Abnormal Cervical Cytology After Allogeneic Bone Marrow Transplantation

Giovanni Negri, MD,1 Martina Herz, ScD,1 Sara Deola, MD,2 Andrea Piccin, MD,2 Marco Casini, MD,2 Bianca Babich, MD,3 Martina Tauber, MD,1 Sergio Messini, MD,4 Maria Raffaella Marucci, ScD,5 and Fabio Vittadello, ScD6

From the 1Department of Pathology, Central Hospital Bolzano, Bolzano, Italy; 2Hematology and BMT Department, Central Hospital Bolzano, Bolzano, Italy; 3Consultant Gynecologist, Bolzano, Italy; 4Department of Gynecology and Obstetrics, Central Hospital Bolzano, Bolzano, Italy; 5Department of Pathology I and II, Spedali Civili-University of Brescia, Brescia, Italy; and 6Explora Research and Statistical Analysis, Padova, Italy.

Key Words: Bone marrow transplantation; Cervical cytology; Pap smear; Busulfan; Atypia

ABSTRACT

Objectives: Allogeneic bone marrow transplantation (BMT) is a procedure mostly used for high-risk hematologic malignances. In women, follow-up protocols after BMT include gynecologic checkups with Papanicolaou (Pap) smears.

Methods: We evaluated 117 Pap smears in 54 women who underwent allogeneic BMT and correlated the smear morphology with the BMT-related medical treatment.

Results: Abnormal Pap smears after BMT were found in 13 (24.1%) women. Four (7.4%) women had at least one smear with atypical squamous cells of unknown significance, six (11.1%) had a low-grade squamous intraepithelial lesion, and three (5.6%) had atypical squamous cells/high-grade lesion cannot be excluded (ASC-H). The three patients with ASC-H showed high-grade atypia mimicking cancer but had a negative follow-up. Nine women, including the three with ASC-H, had undergone a conditioning therapy for BMT that included busulfan. No association between other drugs and therapy-related atypia was found.

Conclusions: Pap smears after BMT show a high incidence of dysplastic lesions. Moreover, conditioning including busulfan is often associated with therapy-related cytologic atypia, which may lead to unnecessary colposcopies and biopsies. Knowledge of the patient’s history and a careful evaluation of the smears are mandatory in these cases.
been described in epithelia of different organs, including the urinary tract, lung, and oral cavity. A few cases were reported also in cervical epithelia. To our knowledge, however, no large study has focused on the relationship between therapy protocols and Pap smear findings after BMT. In our study, we evaluated the Pap smears in 54 women who underwent allogeneic BMT for hematologic malignancies, correlating the Pap smear morphology with the BMT-related medical treatment.

Material and Methods

The study was approved by the review board of the Central Hospital Bolzano, Bolzano, Italy. The study included 117 cervical smears, which were taken after allogeneic BMT in 54 women. The Pap history was available for all women. All women had at least one Pap smear before BMT, none with atypical changes or dysplasia. Eighty-nine samples were conventional smears, 28 were liquid-based cytologies (LBCs; ThinPrep; Hologic, Bedford, MA). BMT had been performed because of acute myeloid leukemia, acute lymphoblastic leukemia, non-Hodgkin lymphoma, multiple myeloma, Hodgkin lymphoma, myelodysplasia, thalassemia, and aplastic anemia in 25, seven, eight, four, one, seven, one, and one patients, respectively. In two patients, two different allogeneic transplants were performed in the same patient, with two different donors. Seven patients had undergone an autologous BMT before receiving the allogeneic transplant.

Conditioning was carried out about 1 week before BMT and included total-body irradiation (TBI)–cyclophosphamide, treosulfan-fludarabine, busulfan-fludarabine, and busulfan-cyclophosphamide in 16, eight, five, and four patients, respectively; these drugs were combined differently in other cases with melphalan, thiotepa, cytarabine, and amsacrine according to standard conditioning regimens. Conditioning radiation therapy included full-dose TBI (1,200 cGy), low-dose TBI (200 cGy), and total lymphoid irradiation in 16, two, and two patients, respectively.

All but one Pap smear had been routinely diagnosed in the Department of Pathology of the Central Hospital Bolzano. One abnormal smear had been sent to our department for consultation. For the study, all cases were reviewed by two cytologists (G.N. and M.H.). Classification of the smears resulted in 3 to 6 months of the low-grade lesion in all cases. The three cytologic follow-up with an HPV test for high-risk HPV. Clinical data, including primary chemotherapy and BMT-related medical treatments, were available in all but one case. This case was excluded from the evaluation of the clinical correlations. In all the other samples, a correlation with the BMT-related medical treatment before the smear sampling was carried out.

Descriptive statistics were calculated for all the demographic and clinical variables, including frequency tables (number, mean, median, SD, minimum, and maximum) for continuous variables; categorical values were summarized in terms of absolute frequencies and percentages.

The statistical significance of differences between two groups of patients was assessed with the Pearson χ² test (or Fisher exact test) for categorical variables, while continuous variables were compared using the Mann-Whitney U test.

For all statistical tests, a P value less than .05 (two-tailed test) was taken to indicate a significant difference. All data were processed using the statistical software SPSS version 18.0 (SPSS, Chicago, IL).

Results

The mean (SD) age of the women was 49.6 (13.5) years (range, 20-74 years). Abnormal cervical smears were diagnosed in 13 (24.1%) women. Overall, of the 117 smears, 23 (19.7%) were classified as abnormal. Four (3.4%) were classified as ASC-US, 16 (13.7%) as LSIL, and 3 (2.6%) as ASC-H. Four (7.4%) of 54 women had at least one ASC-US, six (11.1%) had one LSIL, and three (5.6%) had one ASC-H.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Pap Smears Overall, No. (%)</th>
<th>Women, No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>94 (80.3)</td>
<td>41 (75.9)</td>
</tr>
<tr>
<td>ASC-US</td>
<td>4 (3.4)</td>
<td>4 (7.4)</td>
</tr>
<tr>
<td>ASC-H</td>
<td>3 (2.6)</td>
<td>3 (5.6)</td>
</tr>
<tr>
<td>LSIL</td>
<td>16 (13.7)</td>
<td>6 (11.1)</td>
</tr>
<tr>
<td>Total</td>
<td>117 (100)</td>
<td>54 (100)</td>
</tr>
</tbody>
</table>

ASC-H, atypical squamous cells/high-grade lesion cannot be excluded; ASC-US, atypical squamous cells of unknown significance; LSIL, low-grade squamous intraepithelial lesion.

Table II

Cytologic Classification of the Papanicolaou (Pap) Smears in 54 Women

© American Society for Clinical Pathology

Am J Clin Pathol 2014;142:222-226
DOI: 10.1309/AJCP45KAUSBT0TJX
samples that were classified as ASC-H showed high-grade atypia with obvious nuclear polymorphy and hyperchromasy. Polymorphous naked nuclei were often observed. A dirty background with detritus was particularly obvious in one case. Degenerative changes, when present, did not impair the evaluation of the cellular changes; overall cellularity was high. Without knowledge of the clinical history, these cases would have raised suspicion of invasive squamous cancer. Histologic follow-up included punch biopsies in one case and both punch biopsy and conization in one. All histology specimens were negative for neoplasia. In two histologic samples, an abnormal nuclear enlargement in cervical epithelia was found (Image 1D). In both cases, immunohistochemistry with p16ink4a (Roche, Basel, Switzerland) was negative, and these changes were interpreted as nonneoplastic and therapy related. One case had only a cytologic follow-up and an HPV test for high-risk HPV, which were both negative. One of the women experienced a leukemia relapse in the neck region 3 months after the abnormal Pap test.

The correlation with the BMT-related medical treatment before the smear sampling showed that all three women with ASC-H and cancer-like changes had undergone conditioning with busulfan. Busulfan was used in six additional women who, however, did not show atypical cells in the Pap smear. The mean age was not significantly different between the women treated with busulfan (52.2 years) and the women who had not been treated (49.0 years; P = .486).

In women with cytologic atypia after busulfan, the mean time lapse between BMT and the first abnormal smear was 8.0 (5.5-12.9) months, while in women with busulfan therapy
but no cytologic atypia, the mean time (between BMT and all negative smear sampling) was 13.5 (1.2-34.5) months ($P = .555$). No association between treatment with other drugs and Pap test atypia was found.

**Discussion**

Conditioning before BMT and therapies before and after engraftment follow different protocols that may include chemotherapy as well as, particularly in cases of graft vs host disease, immunosuppressive agents. Since immunosuppressed women are particularly prone to new HPV infections or rapid progression of already existing HPV-related cervical lesions,3,10 Pap smears are included in the follow-up after BMT. The ASBMT guidelines1 recommend a Pap smear every 1 to 3 years. One previous study described a high incidence of HPV-related cervical lesions after BMT.2 Similar to these results, our study shows a high incidence of abnormal cervical smears after BMT, which were diagnosed in 24.1% of the women. Since most of the smears were conventional, the overall incidence of HPV infections in our study was not available. The incidence of cytologically obvious HPV-related squamous intraepithelial lesions, however, was 11.1%, which is significantly higher ($P < .001$) compared with the overall incidence of squamous intraepithelial lesions (2.9%) in our institution. The relatively high incidence of squamous intraepithelial lesions compared with ASC-US, which would be unusual in a primary screening setting, may be because of the selected high-risk population of this study. Moreover, we observed a high incidence of smears with high-grade atypia mimicking cancer but negative follow-up after treatment with busulfan. All these cases had a negative HPV test or a cervical biopsy specimen with nonneoplastic, therapy-related changes and negative p16 immunohistochemistry in the follow-up. Overall, 33.3% of women who had undergone conditioning with busulfan showed high-grade atypia. Busulfan is an already well-known cause of cell atypia in histologic and cytologic specimens.6 One recent study4 describes the occurrence of busulfan-associated keratinocyte dysplasia in up to 16% of skin biopsy specimens. The occurrence of high-grade atypia mimicking cancer in cervical smears after BMT was described in only a few previous studies,3,8,9 but the correlation with the therapy has not yet been studied, to our knowledge, on large samples of cases. It is unclear why only some smears show cytologic atypia whereas others do not. In this study, we correlated the time lapse between BMT and smear sampling with the occurrence of atypia, but we did not find a statistically significant difference between the two groups.

Even though a multidisciplinary approach is a requisite in the management of complex conditions such as BMT, hematologists and gynecologists may not always be aware of the potential therapy-induced diagnostic pitfalls, and cytologists often do not obtain complete clinical data concerning the patient’s history or treatments. In fact, since nowadays, busulfan is only rarely used in non-BMT patients, many cytologists may not even be aware of the related cytologic changes. False-positive smears lead to unnecessary colposcopic and histologic investigations. Moreover, women with a history of BMT are particularly likely to have psychological stress when a further malignancy is supposed. Thus, a history of hematologic malignancy and BMT should always be communicated to the cytologist, and these smears should be evaluated carefully to rule out therapy-related changes. In nonvaginal cytologies, the differentiation between cancer and BMT-related atypia may be very difficult. In their study, Gulbahce et al7 describe that no single cytologic finding was helpful for the differential diagnosis between reactive atypia and adenocarcinoma in bronchoalveolar lavage specimens after BMT. Since cervical neoplasia is almost always HPV related,11 the use of a liquid-based cytology technique for the follow-up after BMT could allow HPV testing or immunocytochemistry with surrogate biomarkers in abnormal samples, thus allowing an effective triage of these women. The lack of complete HPV data in our study and the relatively small number of cases do not allow the formulation of definite recommendations. However, because of the high negative predictive value of these techniques,12 further studies that evaluate the possibility of recommending colposcopy only to women with cytologically atypical samples and a positive ancillary test should be carried out.

In conclusion, Pap smears after BMT have a high incidence of dysplastic lesions and, after conditioning with busulfan, may show therapy-related, cancer-like features, which can lead to false-positive reports with unnecessary colposcopies and biopsies. Knowledge of the patient’s history and a careful evaluation of smears are mandatory in these cases.

**Address reprint requests to Dr Neri: Dept of Pathology, Central Hospital Bolzano, Via Boehler 5, 39100 Bolzano, Italy; email: ginegri@gmail.com.**

**Acknowledgments:** The authors thank Dearbha Duffy, MD (Bolzano), Christiane Rohl, MD (Bolzano), Guido Mazzoleni, MD (Bolzano), Enrico Morello, MD (Brescia), and Irene Cavattoni, MD (Bolzano) for their support.

**References**


