IL-6, IL-8 and TNF-α levels correlate with disease stage in breast cancer patients

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Conflict of interest
None declared

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Abstract

Background. Breast cancer is the most common cancer in Chinese women. Inflammation contributes to tumor progression and can be induced by excessive production of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α). However, how their levels relate to the expression of estrogen receptors (ER), progesterone receptors (PR) and human epidermal growth factor receptor 2 (HER2) by the tumor has not been investigated.

Objectives. The aim of the study is to more fully understand the significance of serum IL-6, IL-8 and TNF-α in breast cancers with different ER, PR and HER2 status.

Material and methods. Preoperative serum samples were collected from 110 patients diagnosed with ductal carcinoma and 30 healthy control subjects. IL-6, IL-8 and TNF-α levels were determined by enzyme-linked immunosorbent assay (ELISA). Associations of cytokine levels with clinical tumor stage were evaluated, and correlations of serum cytokine levels with ER, PR and HER2 expression were determined using the Pearson correlation coefficient.

Results. Serum levels of IL-6 and IL-8 were significantly higher in the subjects with ductal carcinoma than in the controls, and strongly correlated with clinical tumor stage, lymph node metastasis, and ER and HER2 antigen expression ($p < 0.05$). TNF-α levels in stage III carcinoma patients were significantly higher than in the controls ($p < 0.01$) and were associated with lymph node metastasis ($p < 0.01$). A strong positive correlation was found between IL-8 and TNF-α levels in the cancer patients ($p < 0.001$).

Conclusions. The study showed that IL-6, IL-8 and TNF-α levels correlated with clinical disease stage and lymph node metastasis as well as with ER and HER2 antigen expression. Specifically, IL-6 and IL-8 seem to have significant potential as prognostic cancer biomarkers. Analyzing serum cytokine levels might help identify patients with a poor prognosis who may benefit from more aggressive disease management.

Key words: breast cancer, biomarkers, interleukin-6, TNF-α, interleukin-8

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Breast cancer is the most common form of cancer among Chinese women and constitutes a large public health burden. The most common type of breast cancer is ductal carcinoma in situ, which is often associated with inflammation. Cells involved in the inflammatory response are attracted by cytokines and chemokines and may contribute to the development and progression of breast cancer.[1,2] Most cytokines are overexpressed in cancer tissues compared to normal tissues, and overexpression correlates with a poor prognosis.[2–4] Numerous cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α), have been implicated in the initiation and progression of ductal carcinoma.[5–10]

IL-6 is a pro-inflammatory cytokine that has multiple functions.[11] It is involved in the regulation of immune reactions, hematopoiesis and the inflammatory state.[2,11,12] IL-6 has an important role in tumor progression, as it can inhibit the apoptosis of cancer cells and stimulate tumor angiogenesis.[13,14] Clinical studies have shown that serum IL-6 levels were increased in patients with breast cancer, and this increase correlated with tumor stage and poor patient survival.[15,16] IL-8 is a chemokine that has an autocrine and/or paracrine tumor-promoting role, and significant potential as a prognostic and/or predictive cancer biomarker.[7] IL-8 plays a specific role in breast cancer, and increased serum IL-8 levels are associated with positive lymph node status and higher-stage tumors.[15] Finally, TNF-α is a necrotic factor in the tumor microenvironment that promotes tumor growth and migration.[4,8] Elevated circulating TNF-α levels correlate with higher tumor stages and lymph node metastasis.[4,6,17] Furthermore, in ductal carcinomas, the steroid hormone receptors progesterone receptor (PR) and estrogen receptor (ER) may also play important roles in cancer progression; it has been shown that the level of the expression of ER, PR, and human epidermal growth factor receptor 2 (HER2) by tumors is associated with tumor prognosis.[12,17–19] Although the presence of IL-6, IL-8 and TNF-α in breast cancer has been noted by many researchers, whether the levels of these cytokines in the serum are correlated with the PR, ER, and HER2 status of the tumor is not clear.[6,15,16] Furthermore, whether there is a correlation among the cytokine levels themselves has not been addressed previously.

In the present study, to more fully understand the significance of serum IL-6, IL-8 and TNF-α in breast cancers with different ER, PR and HER2 status, cytokine levels were measured in samples isolated from patients with ductal carcinoma and from control subjects. Correlations between the cytokine levels and the clinical stage were then analyzed, as well as ER, PR and HER2 antigen expression.

Table 1. Demographic data of the study participants

Group	Age (years)	Number
Healthy subjects	28–57	30
Ductal carcinoma	35–65	110
HER2 positive	35–63	45
PR positive	37–65	60
ER positive	37–65	55
Stage I	35–47	25
Stage II	30–59	50
Stage III	34–65	35

Material and methods

Sample collection

Samples were collected from 110 female patients (Table 1) diagnosed at Huai'an First People's Hospital (Huai'an, China). The age of the patients ranged from 35 to 68 years. Individuals were eligible if they were primary breast cancer patients who had not received any prior treatment. Patients who presented with additional conditions, such as other malignancies, advanced organ failure or active infection, were also excluded. The patients examined were in clinical stage I, II, or III according to the TNM classification. The clinical diagnosis was routinely confirmed by histopathological examination of the tumor tissue samples. ER, PR and HER2 status was determined at the protein level by immunohistochemistry. The control group was comprised of 30 healthy women. Preoperative serum samples were collected before the initiation of treatment. The samples were stored at -80°C until the analysis. The present study conformed to the ethical standards of the World Medical Association Helsinki Declaration and was approved by the Ethics Committee of Huai'an First People's Hospital Faculty of Medicine. All the patients in the study had signed informed consent forms at Huai'an First People's Hospital.

Measurement of serum cytokine levels

Levels of IL-6, IL-8 and TNF-α in the patients' sera were determined by enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's instructions (R&D Systems, Inc., Shanghai, China).

Statistical analyses

Means and standard deviations were calculated. The data were evaluated using Student's t-test for the patient group relative to the control group, and Tukey's multiple comparison test for more than 2 study groups using

GraphPrism 6.0 software (GraphPad, La Jolla, USA). The values $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$(***) were considered statistically significant. The Pearson correlation coefficient was used to assess correlations between the levels of IL-6, IL-8 and TNF-α in the cancer patients.

Results

Serum IL-6 levels in ductal carcinoma

Serum IL-6 levels in the subjects with ductal carcinoma were significantly higher than those in the healthy women ($p < 0.001$; Fig. 1A). Serum IL-6 levels correlated with the clinical tumor stage and lymph node metastasis. IL-6 concentrations were significantly increased in the patients with stage II and III carcinomas and lymph node metastases ($p < 0.01$; Fig. 1B, 1C).

Next, the relationship between serum IL-6 levels and the ER, PR and HER2 status of ductal carcinomas was analyzed. Levels of IL-6 were significantly higher in the sera collected from patients with ER^+ tumors than in the sera from those with ER^- tumors ($p < 0.001$; Fig. 1D). Interestingly, serum IL-6 levels were significantly higher in the patients with $HER2^-$ ductal carcinomas than in those with $HER2^+$ carcinomas ($p < 0.001$; Fig. 1F). Serum IL-6 levels did not correlate with the PR status of the tumors (Fig. 1E).

Serum IL-8 levels in ductal carcinoma

The serum IL-8 levels in the ductal carcinoma patients were significantly higher than in the control group ($p < 0.001$; Fig. 2A). Serum IL-8 levels also correlated with the clinical tumor stage and lymph node metastasis. IL-8 levels were significantly elevated in the patients with stage II and III carcinomas and lymph node metastases ($p < 0.01$; Fig. 2B, C).It was further found that the IL-8 levels correlated with the ER and HER expression of the tumor tissue in the carcinoma patients ($p < 0.001$; Fig. 2D, F). The levels of IL-8 were significantly higher in ER^- and $HER2^+$ tumor patients than in ER^+ or $HER2^-$ patients. However, the IL-8 levels did not significantly differ between patients with PR^+ and PR^- tumors (Fig. 2E).

Serum TNF-α levels in ductal carcinoma

Although a slight increase in TNF-α levels was observed in the ductal carcinoma patients compared to the control group, this difference was not significant (Fig. 3A). How-

Fig. 1. Serum IL-6 levels: A) in healthy women and in patients with ductal carcinoma; B) in different clinical tumor stages; C) in patients with lymph node metastasis; D) ER positive vs ER negative; E) PR positive vs PR negative; F) HER2 positive vs HER2 negative. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$

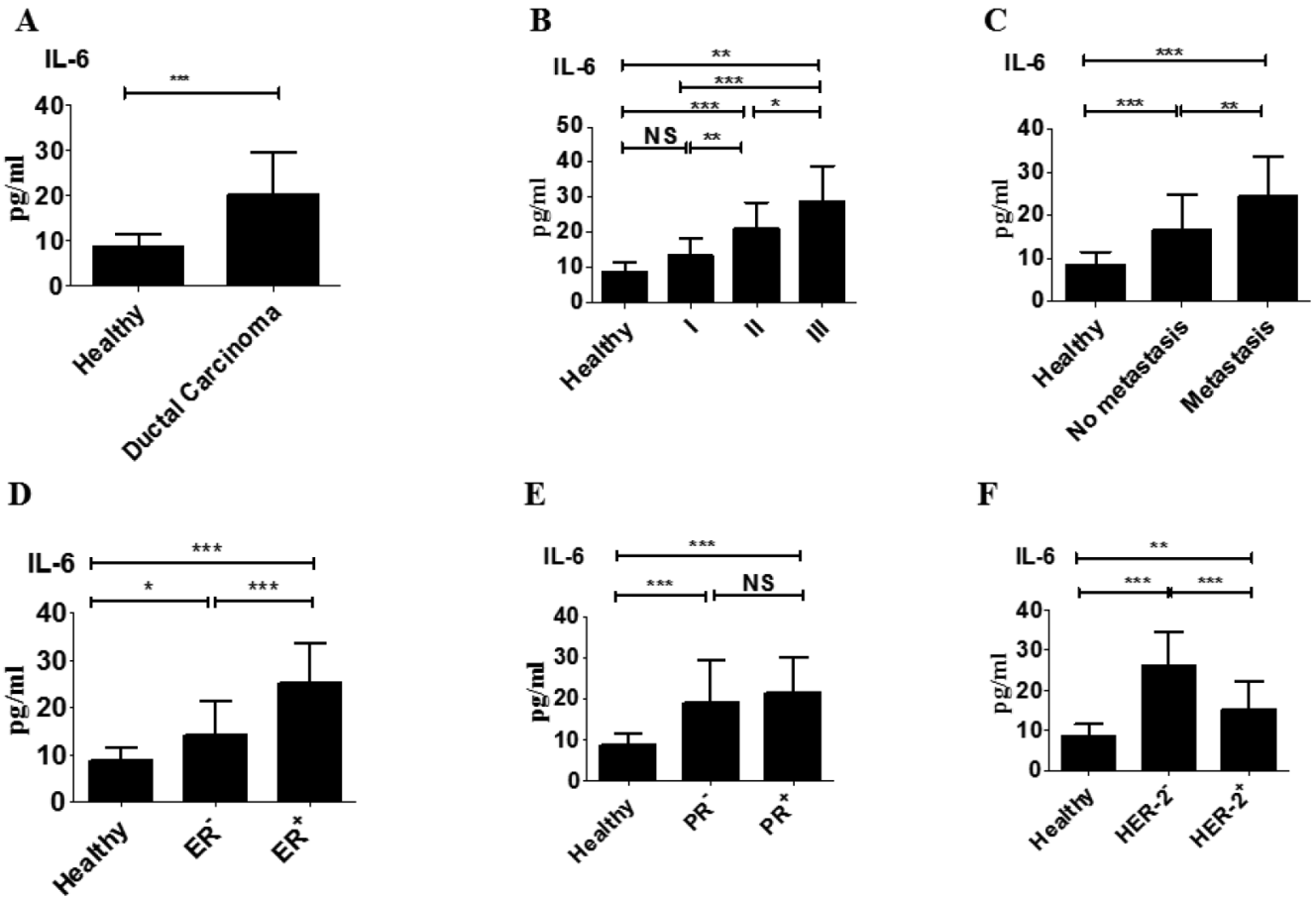


Fig. 2. Serum IL-8 levels: A) in healthy women and in patients with ductal carcinoma; B) in different clinical tumor stages; C) in patients with lymph node metastasis; D) ER positive vs ER negative; E) PR positive vs PR negative; F) HER2 positive vs HER2 negative. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$

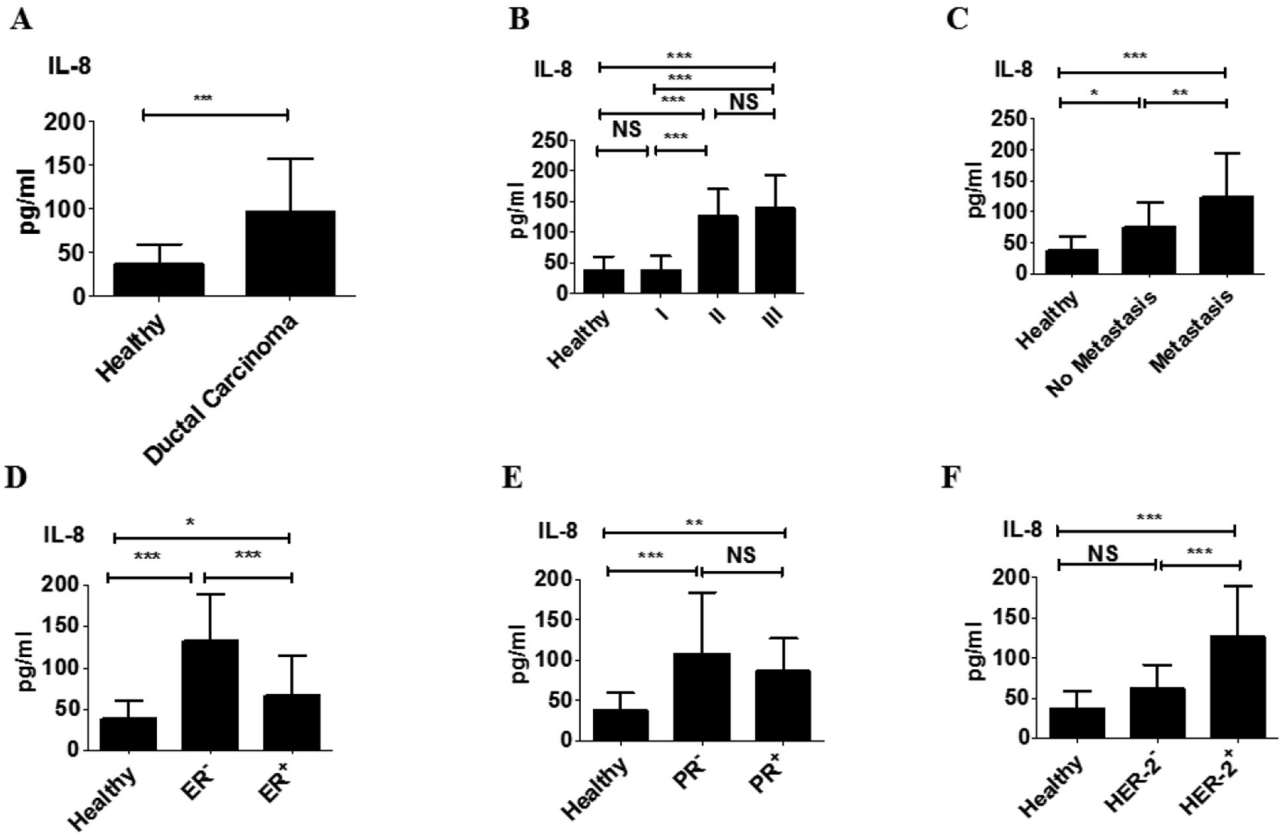


Fig. 3. Serum TNF-α levels A) in healthy women and in patients with ductal carcinoma; B) in different clinical tumor stages; C) in patients with lymph node metastasis; D) ER positive vs ER negative; E) PR positive vs PR negative; F) HER2 positive vs HER2 negative. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.0001$

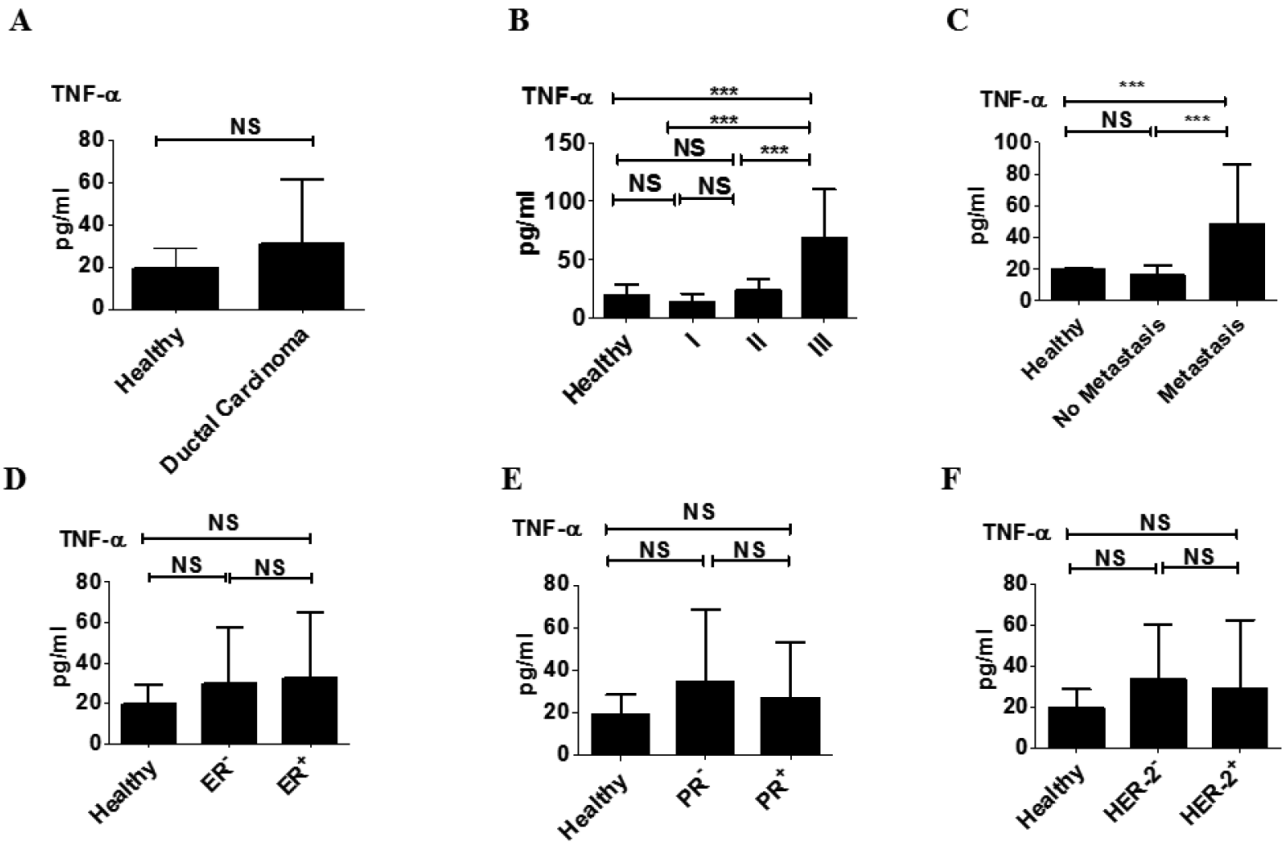
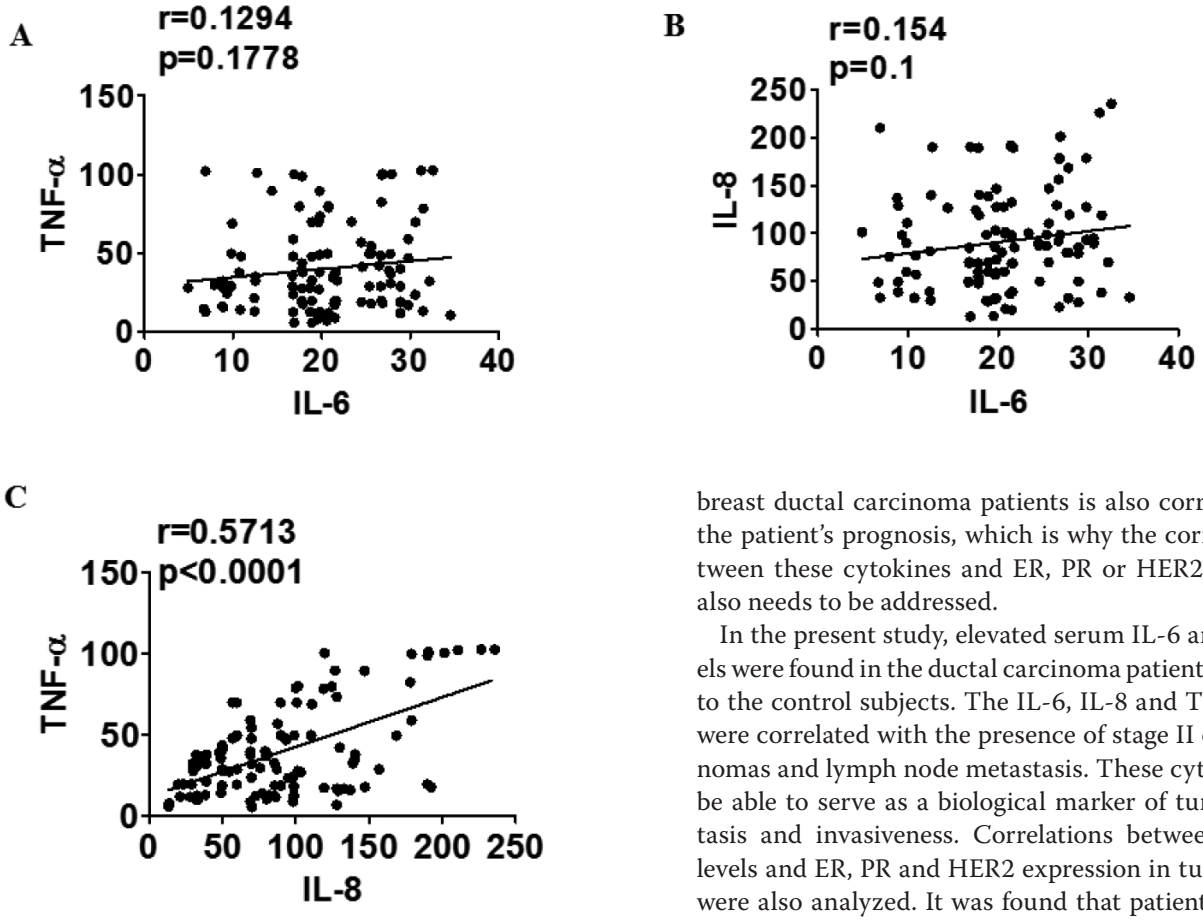


Fig. 4. Correlations between cytokines IL-6, IL-8 and TNF-α: A) IL-6 and TNF-α; B) IL-8 and IL-6; C) IL-8 and TNF-α



ever, the serum TNF-α levels of stage III carcinoma patients were significantly higher than those in the healthy controls ($p < 0.001$; Fig. 3B). Serum TNF-α levels also correlated with clinical tumor stage and lymph node metastasis ($p < 0.001$; Fig. 3B, 3C). TNF-α levels did not correlate with ER, PR or HER2 expression in the tumor tissues (Fig. 3D, 3F).

Correlations between the levels of IL-6, IL-8 and TNF-α in cancer patients

In the samples isolated from the ductal carcinoma patients, a significant positive correlation was found between the levels of TNF-α and IL-8 ($r = 0.8571$, $p < 0.001$; Fig. 4C), but not between TNF-a and IL-6 levels (Fig. 4A). Similarly, no correlation was found between the levels of IL-6 and IL-8 (Fig. 4B).

Discussion

It has been reported that high serum levels of IL-6, IL-8 and TNF-α are correlated with strong tumor invasiveness and poor prognosis.[2,6,7,11,20] However, whether there is a correlation between these cytokines and the tumor stage and lymph node metastasis is still not very clear. Furthermore, the expression of ER, PR and HER2 in breast ductal carcinoma patients is also correlated with the patient's prognosis, which is why the correlation between these cytokines and ER, PR or HER2 expression also needs to be addressed.

In the present study, elevated serum IL-6 and IL-8 levels were found in the ductal carcinoma patients compared to the control subjects. The IL-6, IL-8 and TNF-α levels were correlated with the presence of stage II or III carcinomas and lymph node metastasis. These cytokines may be able to serve as a biological marker of tumor metastasis and invasiveness. Correlations between cytokine levels and ER, PR and HER2 expression in tumor tissues were also analyzed. It was found that patients with ER+ or HER2- tumors have increased serum IL-6 levels versus those with ER- or HER2+ tumors; however, IL-8 levels were higher in ER- and HER2+ tumor patients compared with those with ER+ or HER2- tumors. While ER+ breast cell lines secrete lower IL-6 levels than ER- cells, it is possible to speculate that IL-6 production from breast tumor cells may constitute only a small fraction of total serum IL-6 content. In addition to tumor cells, T cells, macrophages, and B cells are able to secrete IL-6.[13,14,21,22] In the late stage of ductal carcinoma, tumor tissues are infiltrated by numerous T cells and M2 macrophages; these cells produce large amounts of IL-6 and promote the metastasis of breast tumor cells.[23–25] Thus, IL-6 production by immune cells likely accounts for the increased IL-6 serum levels in patients with ER+ ductal carcinomas. For IL-8 production, it has been suggested that ER expression can downregulate IL-8 production in tumor cells, and breast cancer patients who lack ER expression have worse prognoses.[7,26] HER2 is a tumor antigen that is expressed by different tumors (breast cancer, lung cancer, gastric cancer) and can elicit a host immune response; therefore, it can be hypothesized that HER2 expression in breast cancer may directly promote the production of IL-8.[27]

The relationship among cytokine levels was also analyzed, and a strong positive correlation was found between IL-8 and TNF-α. Therefore, IL-8 and TNF-α might act synergistically in the initiation and development of

tumors. However, there was no correlation between IL-6 levels and IL-8 or TNF-α levels. Although tumor cells are able to produce all 3 cytokines, the authors speculate that the main origin of these cytokines in tumor patients is different. Surprisingly, the data from the present study revealed that serum TNF-α levels did not significantly differ between the healthy controls and the ductal carcinoma patients. However, the serum TNF-α levels were elevated in stage III breast cancer patients as compared with those with stage I cancers and the healthy control subjects, meaning that only late stages tumor patients secrete high levels of TNF-α (Fig. 3B). It is interesting to note that TNF-α levels were not correlated with ER, PR or HER2 expression, although there was a strong positive correlation between TNF-α levels and IL-8 levels. The authors speculate that the main TNF-α producing cells (such as macrophages) may not be affected by ER, PR or HER2 expression, which could explain this result.[28,29] Another possibility is that the production of TNF-α is not only correlated with IL-8 levels, but also associated with other factors, such as IL-1β.[4,30]

In this study, a correlation was found between cytokine levels and tumor stages, lymph node metastasis, and the ER, PR and HER2 status of tumors. However, it is still unclear whether the elevated cytokine levels are merely a result of the presence of late stage and/or metastatic tumors or if their elevated levels contribute to the progression of tumors to an advanced clinical stage. More research is needed to investigate how the ER, PR and HER2 expression affects the production of IL-6 and IL-8.

The present study suggests that increased serum levels of IL-6, IL-8 and TNF-α are associated with breast ductal carcinoma. Specifically, the levels of these cytokines correlate with the clinical stage of the disease and with ER and HER2 antigen expression by the tumors. Thus, these cytokines seem to have significant potential as prognostic cancer biomarkers.

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