Association of HER-2/CEP17 Ratio and HER-2 Copy Number With pCR Rate in HER-2-Positive Breast Cancer After Dual-Target Neoadjuvant Therapy With Trastuzumab and Pertuzumab

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Objective: To explore the correlation between HER-2 status and pathological complete response (pCR) in HER-2-positive breast cancer after dual anti-HER-2 neoadjuvant therapy with trastuzumab and pertuzumab.

Methods: A total of 57 HER-2-positive breast cancer patients admitted to the Second Affiliated Hospital of Anhui Medical University and Xijing Hospital Affiliated to Air Force Military Medical University, between January 1, 2019 and September 30, 2020, were enrolled in this multicenter retrospective study. HER-2 status, including HER-2/CEP17 ratio and HER-2/cell number ratio, was detected by FISH. The correlation between HER-2 status/clinicopathological data and pCR was analyzed. The ROC curve was drawn to determine the cutoff value.

Results: IHC assessment revealed 40 (70.18%) patients with IHC 3+ and 17 (29.82%) with IHC 2+. 41/57 (71.93%) patients achieved pCR. FISH revealed that the ratio of HER-2/chromosome 17 centromere (HER-2/CEP17) (p<0.001) and the ratio of HER-2/cell number (p<0.001) was significantly correlated with the pCR rate. ROC analysis showed that patients with an HER-2/CEP17 ratio ≥4.495 or HER-2/cell number ratio ≥11.650 have a high pCR rate after dual anti-HER-2 neoadjuvant therapy, suggesting its predictive significance.

Conclusion: The response to dual-targeted neoadjuvant therapy with trastuzumab and pertuzumab was adequate in hormone receptor-negative, HER-2-positive breast cancer patients. HER-2/CEP17 ratio and HER-2/cell number ratio were crucial for predicting efficacy.

Keywords: breast cancer, HER-2 positive, IHC, FISH, dual-target neoadjuvant therapy
INTRODUCTION

Breast cancer has replaced lung cancer as the world’s most common malignant tumor that severely endangers women’s health (1). Human epidermal growth factor receptor-2 (HER-2)-positive breast cancer accounts for 20–25% of all breast carcinomas and has a poor prognosis and high recurrence rate (2). However, the development of the HER-2-targeting antibody has profoundly improved the outcome in HER-2-positive breast cancers (3).

As monoclonal anti-HER-2 drugs, trastuzumab and pertuzumab, act on two different domains of HER-2, respectively, and synergistically inhibit HER-2-positive breast cancer (4–8). Recently, several randomized trials have also investigated its efficacy in the neoadjuvant setting, with pathological complete response (pCR) as the primary endpoint. In the NeoSphere study, the pCR rate of trastuzumab combined with pertuzumab (dual anti-HER-2 therapy) combined with docetaxel was significantly higher than that in the single-target group (9). The five-year follow-up to the NeoSphere study confirmed that patients achieving pCR had longer disease-free survival (DFS) than those who did not achieve pCR. Despite the improvement of 45.8%, more than 50% of patients did not achieve pCR. Thus, screening patients with high drug sensitivity before neoadjuvant therapy is crucial in HER-2-positive breast cancer patients.

HER-2 is a transmembrane glycoprotein with tyrosine kinase activity that belongs to the epidermal growth factor receptor family. The amplification of the HER-2 gene is the primary mechanism of HER-2 overexpression. The HER-2 status is necessary for anti-HER-2 treatment, based on immunohistochemistry (IHC) to assess the protein overexpression and in-situ hybridization (ISH) to assess gene amplification (10). However, both tests determined whether a tumor is a potential target for anti-HER-2 treatment response in the metastatic setting, which is only achieved in about 50% of cases, while >25% of women treated with neoadjuvant anti-HER-2 therapy relapse within the first ten years of diagnosis (11). The predictive efficacy of HER-2 amplification and expression has not been studied in-depth. Currently, only foreign research data are available on the curative effect of neoadjuvant trastuzumab-based chemotherapy for HER-2 positive breast cancer. This retrospective analysis aimed to evaluate the correlation between HER-2 data and pCR of HER-2-positive breast cancer patients treated with dual-target neoadjuvant therapy of trastuzumab and pertuzumab.

MATERIAL AND METHODS

Patients

Patients with core biopsy confirmed HER-2-positive breast cancer, between January 1, 2019 and September 30, 2020, from the Second Affiliated Hospital of Anhui Medical University and Xijing Hospital Affiliated to Air Force Military Medical University, were enrolled in this retrospective study. All patients were treated with dual-target neoadjuvant therapy after primary tumor and axillary lymph node core biopsy confirmed. The inclusion criteria were as follows: (1) age 18–75-years-old, (2) HER-2-positive non-special type invasive breast cancer; (3) received dual anti-HER-2 neoadjuvant therapy with trastuzumab and pertuzumab; (4) written informed consent. The exclusion criteria were as follows: (1) male gender; (2) metastatic disease at diagnosis; (3) cases with incomplete clinical or pathological data and patients without surgical treatment.

The study protocol was approved by an independent ethics committee of the Second Affiliated Hospital of Anhui Medical University, and all participants provided informed consent (approval number: XY2021-023). The study was conducted in accordance with the Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent before enrollment.

Neoadjuvant Therapy Regimen

All patients were treated with three types of neoadjuvant chemotherapy combined with trastuzumab and pertuzumab regimen for 6–8 cycles after the diagnosis was confirmed by a puncture. Some patients were treated with single-agent docetaxel or paclitaxel combined with trastuzumab plus pertuzumab regimen (THP) for six cycles, while some were treated with docetaxel or paclitaxel combined with carboplatin plus trastuzumab plus pertuzumab regimen (TCPbHP) treatment for six cycles. The other patients were treated with docetaxel or paclitaxel combined with trastuzumab plus pertuzumab regimen for four cycles before or after treatment with doxorubicin or epirubicin combined with cyclophosphamide regimen for four cycles (THP-A(E)C/A(E)C-THP). Surgery was performed after the completion of neoadjuvant treatment.

ER and PR Detection

Estrogen receptor (ER) and progesterone receptor (PR) were detected by immunohistochemistry (IHC). These data were obtained from pathological reports of needle core biopsies and postoperative specimens and evaluated according to the 2018 ASCO/CAP guidelines using FDA-approved analysis (12). The ER and PR positivity is defined as nuclear staining of at least 1% of tumor cells (13).

HER-2 Detection and Analysis

IHC and fluorescence in situ hybridization (FISH) were also used for HER-2 assessment. HER-2 positivity was defined as HER-2 IHC3+, or HER-2 IHC2+ but HER-2 FISH amplified. HER-2 IHC results come from the pathology report. HER-2 IHC score is positive (3+), suspicious (2+), or negative (0 or 1+) (12). IHC 2+ (equivocal) is invasive breast cancer with “weak to moderate complete membrane staining observed in >10% of tumor cells”. Next, we evaluated the expression of HER-2 in all invasive breast cancers through IHC. Typically, HER-2 IHC 3+ cases do not require further testing. All suspected HER-2 (IHC 2+) cases were evaluated by HER-2 FISH. In order to explore the predictive significance of the efficacy, we performed FISH on needle core biopsies before neoadjuvant treatment of all breast cancer patients. It was also performed on paraffinized tissue sections using the HER-2 DNA Dual Probe Kit (Linked-Biotech Pathology, Guangzhou, People’s Republic of China). Furthermore, the HER-2/CEP17 ratio ≥2.0 or an average HER-2 copy number ≥6.0/cell (the ratio...
of HER-2/cell number) was evaluated in FISH (12). HER-2 and CEP17 signals of ≥20 nuclei of tumor cells within invasive tumor areas were measured to determine the HER-2/CEP17 ratio. If the HER-2/CEP17 ratio exceeded 2 or the absolute HER-2 copy number exceeded 6, tumors were considered to be HER-2 FISH amplified, according to the current ASCO/CAP guidelines.

**pCR**

pCR was defined as no residual invasive cancer in the breast and axillary lymph nodes, irrespective of ductal carcinoma in situ residual components (ypT0/is ypN0).

**Statistical Analysis**

Quantitative data were assessed for normality using the Shapiro–Wilk (S-W) method; t-test was used for comparison with normal distribution; the rank-sum test was used for comparison with non-normal distribution. Categorical variables are compared by Fisher’s exact test or χ² test. The multivariate analysis was carried out using multivariable Logistic Regression model. And multivariable Logistic Regression model was fit by a backward selection method with an alpha =0.20 removal criteria. The receiver operator characteristic (ROC) curve was used to determine the cutoff value of the HER-2/CEP17 ratio and HER-2/cell number ratio in FISH detection for the prognostic value. The value corresponding to the maximum Youden index is chosen as the optimal cutoff value. All statistical analyses were conducted using SPSS 23.0 statistical software to analyze the data. The p-values were two-sided, and p<0.05 was considered statistically significant.

**RESULTS**

**Baseline Characteristics**

A total of 57 patients, aged 48.46-years-old, were included in this study. The baseline characteristics were listed in Table 1. 29/57 (50.88%) patients were ER-positive, and 25/57 (43.86%) patients were PR-positive. Patients with pretreatment clinical T stages of T1, T2, and T3 accounted for 4/57 (7.02%), 39/57 (68.42%), and 14/57 (24.56%) patients, respectively. 36/57 (63.16%) patients had pretreated lymph nodes. Among them, 1 (1.75%), 47 (82.46%), and 9 (15.79%) patients had pretreatment puncture pathology grade 1, 2, and 3, respectively. In terms of chemotherapy regimen, 36/57 (63.16%) patients received anthracycline combined with taxane regimen [A(E)C-T/T-A(E)C], 11 (19.30%) patients received taxane combined with carboplatin regimen (TCb), and 10 (17.54%) patients received single taxane regimen (T) treatment.

**HER-2 Status**

IHC assessment revealed that 40 (70.18%) patients were IHC 3+ and 17 (29.82%) patients were IHC 2+. IHC 2+ was further confirmed with positive FISH results. Among 57 patients, the average HER-2/CEP17 ratio was 5.55, the median was 5.25 (range:1.04–15.60), and the average of HER-2 copies/cell (HER-2 signal/cell number) was 15.08, and the median was 15.24 (range: 2.03–25.00). The average HER-2/CEP17 ratio among HER-2 IHC2+ patients and HER-2 IHC3+ patients was 4.17 and 6.23, respectively, and the difference was statistically significant (Z=-9.979, p<0.001). The average of

**TABLE 1 | Baseline characteristics and pCR of 57 patients.**

| Parameter                        | Total (n=57) | pCR (n=41) | Non-pCR (n=16) | pCR rate | χ² | p value |
|----------------------------------|--------------|------------|----------------|-----------|----|---------|
| Age (years)                      | 48.46 ± 10.89| 48.37 ± 11.37| 48.69 ± 9.89 | 0.099     | 0.921 |
| Pretreatment clinical T staging  |              |            |                |           |     |         |
| cT1                              | 4            | 0.07       | 4              | 0.10      | 0  | 0.00    |
| cT2                              | 39           | 0.68       | 28             | 0.68      | 11 | 0.69   |
| cT3                              | 14           | 0.25       | 9              | 0.22      | 5  | 0.31   |
| Pretreatment axillary lymph node status | | | | | | |
| Positive                         | 36           | 0.63       | 25             | 0.61      | 11 | 0.69   |
| Negative                         | 21           | 0.37       | 16             | 0.39      | 5  | 0.31   |
| Chemotherapy                     |              |            |                |           |     |         |
| A(E)C-T/T-A(E)C                  | 36           | 0.63       | 25             | 0.61      | 11 | 0.69   |
| TCb                              | 11           | 0.19       | 8              | 0.20      | 3  | 0.19   |
| T                                | 10           | 0.18       | 8              | 0.20      | 2  | 0.13   |
| Pretreatment puncture pathology grading | | | | | | |
| 1                                | 1            | 0.02       | 0              | 0.00      | 1  | 0.06   |
| 2                                | 47           | 0.82       | 35             | 0.85      | 12 | 0.75   |
| 3                                | 9            | 0.16       | 6              | 0.15      | 3  | 0.19   |
| ER                               |              |            |                |           |     |         |
| Positive                         | 29           | 0.51       | 15             | 0.37      | 14 | 0.88   |
| Negative                         | 28           | 0.49       | 26             | 0.63      | 2  | 0.13   |
| PR                               |              |            |                |           |     |         |
| Positive                         | 25           | 0.44       | 12             | 0.29      | 13 | 0.81   |
| Negative                         | 32           | 0.56       | 29             | 0.71      | 3  | 0.19   |
| HER-2 IHC results                |              |            |                |           |     |         |
| 2+                               | 17           | 0.30       | 11             | 0.27      | 6  | 0.38   |
| 3+                               | 40           | 0.70       | 30             | 0.73      | 10 | 0.63   |
HER-2 copies/cell among HER-2 IHC2+ patients and HER-2 IHC3+ patients was 11.80 and 16.68, respectively, and the difference was statistically significant (Z=-7.604, p<0.001).

**pCR and Its Clinicopathological Association**

Among 57 patients, 41 (71.93%) achieved pCR. The pCR rate for ER-negative and ER-positive subgroups was 92.86% (26/28) and 51.72% (15/29), respectively, and the difference was statistically significant (χ² = 11.937, p=0.001). On the other hand, the pCR rate of PR-negative and PR-positive patients was 90.63% (29/32) and 48.00% (12/25), significantly, and the difference was statistically significant (χ² = 12.630, p<0.001). The pCR rates of pretreatment T clinical stages T1, T2, and T3 were 100.00% (4/4), 71.74% (28/39), and 64.29% (9/14), respectively, and the difference was statistically significant (χ² = 28.472, p<0.001). The pCR rates of pretreatment puncture pathology grade 1, 2, and 3 were 0.00% (0/1), 74.47% (35/47), and 66.67% (6/9), respectively, and the difference was statistically significant (χ² = 30.959, p<0.001). However, there was no statistical correlation between patient age, pretreatment lymph node status and neoadjuvant chemotherapy regimens and pCR rates (Table 1).

Multivariate analysis showed that ER and PR are significant predictors of pCR (Table 2). ER-negative tumors had significantly higher pCR rates compared to ER-positive tumors (odds ratio (OR)=0.074, 95% CI: 0.008–0.687, p=0.022). Compared to PR-positive patients, PR-negative patients had a significantly higher pCR rates (OR=0.179, 95% CI: 0.036–0.897, p=0.036). In multivariate analysis, pretreatment, clinical T stage, and pretreatment pathology were not associated with high pCR rates. It should be noted that 1) there was a strong correlation between HER-2 IHC results with HER2/CEP17 ratio and HER2 copy number; but in this study, there was no statistical correlation between HER-2 IHC results with HER2/CEP17 ratio and HER2 copy number; and 2) it might be related to the small sample size of this study, and we will actively expand the sample size in future research. Therefore HER2/CEP17 ratio and HER2 copy number were not included in the multivariate model.

**HER-2 Status and pCR**

The pCR rate of patients with HER-2 protein overexpression (IHC 3+) was 75.00% (30/40). Conversely, the pCR rate of patients with HER-2 IHC 2+ and HER-2 gene amplification detected by FISH was 64.71% (11/17) and no statistically significant difference was detected in the pCR between IHC 2+ (HER-2 FISH positive) and 3+ patients (χ² = 0.626, p=0.523).

Increased HER-2/CEP17 ratio and HER-2 copy number/cell were associated with a high rate of pCR. Among 41 patients that reached pCR, the average HER-2/CEP17 ratio was 7.27, and the median was 5.85 (range: 3.59–15.60). The average HER-2/CEP17 ratio in 16 patients that did not reach pCR was 3.98, the median was 4.17 (range: 1.04–7.35), and the difference was statistically significant (Z=-4.973, p<0.001). The average number of HER-2 copies/cell (HER-2 signal/cell number) in 41 patients in pCR was 17.13, and the median was 16.10 (range: 10.83–25.00); the average HER-2 copy number in 16 patients with no pCR was 10.55, and the median was 10.00 (range: 2.03–19.27), and the difference was statistically significant (Z=-10.838, p<0.001) (Table 3).

The ROC curve (Figure 1) determined the cutoff value of HER-2/CEP17 ratio in FISH detection, which was 4.955 (area under the curve (AUC)=0.764, p=0.001), and the cutoff value of HER-2 copy number per cell was 11.650 (AUC=0.814, p<0.001). According to the HER-2 copy number cutoff value, the patients were divided into high and low groups. Among ER-positive patients, the pCR rate of patients with high and low HER-2 copy number was 72.73% (16/22) and 14.28% (2/14) respectively, and the difference was statistically significant (χ² = 11.688, p=0.002). Among ER-negative patients, 27 had high HER-2 copy number, and only 1 patient did not reach pCR; moreover, only 1 patient had low HER-2 copy number and did not reach pCR, and the difference was not statistically significant (p=0.071). Among the HER-2 IHC 2+ patients, 12 with high HER-2 copy number achieved pCR, while the pCR rate of patients with low HER-2 copy number was only 11.11% (1/9),

### Table 2 | Multivariate logistic analysis of pCR.

| Uni p value | OR  | β   | SD  | Wald χ² | 95% CI          | p value |
|-------------|-----|-----|-----|---------|-----------------|---------|
| **ER**      |     |     |     |         |                 |         |
| Negative    | 0.001 | 1   | 2.598 | 5.251   | 0.0008-0.687    | 0.022   |
| Positive    | 0.074 |     |       |         |                 |         |
| **PR**      | <0.001 | -1.721 | 0.823 | 4.375   | 0.036-0.897    | 0.036   |
| Negative    | 1    |     |       |         |                 |         |
| Positive    | 0.179 |     |       |         |                 |         |

pCR is assigned a value of 1, and non-pCR is assigned a value of 0; ER-negative is assigned a value of 0, and ER-positive is assigned a value of 1; PR-negative is assigned a value of 0, and PR-positive is assigned a value of 1; the puncture pathology grades 1, 2, and 3 are assigned as 1, 2, and 3, respectively.
and the difference was statistically significant (p<0.001). Among HER-2 IHC 3+ patients, the pCR rate of patients with high and low HER-2 expression was 81.08% (30/37) and 16.67% (1/6), respectively, and the difference was statistically significant (p=0.004).

According to the cutoff value of the HER-2/CEP17 ratio, patients were divided into high and low groups. Among ER-positive patients, the pCR rate of patients with high and low HER-2/CEP17 ratio was 83.33% (15/18) and 16.67% (3/18), respectively, and the difference was statistically significant ($\chi^2 = 7.111$, p=0.018). Among ER-negative patients, the pCR rate of patients with high and low HER-2/CEP17 ratio was 94.12% and 90.91% (10/11), respectively, and the difference was not statistically significant (p=1.000). Among the HER-2 IHC 2+ patients, 8 patients with a high HER-2/CEP17 ratio achieved pCR, and the pCR rate of patients with low HER-2/CEP17 ratio was 38.46% (5/13), with a statistically significant difference (p=0.007). In HER-2 IHC3+ patients, the pCR rate of patients with high and low HER-2/CEP17 ratio was 80.56% (29/36) and 28.57% (2/7), and the difference was statistically significant ($\chi^2 = 7.872$, p=0.007).

**DISCUSSION**

Breast cancer is a heterogeneous disease with diverse phenotypes. About 20–25% of invasive breast cancers have HER-2 gene amplification or overexpression. Both early and metastatic breast cancers with HER-2-positive disease should be treated with a regimen containing anti-HER-2 therapy. The preoperative neoadjuvant therapy containing HER-2-targeted drugs for locally advanced HER-2-positive breast cancer patients reduces the size of primary tumors and axillary tumors, provides patients with surgical opportunities, improves patient prognosis, and identifies patients who may benefit from treatment or who do not respond satisfactorily (14, 15).

Presently, the data of dual-target neoadjuvant therapy in the Asian population is not sufficient. In various clinical studies (9, 16), the Asian population only accounts for about 20%, and the majority of subjects are Caucasians. Pertuzumab has been on the market in China for only 2 years, and no large-scale clinical data on dual-target neoadjuvant therapy in China are available. This is a small-sample real-world study of dual-target neoadjuvant therapy that provides data for domestic patients with HER-2-positive breast cancer adopting dual-target neoadjuvant therapy.

Dual-target neoadjuvant therapy with trastuzumab and pertuzumab blocked homodimer and heterodimer active model of HER-2, blocking the downstream signal transduction and showing promising efficacy against HER-2 positive breast cancer (17–21). In addition to the recurrence rate, the overall survival, DFS, and other indicators for evaluating the efficacy of adjuvant therapy, pCR is a major index for evaluating the efficacy of patients receiving neoadjuvant therapy. In the NeoSphere study, neoadjuvant therapy with trastuzumab, pertuzumab, and docetaxel increased the pCR rate from 24% to 46%, indicating long-term survival benefits (22). The current study represented 71.93% pCR rates for HER-2-positive breast cancers patients, which was obviously higher than the reported data. This phenomenon could be attributed to the following reasons. First, 82.46% of patients used the dual-drug chemotherapy (23.40% of patients used the TCb regimen chemotherapy, 76.60% of patients used the A(E)C-T/T-A(E)C regimen chemotherapy), which improved the efficacy. Second, the patients in this study received 6–8 cycles of neoadjuvant therapy.
therapy, which was higher than the four cycles of neoadjuvant therapy in the NeoSphere study. Compared to the current pCR of about 56–60% in other studies (23–25), the pCR rate in the present study is higher, which might be related to changes in HER-2 pathological evaluation criteria. All patients in this study had onset after 2019, and the 2018 ASCO/CAP guidelines (12) were used to assess the HER-2 status, while most of the current studies are based on the 2015 ASCO/CAP guidelines to assess HER-2 status. The 2018 ASCO/CAP guidelines defined group2 as HER-2-positive in the original guidelines as negative. The new version of the guidelines may have excluded some patients with poor efficacy of anti-HER-2 therapy. In addition, since this was a small sample size retrospective study, there may be population selection bias. We will continue to follow up to observe the long-term survival data and confirm the long-term benefits of a high pCR rate.

Hormone receptor (HR) expression also has a predictive significance for the efficacy of neoadjuvant therapy. In this study, the pCR rate of HR-negative patients was higher than that in positive patients. Similarly, the NeoSphere study showed improved pCR in HR-negative patients (9). André et al. claimed that the crosstalk between HRs and HER-2 signaling pathways might be the putative reason for the low pCR of HR-negative patients (26). Some studies have shown that the pCR benefits of HR-negative patients are correlated with long-term survival benefits (27).

The evaluation of HER-2 status is significant for selecting anti-HER-2 therapy of patients. Reportedly, the consistency of IHC and FISH in assessing HER-2 status was >80%. The inconsistency between the two occurred when HER-2 IHC 2+ (28). Previous studies also showed that IHC 2+ (FISH-positive) patients have a lower pCR rate, which was consistent with our results (29, 30). In the current study, patients with HER-2 IHC 3+ had higher pCR rates than those with IHC ≤2+ (FISH-positive), but the difference was not statistically significant. This phenomenon could be attributed to the small sample size. An accurate prediction of anti-HER-2 efficacy would guide the clinical study (31, 32). According to previous data, the ratio of HER-2/CEP17 in FISH predicts the efficacy of trastuzumab-based anti-HER-2 neoadjuvant therapy and long-term prognosis. The pCR rate was high when the HER-2/CEP17 ratio was higher than 6.0 and 7.0 (33, 34). In another study (23), the critical value that affects the pCR rate is set as HER-2/CEP17 = 4.5 and HER-2/cell number=14. In the current study, the ratio of HER-2/CEP17 and the ratio of HER-2/cell number had a specific correlation with the pCR rate. Patients with an HER-2/CEP17 ratio ≥4.49 or HER-2/cell number ≥11.650 have a high pCR rate, indicating its predictive value.

In conclusion, the response to dual-targeted neoadjuvant therapy with trastuzumab and pertuzumab was better in hormone receptor-negative, HER-2 positive breast cancer patients. Patients with a HER-2/CEP17 ratio ≥4.49 or HER-2/cell number ≥11.650 have a high pCR rate, indicating its predictive value.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by independent ethics committee of the Second Affiliated Hospital of Anhui Medical University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

**AUTHOR CONTRIBUTIONS**

FL contributed to provided financial support. FL and NL contributed to conception, design, administrative support, revised the important knowledge content of the study and ensured the submission adheres to all journal requirements. QJ organized the database, performed the statistical analysis and wrote the first draft of the manuscript. FL, QJ and CG wrote sections of the manuscript. JL, XW, MY, LZ, MH, QL and XJ contributed data acquisition and analysis tools. All authors contributed to the article and approved the submitted version.

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