Assessing Response to New Treatments and Prognosis in Melanoma Patients, by the Biomarker S-100β

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Abstract. *Background/Aim: Malignant melanoma incidence is increasing over the last years, while mortality is strongly decreasing due to improved early detection, close monitoring of patients including disease biomarkers as well as introduction of new therapies. The aim of the present study was to evaluate biomarkers, mainly S-100β in melanoma patients, regarding its ability to assess treatment response, especially to new immunotherapies (anti-BRAF, ipilimumab, anti-PD-1) and evaluation of prognosis of those patients. Patients and Methods: We evaluated both retrospectively and prospectively 137 malignant melanoma patients. Blood biomarker levels were evaluated by conventional ELISA assays. Correlations of marker levels to disease stage, metastases, response to new immunotherapies and survival, were performed. Results: Serum levels of biomarkers, mainly S-100β, were significantly higher in all patients before various therapies were applied (5.1+0.7 μg/L) and decreased thereafter (1.3+0.4 μg/L). Significantly higher levels of S-100β were demonstrated in advanced disease including metastases, (5.95+0.62 μg/L) as opposed to early disease (0.32+0.06 μg/L) and NED patients (0.18+0.03 μg/L). When comparing melanoma deceased patients who had extremely high levels of S-100β, (2.2+0.45 μg/L) we showed significantly lower levels in alive patients (0.26+0.02 μg/L) and certainly in normal controls (0.07+0.02 μg/L). In individual patients, kinetic evaluations showed earlier response to therapy, or recurrence and non-response, as shown only later by CT evaluations. Conclusion: S-100β can serve as a useful biomarker for the assessment of treatment response and prognosis, especially after using new immunological treatments, such as anti-BRAF, ipilimumab or anti-PD1 in malignant melanoma patients. Additional biomarkers, such as LDH, β2M and TK may also serve as part of a biomarkers panel, for improved detection of recurrence and metastasis of melanoma patients.*

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Key Words: Melanoma, biomarkers, treatment response, prognosis.

Melanoma is the most malignant type of skin cancer aggressively metastasizing by lymphatic spread. Its incidence is growing over the last years, however, mortality is decreasing due to campaigns for prevention, early detection, improved imaging methods and new available treatments (1, 2). After primary surgery, approximately half of patients will experience recurrence or metastases spread. Early detection of micro-metastases by proliferation markers, undetected by current imaging methods, should improve overall survival (3, 4).

Biomarkers are important tools to mainly predict and assess response to therapy in various cancer patients. Early detection of melanoma and mainly of its recurrence, by serial evaluations of biomarkers, should improve the clinical outcome, especially after the introduction of new immunological treatments, which are reported to improve survival of melanoma patients (5, 6). The most used serum biomarker over the years, was the non-specific LDH (3, 5, 6), however, additional markers, mainly S-100β and MIA, were lately studied in various combinations (7-11) and compared to PET-CTs, MRIs or USs for diagnostic purposes.

We have previously shown preliminary results on the ability of the biomarker S-100β to distinguish between cutaneous primary non metastatic melanoma and metastatic melanoma patients, characterized by high levels of S-100β (12).

We have also demonstrated the efficacy of tumor markers as S-100β and MIA (13, 14), OPN (15) to early detect liver metastases from uveal melanoma, by significant increases of their serum levels, a long time before established by CTs of those patients. Additional biomarkers shown to also be effective and prognostic in uveal melanoma patients, were TPS (16), CEACAM1 (17) and VEGF (18). Based on our preliminary results (12), we have undertaken this study, part retrospective and part prospective and included melanoma patients treated by new immunotherapies as anti-BRAF, ipilimumab or anti-PD1 (19, 20).

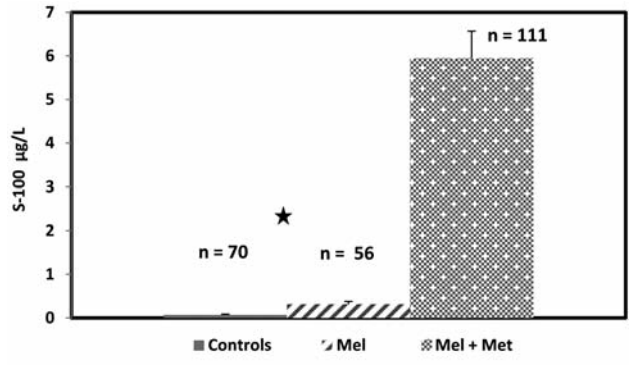

Figure 1. *S-100β levels of metastatic melanoma patients.*

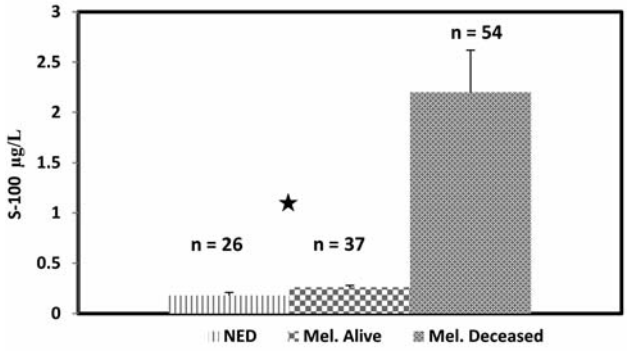

Figure 2. *S-100β levels of various melanoma patients.*

Patients and Methods

Study design. The aims of the present study were to investigate the ability of biomarkers, mainly S-100β, but also TK, β2M and LDH in melanoma patients, to assess their response to therapy and provide prognosis, following new immunological treatments. Serum levels (received after patients' blood centrifugation) of the tumor markers, S-100β mainly, compared to LDH, β2Microglobulin-β2M and Thymidine Kinase-TK (in part of patients), were evaluated by conventional ELISA assays purchased from Diasorin, Italy. Melanoma patients, n=113, were evaluated prior to and after therapy (surgery, chemotherapy, immunotherapy or their combinations) and findings were correlated to clinical status (CTs, stage, metastases and response to treatment). Overall survival was estimated and correlated to high or low initial levels of the tumor markers.

Statistical analysis. Statistical analyses were performed using the SPSS software. All parameters were correlated using *t*-test, Wilcoxon 2 sample test, Kruskal-Wallis test for comparison between groups, and a *p*-value of less than 0.05, was considered statistically significant.

Results

S-100β marker levels were significantly higher in metastatic melanoma patients (5.95+0.62 μg/L), as opposed to low levels in patients without metastases (0.32+0.06 μg/L) and in normal controls (0.07+0.02 μg/L), as shown in Figure 1.

In the retrospective study, S-100β levels were significantly higher in deceased melanoma patients, (2.2+0.45 μg/L) compared to patients alive (0.26+0.02 μg/L) and those with no evidence of disease (NED) (0.18+0.03 μg/L), which are similar and in the same range, as shown in Figure 2.

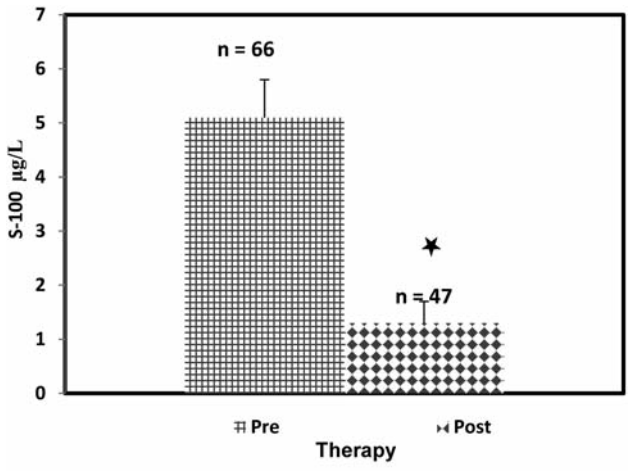

Figure 3. *S-100β levels of melanoma patients following therapy.*

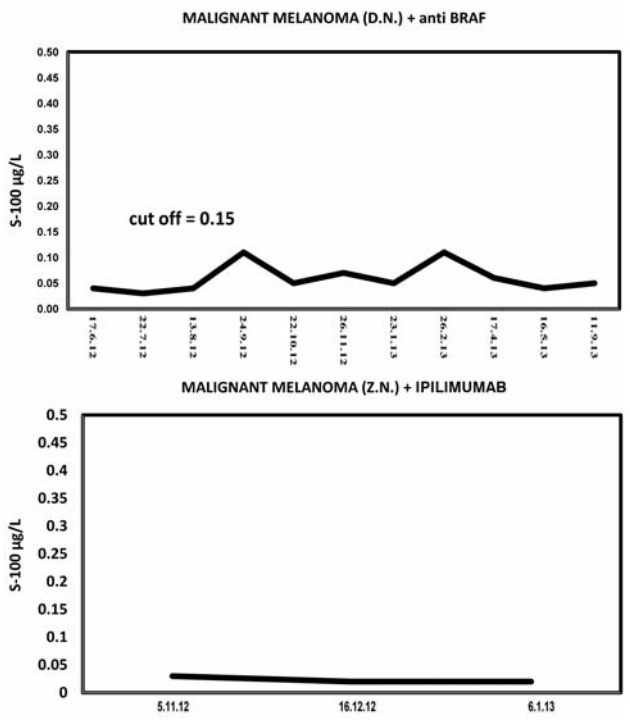

Figure 4. *S-100β levels of patients with stable disease.*

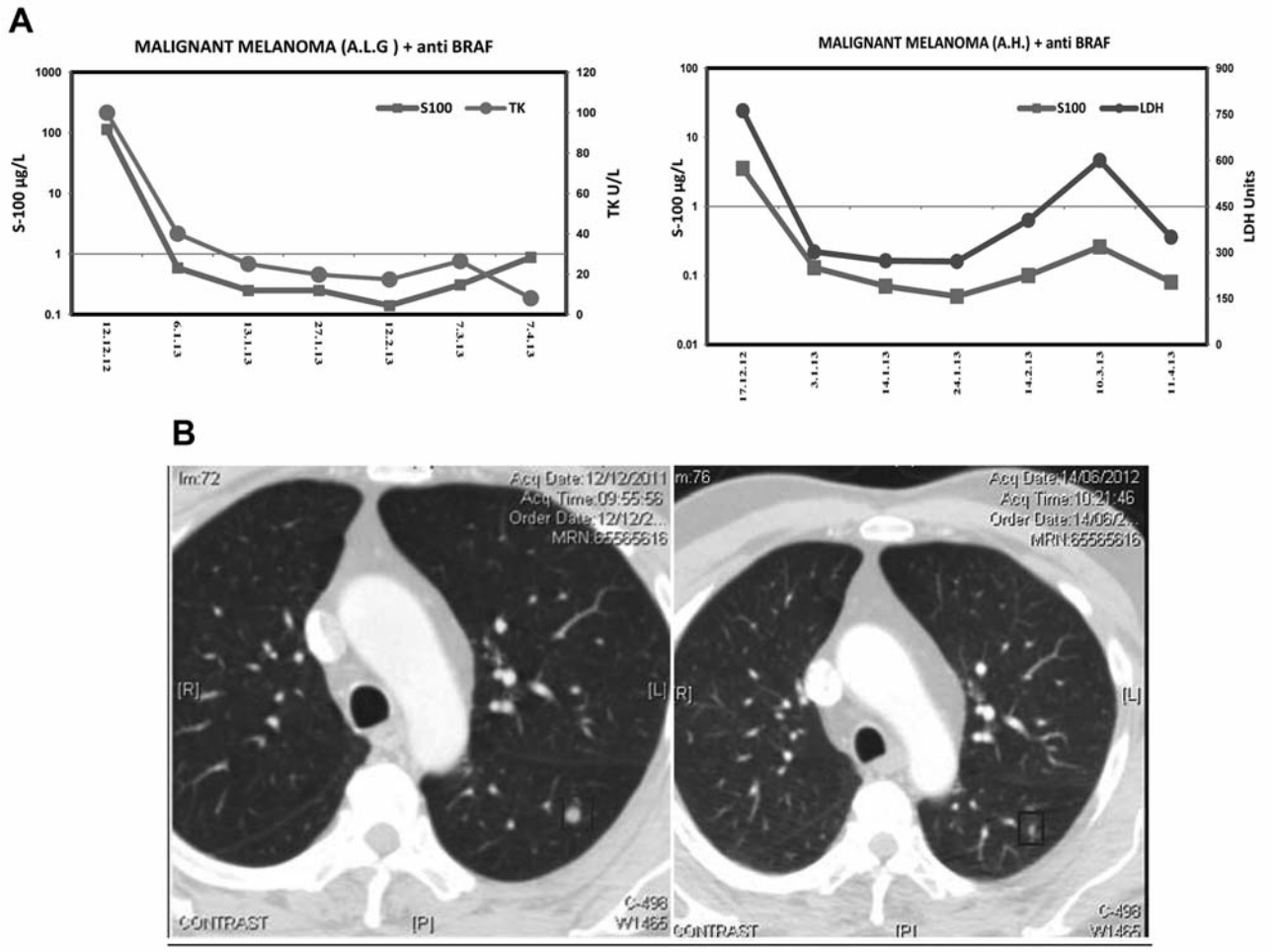

Figure 5. *A. S-100β, TK and LDH levels, in melanoma patients responding to therapy. B. Decreasing tumor volume in a treated melanoma patient.*

Comparing biomarker levels before and following the new immunological therapies Anti-BRAF, ipilimumab and anti-PD1, demonstrated statistically significant higher levels of S-100β pre-therapy, (5.1+0.7 µg/L) that decreased post-therapy (1.3+0.4 µg/L) in responding patients, as shown in Figure 3. These findings show an association of S-100β levels with tumor mass, metastases and activity of the disease, changing according to response to therapy.

Kinetic evaluations of individual patients with the biomarkers S-100β, TK, LDH or β2M, followed-up every 2-3 weeks, before every therapy cycle, are shown in Figure 4 for patients with stable disease, in Figure 5A, for responders to therapy and Figure 5B, CTs, showing decreases in tumor mass, in Figure 6A, for non-responders and disease progression, Figure 6B, CTs demonstrating enlargement of tumor mass. In some patients the trend of increases in 2 or 3 markers was similar, however, is some patients S-100β increased sharply, while LDH was relatively low (350-450 u, under the cutoff of 620 u) and did not increase, as in patient SF, who died 2 weeks following the sharp increase in S-100β levels, after the last point on our graph as shown on Figure 6B.

The significant increases in S-100β levels reflected earlier the disease recurrence, shown later by a computed tomographic scan (CT) for this patient (see raw on the graph), as shown in Figure 6B and in Figure 7.

OS of patients was correlated with high S-100β levels: 17% were alive after 2 years, while patients with low S-100β levels – 58% of them were alive after 2 years.

Discussion

In order to achieve personalized treatment for cancer, biomarkers for determining prognosis, predicting response to therapy and predicting severe toxicity related to treatment,

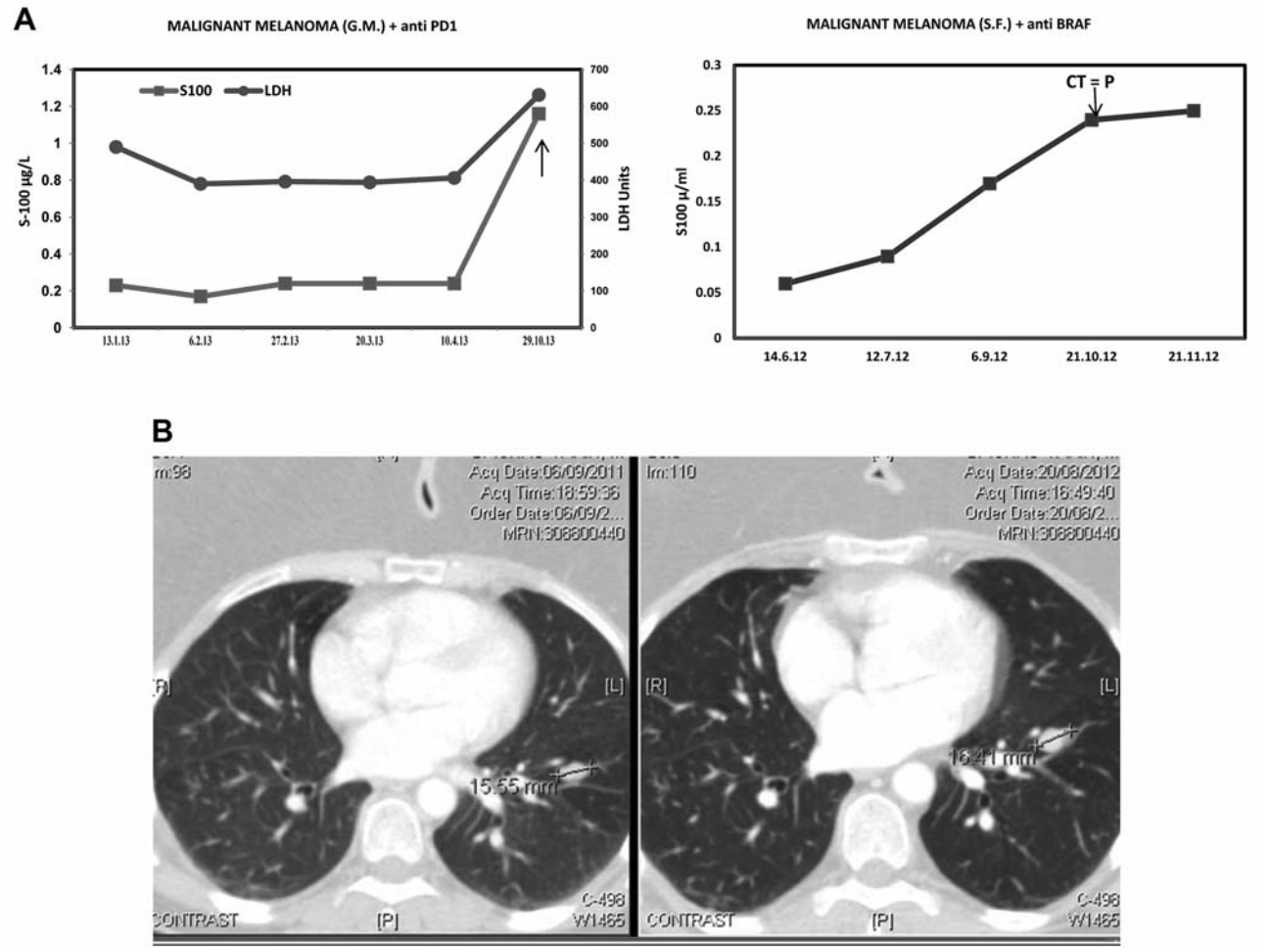

Figure 6. *A. Recurrence in melanoma patients. B. Recurrence, enlargement of tumor, by CT in a melanoma patient.*

are urgently required and expected to increase survival, due to effective and earlier treatment initiation (9, 19).

Various Guidelines for melanoma patients stratification and monitoring using biomarkers, were published and are currently used (9, 20). The NCCN recommends LDH (lactic dehydrogenase) and CBC (complete blood count) every 6 months (2). The UK Guidelines for surveillance of stage IIB, IIC or III melanoma, include chest X-ray, liver US/ CT of chest, abdomen and pelvis, LDH, liver function tests and CBC at baseline and at frequent clinical follow-up. The consensus-based German Guidelines recommend lymph node sonography, S-100β and LDH, for the routine surveillance every 3-6 months and imaging (3). The European Society of Medical Oncology (ESMO) has specific guidelines for frequency of clinical examinations, but has no consensus regarding blood tests, biomarkers or imaging techniques. The Sydney Melanoma Group recommends scheduled follow-up, without requirements of biomarkers. The European Group for Tumor Markers (EGTM) Guidelines include: S-100β mainly, but also a panel including OPN, LDH, CEACAM, β2M, TK (V Barak, In preparation).

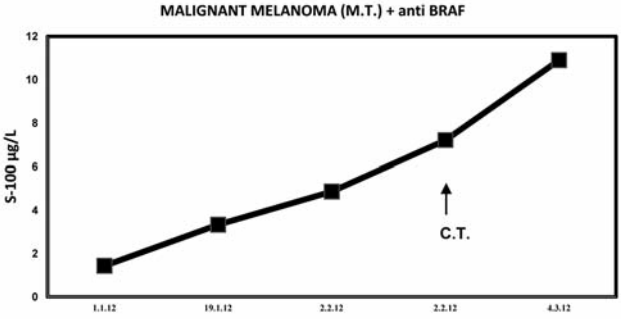

Figure 7. *S-100β levels increase significantly in a melanoma patient unresponsive to anti-BRAF.*

During recent years, new immunotherapies were introduced, including anti - BRAF, ipilimumab, anti - PD1. (1, 19, 20). Therefore, there is a great need to implement biomarkers which should provide an easy, non- invasive and cheap aid, to assess response to those treatments and prognosis of those patients – leading to an improved survival (19, 20).

Concerning our study on the relevance of the biomarkers for monitoring response to therapy, our results demonstrated statistically significant higher levels of S-100β, in all patients before therapy, but also of additional markers as LDH, TK and β2-microglobulin, in part of the patients. In most patients, significant lower levels post-therapy were demonstrated while response lasted. Significant increases in S-100β and/or other biomarkers, demonstrated clearly recurrence and unresponsiveness – which could be taken into consideration for therapy changing/planning.

Our present results are in good concordance to our preliminary observations on high S-100β levels of metastatic patients (12) and to other studies demonstrating the importance of S-100β as a stratification and surveillance marker (5, 7, 9, 11, 21, 22, 23) for melanoma patients. Many studies indicated the importance of using panels of markers, *i.e*. S-100β and MIA (11) or S-100β and LDH (3, 6, 8) for early detection of metastases formation in most melanoma patients. Both biomarkers S-100β and MIA have been shown to be independent prognosticators in melanoma patients. The addition of LDH to these two biomarkers in monitoring patients, did not increase the sensitivity of the former two markers (11). We had similar results in some patients, when only S-100β, but not LDH, increased dramatically in unresponsive patients. Our results on the effectiveness of S-100β in patients treated with anti-BRAF, are in good concordance with results of other groups (11, 23, 24).

We have also previously shown in uveal melanoma that biomarkers, mainly S-100β, MIA, OPN, are sensitive early indicators of disease progression and metastases formation, mainly in the liver (13-18). Additional cytokine markers were also proposed as effective markers in melanoma patients follow-up (25) and those results are in in good concordance to our preliminary yet unpublished results.

In conclusion, we demonstrated that S-100β is a sensitive and useful biomarker for malignant melanoma patients. Significantly higher levels of this biomarker, and also of the other markers applied in some of the patients (LDH, β2M, TK), were associated with active disease, higher stage and metastases. Significant associations were demonstrated between response to therapy (such as first surgery, chemotherapy and immunotherapies including anti-PD1, ipilimumab and anti-BRAF used in recent years) and decreases in marker levels. Significant decreases in marker levels demonstrated positive therapy effects, as also seen in the clinic and later on in patients' CTs, followed by a longer survival. In contrary, significant increases in biomarker levels were the most sensitive predictors of recurrence, metastases and poor prognosis, recorded earlier than in CTs, best correlated to a shorter overall survival. We, therefore, suggest, introducing the S-100β biomarker into routine practice, for close monitoring of melanoma patients, assessment of their response to new therapies and early detection of recurrence- enabling earlier or change of treatment, with improved survival.

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Received August 7, 2015
Revised September 11, 2015
Accepted September 14, 2015