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ORIGINAL ARTICLE

Prospective qualitative and quantitative non-invasive evaluation of intestinal acute GVHD by contrast-enhanced ultrasound sonography

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Intestinal acute GVHD (I-aGVHD) is a life-threatening complication after allografting. Non-invasive bed-side procedures to evaluate extension and treatment response are still lacking. We hypothesized that, during I-aGVHD, contrast-enhanced ultrasound sonography (CEUS) could detect microcirculation changes (MVC) of the bowel wall (BW) and help to monitor treatment response. We prospectively employed CEUS in 83 consecutive patients. Of these, 14 patients with biopsy-proven intestinal GVHD (I-GVHD) were defined as the study group, whereas 16 patients with biopsy-proven stomach GVHD (U-GVHD) without intestinal symptoms, 6 normal volunteers and 4 patients with neutropenic enterocolitis were defined as the control group. All patients were evaluated with both standard ultrasonography (US) and CEUS at the onset of intestinal symptoms, during clinical follow-up and at flare of symptoms. Standard US revealed BW thickening of multiple intestinal segments, useful to determine the extension of GVHD. CEUS showed MVC, which correlated with GVHD activity, treatment response, and predicted flare of intestinal symptoms. US and CEUS findings were superimposable at diagnosis and in remission. CEUS was, however, more sensitive and specific to identify subclinical activity in patients with clinical relevant improvement. These findings were not observed in the control groups. CEUS is a non-invasive, easily reproducible bed-side tool useful to monitor I-aGVHD.

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INTRODUCTION

Intestinal acute GVHD (midgut syndrome, I-aGVHD) is a major cause of non-relapse mortality following an allograft.[1,2] Diagnosis remains problematic for some patients with this pathology in the midgut. Diarrhea volume is generally used to determine the severity of the intestinal involvement, but its clinical reliability is highly limited.[3,4] Overall, non-invasive specific and sensitive techniques to diagnose intestinal GVHD (I-GVHD), to evaluate treatment response and to guide the duration of immunosuppression are still lacking. Standard transabdominal ultrasonography (US) has already been used for its diagnosis and clinical follow-up,[5–7] and, more widely, in a variety of other intestinal diseases,[8–10] including inflammatory bowel diseases.[11–14] Recent studies have highlighted neovascularization in the early stages of GVHD.[15]

We previously reported our experience on the use of contrast-enhanced ultrasound (CEUS) during I-aGVHD that showed contrast enhancement uptake in the bowel wall (BW).[16] Schreyer *et al.*[17,18] have also reported similar findings.

Standard US is routinely employed to evaluate both small and large intestines in a number of intestinal diseases. It is widely employed for both diagnosis and follow-up of inflammatory bowel diseases such as Crohn's disease, where intramural and extramural involvements can accurately be evaluated.[19] Although more rarely, it has also been used for assessing I-GVHD.[20]

In this study, we have evaluated the changes of the BW during GVHD using both standard US and CEUS, which is an easily reproducible ultrasound technique. Real-time microvascular imaging has recently been made possible by novel echo-contrast enhancing agents and low mechanical-index harmonic sonography. Moreover, CEUS has already been extensively used in active Crohn's disease in which the neovascularization of the small BWs has been described.[21–24] Importantly, recent studies have highlighted neovascularization in the early stages of GVHD.[15]

Here, we report the preliminary findings of our prospective study on the role of CEUS as a non-invasive qualitative and quantitative tool to evaluate extension and monitor I-GVHD. Overall, our aims were to evaluate if standard US could detect the extension of I-GVHD; if BW microcirculation changes (MVC) by CEUS correlated with clinical symptoms and treatment response; and, finally, if CEUS enhancement findings were similar to those seen in active Crohn's disease.[25]

PATIENTS AND METHODS

Patients

Between 2008 and 2012, we prospectively evaluated 83 consecutive patients admitted for an allogeneic transplantation, at the Division of Haematology, University of Pisa, Italy. All patients underwent a standard US of the abdominal organs, small and large intestines as part of the pre-

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transplant work-up. All patients who developed post-transplant diarrhea and/or vomiting and/or abdominal cramping underwent transabdominal US of the small and large intestine as described below. The study group consisted of 14/83 patients who developed biopsy-proven I-GVHD. The control group consisted of 16 patients who developed biopsy-proven U-GVHD without intestinal symptoms;[26] 4 patients who developed neutropenic enterocolitis (NEC) after allografting;[27,28] 6 healthy volunteers without any documented diseases. Patient characteristics are summarized in Table 1. Median age was 50 years old (range 22–66 years).

After ruling out bacterial, fungal and viral infections by routine stool cultures and PCR analyses (for herpes viruses, adenovirus, EBV, rotavirus and norovirus), patients with persistent nausea, vomiting and diarrhea underwent endoscopy for histological assessment as previously described by Sale *et al.*[3] and modified by Epstein *et al.*[29] CMV infection was ruled out by immunostaining techniques. Clinical assessment and grading of GVHD was performed according to standard criteria.[30,31] All patients and volunteers provided written informed consent and the study was approved by the Institutional Review Board according to the Declaration of Helsinki.

Standard sonography methodology

Standard (B-mode) US was performed at bed-side with a portable sonographer (Esaote model My Lab 25, Esaote Italia, Florence, Italy) without any preparation, within 24 h from the onset of clinical GVHD symptoms. The entire gastrointestinal tract was submitted to a gray-scale ultrasound examination (B-mode US). The colon was examined from the cecum to the sigmoid colon. The entire small bowel was examined with particular attention to the last portion of the terminal ileum, which is the site most commonly involved in GVHD.[5–7] The following parameters were assessed: (1) bowel wall thickness (BWT) defined as abnormal if $\leqslant$3 mm in the large bowel and $\leqslant$2 mm in the duodenum and small bowel;[32] (2) BW layers: the superficial mucosal interface, the deep mucosa, the submucosa, the muscularis propria and the serosa;[33,34] (3) degree of dilation;[32,35] (4) motility;[32] (5) bowel content defined as gas, food stuff of feces, mixtures of the two, or fluid-filled;[36] (6) presence of haustration or dehaustration;[32,37] and (7) presence/absence of free abdominal fluid in all four quadrants and/or upper abdominal organ pathologies other than GVHD.

CEUS methodology

After standard US, the ultrasound contrast agent was administered i.v. and CEUS was performed on diseased intestine[38] with the same sonographer equipped with contrast-specific real-time imaging technology defined as contrast tuned imaging. A second-generation echo-contrast agent, SonoVue (Bracco, Milan, Italy), was injected as i.v. bolus into an antecubital vein. Briefly, SonoVue is a non-nephrotoxic contrast agent that consists of 2.5-μm-diameter microbubbles stabilized with phospholipids and filled with sulfur hexafluoride that flow through the pulmonary microcirculation and remain within the vascular space.[39] SonoVue is approved in Europe for clinical use and has a wide range of clinical applications.[38,40,41]

Contrast tuned imaging exploits the resonance property of the microbubbles and prevents them from bursting during insonation. This allows for real-time imaging of the microcirculation, without gray-scale echoes, and provides continuous perfusion data on viscera.[25,42]

After injection the contrast agent reaches the intestinal wall in about 10–15 s and its peak concentration after approximately 30 s. In the intestine this 'arterial phase' is followed by the 'venous phase' in which the contrast agent, after distributing to the whole intestinal capillary bed, is exhaled through the lungs.[38] Continuous imaging was recorded from injection throughout the entire arterial and venous phases as previously described by Serra *et al.*[25] Distinct digital cine-clips for basic US and for CEUS scans were stored for computed analysis. Echo-signal intensity of the vascularity of the bowel segments selected with CEUS, defined by the operator as regions of interest (ROI) (Figure 1a), were quantitatively analyzed with a dedicated software (Q-ontrast; e-AMID—Advanced Medical Imaging Development, Italy distributed from Bracco). Q-ontrast generates chromatic maps (Figure 1b) of the ROI perfusion patterns, and automatically compensates for motion artifacts during data acquisition.[43] The Q-ontrast analysis of ROI generated curves representing echo-signal intensity vs time (time intensity curves). For all patients time intensity curve parameters, including the slope of the first ascending tract of the curve, the curve shape, time to peak enhancement, the area under the curve, regional blood flow and mean transit time were recorded (Figures 1c–e).[43]

In all patients, US and CEUS were performed by a physician with 15 years of experience in US, who is a member and teacher of the Italian School of Basic and Emergency Ultrasound (SIUMB) at the University of Pisa.

Statistical analysis

Sensitivity, specificity, positive and negative predictive values were determined to evaluate the diagnostic accuracy of standard US and CEUS. Their point estimates and 95% CIs were reported.

RESULTS

Standard US findings of I-GVHD (study group)

Table 2 illustrates US and CEUS findings, and their correlation with GVHD and clinical outcomes. Standard US invariably revealed increased BWT mostly related to mucosal edema (Figure 2a). Segments involved varied from patient to patient. Of 14 patients,

Table 1. Diagnosis in the study and control group, description of conditioning regimen, GVHD prophylaxis, stem cell source, donor, and number and type of events occurred

Diagnosis	*No. of patients*	*Conditioning*	*GVHD prophylaxis*	*Stem cell source*	*Donor*	*Type and no. of events*
B-ALL	8	Cy/TBI	Cya + short-course MTX	PBSC	Sib ($N=3$), MURD ($N=5$)	I-GVHD ($N=4$), U-GVHD ($N=3$), NEC ($N=1$)
T-ALL	2	Cy/TBI	Cya + short-course MTX	PBSC	MURD ($N=2$)	I-GVHD ($N=1$), U-GVHD ($N=1$)
MM	6	Flu/TBI ($N=2$), Thiotepa/Cy/MEL ($N=3$)	Cya + MMF ($N=2$) Cya + short course MTX ($N=4$)	PBSC	Sib ($N=3$), MURD ($N=3$)	I-GVHD ($N=1$), U-GVHD ($N=5$)
MCL	3	Thiotepa/Cy/MEL	Cya + short-course MTX	PBSC	Sib ($N=1$), MURD ($N=2$)	I-GVHD ($N=1$), U-GVHD ($N=2$)
AML	8	BU/Cy	Cya + short-course MTX	PBSC ($N=6$), BM ($N=2$)	Haplo ($N=2$), sib ($N=2$), MURD ($N=4$)	I-GVHD ($N=4$), U-GVHD ($N=2$), NEC ($N=2$)
sAML	1	BU/Cy	Cya + short-course MTX	PBSC	Sib ($N=1$)	U-GVHD ($N=1$)
MF	3	BU/Cy	Cya + short-course MTX	PBSC	Sib ($N=1$), MURD ($N=2$)	I-GVHD ($N=2$), U-GVHD ($N=1$)
Follicular NHL	3	Thiotepa/Cy/MEL	Cya + short-course MTX	PBSC ($N=2$), BM ($N=1$)	MURD ($N=3$)	I-GVHD ($N=1$), U-GVHD ($N=1$), NEC ($N=1$)
Healthy volunteers	6	NA	NA	NA	NA	NA

Abbreviations: B-ALL = B-cell-ALL; Flu = fludarabine; I-GVHD = intestinal GVHD; MCL = mantle cell lymphoma; MEL = melphalan; MF = myelofibrosis; MM = multiple myeloma; MMF = mycophenolate mofetil; MURD = matched unrelated donor; NA = not applicable; NEC = neutropenic enterocolitis; PBSC = peripheral blood stem cells; PD = progressive disease; T-ALL = T-cell acute lymphoblastic leukemia; U-GVHD, upper gut acute GVHD; VGPR = very good PR. Median age 50 years (range 22–66 years).

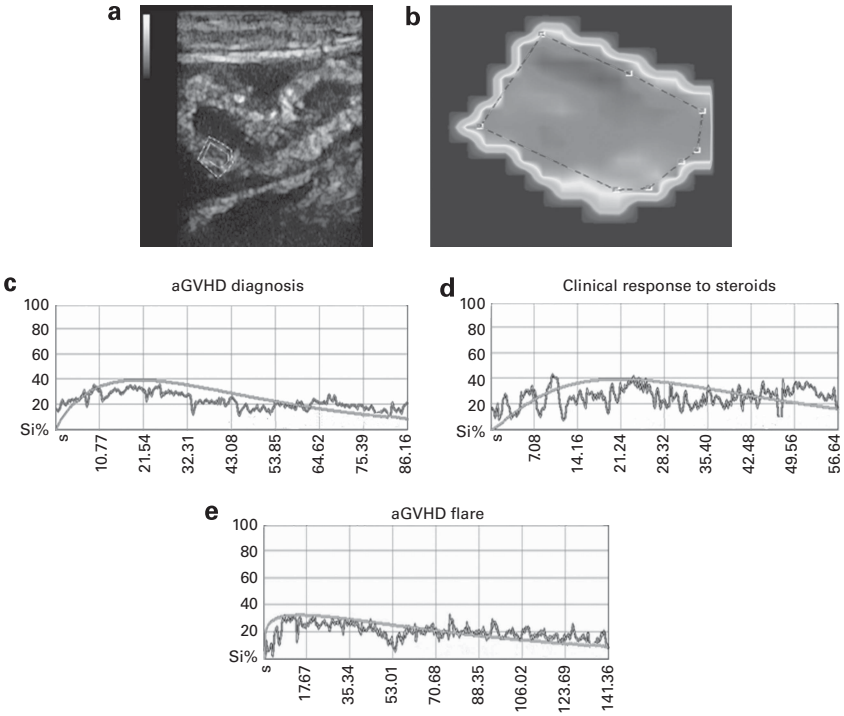

Figure 1. (**a**) CEUS of an intestinal loop with aGVHD. The ROI is indicated by white arrow. (**b**) ROI quantitative analysis of BW vascularity with the Q-ontrast software with the corresponding color map (see text). (**c**) Representative TIC in Pt10 at I-aGVHD diagnosis. The curve has a 'tailing' shape with a mean transit time (MTT) = 46.3 s and AUC = 5.1 cm^2. (**d**) Representative TIC in the same patient (Pt10) after clinical response to steroids: MTT and AUC decreased (MTT = 38.7 s, AUC = 0.25 cm^2). (**e**) Representative TIC in Pt10 at I-aGVHD flare: MTT and AUC increased again (MTT = 92.6, AUC = 2.6 cm^2). A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

9 had more than one site involved at the onset of symptoms. The BW layers could be identified in 11 patients, whereas in the others boundaries were poorly defined.

Standard US findings in the control group

Healthy volunteers ($N = 6$) and U-GVHD ($N = 16$) patients had normal BWT thickness (Figure 3a).[8,32] On the contrary, patients ($N = 4$) with NEC showed BWT as for the study group (I-GVHD) (Figure 3b).[27,28]

CEUS findings in I-GVHD (study group)

CEUS was performed at diagnosis, during treatments and follow-up (Table 2).[38] CEUS was safely performed in all patients, including one with a baseline creatinine level of 4.5 mg/dL.[40] Overall, by Q-ontrast software analysis, a rapid enhancement peak, due to a rich microvascularization of the BW, described by the tailing-curve shape and a long tailing, expressed by a prolonged mean transit time was observed in all patients (Figure 1c).

Findings at diagnosis

Overall, at aGVHD onset, during the arterial phase, CEUS showed three distinct enhancement patterns (EPs) of the bowel microcirculation: EP-1, a complete enhancement of the entire wall section from the mucosal to the serosal layer (Figure 2b); EP-2, enhancement of the entire wall with the exception of the outer border of the muscularis propria (Figure 2c); EP-3, enhancement of the intermediate submucosal layer without enhancement of the outer and inner borders of the BW (Figure 2d). Moreover, a persistent microcirculation enhancement longer than 2 min was seen during the venous washout in 11 of 14 patients.

Follow-up findings

All 14 patients with GVHD were promptly treated with steroids at 2 mg/kg. Salvage treatment in steroid-refractory patients consisted of Infliximab,[4,44,45] Rituximab,[46,47] extracorporeal photophoresis,[48] budesonide[49] alone or in various combinations (Table 2).

In patients who completely responded to treatment with normalization of standard US, CEUS showed similar findings as for healthy volunteers and U-GVHD patients (Figures 3c and d).[38] However, in patients with clinically relevant improvement but no complete response, CEUS showed persistent microvascular enhancement suggesting still active disease, while standard US showed normal findings. In steroid-refractory[50,51] patients, CEUS became normal only in those who achieved complete remission after salvage therapy, whereas it remained unchanged in patients who eventually died of GVHD-treatment-related complications (Table 2). In patients with improvement but no complete remission, US showed a sensitivity of 55% (95% CI: 0.23–0.83) and specificity of 100% (95% CI: 0.19–1) with a positive predictive value of 100% (95% CI: 0.42–1) and negative predictive value of 38% (95% CI: 0.09–0.76), whereas CEUS showed a sensitivity of 100% (95% CI: 0.62–1) and specificity of 100% (95% CI: 0.62–1), with positive and negative predictive values of 100%.

Findings at GVHD flare

Overall, I-aGVHD flared in three patients (Table 2, Figure 1e). The CEUS BW EPs correlated with clinical symptoms. Standard US and CEUS showed normal features in responsive patients. In one patient who only showed a clinical improvement, CEUS showed persistent microvascular changes suggestive of subclinical activity. Qualitative assessment of BW microcirculation enhancement for each patient during follow-up and flare was in accordance with quantitative assessment using time intensity curves. Patients

Table 2. Standard US and CEUS findings at diagnosis, at follow-up and at flare

	Findings at diagnosis				*At 1st follow up*				*At 2nd follow-up*		
Pt	*Standard US involved sites (BWT in mm)*	*CEUS (site examined)*	*GVHD histology/ clinical grade*	*Therapy and outcome*	*Salvage therapy*	*Standard US (involved sites, BWT in mm)*	*CEUS (site examined)*	*Therapy and outcome*	*Standard US*	*CEUS (site examined)*	*Therapy and outcome*
1	T. ileum (5.1), A.colon (5.8)	T. ileum: EP1 (AD)	Severe skin 3, Int 2 III	PDN, steroid refractory	Infliximab (2 doses)	Unchanged	T. ileum: EP1 despite CRI PAD	Continue Infliximab (2 doses); CR	Normal after Infliximab (4 doses)	Normal	Persistent CR
2	T. ileum (7.9); A. colon(8.4); T. colon (4.2); D. colon (6.0)	A. colon: EP1 (AD)	Moderate Int 1 II	PDN CR	NA	Normal	Not done	CR			
3	A. colon (18); T. colon (12.6); D. colon (8.2); S. colon (15.1)	A. colon: EP3 (AD)	Moderate Int 3 III	PDN, steroid refractory	Infliximab (2 doses)	Unchanged	Unchanged PAD	Dead (MOF at D + 61)			
4	T. ileum (5.8); A. colon(6.0)	T. ileum: EP1 (AD)	Severe Int 4 IV	PDN, steroid refractory	Unchanged	Unchanged	Unchanged PAD	Dead (paralytic ileus D + 53)			
5	T. ileum (5.2)	T. ileum:EP2	Mild Int 1 II	PDN, CR	NA	Normal	Not done	CR, D + 280 alive and well			
6	T. ileum (6.0); A. colon(6.2)	T. ileum: EP1 (AD)	Severe Int 4 IV	PDN, steroid refractory	Infliximab (2 doses)	Unchanged	Unchanged PAD	Continue Infliximab (2 doses)	Unchanged	Unchanged PAD	Dead (sepsis D + 73)
7	T. ileum (7.0); A. colon(5.5)	T. ileum: EP1 (AD)	Moderate skin 2, Int 3 III	PDN, steroid refractory	Infliximab (2 doses)	Improved	T. ileum: unchanged; ↓EP1 despite CRI, PAD	Continue Infliximab (2 doses)	Unchanged	Unchanged PAD	Died (MOF D + 84)
8	T. ileum (6.0)	T. ileum: EP2 (active disease)	Mild skin 2, Int 1 II	PDN, CRI	Unchanged	Normal T. ileum (4 mm)	Unchanged: T. ileum; ↓EP2 despite CRI, PAD	Budesonide	Normal	Normal	CR (alive D + 80)
9	T. ileum (5.7); A. colon(5.5)	T. ileum: EP2 (AD)	Moderate Int 1 II	PDN, CR	Unchanged	Normal	NF	Unchanged CR; died of HHV6-encephalitis D + 69			
10	T. ileum (5.9); D. colon (5.5)	T. ileum: EP2 (AD)	Moderate skin 2, Int 1 II	PDN, CRI	Unchanged	Normal	T. ileum; EP2 with CRI PAD	Budesonide	Normal	Normal	CR (D + 144)
11	T. ileum (5.7)	T. ileum: EP2 (AD)	Moderate skin 2,Int 1 II	PDN, CRI	Unchanged	Normal	T. ileum: ↓EP2, PAD	ECP Budesonide; CR	Normal	Normal	
12	A. colon (7.2); T. colon (6.5); D. colon (6.5)	D. colon: EP2 (AD)	Moderate Int 1 II	PDN, CRI	Unchanged	A. and T. colon, normal; D. colon, improved	D. colon: ↓EP2, PAD	Budesonide; CR	Normal	Normal	Persistent CR
13	D. colon (6.1)	D. colon: EP2 (AD)	Moderate Int 1 II	PDN, CRI	Unchanged	Unchanged	Unchanged PAD	ECP, Rituximab	Improved	Unchanged, persistent active disease	Dead (sepsis D + 46)
14	D. colon (7.2)	D. colon: EP2 (AD)	Moderate Int 1 II	PDN, CRI	Unchanged	Unchanged	Unchanged PAD	ECP, Rituximab	Normal	Normal	Dead (relapse D + 146)

Findings at flare

	1st follow-up			*2nd follow-up*		
	Standard US (involved sites, BWT in mm)	*CEUS (site examined)*	*Therapy and clinical outcome*	*Standard US (involved sites, BWT in mm)*	*CEUS (site examined)*	*Therapy and clinical out come*
1	D. colon (7.5)	D. colon: EP2 (AD)	PDN CR	Normal	NF	Persistent CR
2	T. ileum (7.0)	T. ileum: EP2 CRI PAD	Rituximab (4 doses) CRI	Improved	T. ileum: EP2.CRI PAD	Overt flare; Infliximab; dead (sepsis D + 223)
10	T. ileum (5.2)	T. ileum: EP2 (AD)	PDN with CR	Normal	NF	CR; alive and well (D + 171)

Abbreviations: A. colon = ascending colon; AD = active disease; AP = arterial phase; BW = bowel wall; BWT = bowel wall thickening; CEUS = contrast-enhanced ultrasonography; CRI = clinically relevant improvement; D. colon = descending colon; D + = day at follow-up; ECP = extracorporeal photophoresis; ENH = enhancement; EP1 = enhancement pattern 1 (Figure 1); EP2 = enhancement pattern 2 (Figure 2); EP3 = enhancement pattern 3 (Figure 3); Int = intestinal; MOF = multi-organ failure; MTT = mean transit time; NA = not applicable; NF = normal features; PAD = persistent active disease; PDN = prednisone; S. colon = sigmoid colon; T. ileum = terminal ileum; T. colon = transverse colon; Tx = transplant; ↓ = reduced. The table shows the correlation with aGVHD grade, stage, therapy, outcome and cGVHD. Underlined patients depict those in whom during follow-up there were different findings between CEUS and US.

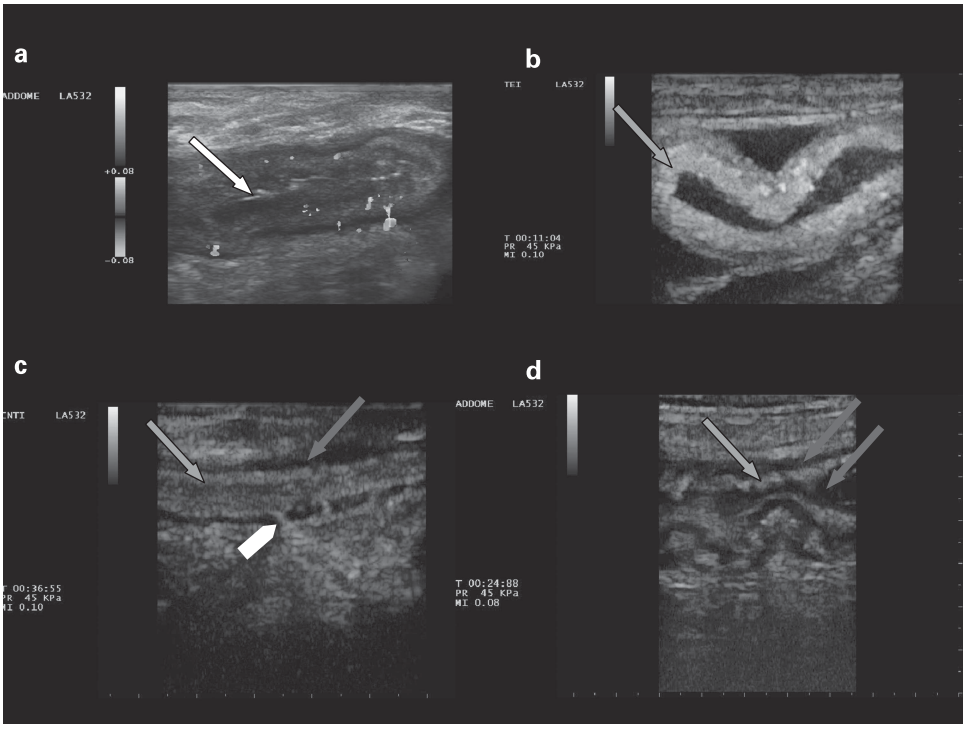

Figure 2. Imaging of aGVHD by color-doppler US and EPs (1, 2, 3) by CEUS. (**a**) Macro-vascularization of the terminal ileum with color-doppler US (arterioles). Increased BWT (5.3 mm), mostly due to mucosal edema. An echo-rich layer is detectable between the mucosal layer and the bowel lumen of the distal ileum (white arrow). (**b**) EP 1: arterial phase enhancement of microcirculation with complete enhancement of the entire wall section from mucosal to the serosal layer (green arrow). (**c**) EP 2: the contrast medium reaches and enhances the BW microcirculation during the arterial phase (green arrows), with absence of enhancement only in the outer border of the muscularis propria (red arrow). The 'Vasa recta' are identified by contrast medium (white arrowhead). (**d**) EP 3: arterial phase enhancement of microcirculation showing absence of enhancement (red arrow) both in the outer and in the inner border of the BW and enhancement of microcirculation only in the intermediate submucosal layer (green arrow). A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

responding to treatment had a decrease in mean transit time and area under the curve (Figures 1c–e).

CEUS findings in the control group

CEUS showed a thin layer of enhancement of BW both in healthy volunteers (Figure 3c) and in U-GVHD control patients (Figure 3d). These findings differed from those in the I-GVHD study group (Figures 2b–d). In patients with NEC, there was a strong arterial phase enhancement, suggestive of BW microvascular changes due to infection-related inflammation (Figures 3e and f).[27] The US and CEUS findings in NEC patients were superimposable.

We found penetration of microbubbles in the bowel lumen, not only in the study group as previously reported,[17,18] but also in one patient with NEC (Figures 3e and f).

DISCUSSION

Acute GVHD and its complications are major causes of non-relapse mortality following an allograft. Given the non-specific symptoms and the significant side effects of intestinal GVHD treatment, a histological diagnosis via endoscopic biopsies is highly recommended and may be required in up to 40% of patients for differential diagnosis.[52] Reliable non-invasive procedures to evaluate its extension and treatment response are still lacking.[3,4]

We hypothesized that CEUS could specifically detect MVC at the onset of GVHD and during its treatment. Our hypothesis partly derives from the observations that intestinal biopsies during acute GVHD show an increased microvessel circulation[53] and that graft-vs-host reactions are associated with increased neovascularization.[15] In our study, standard US mostly revealed abnormal BWT in multiple segments suggestive of extensive intestinal involvement, as shown in a previously published study that employed positron emission tomography (PET).[54] Increased BWT, due to mucosal edema, was consistently observed in both the small intestine and the colon as previously described by Klein *et al*.[5] However, these non-specific findings are also found during several intestinal infections.[27,28]

CEUS clearly showed a microcirculation enhancement of the BWs during the arterial phase, followed by a prolonged venous phase washout. The persistence of the contrast agent in the BWs was expressed by the prolonged mean transit time. Interestingly, the EPs observed were similar to those described in active Crohn's disease.[25] The tailing shape of the washout curve may be explained by a marked inflammatory interstitial edema that may correlate with the pericapillary hemorrhage described in biopsies during active GVHD.[22] The interstitial edema does not prevent the arterial in-flow but slows the circulation in the venules and is likely responsible for the prolonged venous phase washout.[23] Furthermore, GVHD pathophysiology is characterized by neovascularization, mainly driven by vasculogenesis, during its early inflammatory phase. At a later stage, the vasculature itself becomes a target of allo-reactive donor T cells leading to fibrosis and rarefaction of blood vessels.[15] Overall, the increased arterial microvascular enhancement may correlate with an early neovascularization of the BWs[15] and the prolonged venous phase washout may be seen where the inflammatory interstitial edema is more prominent.

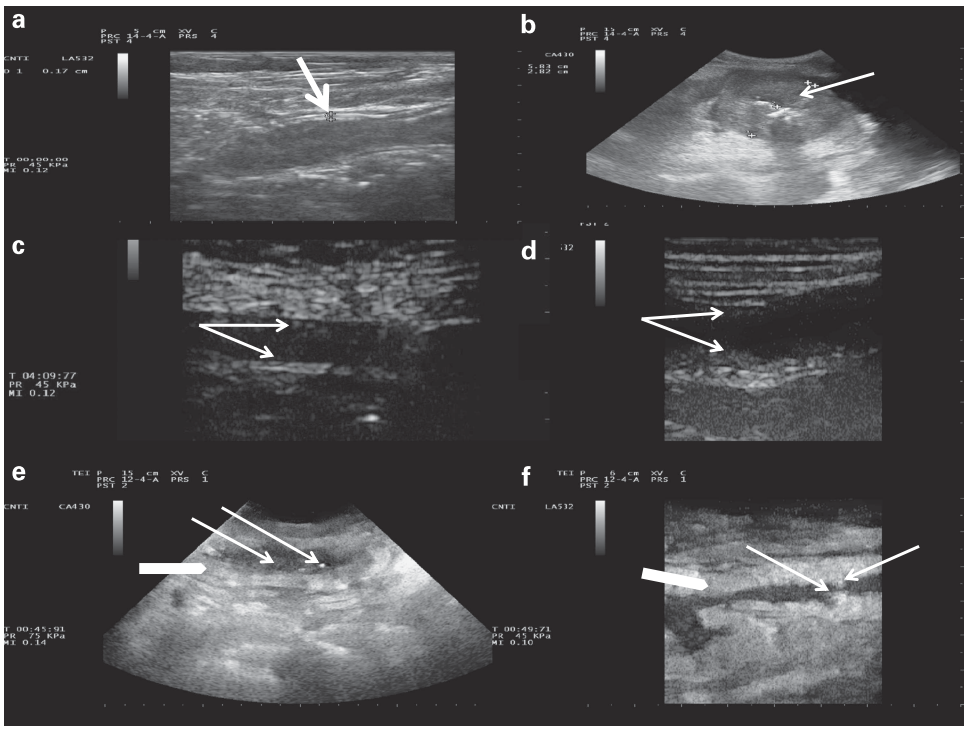

Figure 3. (**a**) Standard US of a normal volunteer showing normal BW thickness (BWT; 1.7 mm, white arrow). (**b**) Standard US in a patient with NEC showing BWT (28.2 mm, white arrow). (**c**) CEUS in a normal volunteer (control group) showing arterial phase enhancement of BW, and normal thickness. (**d**) CEUS in a patient with U-GVHD without I-GVHD (control group) showing arterial phase enhancement of BW, and normal thickness. White arrows show arterial enhancement. (**e**) CEUS with convex probe and (**f**) CEUS with high-frequency linear probe of the same patient with NEC showing BWT and arterial phase enhancement (white arrowhead). There is passage of microbubbles in the lumen (white arrows).

GVHD response to treatments correlates with longer survival.[17] In our study, complete clinical remission, after first-line or salvage treatment, coincided with normalization of both standard US and CEUS. However, patients with clinically relevant improvement without complete resolution of symptoms are a challenging subgroup at high risk of flare. Close follow-up is mandatory. Standard US only allows monitoring of BWT. On the contrary, CEUS allows us to detect microvascular changes at a capillary level, underlying persistent inflammatory activity, also in patients with normal BWT. Though the patient series was small (14 patients in the study group and 16 in the control group), CEUS was significantly more sensitive and specific than standard US in identifying subclinical GVHD activity, predictive of clinical flare, in patients without complete resolution of symptoms. Similarly, Di Sabatino *et al.*[24] described a group of patients with clinically inactive Crohn's disease who showed enhanced CEUS signal intensity. This group of patients developed early relapse. The authors suggested that CEUS findings were suggestive of subclinical disease activity that preceded early relapse.[24] Moreover, Serra *et al.*[25] reported a significant correlation between CEUS EPs and Crohn's disease activity. These observations are similar to what we have seen in our I-GVHD patients with abnormal CEUS findings despite relevant improvement of symptoms. Moreover, US and CEUS allowed to rule out lower intestinal GVHD when patients only presented with U-GVHD. This could be particularly useful in U-GVHD patients who can be treated with lower doses of steroids, reducing their side effects, as compared with patients with midgut symptoms.

CEUS is well tolerated, non-invasive and less expensive than other imaging techniques.[54] Moreover, it is applicable to patients with renal insufficiency, where CT or MRI with contrast media are contraindicated.[55] The reported incidence of severe hypersensitivity or allergic events to CEUS contrast agents is extremely low (0.001%).[40]

In conclusion, though not diagnostic, CEUS showed MVC of the BW that correlated with clinical symptoms of biopsy-proven I-GVHD and its treatment response at diagnosis, at follow-up and at flare.

Importantly, even though US and CEUS findings were superimposable in patients at diagnosis and in complete clinical remission after treatment, CEUS was more sensitive and specific in identifying residual disease activity in high-risk patients with clinically relevant improvement but not complete resolution of symptoms.

Moreover, CEUS provided a quantitative measurement of altered vasculature in segments inaccessible to endoscopic evaluation. Larger prospective studies are warranted to establish the role of the combination of clinical signs and/or symptoms with CEUS findings in the clinical follow-up of biopsy-proven I-GVHD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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