Human Mammaglobin Transcript Amplification for Differential Diagnosis in a Breast Cancer Metastatic to Dura Mater

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Abstract. Background: In breast cancer (BC), metastases to the central nervous system usually arise in women with advanced disease. Diagnosis of leptomeningeal (LM) metastasis is based on neurological symptoms, imaging studies and cytological detection of malignant cells in the cerebrospinal fluid (CSF). However, often these approaches are not sensitive enough to recognize leptomeninges involvement and subsequently to make a diagnosis of LM carcinomatosis. This study investigated the employment of reverse transcriptase-polymerase chain reaction (RT-PCR) for the human mammaglobin (hMAM) gene in a case of BC with cerebral metastases in which the involvement of the leptomeninges was in doubt. Materials and Methods: Amplification of hMAM mRNA was performed from CSF cells by RT-PCR. Results: No amplification of hMAM was obtained from the CSF cells. Conclusion: RT-PCR for human mammaglobin mRNA of the CSF in BC patients with brain metastases may aid clinical determination of LM involvement and consequently the choice of the most effective therapy regimens for affected patients.

In breast cancer (BC), metastases to the central nervous system (CNS) usually arise in women with advanced disease and cause substantial morbidity and mortality. They may occur either within the brain parenchyma, along the leptomeninges (LM), or both. The estimated incidence of clinically evident brain metastases in BC ranges from 10% to 16%, although autopic data suggest that it may be as high as 30% (1, 2). Most likely, this difference is linked to occult asymptomatic lesions in the former group. LM involvement is less common than parenchymal metastasis, with an estimated frequency in BC patients of 2-5% and 3-6% in clinical and autopic series, respectively (3, 4). In patients with primary extraneural malignancies, the intracranial dural compartment can also be involved and the percentage of dural metastases is reported to be comparable to those of the LM. In retrospective studies of BC patients with brain metastases, 78% had multiple intracerebral metastases, 14% had a single metastasis and the remaining 8% had LM metastasis (5). In another retrospective evaluation, the percentage of patients with a solitary metastasis was higher (24%), while typically 4% of them had both parenchymal and LM metastases (6).

CNS metastases can be considered a marker rather than a cause of the limited survival characteristic of the BC patient affected by brain metastases. The prognosis of women with multiple brain metastases is poor compared to those with a solitary lesion. Indeed, for the latter, more therapeutic options are available and include stereotactic radiotherapy and surgery. Nevertheless, despite modern therapeutic approaches LM carcinomatosis remains largely incurable, with a median survival of 12 weeks (7, 8).

Usually, the diagnosis of CNS metastases is based on clinical data (neurological symptoms), supported by magnetic resonance imaging (MRI) and computed tomography (CT). However, often these approaches are not sensitive enough to recognize LM involvement and subsequently to make a diagnosis of LM carcinomatosis. In addition, it is sometimes not easy to determine a diagnosis of parenchymal metastasis rather than LM or vice versa solely by the neurological clinical symptoms.

Despite the use of MRI, cytological detection by haematoxylin-eosin and/or Papanicolaou staining of cytospins of malignant cells in cerebrospinal spinal fluid (CSF) is considered crucial for the final diagnosis of LM carcinomatosis.

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(3, 9). CSF cytology shows higher specificity (about 100%) than MRI analysis (about 77%). However, the false-negative rate of CSF cytology in LM diagnosis is 50% with one lumbar puncture and is mainly due to the low sensitivity of the assay (65% sensitivity increasing to 80% with a second CSF examination) (4, 7).

In recent years, in order to enhance the diagnostic sensitivity of cytology, reverse transcriptase polymerase chain reaction (RT-PCR) amplification of specific tumour cell mRNA transcripts has been proposed if a target cancer-specific gene is available (10, 11). Among the different markers tested in RT-PCR, the human mammaglobin (hMAM) gene appears to be, at present, one of the most promising for detecting micrometastases in BC patients (12).

The hMAM gene was cloned and characterized by Watson and Fleming in 1996 (13). hMAM expression has been used for the indirect detection of micrometastasis in the peripheral blood (PB), lymph node and bone marrow of BC patients, emerging as a specific and highly sensitive marker for the detection of BC cells (12).

We have previously developed a sensitive (one BC cell per 106 normal haematopoietic cells) and specific nested RT-PCR assay for amplifying the hMAM mRNA transcript (14). hMAM RT-PCR assay has also been applied in pleural effusion (15, 16) and increased the BC cell detection rate by 32% compared to cytology, thus confirming that the molecular techniques used for the search of tumour cells are more sensitive than classical cytology (11).

We have also proposed hMAM mRNA amplification in the search for metastatic cells in the CSF of a BC patient with suspected LM carcinomatosis (17). Here, the case of a patient with a recent history of ductal BC who developed neurological symptoms 15 months after surgery and in whom, in addition to classical tests, hMAM mRNA amplification of CSF cells was performed, is described.

In this work, we discuss the fact that this hMAM molecular marker helped to exclude concomitant LM involvement from the final diagnosis of a dural/pachymeningeal metastasis of the present BC patient.

Patient and Methods

The study was approved by the Institutional Ethics Committee and the patient provided informed consent. A 57-year-old postmenopausal woman was investigated for the presence of nodules in her left breast in February 2008. Bilateral mammography, positron-emission tomography (PET)/CT scan and MRI demonstrated large tumor masses located in both the left and right upper quadrant of her breasts (5 cm and 3.5 cm, respectively; not shown). In addition, diffuse metastases in the skeleton were also present.

Histopathology and immunohistochemistry. Cytological examination of CSF sample obtained from the BC patient was performed after conventional Papanicolaou staining. Immunohistochemistry, histology and fluorescent in situ hybridization (FISH) were carried out on 5-μm paraffin sections from breast biopsy samples. Immunohistochemistry (IHC) was performed using a Pathway kit (Ventana Medical Systems, S. A. Strasbourg, France) and the Benchmark XT system (Ventana Medical Systems). The antibodies employed were: clone SP1 for oestrogen receptor (ER), clone 1E2 for progesterone receptor (PGR), clone 30-9 for proliferating nuclear antigen Ki-67/MIB-1 (Ventana Medical Systems).

The HER2 status was assayed using a PathVysion® HER-2 DNA Probe kit (Abbott Laboratories, Downers Grove, IL, USA) as recommended by the manufactures. HER-2 was scored according to the HercepTest guidelines (18).

Both right and left tumour biopsies were found to express the ER and the PGR and about 50% of the cells showed the Ki-67/MIB-1 (not shown). Amplification of the HER-2 gene by FISH was negative in both breast tumour masses (not shown).

The patient received 4 cycles of neoadjuvant CEF chemotherapy (cyclophosphamide 500 mg/m², epirubicine 90 mg/m² and 5-fluorouracil 500 mg/m² on day 1, q3w). In July 2008 the patient underwent bilateral mastectomy. Histological and immunohistochemical analyses of samples from both breasts confirmed the previous diagnosis of a grade III multifocal ductal infiltrating BC. A bone marrow biopsy revealed the presence of metastasis from BC. After surgery, hormonal therapy ( exemestane 25 mg/day) was provided plus zoledronic acid 4 mg i.v. every 4 weeks.

Fifteen months after diagnosis, the patient came to our observation because of various neurological symptoms: diplopia, amblopia in the left eye, paraesthesia, hemiparesis and multiple palsy of the cranial nerves. Since the patient suffered diplopia, which is considered the most common symptom of cranial nerve dysfunction, clinical suspicion of leptomeningeal involvement could not be excluded. In order to acquire more information, an MRI was performed. Neuroimaging revealed an evident enhancement of the dura mater along the cerebral hemispheres and appreciable tissue thickening with some focal nodularity (see Figure 1). There was no substantiation of lesions of the cerebral parenchyma. However, since very mild involvement of the leptomeninges usually does not give reliable imaging, an LM carcinomatosis could not be excluded solely by the MRI. In addition, a clear involvement of cranial bone structures was evident and subsequently, a total body scan confirmed a diffuse infiltration of the bones by cancer cells.

CSF examination and cells. In order to explore the issue of possible LM involvement, a lumbar puncture was performed and CSF was withdrawn for diagnostic purposes and cytochemical indices (cell count, glucose and protein levels). After centrifugation at 1,700 rpm for 10 min, cytological analyses were performed in a routine setting. Mild proteinorrachia (4 g/l) was evident and subsequently, a total body scan confirmed a diffuse infiltration of the bones by cancer cells.
amplifications were performed by Platinum Taq DNA polymerase (Invitrogen) using primers specific for hMAM mRNA and reaction conditions as reported elsewhere (14, 15).

As internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene amplification was carried out for each cDNA sample under conditions identical to those for the first hMAM amplification. The PCR products were separated by standard electrophoresis on 1.5% agarose gel containing ethidium bromide. The MDA-MB415 BC cell line (ICLC, National Institute for Cancer Research, Genova, Italy) was used as the hMAM-positive control. The hMAM-positive samples were confirmed in a second RT-PCR experiment.

Results

The CSF cells of the patient were negative for amplification of hMAM, whereas the MNC from PB of the patient were positive for hMAM expression (Figure 2).

Consequently, a final diagnosis of dural/pachymeningeal metastases with multiple cranial nerve deficits was stated. The patient’s condition improved and the neurological symptoms disappeared. To date (December 2010), the patient is still alive and continuing on hormonal therapy.

Discussion

hMAM transcripts have previously been successfully found in the CSF of a BC patient with LM carcinomatosis (17) and an additional three samples from similarly diagnosed BC patients (Figure 2), but were not expressed in 13 CSF samples from various pathologies (not shown), suggesting that hMAM is a potentially specific and sensitive test to identify BC cells. In addition, the hMAM amplification approach for the detection of malignant cells also helps in overcoming problems related to the number of cells obtained and the repeated CSF samples needed for LM carcinomatosis diagnosis.

The BC patient analysed in this study had come to our attention for a number of neurological symptoms; moreover, the MRI showed thickening of the meningeal layer of the dura and an involvement of the cranial bones, confirmed by the total body scan. However, since BC patients are known to be prone to developing LM carcinomatosis, it was important
to exclude a diagnosis of concurrent LM metastases, since this would have important therapeutic and prognostic implications. Moreover, since a very mild involvement of the leptomeninges usually would not give reliable imaging, LM carcinomatosis could not be excluded solely by MRI. The hMAM amplification test was therefore used in an attempt to improve the diagnostic accuracy.

The CSF sample proved negative for amplified hMAM mRNA. In contrast, the PBMNC were found to express the hMAM gene transcripts (Figure 2), thus implying that the negative finding of the CSF was not due to the absence of hMAM gene expression as has been reported for about 5% of BC (12).

These findings assisted the clinician in excluding tumor spread into the leptomeninges in this BC patient with extensive bone disease and dural metastases. The hypothesis that the solid tumour cells can reach the leptomeninges via direct extension from dural metastases is not unlikely (19). Leptomeningeal involvement in BC patients has important consequences because the survival rate is very low (4, 5). However, BC is also known to be a solid tumour that responds best to therapy and the survival rate can be raised to 4 months with chemotherapy. Thus, certainty of diagnosis may help the clinician in predicting patient prognosis and deciding the therapeutic protocol (supportive comfort care vs. aggressive therapy). Indeed, in contrast to LM carcinomatosis, the median survival in cancer patients with sole intracranial dural metastases was reported to be 9.5 months which compared favorably with the 2-4 months for LM metastases (7, 8). Of note, the current patient was still alive 34 months after diagnosis.

In conclusion, hMAM mRNA amplification is a highly sensitive test for the diagnosis of LM involvement in BC metastatizing to the brain, adding new insights in the difficult diagnostic field of brain metastases and may represent a significant advantage for the clinician. Further studies on a large series of BC patients are needed to validate its usefulness in clinical routine.

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