Peripheral cytotoxic T lymphocyte predicts first-line progression free survival in HER2-positive advanced breast cancer

Xiao-Ran Liu, Jian-Jun Yu, Guo-Hong Song, Li-Jun Di, Han-Fang Jiang, Ying Yan, Xu Liang, Ru-Yan Zhang, Ran Ran, Jing Wang, Han Bai, Shi-Dong Jia, Hui-Ping Li

**Department of Breast Oncology, Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education), Peking University Cancer Hospital & Institute, Fu-Cheng Road No.52, Hai-Dian District, Beijing, 100142, China**

**Haidu Shanghai Medical Sciences, Wang-Yuan Road No.1698, Feng-Xian District, Shanghai, 201499, China**

**A R T I C L E   I N F O**

Article history:
Received 5 July 2020
Received in revised form 6 November 2020
Accepted 9 November 2020
Available online 12 November 2020

Keywords:
Peripheral cytotoxic T lymphocyte
HER2-Positive breast cancer
Circulating tumor DNA
Progression free survival

**A B S T R A C T**

Background: The role of peripheral blood lymphocyte (pBL) in breast cancer has long been studied. However, the predictive role of pBL in advanced breast cancer (ABC) is poorly understood.

Methods: A total of 303 patients with ABC were consecutively recruited at our center between January 2015 and September 2019. At baseline, pBL subtypes were detected in all patients with 229 blood samples available for circulating tumor DNA (ctDNA) detection. pBL was analyzed through flow cytometry. ctDNA-based gene mutations were detected using next generation sequencing. The cutoff value of pCTL was estimated by X-tile software. Progression free survival (PFS) was estimated by Kaplan-Meier curve and Cox hazard proportion regression model, with difference detection by log-rank test.

Results: Median follow-up time of the study was 21.0 months. The median age of diagnosis was 52.0 years. Among the pBL subtypes, only pCTL level was found predictive for PFS in the HER2+ patients whom received anti-HER2 therapy (13.1 vs. 5.6 months, \( P = 0.001 \)). However, the predictive role of pCTL was not found in HR-positive (\( P = 0.716 \)) and TNBC (\( P = 0.202 \)). pCTL high associated with suppressive immune indicators including lower CD4/CD8 ratio (\( P = 0.004 \)) and high level of Treg cell (\( P = 0.004 \)). High occurrence of FGFR1 amplification which has been reported as immune suppressor was also found in HER2+ patients with pCTL high (22.2% vs. 4.3%, \( P = 0.048 \)).

Conclusions: Higher pCTL levels associated with shorter PFS and FGFR1 mutation in HER2+ patients.

© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Breast cancer is the leading cause of malignancy related death in women [1]. Various factors contribute to breast cancer patients’ outcome including age, molecular subtype, tumor grade and stage, and treatment. The clinical relevance of the host immune system in breast cancer has long been studied, especially the role of tumor-infiltrating lymphocytes (TILs). Previous studies showed that TILs can be used as a predictor of response to neo-adjuvant chemotherapy in breast cancer [2,3]. Moreover, several randomized controlled trials revealed that TIL levels have a different prognostic efficacy in various breast cancer subtypes [4,5]. Despite the clinical importance of TILs, metastases from breast cancer have been shown to have a low immune infiltration which undermines the potential prognostic and predictive role of TILs in advanced breast cancer (ABC) patients [6]. Another limitation of TILs in predicting clinical outcome is the spatial heterogeneity, which is currently a main obstacle for manual TIL assessment [3]. Distribution of TILs in a tumor can be affected by many factors such as tumor growth patterns or histological type [7]. For example, the TILs score can be completely different between a tumor with solid growth pattern and a tumor with dissociative growth pattern [4]. Although the

**Abbreviations:** ABCs, advance breast cancer patients; ctDNA, cell free DNA; ctDNA, circulating tumor DNA; DFS, disease free survival; gDNA, genomic DNA; HR, hormone receptor-positive; IHC, immunohistochemistry; OS, overall survival; pBL, peripheral blood lymphocyte; pCTLs, peripheral CD8+ cytotoxic T lymphocytes; PFS, progression free survival; RECIST, Response Evaluation Criteria in Solid Tumors; TNBC, triple-negative breast cancer; TILs, tumor-infiltrating lymphocytes.

* Corresponding author.
** Corresponding author.

E-mail addresses: huipingli2012@hotmail.com (H.-P. Li).

1 Indicates that these authors contribute to this work equally.
international guidelines for the evaluation of TILs were published in 2014, a standardized TILs scoring system with no controversy has not been well developed. Finally, the use of TILs as a predictor is limited for inaccessible metastases such as those in marrow and brain.

Peripheral blood lymphocytes (pBLs) have shown a great potential as a predictor of outcome because of the advantages in minimal invasiveness, dynamic monitoring and high homogeneity. The correlation between pBL count and clinical outcome in ABC patients was first reported in 1976 [8] and later confirmed by two studies showing an associated risk of recurrence [9,10]. Moreover, certain peripheral lymphocyte subtypes such as CD3+ and CD4+ were found to have predictive and prognostic value regarding the survival of patients with ABC [11]. A recent study showed that in patients with ABC who received adoptive T-cell therapy, high level of peripheral suppressor T cells (CD8+/CD28-) was associated with shorter progression-free survival (PFS) and overall survival (OS) [12]. Although, studies have been exploring the predictive value of pBLs in patients with ABC, the progress in this area is still limited. The main obstacle to understand the predictive mechanism of pBL lies in the lack of molecular evidence.

The primary aim of this study was the assessment of the association between different pBL subtypes and PFS in patients with ABC who received first-line of therapy. The molecular features along with the predictive value of certain pBL subtypes were also explored by detecting circulating tumor DNA (ctDNA).

2. Material and methods

2.1. Patient enrollment

All procedures involving human participants met with the criterion of the Peking University Cancer Hospital ethical committee (Approval No. 2016KT47). First-line ABC patients were recruited at Peking University Cancer Hospital between January 2016 and September 2019. The criteria for enrollment were as followed: (1) patient aged 18 years or older; (2) pathologically confirmed recurrent and metastatic breast cancer; (3) no anti-tumor therapy prior to enrollment; (4) eligible to receive standard chemotherapy against HER2 (Abcam PLC, Cambridge, UK) was scored according to an associated risk of recurrence [9,10]. Moreover, CD3+ and CD4+ were found to have predictive and prognostic value regarding the survival of patients with ABC [11]. A recent study showed that in patients with ABC who received adoptive T-cell therapy, high level of peripheral suppressor T cells (CD8+/CD28-) was associated with shorter progression-free survival (PFS) and overall survival (OS) [12]. Although, studies have been exploring the predictive value of pBLs in patients with ABC, the progress in this area is still limited. The main obstacle to understand the predictive mechanism of pBL lies in the lack of molecular evidence.

The primary aim of this study was the assessment of the association between different pBL subtypes and PFS in patients with ABC who received first-line of therapy. The molecular features along with the predictive value of certain pBL subtypes were also explored by detecting circulating tumor DNA (ctDNA).

2.2. Patients follow-up

For each enrolled patients, one post-treatment follow-up and then routine outpatient follow-up every 3 months for imaging based medical assessments to monitor disease progression. The primary endpoint of this study was PFS. PFS was defined from start of therapy to progression of disease or last date of follow-up.
adapters were amplified via PCR. The amplified DNA libraries were then further checked using Bioanalyzer 2100 (UC Davis Genome Center, CA, US) and samples with sufficient yield proceeded to hybrid capture.

Library capture was conducted using biotin labelled DNA probes. In brief, the library was hybridized overnight with the PredicineCARE™ panel (Predicine, Inc., CA, USA) (Supplementary Fig. 1) and bead-captured beads. The unbound fragments were washed away, and the enriched fragments were amplified via PCR amplifications. Similar to library preparation, the purified product was checked on Bioanalyzer 2100 and then loaded into Illumina HiSeq X Ten (Illumina, Inc., San Diego, CA) for next generation sequencing with paired-end 250bp sequencing kits (Illumina, Inc., San Diego, CA).

### 2.7. Statistical analysis

The primary objective of the study was to assess the predictive impact of peripheral T lymphocyte subtype detection at baseline regarding PFS in ABC. Secondary objectives were to evaluate the relationship between peripheral T lymphocytes detection at baseline and ctDNA detection at baseline. Clinical data were obtained from the patient electronic medical recording system. Relationships between ctDNA derived mutational status, Cytotoxic T Cells (CTL) level and clinical characteristics were assessed using the Chi-square test or Fisher exact test, Student t-test or Mann-Whitney U test, and Pearson correlation tests accordingly. All analyses were conducted by SPSS 19.0 version software (IBM Inc., NY, US). Missing data were not included into the analysis. The cutoff value of CTL regarding PFS was calculated using the software of X-tile 3.6.1 version reported by Camp RL et al. [14] (Supplementary Fig. 2). Kaplan-Meier survival analysis and log-rank test was used to compare PFS between different patient cohorts. The Cox proportional hazard regression model was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) of the proportion of CTLs in peripheral blood with confounder adjustment. All tests were two-sided with a significance level of 0.05.

## 3. Results

### 3.1. Patient characteristics

A total of 303 recurrent and initial stage IV breast cancer patients were enrolled in this study. The median follow-up time was 21.0 months (range: 2.0–46.0 months). The lost to follow-up rate was 14.7%. The clinical characteristics of the patients were presented in Table 1. The median age of diagnosis of present cohort was 52.0 years (range: 27–82). Forty-eight patients were stage IV breast cancer at initial treatment and 255 patients showed recurrent breast cancer. All patients received first-line therapy. Out of 303 ABCs, 109 (36.0%) were triple-negative breast cancers (TNBCs), 97 (32.0%) were hormone receptor positive/HER-2 negative (HR+) and 97 (32.0%) were HER2-positive. Hormonal therapy was used for most HR+/HER2- ABCs (65/97, 67.0%), in which six patients were

| Table 1 | Clinical characteristics of the study cohort (n = 303). |
|------------------------|------------------------|------------------------|
| **Clinical characteristics** | **Number of patients (%)** |
| Age at diagnosis (Range: 27–82, median = 52.0) | |
| ≤ 45 years | 114 (37.6) |
| > 45 years | 189 (62.4) |
| **Histological type at primary diagnosis** | |
| Ductal | 261 (86.1) |
| Lobular | 19 (6.3) |
| Others | 23 (7.6) |
| **Immunohistochemistry at primary diagnosis** | |
| HR positive & HER2 negative | 97 (32.0) |
| HER2 positive | 97 (32.0) |
| TNBC | 109 (36.0) |
| **Tumor grade at primary diagnosis** | |
| I | 15 (5.0) |
| II | 200 (66.0) |
| III | 86 (28.4) |
| Unknown | 2 (0.6) |
| **Tumor stage at primary diagnosis** | |
| I | 36 (11.9) |
| II | 116 (38.3) |
| III | 96 (31.7) |
| IV | 48 (15.8) |
| Unknown | 7 (2.3) |
| **Visceral metastasis** | |
| Yes | 182 (60.1) |
| No | 121 (39.9) |
| **Number of metastatic sites** | |
| Single metastatic site | 128 (42.2) |
| Multiple metastatic site | 175 (57.8) |
| **Disease free survival** | |
| ≤ 36.0 months | 146 (47.3) |
| > 36.0 months | 109 (42.7) |
| **Regimens of first-line therapy** | |
| Taxane single agent or Taxane based chemotherapy | 109 (36.0) |
| Trastuzumab and/or pertuzumab plus chemotherapy | 86 (28.4) |
| Trastuzumab and/or pertuzumab plus hormonal therapy | 11 (3.6) |
| Hormonal therapy | 65 (21.4) |
| Others | 32 (10.6) |

*C Visceral metastasis including brain, liver and lung.
*b Multiple lesions occurred in the same organ only count once.*
also combined with Palbociclib (6/97, 6.1%). All HER2-positive ABCs (97/97, 100.0%) received trastuzumab and/or pertuzumab based therapy. Among them, five patients (5/97, 5.2%) received double-targeted therapy of trastuzumab and pertuzumab. Taxane monotherapy or taxane-based chemotherapy was commonly applied for TNBCs (89/109, 81.7%).

3.2. Peripheral CTL was an independent predictive factor of PFS

We first evaluated the association of conventional clinical characteristics with first-line PFS. In our cohort, DFS (HR: 0.627, 95% CI: 0.471–0.833), IHC of primary tumor (HR: 1.189, 95%CI: 1.016–1.393) and tumor grade at primary diagnosis (HR: 1.920, 95% CI: 1.501–2.455) was associated with PFS. Other factors such as age of diagnosis (HR: 0.966, 95%CI: 0.742–1.258), primary tumor stage (HR: 1.138, 95%CI: 0.989–1.310) and visceral metastasis status (HR: 0.816, 95%CI: 0.628–1.061) were not associated with PFS (Table 2).

We next screened out the association of each pBL subtypes with PFS using the X-tile software. Only peripheral CTL was found to be predictive for PFS with a cutoff value of 13.9% ($\chi^2 = 5.093$, $P = 0.024$) (Supplementary Fig. 2). We set the cutoff value of pCTL as 14.0%, and divided the 303 ABCs into the pCTL low group ($\leq 14.0\%$) and pCTL high group ($> 14.0\%$). Univariate survival analysis showed that the peripheral CTL level at baseline successfully predicted PFS (HR = 0.667, 95%CI: 0.496–0.924, $P = 0.023$) (Fig. 1A). After making adjustment for Disease Free Survival (DFS), IHC and tumor grade, multivariate analysis further confirmed that peripheral CTL at baseline was an independent predictive factor of PFS (9.7 months vs. 7.9 months, $P = 0.022$) (Fig. 1B). The gating parameters for pCTL selection are showed in Fig. 1C.

3.3. Peripheral CTL selectively predicts PFS of HER2-positive subtype

It has been reported that different molecular subtypes of breast cancer possess varying immune features. Thus, we sought to evaluate the predictive value of peripheral CTL in each molecular subtype. Univariate survival analysis revealed that no association between pCTL and PFS was observed in HR-positive (10.9 months vs. 13.7 months, $P = 0.716$) and TNBC subgroup (5.9 months vs. 6.4 months, $P = 0.202$) (Fig. 2A and B). However, we found that high pCTL (>14.0%) predicted a shorter PFS in HER2-positive patients (13.1 months vs. 5.6 months, $P = 0.001$) (Fig. 2C). Multivariate analysis adjusted for DFS and tumor grade also showed that the high pCTL level is a worse predictor for PFS in HER2-positive patients (HR = 0.712, 95%CI: 0.532–0.953, $P = 0.004$) (Fig. 2D). Considering that the possibility of uneven distribution of clinical characteristics might perturb the predictive value of pCTL, we performed a comparison according to pCTL level in the HER2-positive subgroup. The data showed that no significant distributional variation of clinical characteristics, especially for those associated with PFS, was found between pCTL low and pCTL high groups (Table 3).

3.4. Suppressive immune status of pCTL high group

In view of the predictive role of peripheral CTL in HER2-positive ABC, we compared the distribution pattern of different pBL subtypes according to pCTL level. Higher levels of CD3+ T cells (P = 0.005), T8 cells (P = 0.002) and regulatory T-cells (Treg cell) (P = 0.004) were found in the pCTL high group (> 14.0%), and lower

### Table 2

Univariate analysis of conventional clinical features at baseline regarding first-line PFS.

| Baseline Clinical Characteristics | n  | Median PFS±SD       | $P$ value$^a$ | HR (95%CI)    |
|----------------------------------|----|---------------------|--------------|--------------|
| Age of diagnosis                 |    |                     |              |              |
| ≤45 years                        | 114| 9.000 ± 1.023       | 0.798        | 0.966 (0.742–1.258) |
| > 45 years                       | 189| 9.000 ± 0.557       |              |              |
| Primary tumor stage              |    |                     |              |              |
| I                               | 36 | 8.300 ± 2.274       |              | 0.070        | 1.138 (0.989–1.310) |
| II                              | 116| 10.400 ± 1.528      |              |              |
| III                             | 96 | 8.200 ± 1.046       |              |              |
| IV                              | 48 | 8.700 ± 0.872       |              |              |
| Unknown                          | 7  |                     |              |              |
| Primary tumor grades             |    |                     | <0.001      | 1.920 (1.501–2.455) |
| I                               | 15 | 18.800 ± 2.068      |              |              |
| II                              | 200| 10.100 ± 0.700      |              |              |
| III                             | 86 | 5.800 ± 0.545       |              |              |
| Unknown                          | 2  |                     |              |              |
| Primary immunohistochemistry     |    |                     |              |              |
| HR positive & HER2 negative      | 97 | 11.000 ± 0.966      | 0.030        | 1.189 (1.016–1.393) |
| HER2 positive                    | 97 | 10.100 ± 0.736      |              |              |
| Triple-negative                  | 109| 6.400 ± 1.010       |              |              |
| Disease free survival            |    |                     |              |              |
| ≤36.0 months                    | 146| 6.900 ± 1.023       | 0.001        | 0.627 (0.471–0.833) |
| > 36.0 months                   | 109| 12.200 ± 1.732      |              |              |
| Visceral metastasis$^b$          |    |                     | 0.126        | 0.816 (0.628–1.061) |
| Yes                              | 121| 9.500 ± 0.804       |              |              |
| No                               | 182| 8.800 ± 0.613       |              |              |
| Number of metastatic sites$^c$   |    |                     | 0.390        | 0.893 (0.689–1.157) |
| Single metastatic site           | 128| 9.200 ± 0.700       |              |              |
| Multiple metastatic sites        | 175| 8.800 ± 0.563       |              |              |

$^a$ $P$ value of K-M survival analysis was calculated by log-rank test.

$^b$ Visceral metastasis includes liver, lung and brain metastasis.

$^c$ Multiple lesions occurred in the same organ only count once.
CD4+/CD8+ ratio ($P = 0.004$) and suppressor T-cell ($P = 0.003$) were also found in the pCTL high group. The rest of the pBL subgroups did not show correlation with pCTL. Detailed information was listed in Table 4. Meanwhile, we also detected the TILs level of 16 HER2-positive ABCs according to their pCTL level (Fig. 3A and B). In pCTL high group, 1 patients (1/6, 16.7%) were confirmed high TIL, 1 patients (2/6, 33.3%) were intermediate TIL, and 3 patients (3/6, 50.0%) were low TIL. In pCTL low group, 5 patients (5/10, 50.0%) were confirmed high TIL, 2 patients (2/10, 20.0%) were intermediate TIL, and 3 patients (3/10, 30.0%) were low TIL. Although we found that low TIL was more frequently observed in pCTL High group, but the result did not reach statistical significance (16.7% vs. 50.0%, $P = 0.144$) (see Fig. 4).

3.5. Amplified FGFR1 gene and mutation of downstream targets in the pCTL high group

In the HER2-positive subgroup, 64 out of 97 patients had ctDNA detection at baseline. The common single nucleotide variants were in TP53 (66.1%), and PIK3CA (36.3%), and the common copy number variations were in ERBB2 (70.1%) (Fig. 3A). For most single nucleotide variants and copy number variations, no distributional difference was found between pCTL high and pCTL low groups. Notably, three copy number gain mutations and one missense mutation of FGFR1 gene was found in the pCTL high group with only one copy number gain mutation in the pCTL low group (22.2% vs. 4.3%, $P = 0.048$). However, similar data were not found for other FGFR family members including FGFR2 ($P = 1.000$), FGFR3 ($P = 0.553$) and FGFR4 ($P = 0.487$). Subsequently, we detected the mutational frequency of the main downstream genes of FGFR1 pathway using our gene panel. These genes include PIK3CA (35.9%), PIK3R1 (3.1%), KRAS (3.1%), BRAF (6.3%) and NF1 genes (7.8%). We defined any gain-of-function mutation of FGFR1, KRAS, BRAF, PIK3CA and PIK3R1 gene and loss-of-function mutation of NF1 gene as FGFR1 pathway hyper-activation. Mutation of the FGFR1 pathway more frequently occurred in the pCTL high group compared with pCTL low group (77.8% vs. 39.1%, $P = 0.011$). The heatmap of mutational pattern for FGFR1 pathway is shown in Fig. 3B.

4. Discussion

The host immune system has a great impact on the disease course of breast cancer. Dysregulation of cellular and/or humoral immunity gives rise to oncogenesis, tumor metastasis and treatment failure [15]. Several studies in ABC have focused on the relationship between clinical outcome and immune factors such as TILs [3]. However, few studies have paid attention to the predictive value of pBL, and the related mechanism has been unclear. To the best of our knowledge, the present study is the first to report the
predictive value of peripheral CTLs regarding PFS, especially in HER2-positive patients who received anti-HER2 therapy. For TILs, the high level of infiltrating CTLs is usually associated with better survival [16]. Unexpectedly, our data showed that a high level of peripheral CTLs indicated shorter PFS in the HER2-positive subgroup (13.1 months vs. 5.6 months, \(P = 0.001\)) (Fig. 2C). Therefore, we examined why peripheral CTLs were a negative predictor of PFS.

Previous studies found that a low level of CD4/CD8 ratio at baseline was associated with no therapeutic response or tumor progression in breast cancer patients who received neoadjuvant chemotherapy [17,18]. In addition, a low level of CD4/CD8 ratio was also associated with impaired cellular immunity [19] and lymph node involvement [20,21]. Other studies showed that high level of Treg cells contributes to immunosuppressive and poor response to chemotherapy and poor clinical outcomes in ABCs [22–25]. Our data showed that pCTL level (cutoff = 14.0%) was negatively associated with CD4/CD8 ratio and positively associated with Treg cells. Furthermore, we also found that lower TIL level was more frequently observed in patients with pCTL high, although this result did not reach statistical level \((P = 0.144)\). This result was consistent with what we found in peripheral blood. Together, these results indicate a possible suppressive immune status in HER2-positive patients with high level of pCTL, which could be part of the reason for the shorter PFS.

The FGFR pathway initiates several downstream pathways that regulate cell proliferation, angiogenesis, migration, and survival in breast cancer [26,27]. FGFR1 is a crucial member of the FGFR family, and FGFR1 mutation was found in approximately 14% of all breast cancer and is associated with worse prognosis among breast cancer patients [28]. We observed a frequent occurrence of FGFR1 mutation in the pCTL high group compared with the pCTL low group (22.2% vs. 4.3%, \(P = 0.048\)), but no differences were observed in FGFR2 \((P = 1.000)\), FGFR3 \((P = 0.553)\) and FGFR4 mutations \((P = 0.487)\). This result indicated a driver mutation role of FGFR1 in HER2-positive patients with high level pCTL. Notably, dysregulation of FGFR pathway also contributes to immune evasion of breast cancer. Previous studies found that myeloid-derived suppressor cells (MDSCs) could activate Treg cells and inhibit CD8 T cells and natural killer cells, leading to immune evasion in the tumor microenvironment [29–31]. MDSC infiltration and tumor angiogenesis are significantly enhanced during mammary tumorigenesis in MMTV-Wnt1/iFGFR1 bi-genic mice in comparison with MMTV-Wnt1 transgenic mice. Tumor regression and disappearance of MDSCs from the residual mammary gland was found in MMTV-Wnt1/iFGFR1 bi-genic mice treated with BGJ398, an FGFR inhibitor [32]. Similarly, AZD4547, another FGFR inhibitor, also successfully reduced MDSCs in the tumor microenvironment and systemic circulation [33]. Thus, hyper-activation of the FGFR pathway will lead to accumulation of MDSCs in the tumor microenvironment and facilitate the immune evasion of breast cancer cells. In our cohort, mutations were found in FGFR1 pathway genes including FGFR1, PIK3CA, PIK3R1, KRAS, BRAF and NF1 genes. When taking...
Table 3
Comparison of clinical characteristics according to pCTL level in HER2 positive subgroup (n = 97).

| Clinical characteristics                  | n   | Peripheral CTL ≤14.0% (n = 67) | Peripheral CTL >14.0% (n = 30) | P valuea |
|------------------------------------------|-----|-------------------------------|--------------------------------|----------|
| Age at diagnosis                         |     |                               |                                 | 0.479    |
| ≤45.0 years                              | 30  | 19 (63.3%)                    | 11 (36.7%)                      |          |
| > 45.0 years                             | 67  | 48 (71.6%)                    | 19 (28.4%)                      |          |
| Tumor grade at primary diagnosis         |     |                               |                                 | 0.126    |
| I                                        | 2   | 1 (50.0%)                     | 1 (50.0%)                       |          |
| II                                       | 69  | 52 (75.4%)                    | 17 (24.6%)                      |          |
| III                                      | 24  | 13 (54.2%)                    | 11 (45.8%)                      |          |
| Tumor stage at primary diagnosis         |     |                               |                                 | 0.907    |
| I                                        | 9   | 6 (66.7%)                     | 3 (33.3%)                       |          |
| II                                       | 36  | 25 (69.4%)                    | 11 (30.6%)                      |          |
| III                                      | 28  | 20 (71.4%)                    | 8 (28.6%)                       |          |
| IV                                       | 21  | 14 (61.9%)                    | 8 (38.1%)                       |          |
| Visceral metastasis                     |     |                               |                                 | 0.819    |
| Yes                                      | 66  | 45 (68.2%)                    | 21 (31.8%)                      |          |
| No                                       | 31  | 22 (71.0%)                    | 9 (29.0%)                       |          |
| Number of metastatic sites              |     |                               |                                 | 0.371    |
| Single metastatic site                  | 36  | 27 (75.0%)                    | 9 (25.0%)                       |          |
| Multiple metastatic site                | 61  | 40 (65.6%)                    | 21 (34.4%)                      |          |
| Disease free survival                   |     |                               |                                 | 0.616    |
| ≤36.0 months                            | 44  | 30 (68.2%)                    | 14 (31.8%)                      |          |
| > 36.0 months                           | 31  | 23 (74.2%)                    | 8 (25.8%)                       |          |
| Regimens of first-line therapy          |     |                               |                                 | 0.761    |
| Trastuzumab and/or pertuzumab plus chemotherapy | 86 | 60 (69.8%) | 26 (30.2%) |          |
| Trastuzumab and/or pertuzumab plus hormonal therapy | 11 | 8 (72.7%) | 2 (27.3%) |          |
| Optimal response of first-line therapy  |     |                               |                                 | 0.082    |
| CR/PR                                   | 38  | 27 (71.1%)                    | 11 (28.9%)                      |          |
| SD                                      | 34  | 26 (76.5%)                    | 8 (23.5%)                       |          |
| PD                                      | 19  | 9 (47.4%)                     | 10 (52.6%)                      |          |

a Visceral metastasis includes brain, liver and lung.
b Multiple lesions occurred in the same organ only count once.
c P value was calculated using Chi-square test or Fisher exact test.

Table 4
Peripheral lymphocyte subtypes distribution according to pTCL level in HER2+.

| Peripheral lymphocyte subtypes (Phenotype) | pTCL (mean ± SD) | P-valuea |
|-------------------------------------------|------------------|----------|
|                                           | ≤14.0% (n ≤ 67)  | > 14.0% (n = 30) |
| CD3+ T-cells (CD3+)                       | 60.31 ± 12.59    | 66.69 ± 8.62    | 0.005  |
| T4 cell (CD3+ & CD4+)                     | 33.44 ± 9.70     | 34.26 ± 8.35    | 0.689  |
| Th cell (CD3+ & CD8+)                     | 24.30 ± 9.51     | 30.73 ± 8.21    | 0.002  |
| CD4+/CD8+ ratio (CD4+/CD8+)               | 1.62 ± 0.81      | 1.22 ± 0.50     | 0.004  |
| Natural killer cell (CD3+ & CD56− & CD16+) | 16.88 ± 10.33    | 13.75 ± 8.69    | 0.151  |
| Natural killer T-cell (CD3+ & CD56− & CD16+) | 6.70 ± 6.47      | 8.34 ± 8.14     | 0.289  |
| B cells (CD3− & CD19+)                    | 14.80 ± 8.22     | 12.65 ± 4.73    | 0.108  |
| Regulatory T-cell (CD4+ & CD25+)          | 3.03 ± 1.66      | 4.35 ± 2.11     | 0.004  |
| Suppressor T-cell (CD8+ & CD28−)          | 21.46 ± 9.24     | 15.66 ± 6.92    | 0.003  |

a P value was calculated using Student t-test or Mann-Whitney U test.

Fig. 3. Immunohistochemistry of ErbB2 in HER2-positive patients with different pCTL level. (A) HER2-positive patient with low level of pCTL. (B) HER2-positive patient with high level of pCTL. Magnification times: 200.
their mutational status as a whole into consideration, we observed a high mutational occurrence of FGFR1 pathway genes in the pCTL high group (77.8% vs. 39.1%, \( P = 0.011 \)). This result indicates a possible immunosuppressive status of the tumor microenvironment in HER2-positive patients with high level of pCTL. This could be, at least, one of the reasons underlying the shorter PFS of patients with high pCTL level. Future work should evaluate the feasibility of combined FGFR inhibitor with anti-HER2 therapy according to pCTL level in HER2-positive patients.

There are several limitations of our study. First, present study was performed in single center using retrospective design and analysis. The records on some clinical parameters were incomplete. Currently, trastuzumab plus pertuzumab is recommended as standard treatment for first line anti-HER2 therapy. However, due to the current medical situation in China, only 6.1% HER2-positive patients of present study cohort received the trastuzumab plus pertuzumab. Thus, a larger prospective study using standard anti-HER2 therapy which focused on the predictive role of pCTL and the mechanism behind was needed in future works. Second, due to the limited number of enrolled patients, an external validation cohort regarding the cutoff value of pCTL in HER2-positive ABCs was not performed in present study. Finally, the dynamic monitoring of pCTL should be considered to better understanding the predictive role of pCTL.

5. Conclusions

Together, our results show that higher pCTL levels were associated with shorter first-line PFS in HER2-positive ABC patients who received anti-HER2 therapy, but no associations were observed with HR-positive and TNBC patients. High levels of pCTL were also associated with FGFR1 mutations in HER2-positive ABC patients. As a minimal invasive approach, evaluation of pCTL level exhibit a great clinical potential in HER2-positive ABC patients.

Declaration of competing interest

The authors declare no potential conflicts of interest.

Acknowledgement

The work was supported by the National Natural Science Foundation of China, and the Grant No. was 81502269.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.breast.2020.11.006.

Additional information

Financial support

The work was supported by the National Natural Science Foundation of China, and the Grant No. was 81502269.

References

[1] Coughlin SS. Epidemiology of breast cancer in women. Adv Exp Med Biol 2019;1152:9–29.
[2] Denkert C, Loibl S, Noske A, Roller M, Muller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response tooadjuvant chemotherapy in breast cancer. J Clin Oncol 2010;28:105–13.
[3] Savas P, Salgado R, Denkert C, Sotiriou C, Darcy PK, Smyth MJ, et al. Clinical relevance of host immunity in breast cancer: from TILs to the clinic. Nat Rev Clin Oncol 2016;13:228–41.
[4] Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer:
