perform an early, pre-clinical diagnosis and predict SOS/VOD after HSCT. Besides, it could be further used to assess

treatment response in adult patients undergoing HSCT and developing SOS/VOD. Moreover, it is non-invasive, bedside method, very well tolerated by patients, reproducible. Also, it provides a standardised quantitative measurement, avoiding radiations (US-based), intravenous contrast agent and bleeding or infections risks. LSM seems to be accurate to differentiate between SOS/VOD and other liver-related HSCT complications. When the multicenter prospective ElastoVOD study will be terminated, the role of LSM, assessed by different US elastography techniques, could be confirmed in SOS/VOD pre-clinical diagnosis.

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APPENDIX

This thesis is based on the following articles:

I. Ravaioli F, Colecchia A, Alemanni LV, Vestito A, Dajti E, Marasco G, Sessa M, Pession A, Bonifazi F and Festi D. **Role of imaging techniques in liver veno-occlusive disease diagnosis: recent advances and literature review**. *Expert Rev Gastroenterol Hepatol. 2019 (IF 2.963)*; 13(5):463–84. doi.org//10.1080/17474124.2019.1588111/

II. Chan SS, Colecchia A, Duarte RF, Bonifazi F, Ravaioli F and Bourhis JH. **Imaging in Hepatic Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome.** *Submitted to Journal.*

III. Bonifazi F, Sessa M, Barbato F, Ravaioli F, Arpinati M, Cavo M, Colecchia A. **Diagnosis and treatment of VOD after allogeneic hematopoietic stem cell transplantation.** *Submitted to Journal.*

IV. Colecchia A, Marasco G, Ravaioli F, Kleinschmidt K, Masetti R, Prete A, Pession A and Festi D. **Usefulness of liver stiffness measurement in predicting hepatic veno-occlusive disease development in patients who undergo HSCT.** *Bone Marrow Transplant. 2017. (IF 4.674)*; 52(3):494–7. doi.org//10.1038/bmt.2016.320/

V. Zama D, Bossù G, Ravaioli F, Masetti R, Prete A, Festi D and Pession A**. Longitudinal evaluation of liver stiffness in three pediatric patients with the veno-occlusive disease.** *Pediatr Transplant. 2019. (IF 1.377)*; 23(5):e13456. doi.org//10.1111/petr.13456/

VI. Colecchia A*, Ravaioli F*, Sessa M, Alemanni VL, Dajti E, Marasco G, Vestito A, Zagari RM, Barbato F, Arpinati M, Cavo M, Festi D and Bonifazi F. **Liver Stiffness Measurement Allows Early Diagnosis of Veno-Occlusive Disease/Sinusoidal Obstruction Syndrome in Adult Patients Who Undergo Hematopoietic Stem Cell Transplantation: Results from a Monocentric Prospective Study**. *Biol Blood Marrow Transplant. 2019.* (*IF: 3.955*); 25(5):995–1003 doi.org//10.1016/j.bbmt.2019.01.019/

These articles are referred to by their Roman numerals in the text.