Clinical Significance of Skin Biopsies in the Diagnosis and Management of Graft-vs-Host Disease in Early Postallogeneic Bone Marrow Transplantation

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Objective: To determine the value of skin biopsies in the management of suspected graft-vs-host disease (GVHD) within 30 days of allogeneic bone marrow transplantation (BMT).

Design: Retrospective study based on review of a BMT database.

Setting: Leukemia/BMT ward of a tertiary care, university teaching hospital.

Patients: One hundred and eighty-seven consecutive patients who received allogeneic BMT between January 1, 1994, and June 30, 1997, at Vancouver General Hospital, Vancouver, British Columbia.

Main Outcome Measures: (1) Skin biopsy frequency for patients with rashes suggestive of acute GVHD; (2) clinical significance of skin biopsy in the management of patients with suspected acute GVHD after BMT; (3) relationship between severity of clinical GVHD and the likelihood to receive GVHD therapy; and (4) relationship between biopsy status or biopsy result and outcome of BMT (acute and chronic GVHD, transplant-related mortality, and overall and event-free survival).

Results: During the early post-BMT period (<30 days after BMT), 88 patients had rashes suggestive of acute GVHD; of these, 51 (58%) underwent skin biopsy to confirm the diagnosis. Skin biopsies were performed more often for higher clinical stages of cutaneous GVHD. There was no significant difference between the patients with positive biopsy findings and those with negative findings, either in the clinical severity of acute GVHD or in likelihood to receive treatment for GVHD. Most (85%) of the patients who underwent biopsies and received GVHD therapy had treatment initiated before skin biopsies were performed or before the results were available. The higher the clinical grade of overall acute GVHD, the more likely it was that the patients were treated for GVHD (P,<.001). The outcome of BMT was not influenced by the skin biopsy status or biopsy result.

Conclusions: The biopsy findings correlated poorly with the clinical severity of skin rash suggestive of acute GVHD soon after BMT. The decision to treat suspected acute GVHD depended not on skin biopsy findings but rather on clinical severity of acute GVHD. In this regard, skin biopsy has a limited role in the management of patients early after allogeneic BMT.

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GRAFT-VS-HOST disease (GVHD) after allogeneic bone marrow transplantation (BMT) is an immunological process in which activated donor lymphocytes mount an attack on recipient tissues. This process involves secretion of cytokines and recruitment of additional inflammatory cells. The severity of acute GVHD is one of the most important prognostic factors that predict outcome of BMT. The likelihood of developing acute GVHD (ie, within 100 days of BMT) depends on a number of factors, including the degree of histoincompatibility, number of T lymphocytes in the graft, patient's age, and the prophylactic immunosuppressive regimen used. Clinically relevant acute GVHD occurs in 35% to 50% of patients given HLA-identical marrow grafts. The skin is often the initial organ to manifest acute GVHD. Clinically, acute cutaneous GVHD is characterized by a maculopapular rash that involves the palms and soles and, as the disease progresses, the upper trunk, neck, cheeks, and ears.

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In addition to the skin, the liver and gastrointestinal system are often involved in acute GVHD. As summarized...
PATIENTS AND METHODS

DATABASE

A BMT database is maintained by the Leukemia/BMT Program of British Columbia at Vancouver General Hospital. For each patient undergoing BMT, the following information is recorded with regard to acute GVHD of the skin, liver, and gut: onset, clinical stage, date and result of biopsies, and therapeutic interventions. In addition, clinical outcome variables are recorded, including (1) peak cutaneous and overall clinical severity of acute GVHD; (2) development of chronic GVHD (limited vs extensive); (3) mortality due to GVHD; and (4) mortality due to other causes such as infection, graft failure, regimen-related toxic effects, and relapse of malignancy. A widely used clinical staging/grading system is used for the construction of the database and is summarized in Table 1 and Table 2.

POLICY FOR DIAGNOSIS AND TREATMENT OF ACUTE GVHD

The diagnosis of acute GVHD (ie, within 100 days of BMT) was based on clinical symptoms and signs as well as the results of tests (ie, liver function, endoscopy, and skin, gut, or liver biopsies). There was no specific guideline to indicate when a skin (or other) biopsy should be carried out, although histologic confirmation of the diagnosis was obtained routinely. Primary treatment of established acute GVHD was with corticosteroids and started within 12 hours of the diagnosis. Once initiated, the duration of treatment was for a minimum of 6 weeks and did not depend on the results of diagnostic tests. Similarly, cyclosporine was generally continued for 3 to 4 months (related donor) or 6 months (unrelated donor) after BMT and then, if the GVHD was quiescent, tapered over a few weeks. The exception to this was for patients deemed to be at high risk for relapse, for whom the cyclosporine therapy was discontinued earlier.

STUDY DESIGN

Patients who developed skin rashes before day 30 after BMT were placed into 1 of 2 groups: those undergoing biopsy and those not. The group undergoing biopsy was divided into 2 subgroups, patients with positive skin biopsy findings and those with negative findings. A “positive” biopsy finding was recorded when the pathology report stated “diagnostic for GVHD” if no histologic grade, or a grade of 2 or higher, was given. The patients were also divided into treated and nontreated groups. Treatment was defined as initiation of anti-GVHD therapy at or after the first clinical diagnosis of acute GVHD.

The following parameters were then analyzed for each patient category: (1) clinical severity of GVHD (skin and overall) at the time of first diagnosis; (2) the skin biopsy frequency; (3) biopsy result; (4) initiation of anti-GVHD therapy as a result of the diagnosis of GVHD; and (5) the BMT outcome (acute and chronic GVHD, transplant-related mortality, and overall event-free survival).

STATISTICAL ANALYSIS

We used χ² and t tests where appropriate to calculate the statistical significance of the GVHD findings. Survival analysis using the Kaplan-Meier method was performed to estimate overall and event-free survival (with death or relapse as events) and the actuarial probabilities of transplant-related mortality and GVHD.

in Table 1 and Table 2, acute GVHD can be graded according to its clinical severity.1,2,7,8 Outcome analyses have established the prognostic value of this staging system in that the higher the overall clinical grade, the higher the transplant-related mortality.9,10

Skin biopsies are frequently used to confirm the clinical diagnosis of acute GVHD. Histologically, acute GVHD of the skin is characterized by varying degrees of damage to the epidermal keratinocytes.11-18 In grade 1, vacuolization of the basal keratinocytes is present; in grade 2, both basal keratinocyte vacuolization and dyskeratotic keratinocytes are present; in grade 3, focal clefing of the basal layer is formed; and in grade 4, the epidermis is totally separated from the underlying dermis.

The interpretation of skin biopsy results after BMT can be difficult, however, because similar histologic findings may result from the high-dose chemotherapy/radiotherapy of the preparatory regimen before transplantation or other drug treatment afterwards.17,19-21 The situation is further complicated by the fact that frequently there is a lack of correlation between clinical features and histologic findings.16,17 For example, the skin in a clinically affected area may seem unremarkable histologically, or clinically uninvolved skin can display basal keratinocyte vacuolization and necrosis consistent with well-established GVHD.17 Therefore, the diagnostic value of skin biopsies has been questioned.13-21

Several investigators have studied the prognostic value of skin biopsy findings after BMT.18,21 In 1 report, dyskeratotic keratinocytes, number of exocytosed lymphocytes, and presence of follicular involvement did not correlate with outcome.18 In another, more recent study, no significance was found in any of the skin histologic parameters analyzed, including necrotic (dyskeratotic) keratinocytes, basal vacuolization, satellitosis, and appendageal involvement.21 These studies suggest that skin biopsies are of little prognostic value.

Despite these controversies, skin biopsies are still performed regularly on patients after BMT, sometimes as “rush” specimens.22 To our knowledge, there have been few studies to define the role, if any, of skin biopsies in the management of such patients. A retrospective review of data from a leukemia/BMT ward between 1994 and 1997 was performed to determine the frequency and clinical utility of skin biopsies within 30 days of BMT in the management of patients with suspected GVHD.
while the biopsy rates for stages 1 and 2 were lower. 10 of 13 patients with stage 3 GVHD underwent biopsy, 

\[ P < 0.05, \chi^2 \text{ analysis} \].

**RESULTS**

**PATIENT POPULATION PROFILE**

A total of 187 patients underwent allogeneic BMT between January 1, 1994, and June 30, 1997, at Vancouver General Hospital. Relevant information was available on all patients. In the early period after BMT (ie, within 30 days), 88 patients developed a rash suggestive of acute GVHD. The analysis centers on these patients. There were 54 men and 34 women, with a median age of 40 years (range, 20-55 years). The indications for BMT were as follows: chronic myeloid leukemia (n=21), acute myelogenous leukemia (n=21), myelodysplastic syndrome (n=13), multiple myeloma (n=10), acute lymphoblastic leukemia (n=9), non-Hodgkin lymphoma (n=9), and other (n=5). The source of marrow was as follows: unrelated donor (n=48), HLA-matched sibling (n=39), and mismatched sibling (n=1). Prophylaxis for GVHD was with cyclosporine and methotrexate (n=69) or T-cell depletion of the allograft and cyclosporine (n=19).

**BIOPSY FREQUENCY**

Eighty-eight patients developed skin rashes suggestive of acute GVHD within 30 days of BMT, and 51 (58%) of them underwent skin biopsy to confirm the diagnosis. The average clinical cutaneous stages and overall grades of acute GVHD are given in **Table 3**. The mean±SEM skin stage (1.71±0.18) and overall grade (2.02±0.12) of the group undergoing biopsy did not differ significantly from that of the group not undergoing biopsy (1.41±0.11 and 1.84±0.12, respectively; \( P > 0.05, \chi^2 \text{ test} \)). All patients with skin stage 4 (n=2) and 10 of 13 patients with stage 3 GVHD underwent biopsy, while the biopsy rates for stages 1 and 2 were lower (44% and 67%, respectively). The group undergoing biopsy was more likely to receive GVHD treatment than the group without biopsy (80% vs 57%; \( P < 0.02, \chi^2 \text{ analysis} \)).

**CLINICAL CORRELATION OF SKIN BIOPSY RESULTS**

The results of skin biopsy were positive in 27 patients and negative in 24. As outlined in **Table 4**, the 2 subgroups did not differ in their average clinical severity of acute GVHD, either in skin-specific stages or in overall grades. The percentage of positive biopsy findings for stages 3 and 4 (50%) was no higher than the lower clinical stages of cutaneous GVHD (57% and 52%, respectively). More importantly, the patients with positive or negative biopsy findings did not differ in their likelihood to receive GVHD treatment. Specifically, 85% of the patients with a positive biopsy finding received anti-GVHD therapy compared with 75% of the patients with a negative finding (\( P > 0.05, \chi^2 \text{ analysis} \)).

**TEMPORAL RELATIONSHIP BETWEEN SKIN BIOPSY AND INITIATION OF GVHD THERAPY**

Of the 51 patients who underwent skin biopsy, 41 received treatment for GVHD. However, in most of the treated cases, therapy began before the biopsy results were available (29 patients) or even before biopsies were performed (7 patients). The biopsy results were available for only 5 patients at the time of therapy initiation. For 3 of these 5 patients, the therapy was initiated when there was clinical deterioration of the GVHD (1 with new development of diarrhea and 2 with worsening of skin GVHD). Only 2 patients (3%) received treatment for GVHD after (presumably because of) positive skin biopsy findings in the absence of other independent indications for GVHD therapy. Both patients had histologic grade 2, clinical stage 2 skin GVHD and overall grade 1 GVHD when the therapy was initiated.

**RELATIONSHIP BETWEEN CLINICAL SEVERITY OF GVHD AND THERAPEUTIC INTERVENTION**

Sixty-two of the 88 patients with skin rashes before day 30 following BMT received treatment for GVHD around the time of diagnosis. Patients in the treated group, compared with the nontreated group, had significantly higher skin GVHD stages (1.67±0.10 vs 1.35±0.12; \( P < 0.05 \)) and
In this retrospective study we set out to determine the frequency and clinical utility of skin biopsy within 30 days of allogeneic BMT in the management of patients with a suspicious rash. We found that more than half of patients suspected to have skin GVHD underwent skin biopsy to confirm the diagnosis, and that the biopsy rates were related to clinical severity of the rash, with moderate to severe rashes more likely to be tested. The biopsy findings, however, did not correlate with the clinical severity of skin and overall GVHD, nor did they have any impact on the likelihood for the patients to receive GVHD therapy. Furthermore, the outcome of BMT was not influenced by whether a biopsy was done or by the result.

Most patients undergoing biopsy who were treated for GVHD had therapy initiated before the biopsy was performed or before the results were available. Indeed, skin biopsy findings were the reason for initiation of GVHD therapy in only 3% of all treated patients. In our patient population, the decision to treat GVHD was dependent on clinical severity of the GVHD rather than on the skin biopsy findings. Similar results were recently reported by another group of investigators who demonstrated that “rush” skin biopsies did not have any clinically significant relevance in the management of patients with GVHD after BMT. Specifically, of 36 biopsy specimens processed on an urgent basis, only 2 resulted in an immediate change in management.

In summary, skin biopsy findings within 30 days of allogeneic BMT do not correlate with clinical severity of GVHD, and skin biopsy seems to play only a minor role in directing a physician to initiate therapy for acute GVHD. Therefore, we suggest that the routine practice of sub-

**Table 3. Clinical Parameters According to Biopsy Status**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Biopsy (n = 51)</th>
<th>No Biopsy (n = 37)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin GVHD stage</td>
<td>1.71 ± 0.12</td>
<td>1.41 ± 0.11</td>
<td>&gt;.05†</td>
</tr>
<tr>
<td>Overall GVHD grade</td>
<td>2.02 ± 0.18</td>
<td>1.84 ± 0.12</td>
<td>&gt;.05†</td>
</tr>
<tr>
<td>Patients treated, No. (%)</td>
<td>41 (80)</td>
<td>21 (57)</td>
<td>&lt;.02‡</td>
</tr>
</tbody>
</table>

*Unless otherwise indicated, data are mean ± SEM. GVHD indicates graft-vs-host disease.
†P value calculated using t test.
‡P value calculated using \( x^2 \) analysis.

**Table 4. Clinical Parameters According to Biopsy Result**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Positive (n = 27)</th>
<th>Negative (n = 24)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin GVHD stage</td>
<td>1.70 ± 0.16</td>
<td>1.71 ± 0.18</td>
<td>&gt;.05†</td>
</tr>
<tr>
<td>Overall GVHD grade</td>
<td>2.00 ± 0.15</td>
<td>2.04 ± 0.18</td>
<td>&gt;.05†</td>
</tr>
<tr>
<td>Patients treated, No. (%)</td>
<td>23 (85)</td>
<td>18 (75)</td>
<td>&gt;.05‡</td>
</tr>
</tbody>
</table>

*Unless otherwise indicated, data are mean ± SEM. GVHD indicates graft-vs-host disease.
†P value calculated using t test.
‡P value calculated using \( x^2 \) analysis.

**Table 5. Outcome of Bone Marrow Transplantation According to Skin Biopsy Status**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Biopsy (n = 51)</th>
<th>No Biopsy (n = 37)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak clinical severity of skin acute GVHD, mean ± SEM</td>
<td>2.16 ± 0.23</td>
<td>1.89 ± 0.17</td>
<td>&gt;.05†</td>
</tr>
<tr>
<td>Peak clinical severity of overall acute GVHD, mean ± SEM</td>
<td>2.83 ± 0.20</td>
<td>2.41 ± 0.15</td>
<td>&gt;.05†</td>
</tr>
<tr>
<td>Acute GVHD (grade II-IV)</td>
<td>85 (70-96) 76 (61-88)</td>
<td>83 (66-95) 61 (38-85)</td>
<td>&gt;.03‡</td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td>72 (57-87) 79 (62-92)</td>
<td>62 (33-83) 57 (25-83)</td>
<td>&gt;.03‡</td>
</tr>
<tr>
<td>Transplant-related mortality</td>
<td>43 (27-64) 53 (34-75)</td>
<td>62 (34-92) 70 (40-98)</td>
<td>&gt;.03‡</td>
</tr>
<tr>
<td>Event-free survival</td>
<td>41 (23-58) 25 (10-43)</td>
<td>43 (27-58) 25 (10-43)</td>
<td>&gt;.03‡</td>
</tr>
<tr>
<td>Overall survival</td>
<td>44 (26-62) 25 (10-43)</td>
<td>45 (26-62) 25 (10-43)</td>
<td>&gt;.03‡</td>
</tr>
</tbody>
</table>

*Unless otherwise indicated, data are actuarial probabilities in percentages (95% confidence interval). GVHD indicates graft-vs-host disease.
†P value calculated using t test.
‡P value calculated using Kaplan-Meier analysis.

**Table 6. Outcome of Bone Marrow Transplantation According to Skin Biopsy Result**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Positive (n = 27)</th>
<th>Negative (n = 24)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak clinical severity of skin acute GVHD, mean ± SEM</td>
<td>2.37 ± 0.53</td>
<td>2.16 ± 0.23</td>
<td>&gt;.05†</td>
</tr>
<tr>
<td>Peak clinical severity of overall acute GVHD, mean ± SEM</td>
<td>2.56 ± 0.21</td>
<td>2.83 ± 0.20</td>
<td>&gt;.05†</td>
</tr>
<tr>
<td>Acute GVHD (grade II-IV)</td>
<td>86 (70-96) 83 (66-95)</td>
<td>82 (61-85) 61 (35-85)</td>
<td>&gt;.03‡</td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td>82 (61-85) 61 (35-85)</td>
<td>71 (46-95) 54 (29-82)</td>
<td>&gt;.03‡</td>
</tr>
<tr>
<td>Transplant-related mortality</td>
<td>43 (27-64) 53 (34-75)</td>
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In our patient population, the decision to treat GVHD was dependent on clinical severity of the GVHD rather than on the skin biopsy findings. Similar results were recently reported by another group of investigators who demonstrated that “rush” skin biopsies did not have any clinically significant relevance in the management of patients with GVHD after BMT. Specifically, of 36 biopsy specimens processed on an urgent basis, only 2 resulted in an immediate change in management.

In summary, skin biopsy findings within 30 days of allogeneic BMT do not correlate with clinical severity of GVHD, and skin biopsy seems to play only a minor role in directing a physician to initiate therapy for acute GVHD. Therefore, we suggest that the routine practice of sub-
jecting patients to such biopsies could be abandoned without compromising their care.

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REFERENCES