Abstract. Aim: To examine the kinetics of the tumor marker levels: osteopontin (OPN), S-100β, melanoma inhibitory activity (MIA) and tissue polypeptide-specific antigen (TPS), and to evaluate their potential for predicting earlier liver metastasis in patients with uveal melanoma (UM). Patients and Methods: Forty-three UM patients who remained disease-free (DF) for at least 10 years, 32 patients with metastatic UM and 53 healthy controls were enrolled. Median and mean levels of the tumor markers OPN, S-100β, MIA and TPS at the time periods of 0-6, 6-12, 12-18, 18-24 and >24 months prior to confirmation of metastasis by liver ultrasound, CT scan and biopsy, served in a box and whiskers analysis and were compared by Students t-test. Trends of changes in marker levels of DF and metastatic UM groups were calculated and compared by ANOVA. Results: The lead-time for predicting metastasis was: 12-18 months both for OPN (p=0.005) and MIA (p=0.37), for S-100β 18-24 months first increase (p=0.5) followed by a second one 0-6 months (p=0.01) and for TPS 18-24 months (p=0.1). The gradient of the trendlines for the metastatic group was significantly steeper for MIA (p=0.02) and S-100β (p=0.018) than for the DF group and not statistically significant for OPN (p=0.168). For TPS, the trendline was negative. The overall increase in the levels of OPN and S-100β was significant, while for TPS and MIA, it was not. Conclusion: Significant increases in OPN and S-100β levels were demonstrated by a major lead time. Trendlines of the metastasis group were steeper than of the DF group predicting liver metastasis. The routine use of those markers in the follow up of UM patients, can enable earlier diagnosis of liver metastasis and effective therapeutic intervention, with an impact on survival.

Uveal melanoma (UM) is the most frequent primary intraocular tumor in adults. It tends to spread metastases first and preferentially to the liver (1, 2). The survival rate of patients with UM based on tumor-related mortality has improved only slightly over the past few decades, in spite of a better local treatment of the intra-ocular tumor (3).

Currently, there is no effective treatment for metastatic UM (4). The metastatic tumor burden is typically high when liver metastases are detected by abnormalities in routine liver function tests or by imaging studies (5). Following metastatic disease, the median survival is about one year or less (6). The current treatment options available today are surgical resection of the metastases or a combination of resection and intra-arterial hepatic chemotherapy (3, 4).

As our group has shown recently, surgically treated patients with a clear margin resection of a single metastasis had an improved survival up to 3.7-fold higher than patients who were not operated on (4).

Therefore, there is a need to develop sensitive tumor markers to screen for and detect UM liver micro-metastases sooner than they are detected by conventional methods.

In cutaneous melanoma, a variety of molecules secreted into the blood have been proposed as markers of tumor progression (7) and serve in diagnostic tests, and were included in the staging strategy (8). In addition to the classic marker lactate dehydrogenase, the AJCC (American Joint Committee on Cancer) also introduced S-100β (8), neuron-specific enolase (NSE) (7) and melanoma-inhibitory activity (MIA) (9) as diagnostic markers.

A comparative study showed serum S-100β to be an independent prognostic marker for stage II and III melanoma. S-100β serum level changes have been reported
to reflect the increase or decrease in tumor burden quickly and were of predictive value for a positive response to chemotherapy and immunotherapy in patients with cutaneous melanoma (7, 8).

We have previously demonstrated four molecules shed into the serum to be reliable markers of metastatic UM: we confirmed prior data on MIA (10) and S-100β (11) in larger groups of patients with UM followed-up for several years. We were the first to show osteopontin (OPN) (12, 13) and TPS (14) to be sensitive markers for metastatic UM.

Levels of these 4 molecules increased significantly from the disease free (DF) state in UM patients prior to confirmation of metastasis by CT (15). Our results regarding OPN were reconfirmed recently by another group (16, 17).

In the present study, we aimed to evaluate the kinetics of changes in the levels of the four serum tumor markers and their potential to identify metastatic disease before imaging of the liver confirmed metastasis in patients with UM.

Patients and Methods

Patient serum samples. Serum samples were obtained from the Ocular Oncology Serum Bank at the Hadassah-Hebrew University Medical Center. The use of patient sera was approved by the Helsinki Committee. Thirty-two samples from patients with metastatic UM, 43 samples from UM patients who were disease-free (DF) for at least 10 years following treatment of the primary tumor, and one sample from each of 53 age and sex-matched controls were used in this study. Among the 32 patients with metastatic UM, marker levels were evaluated before and after detection of metastasis by ultrasonography and CT scan, followed by tissue confirmation by hepatic biopsy.

Blood (7 ml) was drawn from patients at the time of diagnosis of the primary tumor, 2 weeks later – after the primary treatment, and about every 6 months thereafter. After collection, blood was centrifuged for 10 min at 1200 rpm and serum was stored at −20°C.

Serum assays. Serum levels of OPN (R&D Systems, Minneapolis, MN, USA), S-100β (Dia Sorin, Stillwater, MN, USA), MIA (Roche Diagnostics, Mannheim, Germany) and TPS (IDL, Sweden) were evaluated with enzyme-linked immunosorbent assays (ELISA) kits, following the manufacturers’ instructions, as previously described (15).

Statistics. Student’s t-test was used to compare marker serum levels between the groups of patients. The sign-rank test was used for comparing serum levels before and after the development of metastatic UM in the same patients. A p-value of less than 0.05 was considered significant. Trends in changes of tumor marker levels on consecutive visits (about 6-month interval, but different for each patient) before and until the time of metastasis diagnosis, were compared with those found in the DF group, using one way analysis of variance (ANOVA). In view of the time differences for follow-up and marker level evaluations of the various patients, we used time intervals of 6 months and not exact time points. A linear regression trend-line was calculated for every patient and for each marker. The mean slope of the trend-lines of all patients in a group was calculated for each test.

Results

Levels of the four tumor markers OPN, S-100β, MIA and TPS were available for 22.5±15.8 months, ranging from 3 to 63 months, prior to the clinical detection of metastasis.

The development of hepatic metastases was accompanied by elevations in serum levels of markers in all patients, (at least one or two markers increased in each patient), as we have shown in our previous studies (12, 13).

In the present study, we demonstrated increases in the mean and median levels during each half-year period at consecutive visits of patients, as shown by box and whisker analysis: For OPN, there was an increase from 18-24 months to 12-18 months (p=0.19) and another significant increase at the 6-12 months period (p=0.005) prior to the CT scan identifying the metastasis.

For MIA, there was an increase from 18-24 months to 12-18 months (p=0.4) and to 6-12 months (p=0.37), prior to the CT scan confirming the metastasis.

For S-100β, the increase was from 18-24 months to 12-18 months (p=0.5) and a later significant increase to the 0-6 months point (p=0.01) prior to the confirming CT scan.

For TPS, the first increase was from >24 months to 12-18 months (p=0.1), and then a stabilization took place, prior to the CT confirmation of metastasis (Figure 1).

Figure 2 shows the distribution of the slopes of trend-lines in the metastasis group compared to the DF group, for each of the four tumor markers. The trendlines of the metastasis group were significantly steeper than those of the DF group for MIA (p=0.02) and S-100β (p=0.018), whereas for OPN, in spite of an increase in the mean, there was no statistically significant difference. For TPS, the mean trend line for the metastasis group showed a slight decrease in the levels (a negative slope).

Discussion

UM spreads preferentially to the liver as the first target of metastasis (1, 2).

As predictors of metastatic disease, parameters such as histological cell type, largest tumor diameter, tumor location, and specific vasculogenic mimicry patterns are often used (1, 2, 4).

Formerly, measurement of liver enzymes (5) was the standard parameter for follow up and increases in these levels raised the suspicion of metastasis. However, there are reports of patients with normal activity of liver enzymes and diffuse hepatic metastasis (18).

In our present study, we have demonstrated that the serum tumor marker levels increase during metastasis formation in most of our patients. Increased levels were detected as early as 12-18 months prior to CT scan confirmation of metastasis. Although not all median levels of the markers in patients
increased statistically significantly from one visit to another (about every 6 months), the trend of increase was evident. Of the four tumor markers, MIA and S-100β showed significantly increasing levels before the clinical diagnosis of metastasis. OPN increased as well, but the magnitude of increase as measured by the slope of the trendline, cannot be statistically differentiated from changes that were measured in DF patients. To our surprise, TPS levels decreased slightly in patients with metastasis before the clinical diagnosis of metastasis, which lowers its value as a predictive marker.

As was seen in our previous studies (12-14), our present study emphasizes that the non-invasive serum tests are able to detect hepatic metastases from UM earlier than CT, with a high sensitivity and a remarkable lead time of 6-24 months. Therefore, in recent years, we have introduced tumor marker assays as standard of care, in the semi-annual follow up of patients with UM. As our group has recently published, patients with UM who develop isolated single or several hepatic metastases and undergo resection have a 3.7-fold longer survival than those not operated on (4). These findings underline the significance of earlier detection of minimal liver metastasis which can be resected, and which can have an important impact on survival of these patients.

These results are similar to our previous findings shown in breast cancer studies, including the tumor markers CEA, CA 15-3 and TPS, where serial measurements are considered as an essential part of the follow-up of patients (19, 20). The median lead time between the increase of TPS and the development of clinically progressive disease was shown to be approximately 8 months (20). Recently, we demonstrated in a meta analysis that using a panel of the three markers CA 15-3, CEA and TPS in breast cancer patients with no evidence of disease, indicates earlier metastasis formation, enabling effective treatment with an impact on survival (21).
Figure 2. Comparison of trend-line slopes between disease-free (DF) and metastatic patients (Mets). Mean of both groups shown for each of the tumor markers compared by one-way ANOVA. Numerical values are given in the bottom panel.
In addition, in cutaneous melanoma, the S-100β marker is also widely used both for detection of metastatic disease and also for clinical staging and monitoring of treatment response (8).

Additional serum tumor markers are currently being screened by our group, to further increase the sensitivity and specificity of these tests.

Our results on the sensitivity of liver enzymes will be reported in part 2 of this paper. Briefly, we show there that the sensitivity of serum tumor markers is superior to that of liver enzymes and occurs in all patients.

In conclusion, this study demonstrates that tumor marker levels increase before the existence of metastasis can be identified by imaging. Therefore, individual significant serum marker increases, even within the ‘normal’ range of liver enzymes and occurs in all patients.

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References