EVALUATION OF BONE MARROW BIOPSY IN THE DETECTION OF MICROMETASTASES IN BREAST CANCER

Thesis
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Title of the thesis

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Summary:

Key words: Breast cancer, BM micro-metastases, BM disseminated tumor cells, BM Minimal residual disease.

The present study included 51 female patients divided in to two groups: patient and control groups. The patient group composed of 36 primary, apparently non metastatic [stageI, II or III] breast cancer patients, and none of them had received chemotherapy before, while the control group composed of 15 female cases with NHL were included in this study as control group. BMA and Biopsy were performed, CA15.3 levels were measured in both serum and BMA plasma for both patients and control group. BMB sections were stained with both conventional H&E stain as well as immunohistochemically using a panel of monoclonal antibodies directed against Cytokeratin, Mammaglobin and CA15.3 antigens. Cases were considered positive for BM micro-metastases when at least 2 monoclonal antibodies are positive. Morphological examination detected only 4 of 36 [11.1%] cases positive for micro-metastases. IHC staining detected 9 of 36 [25%] cases positive for CK, 15 of 36 [41.7%] cases positive for CA15.3 and 11 of 36 [30.6%] cases positive for MAM McAbs. Total interpretation of IHC staining detected 13 of 36 [36.1%] positive for micro-metastatic breast cancer cells. No significant correlation between BM micro-metastases and regional lymph node involvement. BMB was superior to BMA in the detection of BM-DTCs. The sensitivity of BMB for DTC detection was enhanced by multi-IHC staining of BMB sections. Although there was correlation between serum and BMA plasma CA15.3 levels in both patients and controls group, there was no correlation between neither serum nor BMA plasma CA15.3 levels and the totally interpreted BM micro-metastases with breast cancer cells.

عنوان الرسالة

تقييم عينات النخاع العظمي في اكتشاف الانتشار المبكر لسرطان الثدي

ملخص الرسالة

الكلمات الدالة: سرطان الثدي ، انتشار مبكر في النخاع

تم اجراء الدراسة على ٣٦ مريضة سرطان الثدى فى المراحل الاولى قبل اخذ أى علاج و تضمنت الدراسة ١٥ مريضة بسرطان الغدد الليمفاوية كمجموعة التحكيم. تم سحب و أخذ عينات من النخاع العظمى و سحب عينات فى كل من الدم و بلازما النخاع العظمى CA15.3 من الدم لعمل صورة دم كاملة و قياس دلالات الاورام ومقارنة النتائج فى كل منهما و مقارنتهما مع نتائج مجموعة التحكيم. قطعت عينات النخاع العظمى و تمت كما تم صبغ شرائح النخاع بطريقة الصبغات المناعية للأنسجة و [H&E] صبغتها بالصبغات العادية للأنسجة و Cytokeratin- Mammaglobin and CA15.3 :ذلك باستخدام مجموعة من الأجسام المضادة وهى ذلك للكشف عن خلايا الثدى السرطانية منتشرة فى النخاع.

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LIST OF ABBREVIATIONS

Abs	Antibodies
AJCC	American Joint Committee on Cancer
AML	Acute myeloid leukemia
ALN	Axillary lymph node
ASCO	American Society of Clinical Oncology
ASIS	Anterior superior iliac spine
BC(s)	Breast cancer(s)
BCCs	Breast cancer cells
BM	Bone marrow
BMA	Bone marrow aspiration
BMB	Bone marrow biopsy
BMM	Bone marrow micrometastases
CA15.3	Carcinoma antigen 15-3
CAP	College of American Pathologists
CEA	Carcino embryonic antigen
CGH	Comparative genomic hybridization
CIS	Carcinoma in situ
CK(s)	Cytokeratin(s)
CML	Chronic Myeliod Leukemia
CR-TM	Cancer related thrombotic microangiopathy
CTCs	Circulating tumor cells
DAB	Di-amino-Benzidine
DCIS	Ductal carcinoma in situ
DTCs	Disseminated tumor cells
ECD	Extracellular domain
EGFR	Epidermal growth factor receptor
ELISPOT	Enzyme linked immunospot
EMA	Epithelial membrane antigen
EPISPOT	Epithelial immunospot
ER	Estrogen receptor
FC	Flowcytometry

FDA	Food and Drug Administration
FISH	Fluorecent IN Situ Hybridization
FM	Fluorecent microscope
GVHD	Graft versus host disease
HCL	Hairy cell leukemia
HD	Hodgkin`s disease
HER-2/ECD	HER-2/extracellular domain
HRP	Horse raddish peroxidase
ICC	Immunocytochemistry
IDC	Invasive ductal carcinoma
IGF-1	Insulin like growth factor receptor -1
IHC	Immunohistochemistry
ILC	Invasive lobular carcinoma
IMF	Idiopathic myelofibrosis
IMS	Immunomagnetic selection
LCIS	Lobular carcinoma in situ
LEA	Leucoerythroblastic anemia
LNs	Lymph nodes
MAHA	Microangiopathic hemolytic anemia
MAM	Mammaglobin
McAbs	Monoclonal antibodies
MEIA	Micro-particle enzyme immunoassay
MHC	Major Histocompatibility Complex
MM	Multiple myeloma
MRD	Minimal residual disease
MUC 1	Mucins
MUP	4-Methyl-umbelliferyl phosphate
NCDB	National Cancer Institute Data Base
NHL	Non Hodgkin`s lymphoma
NOS	Not otherwise specified
PB	Peripheral blood
PBS	Phosphate buffer saline
PC	Primary chemotherapy
PCR	Polymerase chain reaction
PIP	Prolactin inducible protein

PR	Progesterone receptor
PSIS	Posterior superior iliac spine
qRT-PCR	Quantitative RT-PCR
RS	Reed Sternberg cells
RT-PCR	Reverse transcription polymerase chain reaction
SD	Standard deviation
SLN	Sentinel lymph node
SPSS	Statistical Package for social science
TM	Thrombotic microangiopathy
TNM	Tumor Node Metastases
TTP	Thrombotic thrombocytopenic purpura
UICC	International Union against Cancer
US	United States
VWF	Von Willebrand factor

INTRODUCTION

Breast cancer is considered a systemic disease in which a hematogeneous tumor cell dissemination, essentially to bone marrow [the host organ for breast cancer metastases], may occur at very early stages of primary tumor development and form an occult isolated tumor cells, that subsequently lead to an overt metastases (Pantel et al, 1999). Disseminated tumor cells, the most precise term are also described by several synonyms as bone marrow micrometastases or minimal residual disease (Vincent-Salmon et al, 2008). Micrometastases are microscopic [smaller than 2 mm] deposits of malignant cells that are segregated spatially from the primary tumor and depend on neovascular [angiogenesis] formation to propagate (Kell et al, 2000).

The presence of bone marrow disseminated tumor cells at primary diagnosis of breast cancer is a strong independent predictive and prognostic factor for unfavorable clinical outcome (Molino et al, 1997 ; Braun et al, 1998 ;Mansi et al, 1999 ; Braun et al, 2000 ; Muller &Schilmok, 2000 ; Simmons et al, 2000 ; Solakoglu et al, 2002 ; Schleiermacher, et al, 2003 ; Braun et al, 2005 ; Muller &Pantel, 2005 ; Landys 2006 ; Bidard et al, 2007 ; Xenidis et al, 2007 ; Fehm et al, 2008).

Bone marrow biopsy has been suggested as independent prognostic tool to improve staging in patients with breast cancer. Ultrastaging of breast cancer patients may identify a substantial subgroup of lymph node -/bone marrow- patients who may not require adjuvant chemotherapy as well as lymph node-/bone marrow+ patients with a decreased survival who may need more aggressive therapies (Fortunato et al, 2006).The current challenge for pathologists is to improve and standardize early detection of disseminated tumor cells. Immunocytochemistry currently remains the gold standard for bone marrow disseminated tumor cells detection with a sensitivity ranging from 1 disseminated tumor cell in 10^5 to 1 in 10^6 leucocytes (Vincent-Salmon et al, 2008).

Several markers have been used to detect disseminated tumor cells in the bone marrow of breast cancer patients (Stathopoulo et al, 2002). To date Cytokeratins have become the most widely accepted protein marker for the detection of epithelial tumor cells in bone marrow (Braun et al, 2000).

Mammaglobin is a breast [cancer] specific marker (Lacroix 2006), that is almost expressed in breast epithelial cells. It is overexpressed in a subset of 70% - 80% of primary and metastatic breast cancer tissues and can be used for identification of breast cancer cells in bone marrow (Zehenter et al, 2004).

Serum CA15.3 tumor marker has a prognostic relevance in early stage breast cancer with an elevated level at the time of primary diagnosis could be a predictor of worse out come at both univariate and multivariate (Ebeling et al, 2002) analysis

Immunocytochemical detection of disseminated tumor cells in the bone marrow of patients with primary breast cancer at surgery will certainly play a role in the near future for risk stratification and monitoring of therapeutic efficacy in clinical management of patients with breast cancer (Vincent-Salmon et al, 2008).