Trends in Liver Function Tests: A Comparison with Serum Tumor Markers in Metastatic Uveal Melanoma (Part 2)

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Abstract. Aim: To compare trends in liver function test (LFT) levels over consecutive visits before detection of liver metastasis (LM) from uveal melanoma (UM) with such trends in the serum tumor markers S-100β, melanoma inhibitory activity (MIA), osteopontin (OPN), and tissue polypeptide-specific antigen (TPS). Patients and Methods: Blood was drawn from 32 patients with metastatic UM and 43 disease-free (DF) patients semi-annually for levels of S-100β, MIA, OPN, and TPS. Abdominal ultrasonography (US) and LFTs were used to detect LM. Median LFT levels were calculated at 6-month intervals prior to the clinical detection of LM. Trends in LFT levels over consecutive visits in the groups were compared with trends in the tumor markers for these groups. Results: Only LDH gave a statistically significant difference between the trends of the metastasis and DF groups (p=0.0041). When calculating the lead time, all of the elevations were non-significant except for gamma glutamyltransferase which showed a statistically significant elevation at time 0, the time of detection of metastasis. LDH showed a rise at 0-6 months before detection, but this was not significant. For the tumor markers, steeper trendlines were shown for the metastasis group for MIA and S-100β, and most of the markers showed a lead time of more than six months, although this was statistically significant only for OPN. Conclusion: Following the dynamics of tumor markers and LFTs may help to find metastatic disease in UM patients before the metastases are detectable by imaging, enabling earlier treatment.
normal limits, when their levels increased to 80% of the upper normal limit (10). Eskelin et al. showed that 33% of 46 patients with metastatic UM had LFTs that were within normal limits (11). In their study, they concluded that liver ultrasonography and LFTs should be performed once or twice yearly for screening purposes (11). The Collaborative Ocular Melanoma Study (COMS) concluded that the use of LFTs results followed by diagnostic tests has high specificity and predictive values but low sensitivity, and that better tests are needed to identify earlier metastatic disease associated with choroidal melanoma (9).

Serum tumor markers have been shown to be a promising tool in the early diagnosis of metastatic UM (14-21). Missotten et al. have recently reported their analysis of the serum tumor markers S-100β and MIA in comparison with LFTs in detecting patients with metastatic uveal melanoma (17). The receiver operating characteristic (ROC) analysis in their study, comparing the metastatic group with the non-metastatic group, showed that LDH, GGT, S-100β, and MIA (in decreasing order) were the best tests to identify metastatic disease. They concluded that a prospective screening study, with a semi-annual determination of LFTs and tumor markers, is needed in order to draw a final conclusion (17).

In this study, we compared the trends in the metastasis group with the trends in the disease-free (DF) group for the LFTs, over sequential visits during a four-year time period. We also show the dynamics of LFTs in the metastasis group, prior to the clinical detection of metastasis.

**Patients and Methods**

*Patients serum samples.* Patients with UM were followed up about every six months at the Ocular Oncology service of the Hadassah-Hebrew University Medical Center. Abdominal ultrasonography and LFTs (ALK-P, AST, ALT, GGT, LDH, TBil) were used to detect metastatic UM to the liver. Diagnosis of liver metastasis was confirmed by computed tomography or biopsy. In addition, at each visit since September 2003, blood was drawn from all patients with UM. After collection, blood was centrifuged for 10 minutes at 1200 rpm and serum was stored at –20°C.

*Serum assays.* Serum levels of tumor markers were evaluated with enzyme-linked immunosorbent assays (ELISA) kits, following the manufacturers’ instructions (S-100β (DiaSorin, Stillwater, MN, USA), MIA (Roche Diagnostics, Manheim, Germany), OPN (R&D Systems, Minneapolis, MN, USA), and TPS (IDL, Bioteck AB, Bromma, Sweden)). The use of patients’ sera was approved by the Hadassah-Hebrew University Medical Center Institutional Review Board.

Two groups of patients were included in this study. The metastasis group included all the patients who developed metastasis from UM at the time at which we began to collect serum for evaluation of tumor markers. The DF group included patients diagnosed with primary UM who had not developed metastasis for at least ten years since the diagnosis of the primary tumor. Results of pre-metastatic levels of serum tumor markers and LFTs were available for 33 patients with metastatic UM. One of these patients was also diagnosed with adenocarcinoma of the colon and was thus excluded from our analyses. Results of tumor markers and LFTs were available for 43 and 40 DF patients, respectively, who were not diagnosed with another malignancy.

LFT levels were grouped by the time periods (about 6 months) previous to the visit at which metastasis was diagnosed (time 0). The median test level at each of these time periods (visits) was calculated and compared with the previous visit. The time (in months) at which an elevation of LFTs was detected (even within the normal range) before the detection of clinical metastasis was termed lead time. All LFT levels were normalized by dividing the result by the upper normal limit of the laboratory that had performed the analysis.

Trends in changes of LFT levels on consecutive visits before and until the time of diagnosis of metastasis were compared with those found in the DF group using one-way analysis of variance (ANOVA). A linear regression trend line was calculated for every patient for each of the tests. The average slope of the trendlines of all the patients in a group was calculated for each test.

*Statistics.* Statistical analysis (regression analysis and ANOVA) was carried out using JMP Statistical Discovery Software 5.0 (SAS Institute, Cary, NC, USA). The Mann-Whitney (independent samples) test was used to compare the median level at each visit to that of the previous visit.

**Results**

Serum tumor markers (OPN, MIA, S-100β, TPS) and LFTs for the metastasis group were available for an average of 22.5±15.8 months (range 3-63 months), and 24±16.3 months (range 3.5-71.5 months), respectively, prior to the detection of metastasis.

Forty-three DF patients were also followed up with serum tumor markers and LFTs; three of these patients did not have three consecutive measurements for LFTs and were thus excluded from the trends calculations. Serum tumor markers and LFTs for the DF group were available for an average of 33.3±4.8 months (range 20-39.3 months), and 30±9 months (range 6-56 months), respectively.

All patients with metastatic UM had metastasis primarily to the liver, except for one patient who had metastasis to the sacrum.

At the time of diagnosis of metastasis, 13 out of 25 patients (52%) had elevated levels (above the upper normal limit, or cutoff value) of at least one of the LFTs, and 27 of 28 (96%) patients had elevated levels above the cut-off, of at least one of the serum tumor markers. The results with subgroups of each test are summarized in Table I.

Figure 1 shows the distribution of the slopes of the trendlines in the metastases group compared to the DF group, for each of the LFTs. Only for LDH there was a statistically significant difference between the trends of the metastasis and DF groups (p=0.0041). The mean trendlines for AST,
ALT, and GGT were negative for the metastasis group. ALK-P was higher for the metastasis group, but this was not statistically significant \((p=0.2223)\). For Tbil, there was no difference between the two groups. Results for the tumor markers are reported separately (20).

Figure 2 shows the distribution of LFT levels at consecutive visits prior to the detection of metastasis (time 0). The median LFT levels for each visit were compared with the median levels of the previous visit. All of the elevations were non-significant, except that for GGT which showed a statistically significant elevation at time 0, meaning no lead time. LDH showed an elevation at 0-6 months before detection, but this rise was not significant. Results for the tumor markers are reported separately (20).

Discussion

Early detection of metastasis from UM is increasingly important as new treatment modalities are being developed for metastatic disease. The serum tumor markers OPN, S-100β, MIA, and TPS have been considered promising in screening for metastatic disease of UM, as levels rise in serum of patients with metastatic disease (14-21). All studies on these tumor markers in UM to date have evaluated the levels at specific time points, namely before and after the development of metastatic disease. An elevated level has been considered as one that is above the cutoff value for the specific marker. In previous studies on tumor markers in breast cancer, including the tumor markers CEA, CA 15-3 and TPS, serial measurements were considered as an essential part of the follow-up of patients with metastatic breast cancer showing clinical progression during the 6-month follow-up period (23). The median lead time between the increase of TPS and the development of clinically progressive disease was approximately 8 months (23). Even though serial changes, or dynamics, have been previously considered, an elevated result has always been considered one which is above the cutoff value for the specific marker. Our recently published study was the first study of markers in UM in which the trends, or dynamics, in the levels of these biomarkers over consecutive visits have been considered, irrespective of whether a result was within the normal range or elevated (20). We showed that levels of the tumor markers begin to increase 6 months and sometimes more than 6 months before the clinical detection of metastases (20).

We should differentiate between elevated levels and elevation dynamics. In our results, we show that at the time of diagnosis of metastasis almost all patients (96%) had elevated levels of at least one of the tumor markers, whereas only 52% had elevated levels of one of the LFTs. This is in correlation with the results in an earlier study that our group performed on LFTs in UM, where 50% of patients had one abnormal LFT at the time of diagnosis of liver metastasis (10). The lead time can also relate to the time of elevation of a test above the cutoff value, or to the time at which there is a rise in the level of a test regardless of its value. When evaluating the dynamics of elevation, when the levels were still within the normal limits, it is evident from our results that most of the markers analyzed had an increasing trend with a lead time of at least 6 months (20). However, for the LFTs, none of the elevations was significant except that for GGT which was elevated at time 0, meaning that there was no lead time. In the study reported by Missotten et al. comparing S-100β and MIA with LFTs in UM, early detection of metastatic disease, levels of markers and LFTs in the metastatic group were measured at one time point after metastasis had been detected. They found that GGT, LDH, S-100β and MIA (in decreasing order) were the best predictors of metastatic disease (17). Their results showed elevated levels of these markers when metastasis had already been clinically diagnosed; they did not evaluate the levels of these tests before the detection of metastasis. In another study of MIA and S-100β in cutaneous malignant melanoma, serial measurements were taken during follow-up of primary melanoma patients, and metastases were detected on average 6 months earlier than by clinical diagnosis (23); elevation was considered when a marker was above the cutoff value (24). In our study, we have shown both the elevation of levels at the time of diagnosis and the dynamics of elevation, with increasing trends in serial measurements before the detection of metastasis.

### Table I. Elevated levels of liver function tests (LFTs) and tumor markers at clinical diagnosis of metastasis.

<table>
<thead>
<tr>
<th>Test</th>
<th>Number of patients</th>
<th>Number of patients with elevated test (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFTs</td>
<td>25 *</td>
<td>13 (52)*</td>
</tr>
<tr>
<td>ALK-P</td>
<td>23</td>
<td>1 (4)</td>
</tr>
<tr>
<td>AST</td>
<td>25</td>
<td>2 (8)</td>
</tr>
<tr>
<td>ALT</td>
<td>24</td>
<td>3 (12)</td>
</tr>
<tr>
<td>GGT</td>
<td>15</td>
<td>6 (40)</td>
</tr>
<tr>
<td>LDH</td>
<td>19</td>
<td>7 (36)</td>
</tr>
<tr>
<td>Tbil</td>
<td>21</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Tumor markers</td>
<td>28*</td>
<td>27 (96)*</td>
</tr>
<tr>
<td>OPN</td>
<td>27</td>
<td>20 (74)</td>
</tr>
<tr>
<td>MIA</td>
<td>18</td>
<td>17 (94)</td>
</tr>
<tr>
<td>S-100β</td>
<td>28</td>
<td>16 (57)</td>
</tr>
<tr>
<td>TPS</td>
<td>27</td>
<td>7 (25)</td>
</tr>
</tbody>
</table>

*The numbers of patients with at least one elevated test (value above the cutoff level) for LFTs and tumor markers.
Figure 1. Trendline slopes comparison between DF and metastatic patients. Mean of both groups shown for each of the tumor markers, compared by one-way ANOVA. Numerical values are given in the bottom panel.
When comparing the trends in the metastasis group with the trends in the DF group, the former group had significantly steeper increases in the levels of MIA, S-100β and LDH, whereas there was no significant difference between the two groups regarding the other markers and LFTs. Furthermore, some of the parameters had a decreasing trend. The regression analysis was performed when there were at least three visits, the last being the date of detection of metastasis in the metastasis group. In some of the cases, there were only three or four time points. We should be able to decide whether a patient needs further evaluation even though we have only three time points to study. If there is an
increase in the levels of the test over consecutive visits, should the patient be sent for further evaluation for detection of early metastasis? Perhaps, only if the slope of the trend is more than or equal to the average found in our results should the patient be sent for further testing. Another option is to ask the patient to return for follow-up earlier, within two months to re-assess the markers. If there is still an increase in levels, then the patient should be sent for imaging.

Neither these tumor markers nor LFTs are specific for liver metastasis of UM. These tumor markers may rise due to other types of carcinoma, infections, or even inflammatory disease (18, 21, 25). LFTs are also influenced by a number of causes other than metastases, such as other liver disease, alcoholism or cholesterol-lowering agents. In this study, we excluded patients who may have had another reason for elevation of markers, such as another carcinoma. But it is impossible to exclude all causes, such as simple infection or consumption of alcohol, which the patient would not necessarily report.

Our study is unique in that patients had a semi-annual follow-up of serum markers and LFTs over a time period of four years. However, our study is a retrospective study. A prospective study of consecutive measurements is needed to attempt to diagnose patients with metastatic disease by detecting increasing trends before elevation of the test above the cutoff values.

The groups of patients in this study are small. This is because there were only a certain number of patients who developed metastasis over the time period studied. The analyses performed on a small group of patients do not necessarily reflect the results from a larger group of patients. Therefore, the analyses should be repeated on a larger group of patients.

In conclusion, we show that the tumor markers studied here provide us with earlier detection of metastasis as compared with LFTs. Following the dynamics of these tumor markers for each patient may help to those with metastatic disease earlier and thus extend their survival.

References

1 Shields JA and Shields CL: Diagnosis and Management of Intraocular Tumors. St. Louis, Mosby, 1983.


