**Brief report**

**Pericapillary hemorrhage as criterion of severe human digestive graft-versus-host disease**

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In an experimental model we demonstrated that endothelial cells of all organs are targets of the alloimmune reaction. Here, in 68 digestive biopsies, we found endothelial lesions by immunohistochemistry and ultrastructure in patients with severe acute graft-versus-host disease (GVHD). In contrast, no such endothelial cell alterations were found either in patients without GVHD or in nongrafted controls. In the biopsies with severe GVHD lesions, ultrastructure showed rupture of the capillary basal membrane and extravasated red blood cells. These pericapillary hemorrhages were highly correlated with GVHD severity. In a separate cohort of 39 patients who underwent an allogeneic transplantation after a nonmyeloablative conditioning, 8 patients had intestinal biopsies. Three of these latter patients had both severe pathologic lesions of GVHD and similar endothelial lesions, thus, strengthening the concept that endothelial lesions are linked to GVHD severity and not to the intensity of the conditioning regimen. (Blood. 2004; 103:4681-4684)

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**Study design**

Between 1996 and 1998, 68 of 258 patients who underwent HSCT developed digestive symptoms requiring an upper digestive endoscopy with biopsies for GVHD diagnosis before day 100. The male-to-female ratio was 41:27, median age was 35 years (range: 7-55 years). Patient, disease, and transplant characteristics of these 68 patients are summarized in Table 1. Conditioning regimen consisted of chemotherapy alone in 30 cases or chemoradiotherapy in 38 cases. GVHD in skin, liver, or gut was clinically graded at the time of digestive biopsy, according to Seattle criteria. Clinical acute GVHD was found in 56 patients (82.3%; grade IV, 16; grade III, 15; grade II, 22; grade I, 3).

Each patient had 4 duodenal biopsies whether or not there was a mucosal lesion detectable by endoscopic examination. Two biopsies were embedded in parafin and 2 processed for ultrastructure. Normal digestive specimens for surgical pieces were taken from 10 healthy patients, as controls. One additional biopsy was systematically screened for microbial and/or viral infections (none of the patients with pericapillary hemorrhage had evidence of digestive infection). Biopsies were taken with a “multibite” biopsy device (Boston Scientific microvasive, reference 1012; Boston, MA). Biopsies for pathologic examination were fixed in AFA (alcohol, formalin, and acetic acid) for 2 hours and further processed for paraffin embedding, or fixed in glutaraldehyde 4% and processed for ultrastructural examination. Patients underwent biopsies in their individual sterile rooms if white blood cell counts were less than 0.5 G/L, and in a surgical room if the counts were higher. If platelet counts were below 50 G/L, systematic transfusion was performed before biopsy. The patients gave their informed consent according to the Declaration of Helsinki.

Histologic assessment of digestive GVHD was performed blindly by 2 different pathologists according to criteria described by Sale et al and modified by Epstein et al: grade I, crypt cell degeneration or epithelial cell apoptosis, without crypt loss; grade II, loss of up to 3 contiguous crypts; grade III, loss of 4 or more crypts without sloughing; grade IV, total sloughing. For the 68 patients, results were as follows: grade IV, 3; grade III, 10; grade II, 15; grade I, 22; no GVHD, 18.

Assessment of capillary damage was based on (1) presence of pericapillary hemorrhages on paraffin sections; (2) rupture of capillary basal membrane on ultrastructure; and (3) alterations of endothelial cell on ultrastructure and immunohistochemistry.


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Results for pericapillary hemorrhages were expressed by the presence or absence of hemorrhage in one of the 3 fields systematically studied at a magnification of ×400 for each biopsy. For ultrastructure, 4 resin-embedded blocks were obtained from 2 biopsies for each patient. The analysis was carried out on a minimum of 4 capillaries on each resin block, focusing on both endothelial cells and basal membranes.

Immunohistochemistry, using antihuman CD31 (Novocastra, Newcastle, United Kingdom) was performed on paraffin sections, with controls, by omitting the first antibody and by using an irrelevant antibody of identical isotype. Counts of damaged endothelial cells were performed on 3 fields at a magnification of ×400 on an Olympus AX 70 microscope, with wide-field eyepiece number 26.5, providing a field size of 0.344 mm², at a magnification of ×400. Endothelial cell lesions were considered when endothelial damage was found on at least 5 of the 50 capillary sections systematically studied per biopsy.

Controls and differential diagnoses

The best control group would have been patients who were without signs and symptoms following myeloablative therapy and allogeneic HSCT. However, for obvious ethical reasons such patients did not have endoscopy and biopsies. Thus, we decided to examine 2 groups of patients as controls: (1) patients who underwent autologous stem cell transplantation and who had biopsies for diagnostic purposes, and (2) patients who received a nonmyeloablative (NMA) conditioning regimen followed by allogeneic HSCT. GVHD-associated thrombotic thrombocytopenic purpura (TTP) was systematically searched. Criteria for TTP included red cell fragmentation, laboratory findings of hemolysis, and de novo or persistent thrombocytopenia caused by consumption, in the absence of disseminated intravascular coagulation. Additional criteria (not mandatory) for TTP were hypertension and renal insufficiency.

Statistical analyses

The 2-sided χ² test was used to compare the grades of clinical and histologic digestive GVHD, with the presence of pericapillary hemorrhages and of endothelial damage. Correlations were statistically significant for P values less than 5 × 10⁻².

Results and discussion

Only one of the 68 patients of this series had intestinal bleeding as an indication for endoscopy. Endoscopic examinations were performed as soon as diarrhea, nausea, or vomiting occurred; thus, intestinal biopsies were taken very early in the course of GVHD. Pericapillary hemorrhages were found in biopsies of 13 patients, preferentially in the upper part of duodenal villi (Figure 1A). Ultrastructure showed ruptures of capillary basal membrane with extravasated red blood cells (Figure 1C). However, capillary lesions were heterogeneous, with, in the same area (Figure 1C), intact capillaries, capillaries with damaged endothelial cells only, and capillaries with damaged endothelial cells and rupture of basal membrane.

Among these 13 patients, 7 underwent transplantation after a total body irradiation–based conditioning and 6 after chemotherapy only. None of these patients had evidence at the time of biopsy of TTP or veno-occlusive disease of the liver. The clinical presentation of the patients with pericapillary hemorrhages differed from the presentation of the patients without pericapillary hemorrhage, since the patients with pericapillary hemorrhage had signs and symptoms of GVHD that were more severe than the patients without pericapillary hemorrhage (Table 1). Major changes (including mucosal ulceration) were found in 4 patients (33%) and minor changes such as edema were found in 5 (41%).

Pericapillary hemorrhages were only found in grade 4 (3/3) and grade 3 (10/10) histologic grades, with a highly significant correlation between hemorrhages and severity of histologic GVHD (grades 0-2 versus grades 3-4, \( P < 4.7 \times 10^{-15}; \chi^2 = 61.66 \)). Considering clinical grade, pericapillary hemorrhages were found in grade IV (8/16), grade III (3/15), and grade II (2/22) cases, but not in grade I cases or in controls (\( P < 4 \times 10^{-3} \)). Twelve of the 13 patients with pericapillary hemorrhages died a mean of 33 days (range: 7-65 days) after the biopsy. Thus, as previously reported, digestive GVHD is an important factor of morbidity and mortality. Its incidence and severity may be underestimated if patients with digestive symptoms following HSCT are not systematically biopsied.\(^{14,15}\)

Pericapillary hemorrhages are the ultimate result of capillary wall damage, and are easy to detect on paraffin sections. However, lesions limited to endothelial cells, without basal membrane rupture, were also observed with anti–CD-31 antibody (Figure 1B) and ultrastructure (Figure 1C). Neither pericapillary hemorrhages nor endothelial damage we detected at the ultrastructural level were associated with thromboses or fibromuscular hyperplasia in vessels of adjacent lamina propria. When compared with histologic grade of GVHD, endothelial lesions were found in all grade 4 (3/3) and grade 3 (10/10) cases,

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**Figure 1. Pathologic features of capillary damage in human digestive graft-versus-host disease.** (A) Blood suffusion (arrow) around capillary loops (white broken lines) in the upper part of duodenal villi. Paraffin section with hematoxylin–eosin stain; original magnification, ×300. (B) High magnification of a capillary, with partial detachment of an endothelial cell (asterisk). Indirect immunoperoxidase with an antibody directed against CD31; original magnification, ×1200. (C) Ultrastructural aspect of close capillary sections in the lamina propria of a duodenal biopsy. 1: capillary section with intact endothelium and basal membrane; 2: capillary section with endothelial cytoplasm of irregular thickness around the capillary lumen, without basal membrane rupture; 3: capillary section with discontinuity in the basal membrane (= – – – – ) and endothelial cytoplasmic cover; and 4: extravasated red blood cells. Insert: higher magnification of basal membrane rupture.
as well as in 5 of 15 grade 2 cases ($P < 2.4 \times 10^{-10}$, $\chi^2 = 40.09$). When compared with clinical grade of GVHD, endothelial lesions were found in 11 of 16 grade IV cases, 4 of 15 grade III cases, and 3 of 22 grade II cases ($P < 5 \times 10^{-4}$).

Endothelial cells are targets of alloimmune reactions in solid organ rejection.16 Endothelial lesions have also been reported in acute GVHD in skin17 or liver.18 We have demonstrated widespread endothelial lesions following allogeneic splenocyte transfer in immunodeficient mice. In this experimental model of pure alloimmune reaction, endothelial lesions were massive and synchronous in all organs.8 However, in humans, biopsies can only be performed for a diagnostic purpose. At the time of biopsy, lesions in target organs associate damages linked to conditioning regimen and GVHD but also signs of wound healing.19 In this series, a striking ultrastructural feature was the coexistence, in the same area, of severely damaged capillary sections with extravasated red blood cells and of normal capillary sections. This suggests that both acute damage and repair processes exist in digestive biopsies performed before day 100. This is in accordance with the recently proposed concept,9 that microvessel loss follows endothelial injury of acute GVHD disease.

Vascular ectasia have been described in patients who received autografts and in radiation-induced endothelial lesions. Thus, our findings raised the question of the specificity of endothelial damage in the context of severe intestinal GVHD. We do believe in this specificity for the following reasons. First, there is a lack of these findings in grade I and II GVHD. Second, there is a lack of such endothelial lesions in intestinal biopsies following autologous stem cell transplantation ($n = 3$). And, most importantly, among a cohort of 39 patients who underwent an allogeneic transplantation after an NMA conditioning (fludarabine and 2 Gy TBI [$n = 34$]), 8 patients had intestinal biopsies. Among these 8 patients, 3 had no evidence of pathologic GVHD, 2 had pathologic evidence of GVHD without endothelial lesions, but 3 patients had both severe pathologic lesions of GVHD and endothelial lesions, thus strengthening the concept that endothelial lesions are linked to the severity of the GVHD process and not to the intensity of the regimen used.

Therefore, it seems important to assess endothelial cell lesions of acute GVHD. This systematic study of digestive biopsies of patients with acute GVHD showed that pericapillary hemorrhages, which are easy to detect on paraffin sections, can be proposed to characterize the severity of microvessel damage during the acute phase of GVHD.

References


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