Prognostic Value of the Disodium Phosphate $^{32}$P Uptake Test in Uveal Melanoma

A Long-term Study

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**Objective:** To evaluate whether nuclear activity as measured by the disodium phosphate $^{32}$P ($^{32}$P) uptake test for uveal melanoma is of prognostic value and corresponds to known prognostic factors.

**Methods:** A retrospective analysis of 121 patients with choroidal and/or ciliary body melanoma, tested with the $^{32}$P uptake test before enucleation between January 1, 1973, and December 31, 1976, at the Leiden University Medical Center. We obtained the 25-year follow-up information of this group of patients and compared the $^{32}$P test results and histopathological variables with the long-term survival rates.

**Results:** The cumulative 5-, 10-, and 20-year survival for melanoma-related death was 81.4%, 73.3%, and 63.9%, respectively. The results of the $^{32}$P uptake test were not significantly correlated with survival ($P = .35$). Of all prognostic factors under study, tumor diameter, cell type, and mitotic count were identified as the most important prognostic markers for uveal melanoma in this group.

**Conclusions:** The $^{32}$P isotope uptake test has no prognostic value for uveal melanoma. Moreover, the results of this study indicate that it is unlikely that cell activity as determined by $^{32}$P uptake involves mitotic activity of the tumor.

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**Uveal Melanoma** is the most common primary malignant intraocular tumor, with an estimated incidence of 6 cases per million per year. This malignancy arises in the choroid, ciliary body, or iris. Primary uveal melanoma can be treated by enucleation or eye-preserving methods, but there is still no effective therapy for metastases from uveal melanoma. Up to 50% of the patients who undergo enucleation die of metastatic disease within 10 to 15 years after diagnosis of choroidal and/or ciliary body melanoma.

To improve survival, it is essential to prevent metastases or to treat uveal melanoma metastases more effectively. To accomplish this, it is necessary to determine reliable prognostic indicators and to develop methods for detecting metastases at an early stage. A better insight into the mechanisms that induce certain tumors to develop metastases and others not may help to develop new therapies.

During the 1950s, the disodium phosphate $^{32}$P ($^{32}$P) isotope uptake test was developed to differentiate benign from malignant lesions of the choroid and ciliary body, because it was considered to be an indicator of nuclear activity. The $^{32}$P radioactive isotope of phosphorus has a half-life of 14.3 days and emits beta particles from unstable nuclei. Phosphate labeled with $^{32}$P is specifically integrated in nucleoproteins during the synthesis of new nuclei. In the $^{32}$P uptake test, $^{32}$P was administered to the patients by intravenous injection. The labeled phosphate accumulated in tissues according to their turnover of phosphate. After administration of the $^{32}$P, melanomas of the choroid showed 5 to 10 times higher radioactivity than adjacent ocular structures, and a relationship between $^{32}$P uptake and nuclear activity was suggested. The uptake of the $^{32}$P isotope in the tumor was said to be a measure of mitotic activity of neoplastic tissue. Several authors studied the potential of using $^{32}$P as a prognostic marker in uveal melanoma, with contradictory outcomes.

Potential complications associated with the $^{32}$P test were ruptures in Bruch’s membrane with subretinal hemorrhage as a direct consequence of probe placement and long-term potential leukemogenic complications in patients with polycythemia. The $^{32}$P test was used for more than a decade as a diagnostic method for uveal melanoma, but it is no longer per-
formed. Today, better and less invasive diagnostic methods are used, mainly ultrasonography.

At the Department of Ophthalmology of the Leiden University Medical Center, Leiden, the Netherlands, the $^{32}$P test was performed for many years and informative data regarding the patients and the tumors, as well as the patients’ follow-up, were registered. Therefore, we were able to obtain a follow-up of more than 25 years with complete information on all patients originally investigated. We evaluated the prognostic value of the $^{32}$P uptake test for uveal melanoma, as this test may give an indication of the importance of nuclear activity in the development of uveal melanoma metastases.

**METHODS**

**PATIENTS**

The population selected for this analysis was a group of 121 patients with choroidal or ciliary body melanoma. The patients underwent the $^{32}$P uptake test at the Department of Ophthalmology, Leiden University Medical Center, between January 1, 1973, and December 31, 1976. The patients were primarily treated by enucleation, and the $^{32}$P uptake test was performed just before or during enucleation. The research protocol followed the tenets of the Declaration of Helsinki. If the probe for radioactivity registered 60% more radioactivity than in a comparable area outside the tumor region of the same eye (ie, 160%), the result was considered positive and highly suggestive of the presence of a melanoma, and the eye was enucleated in the same operating session. If the value remained less than 160%, the eye remained in situ. In general, clinical examination included ophthalmoscopy, fundus drawings, photography, fluorescein angiography, and the $^{32}$P uptake test. Histopathological analysis was performed by one ocular pathologist (D.W.-R.). The clinical, histopathological, and follow-up data were collected and registered at the Oncological Registration at the Leiden University Medical Center. Once every 5 years, the Dutch Population Registry was consulted. The melanoma-related deaths were confirmed by general practitioners or specialists and a copy of the records was sent to the Oncological Registration. For this study, 121 patients were followed up until December 2000.

**HISTOPATHOLOGICAL ANALYSIS**

The following variables were analyzed: cell type (spindle, mixed, or epithelioid); largest tumor diameter in millimeters; condition of Bruch’s membrane (intact or broken); number of mitoses (mitotic rate per 15 high-power fields); degree of pigmentation (none, light, intermediate, or heavy); depth of scleral infiltration (none, <30%, ≥50%, or total thickness or episcleral); intravascular ingrowth of tumor cells (no ingrowth, ingrowth in vessels inside the tumor, ingrowth in vessels outside the tumor, or ingrowth in vessels both inside and outside of the tumor).

**STATISTICAL ANALYSIS**

All clinical, histopathological, and follow-up data of this study were registered in a Paradox database program (version 7.0; Borland Software Corp, Scotts Valley, Calif). In several analyses, the $^{32}$P uptake was dichotomized into 2 groups according to the median: low (<176%) and high (≥176%) $^{32}$P uptake. Tumor characteristics of the 2 groups were compared by means of the χ² test and the t test (Mann-Whitney test). Survival was illustrated by Kaplan-Meier curves and analyzed by Cox proportional hazards model. Univariate Cox proportional hazards modeling was used to evaluate the prognostic value of $^{32}$P uptake and other variables. Multivariate analysis identified the independent significant prognostic variables. Any P values less than .05 were taken as statistically significant.

**RESULTS**

**DESCRIPTION OF POPULATION**

Data from a total of 121 patients examined with the $^{32}$P isotope uptake test were analyzed in this study. All tumors were treated with enucleation. The $^{32}$P test yielded 1 false-positive result: the eye contained a choroidal hemangioma. The mean age at enucleation was 55.4 years (range, 15-93 years). Sixty-five patients (54%) were men and 56 (46%) were women. Some categories of data were not available for all patients; therefore, in some of the results given, total numbers of patients may be less than 121.

No patients were lost during follow-up. At the end of the follow-up time (December 2000), 36 patients (30%) had died of melanoma-related disease, 28 (23%) had died of non–melanoma-related causes, 17 (14%) had died of unknown causes, and 40 (33%) were still alive.

**TUMOR CHARACTERISTICS**

The mean tumor diameter was 10.6 mm, and tumors were classified according to diameter into 3 groups: small (<10 mm), medium (10-15 mm), and large (>15 mm). Fifty-nine tumors (49.6%) were small, 50 tumors (42.0%) were medium sized, and 10 (8.4%) were large. Seventy tumors (58%) were of the spindle cell type, 35 (29%) were of the mixed cell type, and 13 (11%) were of the epithelioid cell type. Three tumors were completely necrotic and cell type could not be determined. In 71 cases (58%), Bruch’s membrane was broken. In 16 tumors (13%), there was no pigmentation; pigmentation was light in 50 (41%), moderate in 40 (33%), and heavy in 14 (12%). In 83 tumors (68%), there was no tumor cell ingrowth in vessels, 20 (17%) had tumor cell ingrowth in vessels within the eye, 11 (9%) in vessels outside the eye, and 7 (6%) both within and outside the eye. Seventy-eight (65.6%) had intrascleral tumor growth and 30 (25.2%) had deep intrascleral tumor growth (Table 1).

**STATISTICAL ANALYSIS**

The mean survival time after enucleation was 19.7 years, with a 95% confidence interval of 17.7 to 21.8 years. The cumulative 5-year survival for melanoma-related death was 81.4%, 10-year survival was 73.3%, and 20-year survival was 63.9%. Thirty-six patients died of melanoma metastases. Figure 1 shows the normal distribution of $^{32}$P uptake. The median uptake was 176%. The prognostic value of the $^{32}$P uptake test was analyzed with the Cox proportional hazards model for death from melanoma. The level of $^{32}$P measurement was not significantly correlated to survival ($P = .35$; relative risk, 1.37). The $^{32}$P isotope uptake test has therefore no prognostic value for uveal melanoma (Figure 2).

In a regression analysis, a significant correlation was found between $^{32}$P uptake and tumor diameter ($P = .03$). Al-
though this correlation was significant, the scatterplot (not shown) did not show a strong connection: the correlation coefficient was only 0.201. In addition, the χ² test and t test were not significant: P = .57 and P = .72, respectively (Table 1).

Therefore, we consider 32P not an accurate predictor of tumor diameter. The relationship between 32P uptake and tumor thickness, mitotic count, cell type, necrosis, condition of Bruch’s membrane, pigmentation of the tumor, intravascular ingrowth, intrascleral growth, and patient sex showed no significant correlations (Table 1).

The presence of spindle cells was associated with improved survival. A higher mitotic count and larger tumor diameter meant a worse prognosis (Figure 3 and Figure 4, respectively). There was a significant correlation between mitotic count and tumor diameter (P = .001; correlation coefficient, 0.32), but not between mitotic count and 32P uptake.

Of all possible prognostic factors analyzed with Cox proportional hazards model, tumor diameter (P = .01; risk per unit, 1.2), cell type (spindle: P < .001, relative risk, 1; epithelioid: P < .001, relative risk, 14.2; mixed, P < .10, relative risk, 2.1), and mitotic count (P < .001; risk per unit, 1.06) were identified as the most important prognostic markers for uveal melanoma in this group (Table 2). Another significant association was seen between intravascular ingrowth and survival (P = .04):
Figure 3. Cumulative survival by mitotic count. Kaplan-Meier analysis showed that survival worsened with increasing numbers of mitoses. Mitotic count was a significant prognostic factor ($P < .001$) by Cox regression analysis. HPF indicates high-power field; plus sign, censored values, resulting from death of a patient or loss to follow-up.

Figure 4. Cumulative survival by tumor diameter. Kaplan-Meier analysis showed that increasing diameter indicated poor prognosis. Diameter was a significant prognostic factor ($P = .001$) by Cox regression analysis. Plus signs indicate censored values, resulting from death of a patient or loss to follow-up.

Table 2. Relative Risks or Risk Factors per Unit of Prognostic Variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis (Independent Factors)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{32}$P uptake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low ($&lt;176%$)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High ($\geq 176%$)</td>
<td>1.4 (0.7-2.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell type</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Spindle</td>
<td>1</td>
<td></td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mixed</td>
<td>2.5 (1.2-5.3)</td>
<td>2.1 (0.9-5.2)</td>
<td>.10</td>
</tr>
<tr>
<td>Epithelioid</td>
<td>4.6 (1.9-11)</td>
<td>14.2 (4.6-44.0)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Necrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial</td>
<td>1.6 (0.6-4.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.4 (0.2-10)</td>
<td></td>
<td></td>
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<tr>
<td>Bruch’s membrane</td>
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<tr>
<td>Intact</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Broken</td>
<td>2.3 (1.0-5.1)</td>
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<td></td>
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<tr>
<td>Pigmentation</td>
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<td></td>
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<tr>
<td>None</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Slight</td>
<td>1.6 (0.4-5.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>3.2 (0.9-11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>1.2 (0.2-5.9)</td>
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<td></td>
</tr>
<tr>
<td>Intravascular growth</td>
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<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Within</td>
<td>1.8 (0.8-4.2)</td>
<td>1.3 (0.4-4.5)</td>
<td>.66</td>
</tr>
<tr>
<td>Outside</td>
<td>3.2 (1.3-7.9)</td>
<td>0.3 (0.07-1.6)</td>
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<tr>
<td>Both</td>
<td>2.4 (0.7-8.1)</td>
<td>0.3 (0.1-1.0)</td>
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<td>Intrasceral growth</td>
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<tr>
<td>None</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial</td>
<td>2.5 (0.6-10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Episceral</td>
<td>1.6 (0.2-12)</td>
<td></td>
<td></td>
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<tr>
<td>Deep</td>
<td>1.8 (0.4-8.4)</td>
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<tr>
<td>Diameter, mm†</td>
<td>1.2 (1.1-1.3)</td>
<td>1.2 (1.1-1.5)</td>
<td>.01</td>
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<tr>
<td>Thickness, mm†</td>
<td>1.1 (1.0-1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitotic rate†</td>
<td>1.05 (1.03-1.07)</td>
<td>1.06 (1.03-1.09)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age, y†</td>
<td>1.005 (0.98-1.0)</td>
<td></td>
<td></td>
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</table>

Abbreviations: CI, confidence interval; $^{32}$P, disodium phosphate $^{32}$P; RR, relative risk.

*Analyzed with the Cox proportional hazards model for death from melanoma.

†Risk factors per unit.
In the present study of 121 uveal melanomas, the $^{32}$P isotope uptake test was not found to be a significant prognostic factor for uveal melanoma. In $\chi^2$ and $t$ test analyses, no significant correlation between $^{32}$P test and other prognostic factors was found. In previous studies, various associations were reported.\(^{13,18,19,21,24}\) Char et al\(^ {18}\) observed a lower $^{32}$P outcome in eyes with a spindle cell tumor as compared with those with mixed and epithelioid cells. Hagler et al\(^ {19}\) studied 82 eyes enucleated for uveal melanoma, with a 5-year follow-up. No significant relationship between $^{32}$P value and either cell type or tumor diameter was observed, but in general, small tumors had lower $^{32}$P values than larger tumors. Van Dijk\(^ {13}\) studied 44 uveal melanomas and observed no correlations between $^{32}$P value and any histologic variables tested. The $^{32}$P test was identified by McLean and Shields\(^ {31}\) as a prognostic indicator, but its prognostic importance was far less than that of the largest tumor dimension or cell type. The survival rate in the group under study was 81% after 5 years, with a maximum follow-up of 8 years. McLean and Shields concluded that the $^{32}$P test could only be used as a prognostic variable in small melanomas when the tumor was not greater than 7 to 10 mm in diameter and 2 to 3 mm thick.\(^ {21}\) In our study, these small melanomas were only a subpopulation of all uveal melanomas.

Studies in which patients were treated for uveal melanoma more than 15 years ago included all sizes of melanomas. First choice of treatment at that time was enucleation, even for small melanomas.\(^ {25,26}\) In more recent studies, mostly material from large melanomas was obtained. This difference in distribution of diameter makes it complicated to compare the results of our study with results of recent survival analyses. Our population contained patients treated with enucleation for even the smallest tumors considered melanomas. This probably explains why the 20-year survival was 64%, which is higher than the average survival of 53% found in other studies reviewed by Mooy and De Jong\(^ {27}\) in 1996.\(^ {27-29}\) The higher survival rate is probably due to the diameter and the cell type of the tumors in this study. Most of the tumors (91.6%) were small to medium sized, an indicator of better survival. Only 11% of the tumors had the epithelioid cell type, a prognostic factor that is associated with a poor survival.

An interesting finding was the significant correlation of intravascular ingrowth with poor prognosis. To our knowledge, this has not been reported in the literature. We could not investigate patterns of periodic acid–Schiff positivity, as described by Folberg et al,\(^ {30}\) since no appropriate tumor material was available.

The presence of epithelioid cells, a high mitotic count, large tumor diameter, and intravascular ingrowth indicated a poor prognosis. Mitotic count was a highly significant prognostic factor ($P<.001$). There was a significant correlation between the mitotic count and the tumor diameter ($P=.001$; correlation coefficient, 0.32). An earlier study of the same population also showed this correlation between size and mitosis. Smaller melanomas have fewer mitoses. This finding confirmed the results of the Collaborative Ocular Melanoma Study Group.\(^ {31}\)

The introduction of radioisotopes in medicine had a big influence in the diagnosis of malignant lesions. Of great benefit is the observation of accumulation of the radioisotopes in vivo and the possibility of direct measurement of the radioactivity in structures of interest. In ophthalmology, this method was once considered of paramount importance in correctly diagnosing and interpreting intraocular lesions to prevent unnecessary enucleations.\(^ {32}\) The $^{32}$P uptake is related to cell activity. According to Van Dijk, the differences in $^{32}$P uptake are due to the mitotic activity of the tumor.\(^ {13}\) In the present analysis, the mitotic count was a highly significant prognostic factor ($P<.001$) but did not correlate with the result of the $^{32}$P uptake test. This confirmed the finding of McLean and Shields,\(^ {21}\) who also could not show a significant correlation between $^{32}$P uptake and the mitotic count. It is therefore unlikely that cell activity as determined by $^{32}$P uptake involves mitotic activity of the tumor. The relationship between $^{32}$P uptake and cellular or nuclear activity of the tumor is therefore still unclear.

Mitotic count is a measure of cell proliferation.\(^ {2}\) A high rate of mitosis means a high rate of cell proliferation, and this indicates that the cell is biochemically very active. Tumor cells that are very active are more aggressive and therefore more malignant. Large melanomas are more malignant.\(^ {37}\) The higher number of mitoses in large melanomas can be an explanation for higher mortality rate. It is therefore surprising that there is no correlation between $^{32}$P uptake and mitotic count. This means

![Figure 5](https://www.archophthalmol.com/article Figures/0145 FIG5.jpg)

**Figure 5.** Cumulative survival by intravascular ingrowth. Intravascular ingrowth was a significant prognostic factor for uveal melanoma. Plus signs indicate censored values, resulting from death of patient or loss to follow-up.
that the biochemical cell activity as measured by $^{32}$P uptake is not related to cell proliferation, as has been thought.

Although the reliability of the $^{32}$P test in some studies was more than 95%, there is no role for the $^{32}$P uptake test as a prognostic test; it has been replaced by ultrasonography.

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