The Role of Microsatellites as a Prognostic Factor in Primary Malignant Melanoma

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Objective: To determine the impact of microsatellites as a prognostic factor in primary cutaneous melanoma.

Design: Retrospective cohort study.

Setting: Tertiary referral center.

Patients: A total of 504 patients with a history of primary melanoma observed for 2 years or having experienced a first relapse.

Main Outcome Measures: Overall survival (OS) and relapse-free survival (RFS).

Results: Forty-five patients had evidence of microsatellites in their primary melanoma. Presence of microsatellites significantly correlated with the presence of several other histologic high-risk factors such as tumor thickness, ulceration, Clark level, vascular factors, and mitotic rate. Univariate analysis demonstrated decreased RFS and OS in patients with microsatellites. Presence of microsatellites was associated with increased locoregional metastasis but not distant metastasis. In multivariate analysis, with the inclusion of 6 other clinical and histologic factors, presence of microsatellites was a significant predictor of RFS but not OS. Patients with clinical macrosatellites had a trend toward worsening OS compared with those with microsatellites.

Conclusions: The presence of microsatellites is intimately tied to other markers of melanoma aggressiveness. Microsatellites appear to predict locoregional relapse and RFS but neither distant metastasis nor OS. These results may have implications for patient care as well as the inclusion of microsatellites in stage III of the current classification.

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Methods

This analysis was approved by the committee on human research, the University of California, San Francisco, institutional review board. The records of 525 patients from the University of California, San Francisco, Melanoma Center database were reviewed for the presence of microsatellites. Selection guidelines for this database have been described previously and include patients with (1) at least 2 years of follow-up or (2) having experienced a first relapse. The range of follow-up was 0.04 to 24.0 years, with a mean follow-up of 5.37 years. The cohort had a median tumor thickness of 1.55 mm. An updated database of our previously defined cohort was used for all analyses. Relapse was defined to include local dermal recurrence and in-transit, regional nodal, and distant metastasis.
Nine clinical factors were included in the database used to conduct this analysis: age, sex, location of primary melanoma, status of regional lymph nodes, site of first recurrence, site of distant metastasis, presence or absence of macrosatellites, date of relapse, and date of death. Nine histopathologic parameters were also evaluated for each patient by a single pathologist (R.W.S) based on evaluation of routine hematoxylin-eosin slides used to make the diagnosis of primary melanoma at the time of the patient’s initial visit to the University of California, San Francisco, Melanoma Center: tumor thickness, histogenetic subtype, Clark level, ulceration, vascular involvement, tumor vascularity, mitotic rate (defined as mitoses per mm²), regression, and microsatellites. The systematic approach to the histopathologic review of primary melanoma has been described previously.

Data on 504 patients out of a total of 525 patients were sufficient complete for analysis. A microsatellite was strictly defined as a discrete nest of tumor cells distinctly separated by a minimum of 0.5 mm (by ocular micrometer) from the main body (vertical growth phase) of the tumor by a layer of collagen or subcutaneous fat. Tumor cells separated by tumor stroma or single cells (by hematoxylin-eosin or using special stains) were excluded from this definition. Microsatellites were not included in the Breslow measurement or Clark level. Care was taken to distinguish between microsatellites and local persistent disease, vascular involvement, local dermal recurrence, and in-transit disease. Macrosatellites were defined as clinically evident tumor nodules in the dermis or fat located between the primary tumor and the regional nodal basin and therefore encompassed both local recurrences and satellite or in-transit disease. Vascular involvement and tumor vascularity were scored as previously described.

### RESULTS

#### MICROSATELLITES AND CLINICAL FACTORS

Forty-five patients (9%) had evidence of microsatellites in their primary tumor in this cohort. Initially, the relationship between sex, age, and location of the primary tumor and microsatellites was explored. A significant association was observed between presence of microsatellites and location of the primary tumor, with most microsatellites occurring in the head and neck region. Fifty-two percent of patients with microsatellites (n = 23) had primary melanomas located in the head and neck region, 27% (n = 12) in the trunk, 18% (n = 8) in the lower extremity, and 2% (n = 1) in the upper extremity regions (P < .001). In contrast, patients with microsatellites were not significantly different in age or sex from control counterparts (data not shown).

#### MICROSATELLITES AND OTHER HISTOPATHOLOGIC FACTORS OF THE PRIMARY TUMOR

The presence of microsatellites correlated with many other known histopathologic factors. Tumors with microsatellites were mostly of the nodular and superficial spreading melanoma subtypes (Table 1). Stratifying the database on thickness revealed increasing frequency of microsatellites with increasing thickness ranges (Table 1). In addition, tumors with microsatellites were more often associated with a high mitotic rate or a higher Clark level. Finally, presence of microsatellites also correlated with ulceration, tumor vascularity, and vascular involvement (Table 1).

#### UNIVARIATE ANALYSIS OF RELAPSE AND SURVIVAL

Kaplan-Meier survival curves were compared in tumors with and without microsatellites. Both relapse-free survival (RFS) and overall survival (OS) were significantly lower in patients with microsatellites (P < .001, data not shown). Presence of microsatellites was also associated with reduced RFS and OS parameters by several other univariate analyses (Table 2). Intriguingly, microsatellites were associated with significantly increased rates of macrosatellite and lymph node metastasis but not distant metastasis. Finally, comparison of
OS curves between patients with microsatellites and those with macrosatellites demonstrated a trend toward lower OS in the macrosatellite group, with 5-year OS of 40.2% and 22.4%, respectively (1-tailed \( P < .07 \)).

MULTIVARIATE ANALYSIS OF MICROSATELLITES AND OTHER PREDICTORS OF OUTCOME

To determine the independent impact of microsatellites on outcome, we performed multivariate analysis of OS and RFS considering the following parameters: sex, location, age, thickness, ulceration, Clark level, and microsatellites (Table 3). After adjusting for the aforementioned features, microsatellites provided independent prognostic information in predicting RFS but not OS. Finally, with the addition of 3 other prognostic factors (nodal status, vascular involvement, and tumor vascularity), tumor thickness emerged as the strongest predictor of OS by stepwise Cox regression analysis, followed by nodal status, vascular involvement, tumor vascularity, location, and age, all of which were also significantly and independently predictive of OS in this analysis (data not shown).

In this study, we analyzed the role of microsatellites in the outcome associated with melanoma. Our data demonstrate increased frequency of relapse and death and decreased RFS and OS rates in patients with microsatellites by univariate analysis. Microsatellites had a stronger impact on in-transit and nodal relapse than on distant metastasis. Significant correlations were observed between microsatellites and other powerful histopathologic risk factors. Patients with microsatellites had a trend toward poorer 5-year survival than patients with microsatellites. Multivariate analysis by Cox regression revealed an independent role for microsatellites in predicting RFS but not OS.

To date, only a handful of studies have investigated the role of microsatellites in predicting outcome.1,3,10,11 Day et al.1 showed a 36% 5-year RFS in patients with microscopic satellites, compared with an 89% RFS in patients without. A later study7 by this group analyzing 20 clinical and histologic factors identified thickness, ulceration, and microsatellites as the combination of variables most predictive of regional nodal metastasis. Likewise, Leon and colleagues3 showed decreased RFS and OS in a matched cohort of 30 patients with microsatellites. The microsatellite group demonstrated a 37% 5-year OS compared with 63% in the nonmicrosatellite group. Cox regression analysis identified microscopic satellites, regression, ulceration, high mitotic rate, tumor-infiltrating lymphocytes, positive lymph node dissection, and location as independent predictors of survival when 12 factors were considered.

Despite these results, the role of microsatellites as an independent predictor of outcome remains unclear.5,7 Only some of the difficulty stems from different definitions and measurements of this histopathologic factor. Rates ranging from 6%12 to 17%1 have been observed among different cohorts, likely owing to differences in measurements of this high-risk feature and the cohort of patients included for analysis. Harrist et al.2 included in their analyses of microsatellites not only true dermal satellites but also tumor emboli within vascular spaces as well as tumor islands potentially contiguous with the main body of the tumor. Other groups, however, have included only true dermal satellites in the definition of microsatellites and considered other entities such as tumor within vasculature as a representation of vascular involvement.5,7 This would potentially explain the higher frequency of microsatellites observed in the Day et al.1 cohort (17%) as well as the robust decrease in OS and RFS observed by these investigators in the microsatellite group. Furthermore, most of the earlier literature on microsatellites has not considered the relationship between this entity and more recently described pathologic markers such as vascular involvement and tumor vascularity. A stepwise Cox regression analysis revealed microsatellites to be less critical in predicting OS and RFS when vascular factors were also included in the model.

Recently, the AJCC has placed microsatellites in the N2c category of the TNM classification, thus placing it in the stage III category of disease.9 Microsatellites were included in stage III based on multiple survival analyses of patients with clinical satellites, local recurrence, and in-transit and nodal metastases and who all demon-

| Table 2. Relationship Between Presence of Microsatellites and Relapse by Univariate Analysis* |
| --- | --- | --- | --- |
| Finding | Microsatellite Absent | Microsatellite Present | \( P \) Value |
| Median relapse-free survival, y | 3.5 | 0.9 | <.001 |
| Median overall survival, y | 4.2 | 3.1 | <.001 |
| Relapsed | 219 (47.7) | 40 (88.9) | <.001 |
| Dead | 167 (23.3) | 29 (44.4) | .006 |
| Macrosatellites | 48 (10.4) | 16 (34.9) | <.005 |
| Regional nodal metastasis | 158 (34.5) | 24 (54.5) | <.02 |
| Distant metastasis | 100 (21.8) | 15 (33.3) | .14 |

*Unless otherwise noted, data are reported as number (percentage) of patients.

<p>| Table 3. Multivariate Analysis of Impact of Microsatellites on Relapse-Free and Overall Survival |
| --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Prognostic Factor</th>
<th>Risk Ratio</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Relapse-Free Survival</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor thickness</td>
<td>1.52</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Microsatellites</td>
<td>1.88</td>
<td>.001</td>
</tr>
<tr>
<td>Ulceration</td>
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<td>.02</td>
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<tr>
<td>Sex</td>
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<td>.02</td>
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<tr>
<td>Clark level</td>
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<td>.05</td>
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<tr>
<td>Age</td>
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<td>.19</td>
</tr>
<tr>
<td>Location</td>
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<tr>
<td><strong>Overall Survival</strong></td>
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<td></td>
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<tr>
<td>Tumor thickness</td>
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<tr>
<td>Age</td>
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<tr>
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strated similar 10-year survival rates of 20% to 40%. The decision to include microsatellites in this same category has mainly been based on the idea that the development of microsatellites, clinical satellites, and in-transit and regional nodal metastases are all continuous biologic events and can be treated as 1 entity. This recategorization will have significant prognostic and therapeutic consequences, especially in regard to adjuvant therapy and clinical trial eligibility.

However, using a database that recapitulated the prognostic impact of thickness and ulceration, we found that microsatellites do not have additional prognostic impact on OS when added to the factors recently analyzed by the AJCC staging committee. In addition, the frequency of distant relapse, a more powerful indicator of disease-specific survival, was not significantly different in patients with and without microsatellites. In this regard, a prior study of 284 patients showed thick tumors with and without microsatellites to have similar overall (locoregional and distant) recurrence rates. Further stratification of relapse into local and distant relapse showed a significantly higher local but not distant recurrence rate in the microsatellite group. By more powerful prognostic factors in our database such as vascular involvement, tumor vascularity, and ulceration have been shown to significantly predict both regional and distant metastasis. Furthermore, there was a trend toward decreased OS among patients with microsatellites compared with those with microsatellites, suggesting that these represent 2 distinct entities.

While the inclusion of ulceration in the latest staging classification was based on its independent impact on survival by Cox regression, the inclusion of microsatellites did not undergo similar validation. A multivariate analysis of our data using the same factors as the AJCC identified tumor thickness, age, location, and ulceration as the strongest predictors of OS. By contrast, presence of microsatellites was predictive of RFS but not OS according to this analysis. Since microsatellites did not have the same prognostic power in determining OS as did other prognostic factors, its automatic inclusion in stage III disease deserves further scrutiny. The observation that microsatellites occurred more frequently in the context of all the other high-risk features considered in this study suggests that by the time microsatellites develop, numerous other high-risk events have already taken place, further reducing its impact in multivariate analysis. Finally, the finding of a trend toward reduced OS in the subgroup of patients with microsatellites may provide further evidence that microsatelites and clinical satellites represent potentially distinct points along the tumor progression pathway of primary melanoma.

In summary, microsatellites seem to impart their influence on outcome through their correlation with locoregional as opposed to distant relapse. Thus, they represent yet another factor that refines the prognosis of primary melanoma, given a range of tumor thickness. In this regard, a previous study showed microsatellites to be 1 of 4 factors (along with mitotic index, vascular invasion, and ulceration) that increase the risk of identifying a positive sentinel lymph node. In view of these findings, we recommend that microsatellites be placed alongside ulceration (and vascular invasion) as a second determinant (ie, in the b subclassification) of the T staging category.

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