Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Criteria derived from serum markers can precisely evaluate axillary status in breast cancer patients

Hangyu Wan, MD,[a,1] Zhenggui Du, MD,[a,b,c,1] Quanyi Long, MD,[a,b] Qing Lü, MD,[a,b] and Hongjiang Li, MD[a,b,*]

[a] Department of Breast Surgery, West China Hospital, Sichuan University, Chengdu, China
[b] Laboratory of Breast Disease, West China Hospital, Sichuan University, Chengdu, China
[c] Laboratory of Pathology, West China Hospital, Sichuan University, Chengdu, China

ARTICLE INFO

Article history:
Received 19 June 2016
Received in revised form
3 August 2016
Accepted 26 August 2016
Available online xxx

Keywords:
Breast cancer
Axillary lymph node metastases
Sentinel lymph node biopsy
Chemokine ligand
Hepatocyte growth factor
Matrix metalloproteinase

ABSTRACT

Background: A noninvasive method to confirm the presence of lymph node metastases (LNM) in breast cancer patients is lacking. This study aimed to identify markers from peripheral blood that have diagnostic value in evaluating axillary LNM.

Methods: We tested 26 factors in serum from 57 patients with resectable breast cancer by the Luminex assay. Differences between node-negative and node-positive patients were assessed. The diagnostic value of the factors was determined by further analyses and a validation test.

Results: Matrix metalloproteinase-1, hepatocyte growth factor, and chemokine ligand 5 were independent risk factors for LNM. However, receiver operating characteristic analysis showed that these factors alone were not ideal predictors. The LNM score (LNMS), derived from combining these markers, correlated significantly with numbers of positive lymph nodes. Patients with LNMS of 0 had few LNM, axillary lymph node dissection (ALND) could be avoided, and sentinel lymph node biopsy (SLNB) was unnecessary. Very high accuracy was achieved for patients with LNMS of 1 with SLNB using only methylene blue, patients with LNMS of 3 required ALND, and patients with LNMS of 2 needed SLNB using both a radioactive isotope and methylene blue, and ALND.

Conclusions: The LNMS derived from matrix metalloproteinase-1, hepatocyte growth factor, and chemokine ligand 5 serum levels identified the axillary lymph node status with high accuracy. Patients with higher LNMS had a greater probability of LNM.

Introduction

Axillary lymph node metastases (ALNM) exist in a proportion of women diagnosed with breast cancer.[1] In both lymph node-negative (NN) and lymph node-positive (NP) breast cancer patients, a randomized clinical trial (NSABP B-04) showed that axillary lymph node dissection (ALND) was not superior to axillary radiotherapy with respect to disease-free survival, distant disease-free survival, and overall survival.[2] Therefore, the value of ALND has shifted from its therapeutic effect to identifying the axillary status that significantly influences postoperative therapies and the prognosis.[3,4]

Sentinel lymph node biopsy (SLNB), using a radioactive isotope and methylene blue dye, can evaluate the axillary

* *Corresponding author*. Department of Breast Surgery, West China Hospital, Sichuan University, 37 Guoxue Street, Chengdu 610041, China. Tel.: +86 28 85422921; fax: +86 28 85422467.
E-mail address: lihongjiang@sohu.com (H. Li).
[1] These authors contribute equally to this study.
0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2016.08.086

lymph node status precisely.[5,6] However, the difficulty of this procedure, and the need for a nuclear medicine department, has limited its availability.[7] In general, SLNB using only the blue dye injection is more viable for clinicians; however, there is a relatively high false negative rate (approximately 20%) that has limited its widespread adoption.[8,9] Therefore, ALND is still performed at most breast cancer centers in China and other developing countries because it remains the easiest way to evaluate the axillary lymph node status of patients.[10-12] However, ALND may cause many complications including lymphedema,[13] axillary web syndrome,[14] sensory morbidity, and infection. Thus, finding a simpler and less harmful method to evaluate the axillary lymph node status is important.

The epithelial-mesenchymal transition (EMT) is regarded as the initial step of invasion and metastasis of cancer cells.[15] Recent studies showed that cytokines, inflammatory mediators, and some other substances found in the tumor microenvironment, play significant roles in the EMT process.[16,17] Matrix metalloproteinases (MMPs), that can degrade the extracellular matrix (ECM) surrounding the tumor and affect growth signals, are also essential to the EMT process.[18,19] Therefore, we speculate that, during the EMT and the subsequent steps leading to cancer cell LNM and other distant metastases, some substances must be released from tumor cells and the surrounding microenvironment to peripheral blood, inducing changes in the types and quantities of these components. If this hypothesis is correct, we should be able to detect differences in the quantity of these components in the blood between individuals with and without ALNM. This information may be helpful to find a new way to diagnose ALNM.

However, the number of different components varying during EMT is large, and it is difficult to determine the quantity of these components individually. Luminex liquid protein chip technology features many advantages over other techniques including high-throughput, flexibility, rapidness, and multiplexing ability.[20,21] Thus, the present study used this technology to identify serum predictors with diagnostic value in LNM before surgery. In addition, we also conducted a validation test to confirm the model's clinical applicability.

Patients and methods

Patients

From May to September 2015, 431 consecutive breast cancer patients received surgical therapies at the Department of Breast Surgery, West China Hospital of Sichuan University. The exclusion criteria include: proved positive lymph node; pathology type were carcinoma *in situ* or other special histology types; received any therapies for tumor before; chose SLNB or breast conserving surgery; with diseases that may affect the levels of serum cytokines (e.g., immune diseases, inflammatory diseases, hepatitis, tumor history, and long-term medication use). After exclusion, 157 patients were enrolled in this study. Fifty-seven patients were assigned to derivation group and 100 patients for validation group (Fig. 1). The study protocol was approved by the Ethics Committee of Sichuan University and relevant institutions for the use of human subjects in research. Written informed consent was obtained from all patients in this study.

Peripheral blood collection and preservation

Venous blood samples (2 mL) were collected from all patients 1 d before surgery and placed in serum separator tubes. The tubes were left at room temperature for 30 min to clot and then centrifuged for 15 min at 1000 × *g* to obtain serum. Serum was stored at −80°C until analysis. Repeated freeze-thawing was avoided before the test. Blood samples used for the carcinoembryonic antigen and CA15-3 tests were collected at the same time but analyzed by the clinical laboratory of the West China Hospital.

Serum cytokine analyses by Luminex technology

The Luminex 200 system was used to evaluate serum from a total of 57 breast cancer patients (28 NN and 29 NP). Levels of the following 26 cytokines were assessed: interleukin (IL)-1b, IL-4, IL-6, IL-12, IL-17a, IFN-γ, chemokine ligand (CCL)2, CCL3, CCL4, CCL5, CCL11, chemokine (C-X-C motif) ligand (CXCL)9, CXCL10, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor, MMP-1, MMP-2, MMP-3, MMP-9, MMP-13, hepatocyte growth factor (HGF), placental growth factor, platelet-derived growth factor (PDGF)-AA, PDGF-BB, epidermal growth factor, and fibroblast growth factor-basic. For technical reasons, these 26 cytokines were measured using three different Luminex panels. Panel 1 included PDGF-BB, epidermal growth factor, MMP-13, fibroblast growth factor-basic, and PDGF-AA. Panel 2 included MMP-1, MMP-2, MMP-3, and CCL5. The remainder of the cytokines was in panel 3. Briefly, panels 1 and 2 were diluted 10-fold, and panel 3 was diluted 2-fold. Fluorescence intensity was determined, and the concentration of each cytokine was calculated based on a standard curve. These values were subsequently converted to pg/mL. The same steps were used in the validation test.

Validation test

A validation test was performed to determine the predictive value of the markers for ALNM. A total of 100 breast cancer patients were enrolled in this part of the study. Sera were collected, and Luminex technology was used to measure the levels of MMP-1, HGF, and CCL5. SLNB using only subdermal (or peritumoral) injection of methylene blue was performed on all 100 patients before modified radical mastectomy. The axilla status and the false negative rate of SLNB were determined by postoperative pathologic examination.

Statistics

Descriptive statistics included means, ranges, standard deviations, and proportions. Categorical data are presented as percentages, and differences between proportions were compared with the chi-square or Fisher's exact tests. Continuous variables were compared using the unpaired Student's *t*-test. Multivariate analysis was performed to identify independent determinants for ALNM (logistic