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# Early detection of acute graft-versus-host disease by wireless capsule endoscopy and probe-based confocal laser endomicroscopy: results of a pilot study

Emmanuel Coron<sup>1,2,\*</sup>, Valerie Laurent<sup>1,\*</sup>, Florent Malard<sup>3,4</sup>, Marc Le Rhun<sup>1</sup>, Patrice Chevallier<sup>3</sup>, Thierry Guillaume<sup>3</sup>, Jean-François Mosnier<sup>5</sup>, Jean-Paul Galmiche<sup>1,2</sup> and Mohamad Mohty<sup>3,4,6</sup>

## Abstract

**Objective:** Acute gastrointestinal graft-versus-host disease (GI-GVHD) is usually diagnosed using endoscopic examinations and biopsies for conventional histology. The aim of this pilot study was to determine whether mini-invasive techniques such as probe-based confocal laser endomicroscopy (pCLE) combined with wireless capsule endoscopy (WCE) could detect early lesions of GI-GVHD prior to symptoms.

**Design:** Fifteen patients undergoing allogeneic haematopoietic stem cell transplantation (allo-HSCT) were prospectively examined with a small bowel WCE, duodenal and colorectal pCLE, and standard biopsies. Per study protocol, all these examinations were scheduled between day 21 and day 28 after allo-HSCT, independently of the presence or absence of digestive symptoms.

**Results:** During follow up, eight patients developed acute GI-GVHD. Sensitivity of WCE, pCLE, and histology were 50, 87.5, and 50%, respectively. Specificity of WCE, pCLE, and histology were 80, 71.5, and 80%, respectively. We showed a positive correlation between the Glücksberg scoring system and WCE ( $\rho = 0.543$ ,  $p = 0.036$ ) and pCLE ( $\rho = 0.727$ ,  $p = 0.002$ ) but not with standard histology ( $\rho = 0.481$ ,  $p = 0.069$ ).

**Conclusions:** The results from this pilot study suggest that novel methods such as pCLE and WCE could be part of a mini-invasive algorithm for early detection of GI-GVHD.

## Keywords

Early diagnosis, graft-versus-host disease, novel imaging techniques, probe-based confocal laser endomicroscopy, wireless capsule endoscopy

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## Introduction

Graft-versus-host disease (GVHD) is the major source of morbidity and mortality after allogeneic haematopoietic stem cell transplantation (allo-HSCT).<sup>1</sup> The diagnosis of acute GVHD is based upon analysis of clinical and laboratory criteria in the appropriate clinical context after excluding other causes.<sup>2</sup>

While severe acute gastrointestinal GVHD (GI-GVHD) is likely the most serious and most potentially fatal form of GVHD, gastrointestinal symptoms are rather common in allo-HSCT patients, but not specific for GI-GVHD. Therefore, endoscopic examination

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with biopsy sampling of the mucosa is usually performed to confirm the diagnosis and exclude differential diagnoses. However, this strategy is hampered by some major limitations. Indeed, GI-GVHD is characterized by a patchy distribution of lesions, which can either affect a short segment or involve the whole gastrointestinal tract. Therefore, available endoscopic examinations such as oesophagogastroduodenoscopy (OGD) and colonoscopy have a limited diagnostic yield since they can only explore a short segment of the small bowel, an area which is frequently involved in GI-GVHD.<sup>3–7</sup> Moreover, usual endoscopic examinations are relatively invasive in such fragile patients. Also, one should bear in mind that endoscopic abnormalities are usually found only in a minority of cases (16–32%)<sup>8</sup> and are usually nonspecific. Thus, additional biopsies are necessary but are deemed to be invasive, especially due to the high risk of bleeding in case of thrombocytopenia.

In the last decade, several novel imaging modalities have been developed to offer extensive and noninvasive explorations of the small bowel as well as alternatives to conventional histology during endoscopic procedures. One method is wireless capsule endoscopy (WCE), which allows a noninvasive examination of the entire small bowel. WCE has been successfully used for the diagnosis of anaemia and occult bleeding, inflammatory bowel disease, and recognition of intestinal tumours.<sup>9</sup> Focusing on patients treated with allo-HSCT, some studies have demonstrated the ability of WCE to detect intestinal lesions suspected to be related to GI-GVHD.<sup>6,10–12</sup> Moreover, these studies reported an excellent negative predictive value to rule out the diagnosis of GI-GVHD.<sup>6,10</sup> Another interesting method is confocal laser endomicroscopy, a high-resolution imaging modality allowing access to in vivo histology at the subcellular level during ongoing endoscopy. Confocal laser endomicroscopy, either using an endoscope-based or a probe-based technology, aims at decreasing the number of standard biopsies and their associated risks, by providing real-time in situ microscopy.<sup>13</sup>

Thus far, only a small single pilot study assessed integrated confocal laser endomicroscopy (iCLE) performances in GI-GVHD patients showing good sensitivity and specificity as compared with conventional histology.<sup>14</sup> However, none of the currently available studies using WCE, iCLE, or probe-based confocal laser endomicroscopy (pCLE) attempted to predict the risk of acute GI-GVHD at an early stage in asymptomatic patients.<sup>6,10–12,14</sup>

Therefore, this pilot prospective study aimed to evaluate the diagnostic value of both WCE and pCLE to detect early lesions suggestive of acute GI-GVHD and to determine whether pCLE and WCE could

detect early lesions of GI-GVHD independently of the presence or absence of symptoms and to assess the diagnostic yield of each method separately and in combination.

## Methods

### *Patients and study design*

Studies described in this report were undertaken in a pilot cohort of 15 consecutive patients who underwent allo-HSCT at the University Hospital of Nantes, Nantes, France. All patients were enrolled in clinical research protocols approved by local ethical committees. Written informed consent was obtained in accordance with the Declaration of Helsinki. All patients received a reduce intensity conditioning regimen.

For inclusion in this study, there were no restrictions in terms of diagnoses or stem cell sources. Patients with ongoing uncontrolled medical condition, prior history of allergy to fluorescein, renal dysfunction, suspected or documented bowel obstruction, known inflammatory bowel disease, or a history of major abdominal intervention were excluded.

### *Clinical and biological assessment*

The standard Seattle criteria were used for the diagnosis of GVHD.<sup>15</sup> Laboratory values, medications, clinical symptoms, and results of physical examination at time of investigation and during follow up after allo-HSCT, as well as virological and bacteriological status, were recorded. Stool culture and searching for *Clostridium difficile* toxin in stool and for *Cytomegalovirus* (CMV) DNA in plasma were systematically performed in order to exclude other differential diagnoses. Clinical and biological data were interpreted and GVHD was scored for each patient by a single allo-HSCT expert (MM), according to the modified Glücksberg grading system (Appendix).<sup>15</sup> Per protocol, the latter expert was blinded to the results of optical examinations (i.e. standard endoscopy, WCE, pCLE, and histology).

### *Endoscopic examination*

Given that the aim of this study was to determine whether pCLE and WCE could detect early lesions of GI-GVHD before the onset of symptoms and whether GVHD occurs at a median of 21 days after allo-HSCT,<sup>16</sup> we chose to perform endoscopic examinations as soon as possible after haematopoietic recovery in patients free of GVHD. Consequently, per protocol, endoscopic examinations were performed between day 21 and day 28 following allo-HSCT. No patient was on

nonsteroidal anti-inflammatory drugs (NSAID) at time of endoscopic examinations. Patients answered questionnaires using a 10-cm visual analogue scale about their tolerance of the procedure, with 10 being perfect tolerance and 0 suggesting worse acceptability.

**Conventional endoscopy.** Endoscopic procedures consisted in a standard upper GI endoscopy (OGD) and a rectosigmoidoscopy, which were performed during a single endoscopic session. Examinations were carried out by a single experienced endoscopist (EC), without general anaesthesia. Patients received 100 mg hydroxyzine (Renaudin, Ixassou, France) orally 1 h prior to the examinations in order to improve the tolerance of the procedures. OGD and rectosigmoidoscopy were performed using a standard gastroscope (EG530) and a standard colonoscope (EC530), respectively (Fujinon, Japan).

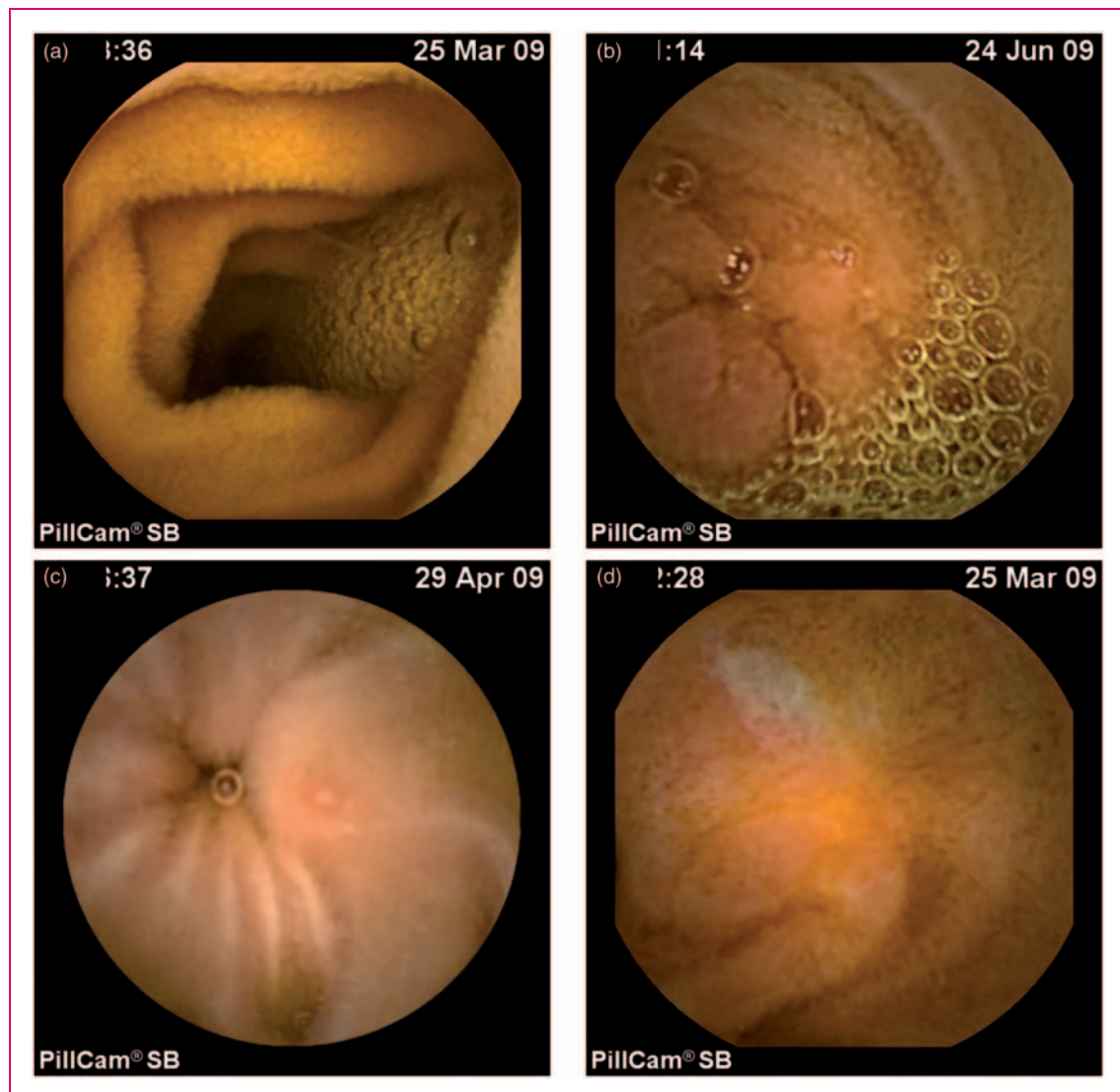
**Wireless capsule endoscopy.** The WCE system consists of three major components: an ingestible videocapsule, a data recorder (together with a recorder belt and a sensor array), and a computer workstation. The small bowel capsule measures 11 × 26 mm and acquires video images from one end of the device (2 frames/s). The capsule transmits the acquired images via a digital, radio frequency, communication channel to the data recorder unit strapped to the patient's waist. The data recorder is an external receiving/recording unit that receives the data transmitted by the capsule. Upon completion of the examination, the physician transfers the accumulated data from the data recorder to the computer workstation for processing and interpretation. For the purpose of this study, we used standard procedures as previously described.<sup>17</sup> For bowel preparation, patients had a 12-h overnight fast and drank 1 l of a polyethylene glycol/electrolyte solution 2 h prior to swallowing the capsule. Then, they were connected to the recording device and swallowed the WCE (Given Imaging, Yoqneam, Israel). The capsule recorded images at a rate of 2 frames/s for a period of 8 h. WCE images were interpreted by a single experienced endoscopist (VL) who knew that the patient had received allo-HSCT, but was blinded to other clinical and biological data. Visual grading of GI-GVHD potential lesions was scored according to the Brand criteria<sup>18</sup> as follows (Figure 1): grade 0, normal; grade 1, mild: loss of vascular marking, and/or mild focal erythema; grade 2, moderate: moderate/severe and/or diffuse erythema, or nodularity; grades 3 and 4, severe: grade 3 showing erosions and/or friable mucosa and grade 4 showing desquamation, ulceration, and denuded mucosa.

**Probe-based confocal laser endomicroscopy.** Probe-based confocal laser endomicroscopy is a new endoscopic

imaging technique, which adapts optical microscopy to 'live' digestive endoscopy. The system (Cellvizio; Mauna Kea Technologies, Paris, France) was installed on an independent rod dedicated to endomicroscopy. It consists of a laser unit, an ultra high-definition endomicroscopic miniprobe, and a computer with a specific software. The laser wave length was set up at 488 nm with low power (15 mW). The endomicroscopic miniprobe is a flexible microscope of 2.6 mm in diameter and 3 m in length. Images were gathered at a rate of 12 images/s. Lateral resolution was 1.4 μm and the field of view was 240 μm. pCLE was performed during the same sessions that OGD and rectosigmoidoscopy. Predetermined areas (i.e. duodenum, sigmoid, and rectum) were systematically explored following conventional endoscopic examination and prior to taking biopsies for conventional routine histology. The pCLE procedure was performed as follows: firstly, 5 ml of 10% fluorescein sodium (Novartis Pharma, Paris, France) was injected intravenously at the beginning of the OGD procedure, and served as a contrast agent to visualize crypt and vessels architecture.<sup>19</sup> Secondly, the confocal mini probe was threaded through the operative channel of the endoscope. With its distal end resting gently on the tissue, it produced images of the digestive mucosa at a subcellular level. All pCLE images were recorded for post-procedure interpretation using specific software (Cellviewer; Mauna Kea Technologies). pCLE images were interpreted by the same endoscopist who performed OGD, rectosigmoidoscopy, and pCLE procedures (EC), independently of other examinations, immediately following acquisition. The definition of normal duodenum and colon by pCLE was based upon the previously described classifications;<sup>20</sup> in contrast, GI-GVHD was defined by the presence of one or more abnormal criteria, either in the duodenum or in the colon. These criteria were as follows (Figure 2, Supplementary Movie File, available online): (a) abnormal microvessel network; (b) mild increase in fluorescein intensity in the lamina propria; (c) distorted villi (duodenum) or crypts (colon); (d) major increase in fluorescein intensity in the lamina propria; and (e) major architectural changes defined by the destruction of villi (duodenum) or crypts (colon). The severity of GI-GVHD was graded as follows: mild when one criterion a or b was present, as moderate either when criterion c was present or a combination of criteria a and b were met, and as severe when criteria d or e were present.

### Conventional histology

During endoscopy, any visible abnormality was noted and biopsies taken. In addition, systematic samples were taken in the sigmoid (at 40 cm distally to the



**Figure 1.** Small bowel lesions of GI-GVHD detected by wireless capsule endoscopy. (a) Normal jejunum. (b) Focal oedema and enanthaematous aspect of the jejunum. (c) Aphthoid erosion in the proximal ileum. (d) Large superficial ulceration of the ileum.

anus) and in the rectum. All biopsies were processed as best standard of care including haematoxylin and eosin staining and additional immunohistochemical testing for the presence of CMV. Histological sections were interpreted by a single expert GI pathologist (JFM) who was also blinded to all the other above results. Histological sections were graded on haematoxylin and eosin-stained sections according to the grading system described by Sale et al.<sup>21</sup> Of note, none of the patients analysed in this study had evidence of viral infection.

### Statistics

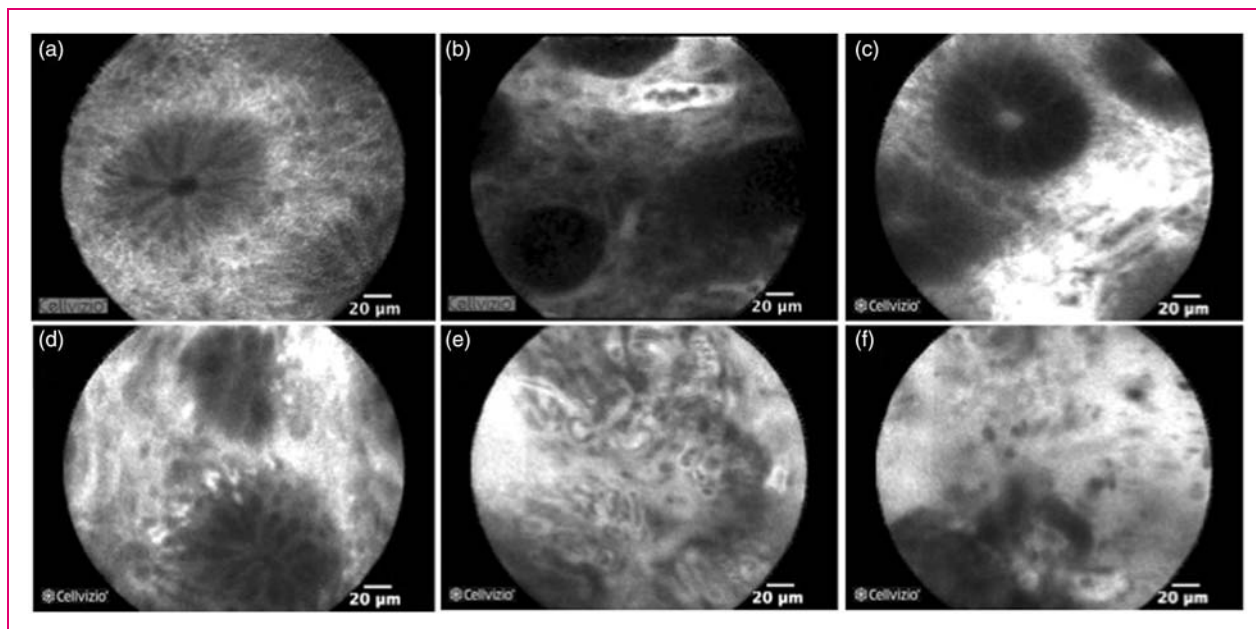
All statistical analyses were performed with GraphPad Prism version 5.00 for Windows (GraphPad Software).

Data were expressed as mean  $\pm$  standard deviation. Results were analysed on an intention-to-diagnose basis (i.e. technical failures were considered as a negative result). Diagnostic performances were expressed as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Correlation was assessed by the Spearman rank test.

### Results

#### *Study population and diagnosis of acute GVHD*

Patients, donors and allo-HSCT characteristics of these patients are summarized in Table 1. In this cohort, according to the modified Glücksberg criteria, eight out of 15 patients developed acute GVHD, which was



**Figure 2.** Probe-based confocal laser endomicroscopy patterns observed in GI-GVHD patients following intravenous injection of fluorescein. (a) Normal sigmoid. (b) Abnormal microvessel network with dilation of a microvessel surrounding a colonic crypt (mild GVHD). (c) Mild increase in fluorescein intensity in the lamina propria (mild GVHD). (d) Distorted crypts (moderate GVHD). (e,f) Major architectural changes showing proliferation and dilation of microvessels in the lamina propria, major fluorescein extravasation, and complete destruction of the colonic crypt architecture (severe GVHD).

classified grade I ( $n=3$ ), II ( $n=4$ ), and IV ( $n=1$ ). GVHD occurred at a median of 13 days (range 5–53 days) after endoscopic examinations. Grade I patients had isolated cutaneous symptoms. Grade II patients had either isolated cutaneous symptoms ( $n=2$ ) or cutaneous and digestive symptoms ( $n=2$ ). The patient with grade IV acute GVHD had cutaneous and digestive symptoms as well as liver abnormalities. The clinical outcome was favourable among grade I patients without additional immunosuppressive therapy. Grade II patients improved following initiation of therapy with 2 mg/kg/day prednisolone. The patient with grade IV acute GVHD died at week 10 following allo-HSCT despite high dose corticosteroid therapy and intensive supportive care.

### Endoscopic examination

The results of WCE, pCLE, and histology for each patient are shown in Table 1. No complication related to standard endoscopic examinations, WCE, pCLE, or biopsy were encountered. Visual analogue scale scores were  $8.8 \pm 1.0$ ,  $5.5 \pm 1.1$ , and  $6.6 \pm 1.9$  for VCE, OGD, and rectosigmoidoscopy, respectively.

**Wireless capsule endoscopy.** Wireless capsule endoscopy was performed in 15 patients but no image was captured in one patient (patient 11) because of a technical failure, therefore leaving 14 patients for small

bowel analysis. WCE detected endoscopic lesions suspicious of acute GI-GVHD in four out of eight patients who developed acute GVHD during follow up. Patients misclassified as ‘normal’ by WCE were grade I ( $n=2$ ) and grade II ( $n=2$ ) in the modified Glücksberg classification. The sensitivity, specificity, PPV, and NPV of WCE for the diagnosis of any grade of GVHD were 50, 86, 80, and 60%, respectively (Table 2).

**Probe-based confocal laser endomicroscopy.** A total of 56 pCLE videosequences and 84 biopsies were obtained from the same sites. In one patient, technical failure occurred resulting in the absence of recorded data for both duodenal and rectosigmoid evaluation. Fourteen patients had duodenal ( $n=12$ ) or rectosigmoid ( $n=14$ ) pCLE examination (two patients refused to have an OGD). pCLE accurately identified seven out of eight patients who developed symptoms of GVHD during follow up, but misclassified as ‘normal’ one patient who developed a grade II acute GVHD. The sensitivity, specificity, PPV, and NPV of pCLE for the diagnosis of any grade of GVHD were 87, 71, 77, and 83%, respectively (Table 3).

### Conventional histology

Conventional histology correctly diagnosed a ‘severe GI-GVHD’ in the patient who developed grade IV acute GVHD. This patient had severe histological

**Table 1.** Patients, transplant characteristics, and acute GI-GVHD grading

Patient	Age (years)	Gender	Diagnosis	Stem cell source	HLA matching	GVHD prophylaxis	Acute GVHD clinical grade			Lesion	
							Overall	GI	Histological	WCE	pCLE
1	48	F	NHL	PBSC	MRD	CsA + ATG	II	0	0	3	Moderate
2	49	F	MM	PBSC	MUD	CsA + MTX	0	0	0	0	Mild
3	43	F	AML	PBSC	MRD	CsA + ATG	0	0	0	1	Mild
4	57	M	CLL	PBSC	MUD	CsA + MMF	IV	4	4	3	Severe
5	52	F	NHL	PBSC	MRD	CsA + ATG	0	0	1	0	0
6	52	M	AML	PBSC	MRD	CsA + ATG	I	0	0	0	Mild
7	40	M	ALL	PBSC	MUD	CsA + MMF + ATG	II	1	1	0	Moderate
8	54	F	AML	PBSC	MRD	CsA + ATG	II	0	0	0	0
9	21	M	AA	PBSC	MUD	CsA + MMF + ATG	0	0	0	0	0
10	29	M	HD	PBSC	MUD	CsA + MMF	II	1	3	2	Moderate
11	51	F	MM	PBSC	MUD	CsA + MTX	0	0	0	Technical failure	0
12	52	M	AML	PBSC	MRD	CsA + MMF	I	0	0	1	Moderate
13	46	M	MPN	BM	MRD	CSA + ATG	0	0	0	0	0
14	46	M	AML	UCB	MISUD	CSA + MMF	0	0	Not performed	0	Technical failure
15	60	M	MM	PBSC	MUD	CsA + MTX	I	0	1	0	Mild

AA, aplastic anaemia; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; ATG, antithymocyte globulin; BM, bone marrow; CLL, chronic lymphoid leukaemia; CsA, cyclosporin A; F, female; GI, gastrointestinal; GVHD, graft-versus-host disease; HD, Hodgkin disease; HLA, human leukocyte antigen; M, male; MISUD, mismatched related donor; MM, multiple myeloma; MMF, mycophenolate mofetil; MPN, myeloproliferative neoplasm; MRD, matched related donor; MTX, methotrexate; MUD, matched unrelated donor; NHL, non-Hodgkin lymphoma; PBSC, peripheral blood stem cell; pCLE, probe-based confocal laser endomicroscopy; UCB, umbilical cord blood; WCE, wireless capsule endoscopy.

**Table 2.** Correlation between Glücksberg and WCE grades according to the severity of graft-versus-host disease

Glücksberg grade	WCE grade			
	0	Mild (1)	Moderate (2)	Severe (3-4)
0	6	1	0	0
Mild (I)	2	1	0	0
Moderate (II)	2	0	1	1
Severe (III-IV)	0	0	0	1

Values are *n*. Wireless capsule endoscopy grade according to Brand classification.<sup>18</sup> Sensitivity of WCE was 50% and specificity was 86%. Spearman test showed a significant positive correlation between both scoring systems ( $\rho = 0.543, p = 0.036$ ).

**Table 3.** Correlation between Glücksberg and pCLE grades according to the severity of graft-versus-host disease

Glücksberg grade	pCLE grade			
	0	Mild (1)	Moderate (2)	Severe (3-4)
0	5	2	0	0
Mild (I)	0	2	1	0
Moderate (II)	1	0	3	0
Severe (III-IV)	0	0	0	1

Values are *n*. Sensitivity of pCLE was 87% and specificity was 71%. Spearman test showed a significant positive correlation between both scoring systems ( $\rho = 0.727, p = 0.002$ ).

lesions consisting in large areas of complete destruction of the duodenal villi and the colonic crypts. The remaining crypts were atrophic with apoptotic changes. Conventional histology did not find any sign of acute GI-GVHD in biopsies for 4/15 patients who developed grade I ( $n = 2$ ) and grade II ( $n = 2$ ) acute GVHD, but without clinical symptoms of GVHD at time of biopsy.

In addition, histology concluded to grade 1 acute GVHD in one patient that was classified as ‘no GVHD’ according to the modified Glücksberg system. The sensitivity, specificity, PPV, and NPV of conventional histology for the diagnosis of any grade of GVHD were 50, 86, 80, and 60%, respectively (Table 4).

**Table 4.** Correlation between Glücksberg and standard histology grades according to the severity of graft-versus-host disease

Glücksberg grade	Histology grade			
	0	Mild (1)	Moderate (2)	Severe (3-4)
0	6	1	0	0
Mild (I)	2	1	0	0
Moderate (II)	2	1	0	1
Severe (III-IV)	0	0	0	1

Values are *n*.

Sensitivity of histology was 50% and specificity was 86%. Spearman test showed no correlation between both scoring systems ( $\rho=0.481$ ,  $p=0.069$ ).

**Table 5.** Correlation between pCLE and standard histology grades according to the severity of graft-versus-host disease

pCLE grade	Histology grade			
	0	Mild (1)	Moderate (2)	Severe (3-4)
0	5	1	0	0
Mild (1)	3	1	0	0
Moderate (2)	2	1	0	1
Severe (3-4)	0	0	0	1

Values are *n*.

Spearman test showed no correlation between both scoring systems ( $\rho=0.486$ ,  $p=0.066$ ).

### Comparison between methods

The detailed results of WCE, pCLE, and histology according to the grade of acute GVHD are reported in Table 1. There was a highly significant positive correlation between the modified overall Glücksberg score, and the WCE findings ( $\rho=0.543$ ,  $p=0.036$ ) and between the modified overall Glücksberg score and the pCLE data ( $\rho=0.727$ ,  $p=0.002$ ) (Tables 2 and 3). In contrast, no statistically significant correlation was noted between the modified overall Glücksberg score and the histology results ( $\rho=0.481$ ,  $p=0.069$ ), between the pCLE data and the histology results ( $\rho=0.486$ ,  $p=0.06$ ), or between the WCE data and the histology results ( $\rho=0.283$ ,  $p=0.31$ ) (Tables 4, 5, and 6). All methods (WCE, pCLE, and histology) accurately identified and classified as 'severe' the patient who developed a severe grade IV GVHD.

### Discussion

This pilot study demonstrates the feasibility, tolerance, and safety of a combined minimally invasive endoscopic approach in patients at risk of GI-GVHD after

**Table 6.** Correlation between WCE and standard histology grades according to the severity of graft-versus-host disease

WCE grade	Histology grade			
	0	Mild (1)	Moderate (2)	Severe (3-4)
0	7	3	0	0
Mild (1)	2	0	0	0
Moderate (2)	0	0	0	1
Severe (3-4)	1	0	0	1

Values are *n*.

Spearman test showed no correlation between both scoring systems ( $\rho=0.283$ ,  $p=0.31$ ).

allo-HSCT. Both WCE and pCLE were able to detect early mucosal lesions in asymptomatic allo-HSCT patients, albeit with different diagnostic performances. Despite the relatively small number of included patients, pCLE was likely to be sensitive while WCE had good specificity. Moreover, the results obtained with these novel technologies were better correlated with the classical modified Gluckberg severity score compared to the results obtained with conventional histology.

Results from the current pilot prospective analysis provide new insights with respect to several aspects: (i) it combined two novel minimally invasive imaging modalities (i.e. WCE and pCLE), while previous studies assessed the diagnostic yield of WCE or pCLE separately; (ii) WCE and pCLE were performed early after allo-HSCT with a prediction rather than a diagnostic goal; and (iii) the majority of the current patients (87%) were asymptomatic at time of examination (in contrast to previous studies, which enrolled patients with diarrhoea or abdominal pain). However, the size of the current study was limited and did not allow for depicting reliable and final conclusions as for sensitivity, specificity, PPV, and NPV. These calculations were only performed for exploratory purposes. WCE had a sensitivity of only 50% for the detection of early lesions of GI-GVHD. Although WCE usually allows visualization of the entire small bowel, this lack of sensitivity is not surprising given the early timing of WCE and the subtle mucosal abnormalities suggestive of GI-GVHD contrasting with more severe lesions reported in symptomatic patients.<sup>6,10,12</sup> Nevertheless, WCE appeared to be more sensitive than standard histology to detect early stages/asymptomatic acute GI-GVHD. One major issue with WCE is that lesions found in GVHD patients are quite nonspecific. Indeed, erythema, denudation, and small ulcers have been reported in patients receiving NSAID and even in some healthy subjects.<sup>22</sup> In addition, it may be difficult to discriminate between lesions of GI-GVHD and lesions induced



by opportunistic infections.<sup>23</sup> However, in the specific context of allo-HSCT, abnormalities detected by WCE could very accurately predict GI-GVHD while their absence cannot rule out this possibility.

In contrast to WCE, pCLE appeared as the most sensitive technique to detect early acute GI-GVHD lesions. Its performance is also better than standard histology. Conversely, pCLE showed a relatively poor specificity for the diagnosis of acute GI-GVHD. Only a single previous study, Bojarski et al.,<sup>14</sup> reported both high sensitivity and specificity for CLE as compared to standard histology; however, there were differences from our study on three important points. The first is that Bojarski et al. performed their study in symptomatic patients, with diarrhoea being the predominant symptom, while no patient had GI symptoms at time of examination in our study. The second difference concerns the technique used for endomicroscopy, not only in terms of technology but also the type of contrast agent used and access route. Indeed, these authors used iCLE onto a colonic mucosa prestained with both intravenously administered fluorescein combined with topical application of acriflavin. Compared to our technique, the technique used by Bojarski et al. might increase the number of subcellular details since iCLE is slightly superior to pCLE in terms of lateral resolution and acriflavin allows for studying the nuclei. However, our study focused on the vascular compartment and dynamic changes seen with videosequences of the mucosa obtained with pCLE. In addition, we examined both the duodenum and the colon (including rectum) while Bojarski et al. only performed colonoscopies. However, whether biopsies obtained from the small intestine are superior to the ones obtained from the colon to diagnose acute GI-GVHD remains to be determined. The third difference is the gold standard that was used to establish the diagnostic value of CLE. In the study from Bojarski et al., iCLE was compared to standard histology while we compared pCLE to the Glücksberg scoring system. Our choice was driven by the fact that the Glücksberg scoring system is a common clinico-biological assessment widely used in routine practice by the allo-HSCT community to diagnose acute GVHD and manage patients. Also, we thought to establish the different correlations using the Glücksberg overall grade, because it is likely that intestinal lesions may reflect the general immune all-or-active status of the patient.

An important limitation is that the confocal classification used in this study has not been externally validated but has been adapted from a previous international classification describing CLE patterns in inflammatory bowel diseases.<sup>20</sup> Indeed, previous studies assessing CLE in GVHD patients used acriflavin in order to identify the apoptotic cells associated with

GVHD. However, acriflavin is not approved for in vivo use in France and in many countries, thus could not be applied in our patients. Instead, we chose to focus our study on other parameters such as the vascular compartment and dynamic changes seen with videosequences of the mucosa obtained with pCLE. Indeed strong data suggest that neovascularization is of major importance in the pathophysiology of acute GVHD.<sup>23</sup> Also, most pCLE classifications, such as the Miami classification<sup>20</sup> used for the diagnosis of other precancerous or inflammatory lesions, did not try to superimpose pCLE and standard histology criteria but rather developed novel diagnostic criteria such as extracellular intensity of fluorescein, as in our study. However, in this study we did not use specific software tools, which might be helpful to perform quantitative analysis of the brightness of the lamina propria in order to provide more objective analysis. Last but not least, discordance between the Glücksberg scoring system and standard histology has been reported,<sup>4</sup> emphasizing the need for novel staging criteria. In our study, it is interesting to note that there are discrepancies between the pCLE and standard histology results (Table 5). For instance, standard histology was not able to predict GVHD onset in three grade I patients without clinical sign of GI-GVHD, whereas they were correctly identified by pCLE, suggesting that these patients probably had a very early GI-GVHD. However, one must acknowledge that the so-called 'screening' biopsies as performed in the current study are not standardized so far, and previous histological criteria were developed for diagnosis of established GVHD but not for prediction as aimed here. Another limitation is that we did not directly compare physical and optical biopsies, since it is almost impossible to target exactly the same spot due to the small field of view of pCLE. However, physical biopsies were performed in the same macroscopic area, within approximately 5–10 mm range from the pCLE probe apposition.

Another potential explanation for the discordance between pCLE and standard histology is that pCLE screens larger areas of mucosa than standard histology and combines functional and morphological criteria rather than morphological criteria alone. Indeed, data suggest that neovascularization is a major prognostic factor in the setting of acute GVHD.<sup>24</sup> Regarding the discriminating ability between grades 0–I and grades II–IV, which is a critical clinical issue in terms of acute GVHD management decision making, it is noteworthy that four out of five patients with grades II–IV GVHD were accurately diagnosed by WCE combined with pCLE.

Obviously, standardization, and validation testing are still needed. However, these results suggest that a new diagnostic algorithm consisting in performing

WCE first might be proposed. In case of abnormalities and after excluding other causes such as concomitant intake of NSAIDs, diagnosis of GI-GVHD could be accepted with a high degree of confidence and management adapted accordingly. If WCE is normal then we suggest performing pCLE without biopsy to further adapt the treatment strategy. In our series, such strategy allowed for an accurate staging in all but one case, while adding histology did not improve the diagnostic yield. Early detection of acute GVHD lesions prior to symptom onset is crucial. Acute GVHD remains the major source of morbidity and mortality after allo-HSCT.<sup>1</sup> So far the diagnoses rely almost entirely on the presence of clinical symptoms and should be confirmed by biopsy of the involved target organ.<sup>2</sup> Early detection of GVHD prior to symptom onset has important therapeutic consequences including more stringent monitoring and/or preventive care. If several acute GVHD biomarkers are promising, they remain to be validated.<sup>25</sup> WCE and especially pCLE represent a promising alternative to these biomarkers for early detection of acute GI-GVHD.

Finally, the current data established the safety and tolerance of these new endoscopic approaches in such fragile patients. Both WCE and pCLE were well tolerated and no significant complications occurred, especially following fluorescein administration, even if pCLE added 5–10 minutes to the standard endoscopic examination. As a matter of fact, larger controlled and multicentre series will be needed to establish the routine use of these novel endoscopic procedures. Also, such studies should pay a special attention to interpretation of early mucosal/vascular lesions detected by pCLE, which can be challenging as our current study did not attempt to assess the interobserver agreement of pCLE criteria. Despite these limitations, we believe that the current pilot findings can pave the way for a new diagnostic algorithm combining WCE first and pCLE in case of a negative WCE result in order to detect early lesions of acute GI-GVHD, allowing for improved therapeutic decisions in high-risk allo-HSCT patients.

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#### Appendix The acute GVHD clinical grading system and the modified Glücksberg grading system

Acute GVHD clinical grades	Skin	Liver	Gastrointestinal
1	Rash <25% of body surface	Bilirubin: 10–30–mg/l	Diarrhoea >500 ml per 24 h or nausea and vomiting
2	Rash 25–50% of body surface	Bilirubin: 30–60 mg/l	Diarrhoea >1000 ml per 24 h
3	Diffuse rash	Bilirubin: 60–150 mg/l	Diarrhoea >1500 ml per 24 h
4	Epidermolysis bullosa	Bilirubin: >150 mg/l	Severe abdominal pain or GI bleeding
Glücksberg grades			
I	1–2	0	0
II	1–3	1	1
III	2–3	>2	>2
IV	≥2	≥2	≥2