The preoperative plasma fibrinogen level is an independent prognostic factor for overall survival of breast cancer patients who underwent surgical treatment

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A B S T R A C T

Background: Previous studies have suggested that plasma fibrinogen contributes to tumor cell proliferation, progression and metastasis. The current study was performed to evaluate the prognostic relevance of preoperative plasma fibrinogen in breast cancer patients.

Method: Data of 2073 consecutive breast cancer patients, who underwent surgery between January 2002 and December 2008 at the Sun Yat-sen University Cancer Center, were retrospectively evaluated. Plasma fibrinogen levels were routinely measured before surgeries. Participants were grouped by the cutoff value estimated by the receiver operating characteristic (ROC) curve analysis. Overall survival (OS) was assessed using Kaplan–Meier analysis, and multivariate Cox proportional hazards regression model was performed to evaluate the independent prognostic value of plasma fibrinogen level.

Results: The optimal cutoff value of preoperative plasma fibrinogen was determined to be 2.83 g/L. The Kaplan–Meier analysis showed that patients with high fibrinogen levels had shorter OS than patients with low fibrinogen levels (p < 0.001). Multivariate analysis suggested preoperative plasma fibrinogen as an independent prognostic factor for OS in breast cancer patients (HR = 1.475, 95% confidence interval (CI): 1.177–1.848, p = 0.001). Subgroup analyses revealed that plasma fibrinogen level was an unfavorable prognostic parameter in stage II–III, Luminal subtypes and triple-negative breast cancer patients.

Conclusion: Elevated preoperative plasma fibrinogen was independently associated with poor prognosis in breast cancer patients and may serve as a valuable parameter for risk assessment in breast cancer patients.

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Introduction

Breast cancer is by far the most common cancer and the main cause of cancer death in women worldwide. According to the National Cancer Institute, there were more than 230,000 cases of female breast cancer in 2014, and more than 40,000 women have died of breast cancer [1]. The prognosis of breast cancer patients is influenced by numerous factors, such as TNM staging, intrinsic subtypes, age, and gender [2,3]. Recurrence and metastasis remain a great challenge for cure despite the excellent outcomes of early-stage breast cancer after standard treatments [4].

Recently, much attention has been given to the association between hypercoagulation and the progression of malignancies. Increasing data indicated that the members of the coagulation cascade are activated during cancer progression, and associated with tumor stage, response to chemotherapy and prognosis [5]. Fibrinogen is one of the important indicators of coagulation. It is a 340-kDa glycoprotein mainly synthesized by hepatocytes and converted to insoluble fibrin by activated thrombin [6,7].

Fibrinogen is involved in a number of biological processes that are regulated by different cytokines, such as vascular endothelial growth factor (VEGF), interleukin-1 (IL-1) beta, and fibroblast
growth factor (FGF) –2 [8–10]. Previous research has demonstrated that an increased level of plasma fibrinogen is frequently observed in cancer patients and that fibrinogen plays a vital role in tumorigenesis and progression, including stroma formation, angiogenesis, and hematogenous metastasis [11,12]. Elevated level of preoperative plasma fibrinogen has been associated with poor prognosis in patients with malignancies, such as lung cancer [13,14], colorectal cancer [15,16], pancreatic cancer [17], prostate cancer [18] and renal cell carcinoma [19].

However, few studies have evaluated the significance of preoperative plasma fibrinogen level in breast cancer patients as a predictor of survival after surgical treatment. The aim of this retrospective clinical study was to investigate the association between the plasma fibrinogen level and the prognosis of breast cancer patients.

Materials and methods

Study population

Patients with histologically diagnosed of breast cancer between January 2002 and December 2008 in the Sun Yat-sen University Cancer Center (SYSUCC) were retrospectively reviewed. Other inclusion criteria were as follows: (1) received surgical treatment and (2) female patients. Exclusion criteria were as follows: (1) received neoadjuvant chemotherapy before surgery; (2) received surgical treatment before admission; (3) with previous or coexisting cancers other than breast cancer; (4) confirmed metastasis; (5) concomitant conditions influencing plasma fibrinogen level, such as liver disease, blood coagulation disorders and daily antiplatelet or anticoagulant treatment before surgery; (6) not enough data can be extracted. All patients were followed up to December 31, 2014 or until death from any cause.

Clinical data collection

Baseline characteristics including age, menstrual status, pathological diagnosis, histologic grade, axillary lymph node status, hormone receptor and human epidermal growth factor receptor-2 (HER-2) status, surgery type, family history, date of last follow-up or death and preoperative plasma fibrinogen level were collected. Fibrinogen levels were measured as part of the routine clinical evaluation prior to surgery by the Clauss method using Dade Thrombin Reagent™ and a Sysmex CA-7000 automated coagulometer (Sysmex, Kobe, Japan). Plasma fibrinogen levels between 1.80 and 4.00 g/L were considered to be normal. The clinical stages of the disease were determined by TNM staging system according to the American Joint Committee on Cancer (AJCC), and the cancer subtypes were classified as follows: Luminal A subtype (estrogen receptor positive (ER+) and progesterone receptor positive (PR+), HER-2–), luminal B subtype (ER+ and/or PR+, HER-2+), HER-2 overexpressing subtype (ER–, PR–, HER-2+) and triple-negative subtype (ER–, PR–, HER-2–). The follow-up of patients was performed through outpatient medical records, telephone or letters by the “Department of Follow-up & Medical Record Management”.

Statistical analyses

Preoperative plasma fibrinogen values were expressed using mean and standard deviations (SD), and categorical data were described using numbers and percentages. Kolmogorov–Smirnov test was performed to test the normal distribution of the data. The correlation between patients’ characteristics and preoperative plasma fibrinogen was evaluated by unpaired t-test or one-way analysis of variance (ANOVA), and differences between categories were examined using the Chi-squared test. The endpoint assessed was overall survival (OS), calculated from the time of pathological diagnosis to date of death from any cause or the last follow-up. SPSS 19.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses in the present study. The clinical significance and the cutoff value for the continuously coded variable preoperative plasma fibrinogen level were determined by the ROC curve analysis, and patients were categorized into two groups according to the cutoff value to assess the risk of death. Kaplan–Meier method was carried out for survival analyses and the differences between the groups were assessed by log-rank test. Factors examined in the univariate analysis were age (<35 vs. >35 years), menstrual status, tumor type, histologic grade, tumor size, lymph node status, hormone receptor status, HER-2 status, surgical method, family history and plasma fibrinogen level. Univariate and multivariate analyses (Cox proportional hazards model) were performed to identify the independent variables associated with OS. Hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) estimated from the Cox analysis were regarded as relative risks, and a two-tailed p value < 0.05 was considered significant.

Results

Patient characteristics

A total of 2073 consecutive patients with histopathologically diagnosed breast cancer in SYSUCC were enrolled after eligibility review. The selection process is shown in Fig. 1. The median follow-up time was 75 months (range 3–143 months), and death occurred in 329 (15.9%) of the 2073 breast cancer patients. The median age of the enrolled patients was 49.0 years (range: 22–96 years), and 189 (9.1%) patients were aged below 35 years. Patient characteristics and correlations between preoperative plasma fibrinogen level and clinicopathological parameters are shown in Table 1. The mean level of preoperative plasma fibrinogen level was 2.88 ± 0.68 g/L, and it was associated with age and menstrual status (both p < 0.001, Table 1). Middle-aged and elderly (>35 years old) and postmenopausal patients had higher preoperative plasma fibrinogen level. No correlation was observed between fibrinogen level and histologic grades, tumor size, lymph node status, hormone receptor status, HER-2 status and family history (all p > 0.05). Moreover, patients who experienced poor outcome had significantly increased preoperative fibrinogen level compared to patients with better prognosis (p < 0.001).

Cutoff value of preoperative plasma fibrinogen

ROC curve analysis was performed to determine the optimal cutoff value of preoperative plasma fibrinogen level. The area under

![Fig. 1. Flow chart of the patient selection.](image-url)
were categorized as low-fibrinogen were classified as high-fibrinogen group, while the remaining 983 fibrinogen group. The chi-squared test indicated that, compared with the low-fibrinogen group, patients in the high-fibrinogen group suffered more axillary lymph node metastases and worse outcome (p = 0.003 and < 0.001, respectively).

### Association of preoperative plasma fibrinogen with survival

The 10-year OS rate was 78.0% for all 2073 patients, and the mean survival time was 124.9 (95%CI: 123.0–126.8) months. The Kaplan–Meier curve indicated that patients in the high-fibrinogen group had worse OS rate than those in the low-fibrinogen group (mean survival time: 120.4 vs. 128.7 months, P < 0.001; Fig. 3).

In breast cancer patients, the preoperative plasma fibrinogen level was found to be associated with OS, along with other variables such as age, menstrual status, histologic grade, tumor size, lymph node status, hormone receptor status, HER-2 status and surgical methods, in the univariate analysis (Table 2, all P < 0.05). However, preoperative plasma fibrinogen was identified as an independent prognostic factor for OS in the multivariate analysis using the Cox proportional hazard model (HR = 1.848, 95%CI: 1.717–2.000, P < 0.001). Reduction in duration of survival in breast cancer patients associated with elevated preoperative plasma fibrinogen levels. In addition, menstrual status, tumor size (T3), lymph node status, ER, and HER-2 were independent prognostic factors for OS.

Multivariate analysis stratified by clinical stages demonstrated that the elevated preoperative plasma fibrinogen level was significantly associated with worse OS in stage II–III breast cancer patients (HR = 1.610 and 1.598, respectively, both P < 0.05, Table 3), and the clinical outcome of stage I patients with elevated fibrinogen level was not significantly different from those with low fibrinogen level (P = 0.659). Meanwhile, the preoperative fibrinogen level was indicated as a risk factor for patients with breast cancer of Luminal A, Luminal B, and triple negative subtypes (all P < 0.05, Table 3), and there was a trend in HER-2 overexpressed subtype, but the significance could not be proven (P = 0.287).
In the present study, the survival condition of 2073 breast cancer patients was retrospectively analyzed and the association between preoperative plasma fibrinogen levels and the clinical outcome in breast cancer patients was examined. The main findings of this study are as follows: (1) preoperative plasma fibrinogen level is an independent prognostic factor in breast cancer patients; (2) elevated fibrinogen levels are associated with poor clinical outcome in patients with breast cancer of Luminal A, Luminal B and triple negative subtypes.

The cutoff value of fibrinogen in the present study was 2.83 g/L and the patients enrolled were categorized into low- or high-fibrinogen groups. Compared with the low-fibrinogen group, the mean survival time of the high-fibrinogen group was shorter (120.4 vs. 128.7 months, \( p < 0.001 \)), and the overall 10-year survival rates were 83.6% and 72.7% (\( p < 0.001 \)), respectively, in both groups. Patients with elevated plasma fibrinogen (>2.83 g/L) levels had 1.475 times the risk of death in those with low fibrinogen levels.

Tumor progression is the consequence of complex interactions between tumor cells and the host environment and inflammatory response [23]. The metastatic cancer cells leave the primary tumor site, migrate and circulate through the bloodstream, adhere to the vasculature of the target organ, invade the surrounding tissue and establish a blood supply at the new metastatic site [24–26]. The clotted plasma and platelets collectively stabilize the circulating cancer cells by thrombus formation, thereby facilitating the attachment and spread of these cells in the vasculature of the distant target organs.

Fibrinogen is one of the important coagulative factors and systemic inflammatory markers detected in the plasma; it enhances the progression and invasive potential of tumor cells through several possible mechanisms. Fibrinogen is deposited around solid tumors and it provides a stable framework to the tumor extracellular matrix. It also serves as a scaffold to support binding of growth factors, such as fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF), to tumor cells, and thus promotes tumor proliferation and stimulates angiogenesis [11,27,28]. Moreover, plasma fibrinogen and tumor cells interact with each other. A large number of fibrinogen receptors such as intercellular adhesion molecule 1 (ICAM-1) and \( \alpha \)-\( \beta \) integrin are present on the tumor cell surface; thus, fibrinogen acts as a bridging factor between the tumor and host cells and enhances the endothelial adhesion of tumor cell emboli in the vasculature of target organs, leading to the occurrence of metastasis [29]. The release of tumor cells from the primary tumor into the cardiovascular system is the initial step of metastasis [26]. Fibrinogen promotes \( \beta \)-integrin-mediated adhesion of tumor cells to platelets, and the platelet–tumor cell aggregates thus formed could shield tumor cells from the innate immune response, thereby leading to an increase in the number of metastatic cells [30–32]. Previous study revealed that pulmonary metastases were markedly reduced in fibrinogen–deficient mice after intravenous injection of cancer cells, demonstrating the vital role of fibrinogen in the process of metastasis [33]. Furthermore, anticoagulants, such as warfarin and heparin, have antitumor and antimetastatic effects in vivo and in vitro [34,35].

Plasma fibrinogen, an acute-phase protein, is found to be at an elevated level during malignancy or systemic inflammation [36]. The high fibrinogen level in cancer patients may be associated with the tumor-derived humoral factors, such as macrophage colony-stimulating factor, IL-1, and IL-6 [37–39]. In addition, previous studies have shown that several procoagulant factors were synthesized and overexpressed in tumor cells [40], and high levels of fibrinogen could also be endogenously synthesized by the epithelial tumor cells.

Due to the retrospective nature of the current study, some limitations exist. First, a selection bias cannot be excluded even

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Discussion

Elevated preoperative plasma fibrinogen level has been determined to be an unfavorable prognostic factor in some malignancies, such as digestive system neoplasms [15–17], gynecologic neoplasms [20], urologic neoplasms [21] and soft-tissue sarcoma [22]. Recent studies had shown that fibrinogen levels were associated with disease development, metastasis and poor prognosis.
were performed to minimize the bias. Second, high though consecutive patients were included and eligibility criteria were performed to minimize the bias. Second, high fibrinogen levels may be associated with a higher risk of thromboembolism events in cancer patients, which may influence patients' survival, and not enough data can be extracted due to the low occurrence (3/2073) in the present study cohort. Moreover, the enrolled patients underwent surgical treatment by multiple surgeons.

In conclusion, it was the first report demonstrating that preoperative plasma fibrinogen level was an independent prognostic factor in breast cancer patients who underwent surgical treatment. This parameter may help clinicians assess the risks of metastasis and death in breast cancer patients. Further prospective trials are needed to confirm its prognostic significance.

**Ethics statement**

The study protocol was approved by the independent ethical committee/institutional review board of SYSUCC, and written informed consent about the treatment and researchable use of the clinical data was obtained from each participant prior to surgery. All patient data were anonymous and de-identified prior to analysis.

**Conflict of interest statement**

The authors have no conflicts of interest to declare.

**Acknowledgments**

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**References**


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**Table 2**

Univariate and multivariate analyses of preoperative plasma fibrinogen for OS in breast cancer patients.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Age</td>
<td>1.011 (1.001–1.021)</td>
<td>0.036</td>
</tr>
<tr>
<td>Menstrual status</td>
<td>0.725 (0.575–0.915)</td>
<td>0.007</td>
</tr>
<tr>
<td>Tumor type</td>
<td>0.808 (0.555–1.175)</td>
<td>0.265</td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>0.738 (0.564–0.967)</td>
<td>0.028</td>
</tr>
<tr>
<td>G3</td>
<td>1.300 (0.997–1.697)</td>
<td>0.053</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>1.533 (1.222–1.975)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>T3</td>
<td>3.970 (2.747–5.736)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymph node status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>2.041 (1.487–2.800)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N2</td>
<td>3.747 (2.723–5.155)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>N3</td>
<td>8.328 (6.144–11.288)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ER</td>
<td>0.543 (0.437–0.673)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PR</td>
<td>0.594 (0.477–0.740)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HER-2</td>
<td>2.028 (1.615–2.546)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Surgical method</td>
<td>0.321 (0.152–0.680)</td>
<td>0.003</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1 (reference)</td>
<td></td>
</tr>
<tr>
<td>BC or OC</td>
<td>1.121 (0.876–1.434)</td>
<td>0.363</td>
</tr>
<tr>
<td>Other carcinomas</td>
<td>1.100 (0.792–1.527)</td>
<td>0.570</td>
</tr>
<tr>
<td>Plasma fibrinogen</td>
<td>1.445 (1.261–1.657)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviation: OS overall survival, HR hazard ratio, CI confidence interval, ER Estrogen receptor, PR Progesterone receptor, HER-2 Human epidermal growth factor receptor-2, BC breast carcinoma, OC ovarian carcinoma.

**Table 3**

Prognostic impact of preoperative plasma fibrinogen in various clinical stages and intrinsic subtypes.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR(95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Clinical stagesa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1.061 (0.476–2.363)</td>
<td>0.886</td>
</tr>
<tr>
<td>II</td>
<td>1.643 (1.151–2.347)</td>
<td>0.006</td>
</tr>
<tr>
<td>III</td>
<td>1.615 (1.191–2.189)</td>
<td>0.002</td>
</tr>
<tr>
<td>Intrinsic subtypesb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>1.913 (1.212–3.018)</td>
<td>0.005</td>
</tr>
<tr>
<td>Luminal B</td>
<td>1.708 (1.211–2.403)</td>
<td>0.002</td>
</tr>
<tr>
<td>HER-2 overexpressed</td>
<td>1.620 (0.965–2.719)</td>
<td>0.068</td>
</tr>
<tr>
<td>Triple-negative</td>
<td>2.866 (1.485–5.533)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

a Tumor size and lymph node status were not involved in the multivariate analyses.
b Hormone receptor status and HER-2 status were not involved in the multivariate analyses.


