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Clinical value of combined determination of plasma L-DOPA/tyrosine ratio, S100B, MIA and LDH in melanoma

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ABSTRACT

Aim of the study: L-DOPA/tyrosine ratio (an index of tyrosinase activity), melanoma antigens S100B and MIA, lactate deshydrogenase (LDH) and their combinations were evaluated for clinical value as tumour markers in melanoma.

Methods: Blood samples were obtained in 170 melanoma patients (stage I–II: $n = 57$, III: $n = 54$, IV: $n = 59$) at inclusion and in a sub-group of 82 subjects during follow-up for up to 4 years. Laboratory analyses were performed by HPLC (L-DOPA, L-tyrosine), immunoassays (S100B, MIA) and colourimetry (LDH).

Results: All markers, except LDH, were elevated in stage IV versus other stages. S100B and MIA highly correlated, especially in stage IV (r_s: 0.849, $p < 0.001$). The combination of L-DOPA/tyrosine ratio with S100B displayed the highest sensitivity/specificity (73/70%) to confirm stage III–IV or stage IV alone (69/75%) (ROC optimised cut-off). Only the L-DOPA/tyrosine ratio significantly increased (+36% over 5 months, $p = 0.001$) during progression from stage I–III to higher stages. S100B, MIA and LDH, but not the L-DOPA/tyrosine ratio, responded to progression towards death in stage IV. All markers exhibited a prognostic value in deceased patients ($n = 44$); S100B and MIA were the best predictors of survival time by Cox proportional-hazards regression.

Conclusion: The combination of plasma L-DOPA/tyrosine ratio and S100B appears an attractive approach for the biological follow-up of melanoma patients.

1. Introduction

A wide range of molecules has been investigated for potential use as serologic tumour marker in melanoma. They all have limited roles in screening and early diagnosis due to limited sensitivity.[1] In metastasised disease, however, specific mRNAs (like tyrosinase mRNA),[2] antigens S100B and MIA (*Melanoma Inhibitory Activity* protein)[3–8] and metabolites of the melano-

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genesis pathway (like 5-S-Cysteinyldopa and L-DOPA)[2,9–14] have demonstrated a correlation with tumour burden, successful therapy, disease progression and/or prognosis. In stage IV,[7] however, S100B and MIA seem to provide no additional useful information to the non-specific lactate dehydrogenase (LDH), with respect to clinical outcome. This marker of cell death has been introduced in the revised AJCC melanoma TNM staging system (M3: elevated serum LDH).[15,16]

The aim of this prospective study was to evaluate four serologic markers for clinical value in melanoma: the L-DOPA/tyrosine ratio, melanoma antigens S100B and MIA, the non-specific LDH, and their different combinations. Their concentrations were measured in the plasma of 170 stage I–IV melanoma patients at inclusion and for a sub-group of them ($n = 82$) during follow-up for up to 4 years. Results are interpreted with regard to disease stage and progression, presence and number of metastases, and survival.

2. Material and methods

2.1. Patients and blood sampling

A group of 170 melanoma patients (91 males, 79 females, median age: 58 years, 25th–75th percentiles: 50–69 years) diagnosed between 01/2000 and 12/2004 in the dermatology departments of Saint-Louis hospital (Paris, France) prospectively entered this study. Exclusion criteria were: absence of staging at inclusion, haemolysed blood samples, and one or more serologic tumour marker missing.

Staging was performed using the tumour-node-metastasis (TNM) system of the American Joint Committee on Cancer Classification (AJCC).[15] At inclusion, there was 57 stage I–II 'localised melanoma' (T1-4N0M0), 54 stage III 'regional metastases' (anyTN1,2M0), and 59 stage IV 'distant metastases' (anyT,anyN,anyM). In 35 stage IV patients, the number and anatomic localisation of metastases was clearly identified by imaging techniques. A blood sample was drawn by venous puncture in 7 mL glass tubes with lithium heparinate as an anticoagulant (Becton Dickinson, Meylan, France). Blood was centrifuged (3000*g* for 10 min at +4 °C) and plasma separated in two aliquots stored at −80 °C before analysis.

Patients were treated by tumour excision, lymphadenectomy, immunotherapy (interferon) or chemotherapy according to their disease stage and progression.[17] Follow-up (for up to 4 years) was obtained in 82 subjects including clinical examination, abdominal ultrasounds, chest X-rays, CT-scans, standard chemistry blood tests and tumour markers (total number of blood samples: $n = 331$). In stage I–III, disease progression was defined as a move to higher stage(s); impact on tumour markers was evaluated within the shortest blood sampling interval available (median: 150 days, 25th–75th percentiles: 106–245). Patients receiving treatments at the time of sampling (chemotherapy: $n = 7$, immunotherapy: $n = 1$) were excluded from this analysis. Marker levels in stable disease (no change from initial staging) were assessed within the longest sampling interval (329 days, 147–523). In stage IV, influence of disease progression and stability on serologic markers was evaluated using the last two blood samples before death (interval: 84 days, 28–284) and the longest preceding period within this stage (99 days, 39–312), respectively. A total of 44 patients deceased from melanoma within the study period (survival time from inclusion: 260 days, 55–429).

This study was in accordance with the ethical standards of the Helsinki declaration of 1975 as revised in 1983; an informed consent was obtained from each patient.

2.2. Serologic marker analysis

Within 2 months from sampling, analysis were performed in a blind fashion between the two laboratories measuring L-DOPA, L-tyrosine, LDH, and melanoma antigens (S100B, MIA), respectively.

2.2.1. L-DOPA and L-tyrosine

Serologic L-DOPA and L-tyrosine analysis have been previously developed in our laboratory.[13] Briefly, 1 mL of plasma is treated by alumina extraction for L-DOPA and 1 mL deproteinised by 1 M trichloracetic acid for L-tyrosine. Separation is obtained by HPLC (515 HPLC pump, Waters, Milford, USA) on a C_{18} reversed-phase analytical column (150 × 4 mm internal diameter) filled with 5 μm Lichrospher particles (Merck, Darmstadt, Germany). L-DOPA is measured using a 5100 A coulometric electrochemical detector equipped with an analytical cell operating in oxidative mode (potential set at +0.35V) (ESA, Bedford, USA). L-tyrosine is measured using a RF 535 fluorimetric detector (Shimadzu, Kyoto, Japan) (excitation at 275 nm, emission at 305 nm). All procedures are carried out at room temperature with a total analysis time of about 3 h. Method precision was: CV <2.5% (intra-assay) and <4.6% (inter-assay) for L-DOPA and L-tyrosine with a detection limit of 0.25 nM for L-DOPA and 2.5 nM for L-tyrosine.[13] The L-DOPA/tyrosine ratio is calculated for each sample and the upper normal cut-off derived from a group of 35 healthy subjects is 16.0×10^{-5}.[13]

2.2.2. Melanoma antigens

S100B plasma concentration was measured using the monoclonal two-site immunoluminometric assay LIA-mat® Sangtec® 100 (Sangtec Medical, Stockholm, Sweden) on a Berilux 400® analyser (Dade-Behring, Eschborn, Germany).[12,14] According to the manufacturer, the detection limit is 0.02 μg/L, the calibration curve is linear until 20 μg/L and the upper normal cut-off is 0.12 μg/L.

Melanoma Inhibitory Activity (MIA) protein was measured in plasma using a quantitative ELISA kit (Roche Diagnostics, Mannheim, Germany). Absorbance was measured in duplicates at 405 nm on a microtitre plate reader. According to the manufacturer, the detection limit is 0.5 μg/L, the calibration curve is linear until 50 μg/L and the upper normal cut-off is 7.5 μg/L.

2.2.3. Lactate dehydrogenase

LDH activity was measured by a colourimetric assay adapted on a Modular® multi-parametric analytical system (Roche Diagnostics, Meylan, France). According to the manufacturer, the upper normal cut-off is 439 UI/L.

2.3. Statistical analysis

Statistical analyses were conducted with Sigmastat® (Jandel Scientific, San Jose, USA) and Medcalc® (Medcalc, Mariakerke,