Contrast enhanced computed tomography is indicative for angiogenesis pattern and display prognostic significance in breast cancer

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Abstract

Background: The Prognostic value of microvessel density in cancer remains unclear. Recent studies have suggested that the uneven distribution of microvessels in tumours caused the variation in sample selection which led to different prognostic outcome. The enhancement pattern of Contrast-enhanced computed tomography (CECT) is determined in part by the microvessel distribution in solid tumors. Therefore, survival analysis of tumors grouping by the enhancement pattern and the pattern of microvessel distribution is important.

Methods: Survival analysis grouped by the tumor enhancement pattern and the microvessel distribution was carried out in 255 patients with invasive ductal carcinoma.

Results: There were significant differences in overall survival (OS) and disease-free survival (DFS) among the homogeneous, heterogeneous and peripheral enhancement groups. There were significant differences between OS and DFS groups with uniform and uneven distributions of microvessels.

Conclusions: The distribution of microvessels in a tumor is a potential prognostic indicator in patients with breast cancer, and can be assessed by CECT prior the operation.

Background

Angiogenesis is the formation of new blood vessels from the endothelium of the existing vasculature. When a new tumor reaches 1–2 mm in size, its growth requires the induction of new blood vessels, which may lead to the development of metastases via the penetration of malignant cells into the blood circulation [1]. Microvessel density (MVD) assessment was once considered a useful indicator in the selection of those node-negative patients with breast carcinoma who are at high risk to have occult metastasis at presentation [2], and was also an commonly used important technique to quantify angiogenesis in other solid tumours [3]. However, its prognostic value remains unclear. The majority of published studies have shown a positive correlation between intratumoral MVD and prognosis in solid tumours [4], but not all studies have demonstrated such association, and this may be attributed to the significant differences in sample collection, immunostaining techniques, vessel counting and statistical analysis, although a number of biological differences may also account for the discrepancy [5]. Recently, it has been accepted that the discrepancy is due to the undifferentiated vessel density caused by variation of sample selection [6], and some researchers even began to apply computing analysis to quantify vascular properties pertaining to size, shape and spatial distributions in photographed fields of CD34 stained sections [7]. Contrast-enhanced computed tomography (CECT)-based criteria improve the diagnostic accuracy of sentinel lymph node metastases and are useful for evaluating the axillary status in patients with early-stage breast cancer [8]. The enhancement pattern of computed tomography (CT) is determined in part by the distribution of microvessels in solid tumours [9]. Therefore, it is important to carry out survival analysis grouping by the enhancement pattern and the pattern of microvessel distribution.

Methods

Study population

Between January 2008 and December 2011, a total of 259 patients with invasive ductal carcinoma (IDC) were
were used in luminal A tumours. Five markers were used in serial sections from each case; EGFR and Ki67 antibodies were used for immunohistochemical studies on serial tissue retrieval. Antibodies recognizing the ER, PgR, and HER2 growth factor receptor (EGFR), which did not need retrieval, were used for all staining except for the epidermal growth factor receptor (EGFR), which did not need retrieval. Antibodies recognizing the ER, PgR, and HER2 were used for immunohistochemical studies on serial tissue sections from each case; EGFR and Ki67 antibodies were used in luminal A tumours. Five markers were assessed: ER, PgR, HER2, and EGFR, which were used for breast carcinoma subtypes, and Ki67, which was used to divide luminal A tumours into two groups. The primary antibodies used in this study include ER (SP1, 1:200 dilution; ZETA), PgR (SP2, 1:200 dilution; ZETA), HER2 (CB11, 1:100 dilution; Invitrogen, Carlsbad, CA, USA), EGFR (SP9, 1:100 dilution; Invitrogen), Ki67 (K-2, 1:100 dilution; Invitrogen) and antiCD34 (class II, clone QBEnd 10, Dako-Cytomation, Glostrup, Denmark, dilution 1:50). Immunostaining was scored in a double-blinded manner by two different pathologists who were blinded to the clinicopathologic characteristics and outcome of each patient. For each antibody, the location of immunoreactivity, percentage of stained cells, and intensity were determined. The evaluation of protein expression was determined as mean ± SEM from each individual case. ER and PgR staining was assessed by Allred scoring, with positive scores ranging from 2 to 8 [10]. EGFR staining was considered positive if any cytoplasmic and/or membrane staining was observed. HER2 (IHC) was defined as strong whole membrane staining in >30% of the tumor cells, and Ki67 status was expressed in terms of percentage of positive cells, with a threshold of 14% of positive cells [11]. Fluorescence in situ hybridization (FISH) analysis was performed on IHC + tumours using the PathVysion HER2 DNA Probe Kit (Vysis, Downers Grove, IL, USA). HER2-positive staining was defined as FISH-positive, and HER2-negative staining was defined as IHC 0 or negative FISH results.

Clinicopathological subtypes

The clinical pathological subtypes of breast cancer were described, and were best matched with gene expression patterns [12]. Briefly, the subtype definitions are as follows: luminal A (ER+ and/or PgR+ and HER2- and/or Ki67 < 14%), luminal B (ER+ and/or PgR+ and HER2+ and/or Ki67 ≥ 14%), HER2 overexpression (ER-, PgR-, and HER2+), triple-negative (ER-, PgR-, HER2-).

Contrast-enhanced computed tomography and tumor enhancement patterns

All CECT examinations were performed on a 64-detector row scanner (Siemens, Germany, Definition 2008 G H-SP), with the patients lying in prone position and with both arms spread out from the body. Bilateral whole breast scanning was performed within a single breath-hold with 1-mm detector raw collimation for breast cancer screening. The technical parameters were standardized as follows: 120 kV, 36 mA and 3-mm-thick contiguous section. CT images from the lower edge of breast to neck were obtained, for which 80 mL of non-ionic contrast material (Omnipaque 350, Cork, Ireland) was injected intravenously at a flow rate of 2.5 mL/s. Postcontrast CECT scanning was initiated 30 s after the start of contrast. The delay
between the initiation of injection and evaluation of contrast enhancement was 60 s for early-phase imaging and 90 s for late-phase imaging. Most of the breast malignant tumor in the CECT performance had tissue fortified; only a few were not strengthened. According to CECT imaging performance morphology, the enhanced patterns of the breast tumours were classified by into peripheral enhancement, heterogeneous enhancement, homogeneous enhancement and centric enhancement [Figure 1A]. Peripheral enhancement is similar to ring strengthening, in which mainly the surrounding area of neoplasm is fortified. The CT value difference between the surrounding area and the central area is more than 10 Hounsfield units (HU). Heterogeneous enhancement means that there is an obvious difference of reinforcement in the various areas of the tissue, and the CT value difference is more than 10 HU. Homogeneous enhancement means that there is no obvious difference in reinforcement in the

**Figure 1 Distribution of microvessels. A. CECT images. B. Immunohistochemical images.**

**Figure 2 Diagram of tumor partition.** Illustration: It was the main aim to lay the chest flat so that the edge of tumor must be located between the tumor and the normal breast tissue.
Table 1 Patient characteristics and survival analysis (by clinicopathological subtype)

| Characteristic                   | Luminal A (n = 119) | Luminal B Ki67+ (n = 52) | Luminal B HER2 + (22) | HER2 overexpression (16) | TNBC (46) | Statistics | P    |
|----------------------------------|---------------------|--------------------------|-----------------------|--------------------------|-----------|------------|------|
| Age (years)                      | 51.67 ± 10.10       | 50.88 ± 8.16             | 55.41 ± 8.98          | 52.13 ± 6.49             | 51.91 ± 10.90 | 0.896      | 0.467|
| Menopause                        |                     |                          |                       |                          |            |            |      |
| Postmenopausal                   | 56                  | 24                       | 14                    | 7                        | 22        |            |      |
| Premenopausal                    | 63                  | 28                       | 8                     | 9                        | 24        |            |      |
| Family history                   |                     |                          |                       |                          |            |            |      |
| No                               | 109                 | 49                       | 21                    | 16                       | 42        |            |      |
| Yes                              | 10                  | 3                        | 1                     | 0                        | 4         |            |      |
| Diameter                         | 2.15 ± 0.98         | 2.32 ± 0.94              | 2.43 ± 1.07           | 3.09 ± 1.49              | 2.50 ± 1.18 | 0.892      | 3.292|
| Quadrant                         |                     |                          |                       |                          |            |            |      |
| Areolar                          | 3                   | 1                        | 0                     | 0                        | 1         |            |      |
| Inner upper                      | 28                  | 8                        | 0                     | 2                        | 7         |            |      |
| Inner lower                      | 14                  | 5                        | 1                     | 2                        | 3         |            |      |
| Outer lower                      | 21                  | 11                       | 3                     | 2                        | 6         |            |      |
| Outer upper                      | 53                  | 27                       | 18                    | 10                       | 29        |            |      |
| Enhancement patterns             |                     |                          |                       |                          |            |            |      |
| Homogeneous                      | 40(33.6%)           | 18(34.6%)                | 7(31.8%)              | 5(31.3%)                 | 12(26.1%) |            |      |
| Heterogeneous                    | 71(59.7%)           | 29(55.8%)                | 8(36.4%)              | 3(18.8%)                 | 10(21.7%) |            |      |
| Peripherals                      | 8(6.7%)             | 5(9.6%)                  | 7(31.8%)              | 8(50.0%)                 | 24(52.2%) |            |      |
| Difference of MVD (Edge - Center)| 3.12 ± 6.26         | 3.18 ± 7.95              | 7.61 ± 8.53           | 7.98 ± 8.97              | 10.23 ± 8.72 | 0.892      | 3.292|
| Grade of DMVD                    |                     |                          |                       |                          |            |            |      |
| Uniform distribution             | 102(85.7%)          | 44(84.6%)                | 14(63.6%)             | 6(37.5%)                 | 21(45.7%) |            |      |
| Uneven distribution              | 17(14.3%)           | 8(15.4%)                 | 8(36.4%)              | 10(62.5%)                | 25(54.3%) |            |      |
| Histological grade               |                     |                          |                       |                          |            |            |      |
| I                                | 37(31.1%)           | 16(30.8%)                | 0(0%)                 | 16(31.3%)                | 9(19.6%)  |            |      |
| II                               | 77(64.7%)           | 20(55.8%)                | 16(72.7%)             | 10(62.5%)                | 34(73.9%) |            |      |
| III                              | 5(4.2%)             | 7(13.5%)                 | 6(27.3%)              | 5(31.3%)                 | 3(6.5%)   |            |      |
| Cancer thrombosis                |                     |                          |                       |                          |            |            |      |
| Negative                         | 89                  | 41                       | 11                    | 10                       | 34        |            |      |
| Positive                         | 30                  | 11                       | 11                    | 6                        | 12        |            |      |
| Nodal metastasis                 |                     |                          |                       |                          |            |            |      |
| Negative                         | 60                  | 24                       | 8                     | 3                        | 23        |            |      |
| Positive                         | 59                  | 28                       | 14                    | 13                       | 23        |            |      |
| Number of metastatic nodes       | 2.09 ± 4.16         | 2.94 ± 5.01              | 6.77 ± 9.63           | 11.88 ± 11.37            | 4.89 ± 9.70 | 0.892      | 3.292|
| Clinical stage                   |                     |                          |                       |                          |            |            |      |
| I                                | 33(27.7%)           | 15(38.8%)                | 1(4.5%)               | 0(0%)                    | 10(21.7%) |            |      |
| IIA                              | 38(31.9%)           | 11(21.2%)                | 10(45.5%)             | 4(25.0%)                 | 13(28.3%) |            |      |
| IIB                              | 39(32.8%)           | 21(40.4%)                | 4(18.2%)              | 4(25.0%)                 | 16(34.8%) |            |      |
| IIIA                             | 7(5.9%)             | 3(5.8%)                  | 3(13.6%)              | 3(18.8%)                 | 2(4.3%)   |            |      |
| IIIB                             | 10(8.8%)            | 2(3.8%)                  | 2(9.1%)               | 16(6.3%)                 | 1(2.2%)   |            |      |
| IIIC                             | 1(0.8%)             | 0(0%)                    | 2(9.1%)               | 4(25%)                   | 4(8.7%)   |            |      |
| IV                               | 0(0%)               | 0(0%)                    | 0(0%)                 | 0(0%)                    | 0(0%)     |            |      |
| P53 (%)                          | 27.76 ± 30.19       | 32.65 ± 34.17            | 41.55 ± 30.32         | 30.19 ± 32.23            | 35.50 ± 35.33 | 0.892      | 3.292|
various areas of the tissue, and the CT value difference is less than 10 HU. In centric enhancement, mainly the central area of the neoplasm is reinforced and the CT value difference between the central area and the surrounding area is more than 10 HU. Four patients failed to enter the study for the purpose of statistical relevance, including three patients without tumor strengthened image and one patient with centric enhancement, therefore 255 patients were ultimately enrolled in this study based on the classification of peripheral, heterogeneous and homogeneous enhancement. All patients signed the Informed Consent for contrast medium hypersensitivity and the radiation dose was 9 Smv.

| Operation  | 5.455  | 0.244 |
|------------|--------|-------|
| Mastectomy | 102    | 47    |
| Tumorectomy| 17     | 5     |

| Chemotherapy program | 49.253  | 0.002 |
|----------------------|---------|-------|
| Not performed        | 1(0.8%) | 0(0%) |
| CMF                  | 2(1.7%) | 0(0%) |
| CAF or AC            | 42(35.3%) | 17(32.7%) |
| CEF or EC            | 21(17.6%) | 12(23.1%) |
| T or TC or TP        | 42(35.3%) | 15(28.8%) |
| TAC or A-T           | 10(8.4%) | 8(15.4%) |

| Radiotherapy | 6.682  | 0.154 |
|--------------|--------|-------|
| Not performed | 67     | 27    |
|Performed     | 52     | 25    |

| Endocrine therapy | 262.436  | 0.000 |
|-------------------|----------|-------|
| Not performed     | 0(0%)    | 0(0%) |
| TAM               | 75(63.0%) | 32(61.5%) |
| LHRH              | 11(9.2%) | 7(13.5%) |
| AI                | 33(27.7%) | 13(25.0%) |

| Targeted therapy | 33.619  | 0.000 |
|------------------|---------|-------|
| Not performed    | 119(100%) | 52(100%) |
| Performed        | 0(0%)    | 0(0%) |

| Overall survival | 99.2%  | 98.1% |
|------------------|--------|-------|
| Event            | 0%     | 0%    |
| Deaths           | 0%     | 0%    |

| Lost to follow-up | 0      | 0     |
|-------------------|--------|-------|
| Median survival time | 54.0 | 48.0 |

| Disease-free survival | 98.3%  | 94.3% |
|-----------------------|--------|-------|
| Event                 | 2      | 3     |
| Local recurrence      | 0      | 0     |
| Contralateral breast cancer | 1 | 1 |
| Lung metastasis       | 0      | 0     |
| Hepatic metastasis    | 0      | 0     |
| Brain metastasis      | 0      | 0     |
| Multi-organ           | 1      | 1     |
| Lost to Follow-up     | 0      | 0     |

| Disease-free survival | 54.0  | 48.0 |
|-----------------------|-------|------|
| Follow-up time        | 4.741 | 0.001 |
| Median                | 23.0  | 25.5 |

| Range | 12.59 | 12.49 |

Table 1 Patient characteristics and survival analysis (by clinicopathological subtype) (Continued)
**Figure 3** (See legend on next page.)

| Parameters                      | Death         | Tumor Progression |
|---------------------------------|---------------|-------------------|
|                                 | Sig.          | EXP(B)            | Sig.          | EXP(B)          |
| Age                             | 0.395         | 0.846             | 0.114         | 0.953           |
| Menopause                       | 0.916         | 1.116             | 0.333         | 0.676           |
| Family History                  | 0.235         | 1.121             | 0.174         | 0.199           |
| Tumor Diameter                  | 0.219         | 1.947             | 0.029         | 1.596           |
| Quadrant                        | 0.252         | 1.159             | 0.383         | 1.253           |
| Pattern of Enhancement (CT)     | 0.255         | 4.535             | 0.000         | 10.311          |
| Histological Grade              | 0.416         | 0.511             | 0.195         | 1.711           |
| Cancer Thrombosis               | 0.228         | 0.331             | 0.793         | 0.839           |
| Lymph Node metastasis           | 0.037         | 9.384             | 0.020         | 4.542           |
| ER                              | 0.243         | 0.361             | 0.594         | 0.723           |
| PgR                             | 0.419         | 0.516             | 0.155         | 0.405           |
| HER2                            | 0.042         | 4.091             | 0.047         | 2.915           |
| Ki67                            | 0.352         | 2.197             | 0.554         | 3.538           |
| P53                             | 0.047         | 0.999             | 0.042         | 1.001           |
Tumor samples and distribution of tumor microvessel density

The largest section of the tumor, which was parallel to the chest wall and more than 3 mm thick, was obtained by open surgery. The center and edge of the tumor were determined by naked eye, and the weight of each specimen was more than 30 mg [Figure 2]. All samples were stored in the freezer (-86°C) after quick-freezing in liquid nitrogen. MVD was evaluated by immunohistochemical staining of tumor vessels for CD34 in whole tissue sections. Any immunopositive single cell or cluster of cells, clearly separated from adjacent clusters and from the background, with or without a lumen, was considered to be an individual vessel. Microvessels in the five most vascularized areas in a 200× magnification field (0.74 mm²) were counted simultaneously by two observers, and the average value of the five fields was calculated. The difference in MVD (DMVD) of each sample was the discrepancy from the average MVD at the edge minus that at the center of the tumor. If the discrepancy was less than 10 microvessels, the distribution of MVD was considered uniform; if the discrepancy was greater than or equal to 10, the distribution was considered uneven [Figure 1B].

Statistics

All statistical analyses were carried out using SPSS software (version 17.0 for Windows). Grouping criteria include clinicopathological subtypes, patterns of tumor enhancement, and distribution of MVD. The correlation analyses among various groups and the various biological factors were examined by the X² test or ANVOA analysis. For the survival analysis, Kaplan–Meier curves were constructed for overall survival (OS) and disease-free survival (DFS). The log-rank test was used to compare survival differences among the groups. Cox proportional hazards models were used to calculate relative risk accounting for covariates. P values less than 0.05 were considered statistically significant.

Results

Survival analysis grouping by clinicopathological subtype

One hundred and nineteen patients were classified as luminal A, 52 patients as luminal B with positive Ki67, 22 patients as luminal B with HER2 over-expression, 16 patients as HER2 over-expression, and 46 as triple-negative breast cancer. The characteristics of the 5 groups are listed in Table 1. There were significant differences in tumor diameter, patterns of tumor enhancement, DMVD (edge-center), grade of DMVD, histological grades, number of metastatic nodes, clinical stage, chemotherapy program, and in targeted therapy among the groups (P < 0.05) [Table 1]. With a follow-up period of 12 to 59 months, the actual OS of luminal A, luminal B with positive Ki67, luminal B with HER2 over-expression, HER2 over-expression, and of TNBC groups was 99.2%, 98.1%, 86.4%, 87.5%, and 91.3%, respectively, and there was significant difference among the groups (P = 0.024) [Table 1]. The median survival time of patients with luminal A, luminal B with positive Ki67, luminal B with HER2 over-expression, HER2 over-expression, and TNBC was 54, 48, 36, 29.4, and 35.2 months, respectively, and there was a significant difference among the groups (P = 0.000) [Table 1]. The actual DFS of luminal A, luminal B with positive Ki67, luminal B with HER2 over-expression, HER2 over-expression, and TNBC was 98.3%, 94.3%, 72.7%, 75.0%, and 82.6%, respectively, and there was a significant difference among the groups (P < 0.05) [Table 1]. With a follow-up period of 12 to 59 months, the actual OS of luminal A, luminal B with positive Ki67, luminal B with HER2 over-expression, HER2 over-expression, and TNBC groups was 99.2%, 98.1%, 86.4%, 87.5%, and 91.3%, respectively, and there was significant difference among the groups (P = 0.024) [Table 1]. The median survival time of patients with luminal A, luminal B with positive Ki67, luminal B with HER2 over-expression, HER2 over-expression, and TNBC was 54, 48, 36, 29.4, and 35.2 months, respectively, and there was a significant difference among the groups (P = 0.000) [Table 1]. At the same time, significant differences were observed among the curves for OS and DFS (P = 0.000; P = 0.000) [Figure 3A].

Cox proportional hazards model (including CT tumor enhancement patterns)

Fourteen independent biological factors were used to build a COX proportional hazard model for death and tumor progression, including the age, history of menopause, family history, tumor diameter, quadrant, patterns of CT enhancement, histological grade, cancer thrombosis, lymph node metastasis, and tumor markers. There were significant differences in the patterns of tumor enhancement, lymph node metastasis, and HER2 between death (P < 0.05) and Exp (B) (expose of the B coefficient), namely 4.555, 9.384 and 4.091, respectively. There were significant differences in the tumor diameter, patterns of tumor enhancement, lymph node metastasis, and HER2 between tumor progression (P < 0.05) and Exp (B), namely 1.596, 10.311, 4.542 and 2.910, respectively [Figure 3B].

Survival analysis grouping by tumor enhancement patterns

Eighty-two patients were classified as homogeneous enhancement, 121 patients as heterogeneous enhancement,
Table 2 Characteristics of patients and survival analysis (by the patterns of tumor enhancement)

| Characteristic                  | Homogeneous enhancement (n = 82) | Heterogeneous enhancement (121) | Peripheral enhancement (52) | Statistics | P     |
|--------------------------------|----------------------------------|---------------------------------|----------------------------|------------|-------|
| Age (years)                    | 51.35 ± 8.51                     | 52.24 ± 10.37                   | 52.00 ± 9.53               | 0.210      | 0.811 |
| Menopause                      |                                  |                                 |                            |            |       |
| Postmenopausal                 | 41                               | 54                              | 28                         | 1.388      | 0.499 |
| Premenopausal                  | 41                               | 67                              | 24                         |            |       |
| Family history                 |                                  |                                 |                            | 1.095      | 0.579 |
| No                             | 76                               | 111                             | 50                         |            |       |
| Yes                            | 6                                | 10                              | 2                          |            |       |
| Diameter                       | 2.08 ± 0.91                      | 2.31 ± 1.04                     | 2.78 ± 1.28                | 7.028      | 0.001 |
| Quadrant                       |                                  |                                 |                            | 12.629     | 0.125 |
| Areolar                        | 2                                | 1                               | 2                          |            |       |
| Inner upper                    | 13                               | 29                              | 3                          |            |       |
| Inner lower                    | 11                               | 9                               | 5                          |            |       |
| Outer lower                    | 13                               | 22                              | 8                          |            |       |
| Outer upper                    | 43                               | 60                              | 34                         |            |       |
| Difference of MVD (Edge - Center) | -0.42 ± 5.72                      | 3.73 ± 4.67                     | 17.02 ± 3.88               | 211.273    | 0.000 |
| Grade of DMVD                  |                                  |                                 |                            | 179.854    | 0.000 |
| Uniform distribution           | 77(93.9%)                        | 110(90.9%)                      | 0(0%)                      |            |       |
| Uneven distribution            | 5(6.1%)                          | 11(9.1%)                        | 52(100%)                   |            |       |
| Histological grade             |                                  |                                 |                            | 2.021      | 0.732 |
| I                              | 22                               | 31                              | 10                         |            |       |
| II                             | 51                               | 80                              | 35                         |            |       |
| III                            | 9                                | 10                              | 7                          |            |       |
| Cancer thrombosis              |                                  |                                 |                            | 2.910      | 0.233 |
| Negative                       | 60                               | 92                              | 33                         |            |       |
| Positive                       | 22                               | 29                              | 19                         |            |       |
| Nodal metastasis               |                                  |                                 |                            | 1.588      | 0.452 |
| Negative                       | 25                               | 61                              | 22                         |            |       |
| Positive                       | 47                               | 60                              | 30                         |            |       |
| Number of metastatic nodes     | 3.80 ± 6.88                      | 2.51 ± 5.30                     | 6.73 ± 10.17               | 6.540      | 0.002 |
| Clinical stage                 |                                  |                                 |                            | 19.007     | 0.040 |
| I                              | 20(24.4%)                        | 32(26.4%)                       | 7(13.5%)                   | 31.203     | 0.000 |
| IIA                            | 25(30.5%)                        | 37(30.6%)                       | 14(26.9%)                  | 34.485     | 0.000 |
| IIB                            | 29(35.4%)                        | 38(31.4%)                       | 17(32.7%)                  |            |       |
| IIIA                           | 4(4.9%)                          | 10(8.3%)                        | 4(7.7%)                    |            |       |
| IIIB                           | 2(2.4%)                          | 2(1.7%)                         | 3(5.8%)                    |            |       |
| IIIC                           | 2(2.4%)                          | 2(1.7%)                         | 7(13.5%)                   |            |       |
| IV                             | 0(0%)                            | 0(0%)                           | 0(0%)                      |            |       |
| ER                             |                                  |                                 |                            |            |       |
| Negative                       | 29(35.4%)                        | 32(26.4%)                       | 37(71.2%)                  |            |       |
| Positive                       | 53(64.6%)                        | 80(73.6%)                       | 15(28.8%)                  |            |       |
| PgR                            |                                  |                                 |                            |            |       |
| Negative                       | 24(29.3%)                        | 29(24.0%)                       | 36(69.2%)                  |            |       |
|                | Positive (%) | Negative (%) | Overall (%) |
|----------------|--------------|--------------|-------------|
| HER2 Positive  | 58(70.7%)    | 92(76.0%)    | 16(30.8%)   |
| HER2 Negative  | 70(85.4%)    | 110(90.9%)   | 37(71.2%)   |
| Positive       | 12(14.6%)    | 11(9.1%)     | 15(28.8%)   |
| Negative       | 53(64.6%)    | 77(63.6%)    | 18(34.6%)   |
| Ki67 Positive  | 29(35.4%)    | 44(36.4%)    | 34(65.4%)   |
| Ki67 Negative  | 33.98 ± 3.52 | 30.35 ± 3.13 | 30.27 ± 3.33 |
| P53 (%)        | 0.357        | 0.700        |             |
| Clinicopathological subtypes |             |             | 59.901      |
| Luminal A      | 40(48.8%)    | 71(58.7%)    | 8(15.4%)    |
| Luminal B (Ki67+) | 18(22.0%)  | 29(24.0%)    | 5(9.6%)     |
| Luminal B (HER2+) | 7(8.5%)    | 8(6.6%)      | 7(13.5%)    |
| HER2 overexpression | 5(6.1%)    | 3(2.5%)      | 8(15.4%)    |
| TNBC           | 12(14.6%)    | 10(8.3%)     | 24(46.2%)   |
| Operation      | 4.885        | 0.087        |             |
| Mastectomy     | 72           | 106          | 51          |
| Tumorectomy    | 10           | 15           | 1           |
| Chemotherapy program |             |             | 14.344      |
| Not performed  | 0            | 1            | 1           |
| CMF            | 1            | 1            | 0           |
| CAF or AC      | 23           | 42           | 7           |
| CEF or EC      | 19           | 27           | 16          |
| T or TC or TP  | 27           | 34           | 15          |
| TAC or A-T     | 12           | 16           | 13          |
| Radiotherapy   |              |              | 1.560       |
| Not performed  | 40           | 69           | 26          |
| Performed      | 42           | 52           | 26          |
| Endocrine therapy |           |              | 53.381      |
| Not performed  | 17(20.7%)    | 13(10.7%)    | 32(61.5%)   |
| TAM            | 38(46.3%)    | 68(56.2%)    | 13(25.0%)   |
| LHRH           | 7(8.5%)      | 8(6.6%)      | 3(5.8%)     |
| AI             | 20(24.4%)    | 32(26.4%)    | 4(7.7%)     |
| Targeted therapy |             |              | 2.329       |
| Not performed  | 79           | 119          | 52          |
| Performed      | 3            | 2            | 0           |
| Overall survival | 100%        | 95.9%        | 88.5%       |
| Event          | 0            | 5            | 6           |
| Deaths         | 0            | 5            | 5           |
| Lost to follow-up | 0           | 0            | 1           |
| Median survival time | 54.0       | 54.0         | 42.0        |
| Disease-free survival | 100%       | 95.9%        | 65.4%       |
| Event          | 0            | 5            | 18          |
| Local recurrence | 0           | 0            | 1           |
| Contralateral breast cancer | 0          | 0            | 4           |
and 52 as peripheral enhancement. The characteristics of the groups are listed in Table 2; there were significant differences in tumor diameter, DMVD (edge-center), grade of DMVD, number of metastatic nodes, clinical stage, ER, PgR, Her2, Ki67, clinicopathological subtypes, and endocrine therapy among the groups (P < 0.05) [Table 2]. With a follow-up period of 12 to 59 months, the actual OS of the homogeneous enhancement, heterogeneous enhancement, and peripheral enhancement groups was 100%, 95.9%, and 88.5%, respectively, and there was a significant difference among the groups (P = 0.018) [Table 2]. The median survival time of the homogeneous enhancement, heterogeneous enhancement, and peripheral enhancement groups was 54, 54, and 42 months, respectively, and there was a significant difference among the groups (P = 0.014) [Table 2]. The actual DFS of the homogeneous enhancement, heterogeneous enhancement, and peripheral enhancement groups was 100%, 95.9%, and 65.4%, respectively, and there was a significant difference among the groups (P = 0.000) [Table 2].

**Cox proportional hazards model (including MVD distribution)**

Fourteen independent biological factors were used to build a COX proportional hazard model for death and tumor progression, including age, history of menopause, family history, tumor diameter, quadrant, grade of DMVD, histological grade, cancer thrombosis, lymph node metastasis, and tumor markers. There were significant differences in the grade of DMVD and lymph node metastasis between death (P < 0.05) and Exp (B) (expose of the B coefficient), namely 62.369 and 19.393, respectively. There were significant differences in age, grade of DMVD, lymph node metastasis, and Ki67 between tumor progression (P < 0.05) and Exp (B), namely 0.905, 112.292, 4.827 and 4.180, respectively [Figure 3C].

**Survival analysis grouping by grade of DMVD**

Distribution was classified as uniform in 187 patients and as uneven in 68 patients. The characteristics of the two groups are listed in Table 3. There were significant differences between groups in tumor diameter, patterns of tumor enhancement, DMVD (edge-center), number of metastatic nodes, clinical stage, ER, PgR, Her2, Ki67, clinicopathological subtypes, chemotherapy, and endocrine therapy (P < 0.05) [14,15]. The results of our study show that only 18.8% of patients with the HER2 over-expression subtype and only 9.1% of patients with luminal B (HER over-expression) subtype received targeted therapy. Therefore, the curves for OS and DFS in the patients with HER2 over-expression (luminal B HER2+ and HER2 OE) were similar to those of the TNBC group and lower than those of other groups (luminal A and

| Lung metastasis | 0 | 0 | 2 |
| Hepatic metastasis | 0 | 0 | 5 |
| Brain metastasis | 0 | 1 | 2 |
| Multi-organ | 0 | 4 | 3 |
| Lost to follow-up | 0 | 0 | 1 |
| Disease-free survival | 54.0 | 54.0 | 29.5 |
| Follow-up time | 2.967 | 0.053 |
| Median | 21.0 | 25.0 | 21.0 |
| Range | 12.56 | 12.59 | 12.44 |

**Table 2 Characteristics of patients and survival analysis (by the patterns of tumor enhancement) (Continued)**

**Discussion**

According to a recent report from Morocco, TNBC, particularly the basal-like subgroup, has the poorest prognosis among the clinicopathological subtypes [13]. The HER2 over-expression subtype has an equally poor prognosis among Chinese women [14,15]. The results of our study show that only 18.8% of patients with the HER2 over-expression subtype and only 9.1% of patients with luminal B (HER over-expression) subtype received targeted therapy. Therefore, the curves for OS and DFS in the patients with HER2 over-expression (luminal B HER2+ and HER2 OE) were similar to those of the TNBC group and lower than those of other groups (luminal A and
| Characteristic                      | Uniform distribution (DMVD < 10) (n = 187) | Uneven distribution (DMVD > 10) (68) | Statistics | P   |
|-----------------------------------|-------------------------------------------|-------------------------------------|------------|-----|
| Age (years)                       | 51.86 ± 9.55                              | 52.04 ± 9.84                        | -0.138     | 0.890|
| Menopause                         |                                           |                                     | 0.389      | 0.572|
| Postmenopausal                    | 88                                         | 35                                  |            |     |
| Premenopausal                     | 99                                         | 33                                  |            |     |
| Family history                    |                                            |                                     | 0.012      | 1.000|
| No                                | 174                                        | 63                                  |            |     |
| Yes                               | 13                                         | 5                                   |            |     |
| Diameter                          | 2.21 ± 0.95                                | 2.67 ± 1.31                        | -3.061     | 0.002|
| Quadrant                          |                                            |                                     | 5.680      | 0.224|
| Areolar                           | 3                                          | 2                                   |            |     |
| Inner upper                       | 39                                         | 6                                   |            |     |
| Inner lower                       | 17                                         | 8                                   |            |     |
| Outer lower                       | 32                                         | 11                                  |            |     |
| Outer upper                       | 96                                         | 41                                  |            |     |
| Patterns of enhancement           |                                            |                                     | 179.854    | 0.000|
| Homogeneous                       | 77(41.2%)                                  | 5(7.4%)                            |            |     |
| Heterogeneous                     | 110(58.8%)                                 | 11(16.2%)                          |            |     |
| Peripheral                        | 0(0%)                                      | 52(76.5%)                          |            |     |
| Difference of MVD (Edge - Center) | 1.29 ± 5.03                                | 15.60 ± 4.27                        | -20.873    | 0.000|
| Histological grade                |                                            |                                     | 2.105      | 0.349|
| I                                 | 50                                         | 13                                  |            |     |
| II                                | 120                                        | 46                                  |            |     |
| III                               | 17                                         | 9                                   |            |     |
| Cancer thrombosis                 |                                            |                                     | 1.891      | 0.204|
| Negative                          | 140                                        | 45                                  |            |     |
| Positive                          | 47                                         | 23                                  |            |     |
| Nodal metastasis                  |                                            |                                     | 0.174      | 0.777|
| Negative                          | 88                                         | 30                                  |            |     |
| Positive                          | 99                                         | 38                                  |            |     |
| Number of metastatic nodes        | 2.91 ± 5.97                                | 6.21 ± 9.42                        | -3.302     | 0.001|
| Clinical stage                    |                                            |                                     | 18.458     | 0.002|
| I                                 | 46(24.6%)                                  | 13(19.1%)                          |            |     |
| IIA                               | 60(32.1%)                                  | 16(23.5%)                          |            |     |
| IIB                               | 64(34.2%)                                  | 20(29.4%)                          |            |     |
| IIIA                              | 11(5.9%)                                   | 7(10.3%)                           |            |     |
| IIIB                              | 2(1.1%)                                    | 5(7.4%)                            |            |     |
| IIIC                              | 4(2.1%)                                    | 7(10.3%)                           |            |     |
| IV                                | 0(0%)                                      | 0(0%)                               |            |     |
| ER                                |                                            |                                     | 18.731     | 0.000|
| Negative                          | 57(30.5%)                                  | 41(60.3%)                          |            |     |
| Positive                          | 130(69.5%)                                 | 27(39.7%)                          |            |     |
| PgR                               |                                            |                                     | 26.314     | 0.000|
| Negative                          | 48(25.7%)                                  | 41(60.3%)                          |            |     |
Table 3 Characteristics of patients and survival analysis (by grade of DMVD)  

|                   | Positive | Negative | \(p\)-Value |
|-------------------|----------|----------|-------------|
| **HER2**          | 139(74.3%) | 167(89.3%) | 0.003 |
| **Ki67**          | 10.786 0.003 | 9.786 0.003 |  |
| Negative (<14%)   | 120(64.2%) | 167(89.3%) | 0.003 |
| Positive (>14%)   | 67(35.8%) 9.786 0.003 | 20(10.7%) 9.786 0.003 |  |
| **P53 (%)**       | 32.08 ± 31.58 0.479 0.633 | 29.90 ± 33.94 0.479 0.633 |  |
| **Clinicopathological subtypes** | 42.300 0.000 |  |
| Luminal A         | 102(54.5%) | 167(89.3%) | 0.003 |
| Luminal B (Ki67+) | 44(23.5%) 9.786 0.003 | 8(11.8%) 9.786 0.003 |  |
| Luminal B (HER2+) | 14(7.5%) 9.786 0.003 | 8(11.8%) 9.786 0.003 |  |
| HER2 overexpression | 6(3.2%) 9.786 0.003 | 10(14.7%) 9.786 0.003 |  |
| TNBC              | 21(11.2%) 9.786 0.003 | 25(36.8%) 9.786 0.003 |  |
| **Operation**     | 0.819 0.485 |  |
| Mastectomy        | 166 | 166 |  |
| Tumorectomy       | 21 | 21 |  |
| **Chemotherapy program** | 17.357 0.004 |  |
| Not performed     | 0(0%) | 0(0%) |  |
| CMF               | 2(1.1%) 9.786 0.003 | 0(0%) 9.786 0.003 |  |
| CAF or AC         | 60(32.1%) 9.786 0.003 | 12(17.6%) 9.786 0.003 |  |
| CEF or EC         | 43(23.0%) 9.786 0.003 | 19(27.9%) 9.786 0.003 |  |
| T or TC or TP     | 59(31.6%) 9.786 0.003 | 17(25.0%) 9.786 0.003 |  |
| TAC or A-T        | 23(12.3%) 9.786 0.003 | 18(26.5%) 9.786 0.003 |  |
| **Radiotherapy**  | 2.012 0.160 |  |
| Not performed     | 104 | 104 |  |
| Performed         | 83 | 83 |  |
| **Endocrine therapy** | 38.443 0.000 |  |
| Not performed     | 27(14.4%) 9.786 0.003 | 35(51.5%) 9.786 0.003 |  |
| TAM               | 99(52.9%) 9.786 0.003 | 20(29.4%) 9.786 0.003 |  |
| LHRH              | 13(7.0%) 9.786 0.003 | 5(7.4%) 9.786 0.003 |  |
| AI                | 48(25.7%) 9.786 0.003 | 8(11.8%) 9.786 0.003 |  |
| **Targeted therapy** | 0.116 1.000 |  |
| Not performed     | 183 | 183 |  |
| Performed         | 4 | 4 |  |
| **Overall survival** | 99.5% 0.479 0.633 | 85.3% 0.479 0.633 |  |
| Event             | 1 | 1 |  |
| Deaths            | 1 | 1 |  |
| Lost to Follow-up | 0 | 0 |  |
| **Median survival time** | 54.0 14.885 0.000 | 54.0 14.885 0.000 |  |
| Disease-free survival | 99.5% 0.479 0.633 | 67.6% 0.479 0.633 |  |
| Event             | 1 | 1 |  |
| Local recurrence  | 0 | 0 |  |
| Contralateral breast cancer | 0 | 0 |  |
luminal B (Ki67). We found that the patterns of the tumor enhancement, lymph node metastasis and HER2 are significant relative risk factors for death and tumor progression.

CECT remains a cost-effective means to assess the status of axillary lymph nodes among patients with breast cancer despite the progress of positron emission tomography/computed tomography (PET/CT) and magnetic resonance imaging (MRI) [16,17]. Beginning in January 2008, most surgeons in our institution gradually adopted preoperative CECT for assessment of axillary lymph nodes. However, a recent study suggests that tumor vascularity is a potential predictor of treatment outcomes in metastatic renal cell carcinoma, and that CECT is correlated significantly with microvessel density [18]. In our study, if the pattern of tumor enhancement was replaced by the grade of DMVD in the Cox model, the grade of DMVD and lymph node metastasis were significant relative risk factors for death, and age, grade of DMVD, lymph node metastasis and Ki67 were significant relative risk factors for tumor progression.

We carried out survival analysis according to the patterns of tumor enhancement, and found that the tumors with peripheral enhancement had the poorest prognosis and tumors with homogeneous enhancement had the best prognosis. We then conducted survival analysis according to the distribution of MVD, and found that tumors with blood vessels concentrating on the edge had the poorest prognosis compared to other tumors. Therefore, our findings suggest that the distribution of microvessels in breast cancer may determine the prognosis.

About a decade ago, Linder et al. demonstrated that angiogenesis in pancreatic tumors was not uniform, and that the tumor cells with more microvessels had greater proliferation capacity than those with fewer microvessels [19]. The uneven distribution of MVD is most likely the reasonable explanation for the differences in the prognostic value of MVD reported in different studies [20-22]. Therefore, we are confident that the distribution of microvessels in a tumor is a useful indicator for prognosis among the breast cancer patients, and can be assessed preoperatively by CECT.

Conclusions
The distribution of microvessels in a tumor is a potential prognostic indicator in patients with breast cancer, and can be assessed by preoperative by CECT.

Competing interests
All authors declare no competing interests.

Authors’ contributions
Li and ZY carried out the Immunohistochemical stain, participated in data analysis and drafted the manuscript. GY, JS and GJ participated in the follow-up. LJ and ZW participated in the design of the study and performed the statistical analysis. ZY conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

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