ABSTRACT
The objective was to study the relationship among Her-2, Ki-67, p53 expression and the clinicopathologic characteristics of breast cancer in the patients of northern China. Expression of Her-2, Ki-67, p53 and clinical characteristics of 260 breast cancer patients were retrospectively studied. Her-2 overexpression led to higher incidence rates of infiltrating ductal carcinoma and axillary lymph node metastasis, bigger diameters of the primary tumors, later pTNM staging, and a lower incidence rate of ductal carcinoma in situ \((p < 0.05)\). High expression of ER and PR led to fewer patients classified histologically in higher grade \((p = 0.001)\), while high expression of Ki-67 and p53 caused more patients classified histologically in higher grade \((p = 0.001)\). In patients histologically classified in grade 1 and 2, the expression of Ki-67 and p53 was significantly \((p = 0.001)\) higher, and the expression of ER and PR was significantly lower, in Her-2 positive patients than Her-2 negative patients. Breast cancer with Her-2 overexpression was more likely to recur and metastasize than Her-2 negative breast cancer. Higher coincidence of high expression of p53 and Ki-67 with Her-2 overexpression and more progressed tumors suggested that in addition to p53, Ki-67 might also be a prognostic biomarker of breast cancer.

KEYWORDS
breast cancer; clinicopathologic characteristic; Her-2 overexpression; Ki-67; p53

Introduction
Breast cancer is globally the leading cause of death in women and ranks second in cancer-related mortality.\(^1\) Incidence rates of breast cancer in most regions of the world, especially in developing nations, are increasing.\(^2,3\) In China, breast cancer–related death is the fourth among all other cancers in women, and the incidence rate of breast cancer in urban areas is higher than in rural areas. There are clear differences in clinicopathological characteristics of breast cancer between central China and Western countries.\(^4\)

The human epidermal growth factor receptor 2 gene (Her-2/neu) is a member of HER family and encodes a receptor of molecular mass 185 kDa. These receptors are single transmembrane proteins consisting of an intracellular tyrosine binding domain with various tyrosine phosphorylation sites\(^5,6\) and an extracellular domain for ligand binding and a cytoplasmic tail.\(^7\) After dimerization, Her-2 induces various cellular functions such as cell growth, differentiation and survival through a different cascade. Through MAPK and PI3K signaling pathways, Her-2 prevents apoptosis and promotes cell proliferation.\(^8,9\) Her-2 overexpression leads to an aggressive form of breast cancer,\(^10\) which comprises 25% of all breast cancer cases.\(^11\) The Her-2 positive breast cancer patients have a lower survival rate than patients without Her-2 overexpression. Her-2 has been used as a predictive and prognostic biomarker of breast cancer.\(^12-14\)

P53, encoded by gene Tp53, is a transcription factor that as a tumor suppressor regulates the cell cycle. Mutation in the p53 genes causes the formation of proteins that are more stable than the wild type protein; the mutant proteins accumulate and can be analyzed by immunohistochemistry.\(^15,16\) The human p53 is a nuclear phosphoprotein composed of 393 amino acids. It represses or activates gene expression through binding at many sites of chromatin.\(^17,18\) Its level is low due to a shorter half-life in unarrested cells, but under stress conditions such as DNA damage, it is stabilized by posttranslational modifications.\(^19\) P53 controls cellular functions of cell cycle control, DNA repair, apoptosis, angiogenesis, and cellular stress response through targeting genes such as Mdm2, WAFI/CIPI,
WPI, BAX, PIG3, FASL, CSR, P21, etc. P53 is regulated at different levels. The human p53 gene encodes many isoforms of p53 protein through alternative splicing, alternative promoter usage and alternative initiation of translation. Many mutations in the Tp53 gene have been found in breast cancers and compose approximately 20–40% of all cases depending upon tumor size and stage of the disease. Mutation in the Tp53 gene seems to be an early event in breast tumorigenesis.

Progesterone receptors (PR) are encoded by the gene PGR and consist of 933 amino acids. Two progesterone receptors, PRA and PRB, are transcribed by the same gene using alternative promoters. PRA and PRB are identical except that the N-terminal of PRB contains an extra 164 amino acids. PRA represses the activity of PRB, while PRB is a major activator of transcription factors. During tumorigenesis, the ratio of PRA to PRB alters, resulting in more PRA than PRB. PR expression is a marker for normal estrogen receptor (ER) functions. Breast cancer patients with PR and ER show better prognosis and response to endocrine therapy than patients that lack these receptors.

Ki-67 is a nuclear protein of molecular mass 359 kDa and is commonly used for the detection and quantification of proliferating cells. An increase in its expression is associated with cell growth. It is commonly used as a diagnostic marker in various cancers including breast cancers.

It was found that Her-2 and Tp53 genes were both present on chromosome 17 and there was a strong association between Her-2 overexpression and p53 mutations in breast cancer. ER and PR were negatively correlated with Her-2 overexpression. The association between Her-2 and Ki-67 in breast cancer patients makes Ki-67 an emerging biomarker for breast cancer. This study retrospectively compared 160 cases of Her-2 overexpressing breast cancer patients with 100 cases of Her-2 negative patients in terms of clinicopathological characteristics and the expression of p53 and Ki-67, and further uncovered the connection among Her-2, p53 and Ki-67.

Results

Effect of Her-2 overexpression on clinic pathological characteristics

The demographic, clinic and pathological characteristics were compared between Her-2 positive and negative patients (Table 1). There were no significant differences in age (p = 1.000), onset time of breast cancer related to menopause (p = 0.130), tumor location (p = 0.430), first symptoms including the formation of lumps in breasts, nipple discharge, formation of axillary lumps and calcification (p = 0.550), and vessel carcinoma embolus (p = 0.126). The incidence rate of infiltrating ductal carcinoma was significantly higher (88.68% vs. 76.53%, p = 0.002) while the incidence rate of ductal carcinoma in situ was significantly lower (11.32% vs. 23.47%, p = 0.002) in Her-2 positive breast cancer patients than in Her-2 negative patients. The diameters of the primary tumors were significantly larger in Her-2 positive patients than the Her-2 negative patients (p = 0.036), with more T2 and T3 tumors in the Her-2 positive group than the Her-2 negative group. There was a significantly higher incidence rate of axillary lymph node metastasis in Her-2 positive patients than in Her-2 negative patients, with

| Variables                                           | HER-2 negative n = 99 | HER-2 positive n = 159 | P   |
|-----------------------------------------------------|-----------------------|------------------------|-----|
| Age(year), mean±sd.                                | 50.06 ± 13.77         | 49.23 ± 10.45          | 1.00|
| Onset time, n (%)                                   | 51(51.52)             | 97(61.01)              | 0.134|
| Pre-menopause                                       | 48(48.48)             | 62(38.99)              |     |
| Tumor location, n (%)                               | 43(43.43)             | 81(50.94)              | 0.430|
| Right                                              | 55(55.56)             | 77(48.43)              |     |
| Bilateral                                           | 1(1.01)               | 1(0.63)                |     |
| First symptoms                                      | 92(92.93)             | 152(95.60)             | 0.550|
| Vessel carcinoma embolus, n (%)                    | 90(90.91)             | 134(84.28)             | 0.126|
| No                                                  | 9(9.09)               | 25(15.72)              |     |
| Pathological type, n (%)                            | 75(76.53)             | 141(88.68)             | 0.002|
| Other                                               | 23(23.47)             | 18(11.32)              |     |
| Histological grade, n (%)                           | 14(14.14)             | 6(3.77)                | 0.001|
| 2                                                   | 57(57.58)             | 81(50.94)              |     |
| 3                                                   | 28(28.28)             | 72(45.28)              |     |
| Diameter of lumps, n (%)                            | 58(58.59)             | 67(42.14)              | 0.036|
| T1                                                  | 37(37.37)             | 82(51.57)              |     |
| T2                                                  | 4(4.04)               | 10(6.20)               |     |
| axillary lymph node metastasis, n (%)               | 66(66.67)             | 78(49.06)              | 0.024|
| N0                                                  | 20(20.20)             | 45(28.30)              |     |
| N2                                                  | 4(4.04)               | 19(11.95)              |     |
| N3                                                  | 9(9.09)               | 17(10.69)              |     |
| pTNM staging, n (%)                                 | 42(42.42)             | 44(27.67)              | 0.033|
| 0–1                                                 | 45(45.45)             | 83(52.20)              |     |
| III                                                | 12(12.12)             | 32(20.13)              |     |
more N1 (28.30% vs. 20.20%, p = 0.024) and N2 (11.95% vs. 4.04%, p = 0.024) cases in Her-2 positive patients than those who were Her-2 negative. There were significantly more patients classified histologically as higher grade in the Her-2 positive group than in the Her-2 negative group (grade 3, 45.28% vs. 28.28%, p = 0.001). There was a significant difference in pTNM staging (p = 0.033); more Her-2 positive patients than Her-2 negative patients were in stage II (52.20% vs. 45.45%) and stage III (20.13% vs. 12.12%).

**Effect of ER, PR, Ki-67 and p53 on clinicopathological characteristics**

The effects of ER expression (Table 3), PR expression (Table 4), Ki-67 expression (Table 2) and p53 expression (Table 5) on clinicopathological characteristics were studied through comparisons in breast cancer patients. ER (Table 3, p = 0.187), PR (Table 4, p = 0.589), Ki-67 (Table 2, p = 0.076) and p53 (Table 5, p = 0.523) expression did not influence the incidence rate of infiltrating ductal carcinoma in breast cancer patients. There were significantly fewer patients classified histologically as higher grade in the ER positive group than in the ER negative group (grade 3, 27.95% vs. 56.70%, p = 0.001) (Table 3) and in the PR positive group than in the PR negative group (grade 3, 29.33% vs. 51.85%, p = 0.001) (Table 4). Furthermore, there were significantly more patients classified histologically as higher grade in the Ki-67 high expression group than in the low expression group (grade 3, 45.08% vs. 18.75%, p = 0.001) (Table 2) and in the p53 positive group than in the p53 negative group (grade 3, 47.41% vs. 31.69%, p = 0.015) (Table 5). There was no significant difference in pTNM staging between the ER positive and ER negative groups (Table 3), the PR positive and PR negative groups (Table 4), the Ki-67 high expression and low expression groups (Table 2), or the p53 positive and p53 negative groups (Table 5).

### Table 3. Comparison of pathological type, histological grade and pTNM staging between ER negative and positive breast cancer patients.

| Variables                  | ER negative n=97 | ER positive n=161 | P   |
|----------------------------|------------------|-------------------|-----|
| Pathological type, n (%)   |                  |                   |     |
| IDC                       | 85(87.63)        | 131(81.37)        | 0.1867 |
| Other                     | 12(12.37)        | 30(18.63)         |     |
| Histological grade, n (%) |                  |                   |     |
| 1                         | 4(4.12)          | 6(3.74)           | <0.001 |
| 2                         | 38(39.18)        | 100(62.11)        |     |
| 3                         | 55(56.70)        | 45(27.75)         |     |
| pTNM staging, n (%)       |                  |                   |     |
| 0–I                       | 25(25.77)        | 61(37.89)         | 0.0923 |
| II                        | 56(57.73)        | 72(44.72)         |     |
| III                       | 16(16.49)        | 28(17.39)         |     |

*No Ki-67 data available for one case of patient.*

### Table 4. Comparison of pathological type, histological grade and pTNM staging between PR negative and positive breast cancer patients.

| Variables                  | PR negative n=108 | PR positive n=150 | P   |
|----------------------------|-------------------|-------------------|-----|
| Pathological type, n (%)   |                   |                   |     |
| IDC                       | 92(85.19)         | 124(82.67)        | 0.589 |
| Other                     | 16(14.81)         | 26(17.33)         |     |
| Histological grade, n (%) |                   |                   |     |
| 1                         | 6(5.56)           | 14(9.33)          | 0.001 |
| 2                         | 46(42.59)         | 92(61.33)         |     |
| 3                         | 56(51.85)         | 44(29.33)         |     |
| pTNM staging, n (%)       |                   |                   |     |
| 0–I                       | 29(26.85)         | 57(38.00)         | 0.091 |
| II                        | 62(57.41)         | 66(44.00)         |     |
| III                       | 17(15.74)         | 27(18.00)         |     |

**Difference in the expression of Ki-67, ER, PR and P53 between HER2 positive and negative breast cancer patients**

The difference of the expression of Ki-67, ER, PR and p53 between Her-2 positive and negative breast cancer patients was also studied (Table 6).

In histological grade 1 and 2 breast cancer patients, the expression of Ki-67 was significantly higher in Her-2 positive patients than Her-2 negative patients (p = 0.001), with higher coincidence rates of Her-2 positive/Ki-67 high expression than Her-2 positive/Ki-67 low expression (86.21% vs. 13.79%) and Her-2 negative/Ki-67 low expression than Her-2 negative/Ki-67 high expression (86.21% vs. 13.79%).

### Table 2.

| Variables                  | Ki-67 low expression n=64 | Ki-67 high expression n=193 | P   |
|----------------------------|---------------------------|-----------------------------|-----|
| Pathological type, n (%)   |                           |                             |     |
| IDC                       | 49(76.56)                 | 166(86.01)                  | 0.076 |
| Other                     | 15(23.44)                 | 27(13.99)                   |     |
| Histological grade, n (%) |                           |                             |     |
| 1                         | 10(15.63)                 | 10(5.18)                    | 0.001 |
| 2                         | 42(65.63)                 | 96(49.74)                   |     |
| 3                         | 12(18.75)                 | 87(45.08)                   |     |
| pTNM staging, n (%)       |                           |                             |     |
| 0–I                       | 27(42.19)                 | 59(30.57)                   | 0.202 |
| II                        | 29(45.31)                 | 99(51.30)                   |     |
| III                       | 8(12.50)                  | 35(18.13)                   |     |

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**Table 5. Comparison of pathological type, histological grade and pTNM staging between PR negative and positive breast cancer patients.**

| Variables                  | PR negative n=108 | PR positive n=150 | P   |
|----------------------------|-------------------|-------------------|-----|
| Pathological type, n (%)   |                   |                   |     |
| IDC                       | 92(85.19)         | 124(82.67)        | 0.589 |
| Other                     | 16(14.81)         | 26(17.33)         |     |
| Histological grade, n (%) |                   |                   |     |
| 1                         | 6(5.56)           | 14(9.33)          | 0.001 |
| 2                         | 46(42.59)         | 92(61.33)         |     |
| 3                         | 56(51.85)         | 44(29.33)         |     |
| pTNM staging, n (%)       |                   |                   |     |
| 0–I                       | 29(26.85)         | 57(38.00)         | 0.091 |
| II                        | 62(57.41)         | 66(44.00)         |     |
| III                       | 17(15.74)         | 27(18.00)         |     |
Ki-67 high expression (56.34% vs. 43.66%). The expression of p53 was also significantly higher in Her-2 positive patients than Her-2 negative patients (p < 0.001), with higher coincidence rates of Her-2 positive/p53 positive than Her-2 positive/p53 negative (54.72% vs. 45.28%) and Her-2 negative/p53 negative than Her-2 negative/p53 positive (70.71% vs. 29.29%). Finally, ER expression was significantly lower in Her-2 positive patients than Her-2 negative patients (p < 0.001) with a higher coincidence rate of Her-2 negative/ER positive than Her-2 negative/ER negative (79.80% vs. 20.20%), and PR expression was significantly lower in Her-2 positive patients than Her-2 negative patients (p < 0.001) with a higher coincidence rate of Her-2 negative/PR positive than Her-2 negative/PR negative (74.75% vs. 25.25%) (Table 6).

### Discussion

This study retrospectively analyzed 260 breast cancer patients of northern China for the expression of Her-2, PR, ER, p53, Ki-67 and clinicopathological characteristics. It was found that the expression of Her-2, PR, ER, p53 and Ki-67 influenced the clinicopathological characteristics of breast cancer. The effect of the expression of Her-2, PR, ER, Ki-67 and p53 on clinicopathological characteristics suggests that these genes may be used as predictive and/or prognosis biomarkers for breast cancer. The coincidence of Her-2

### Table 5. Comparison of pathological type, histological grade and pTNM staging between p53 negative and positive breast cancer patients.

| Variables                              | P53 negative | P53 positive | P  |
|----------------------------------------|--------------|--------------|----|
| Pathological type, n (%)               |              |              | 0.523 |
| IDC                                   | 117(82.39)   | 99(78.95)    |    |
| Other                                 | 25(17.61)    | 17(14.66)    |    |
| Histological grade, n (%)             |              |              | 0.015 |
| 1                                     | 15(10.56)    | 5(4.31)      |    |
| 2                                     | 82(57.75)    | 56(46.28)    |    |
| 3                                     | 45(31.69)    | 51(40.41)    |    |
| pTNM staging, n (%)                   |              |              | 0.115 |
| 0                                    | 49(34.51)    | 37(31.90)    |    |
| 1                                    | 75(52.82)    | 53(45.69)    |    |
| 2                                    | 18(12.68)    | 26(22.41)    |    |

Note: No Ki-67 data available for one case of patient.

### Table 6. Comparison of the expression of Ki-67, ER, PR and p53 between Her-2 positive and negative breast cancer patients.

| Variables | HER-2- tive | HER-2 + tive | P  |
|-----------|-------------|--------------|----|
| HER-2- tive | n = 71 (%)  | n = 87 (%)   |    |
| HER-2 + tive | n = 28 (%)  | n = 72 (%)   |    |
| P          | 0.593       |              |    |
| HER-2- tive | n = 99 (%)  | n = 159 (%)  |    |
| HER-2 + tive | n = 99 (%)  | n = 159 (%)  |    |

Note: No Ki-67 data available for one case of patient.
overexpression with Ki-67 high expression, p53 positive, PR negative and ER negative indicates that there may be some regulatory relationship between Her-2 and these genes in signaling transduction pathways.

**Role of Her-2, PR, ER, Ki-67 and p53 in breast cancers**

In this study, it was uncovered that Her-2 overexpression is connected with a higher incidence rate of infiltrating ductal carcinoma, a lower incidence rate of ductal carcinoma in situ, larger primary tumor diameters, a higher incidence rate of axillary lymph node metastasis, a higher histological grading, and a later pTNM staging in breast cancer patients, which is in agreement with previous reports.1,37-47 Our observations support that Her-2 overexpression leads to aggressive forms of breast cancer.11 P53 is a transcription factor that is encoded by the Tp53 gene and regulates the cell cycle as a tumor suppressor. Mutations in the p53 genes cause the formation of stabilized proteins, which accumulate and can be analyzed by immunohistochemistry.15,16 Our finding that p53 positive caused breast carcinoma to progress to a higher histological grade, which is in agreement with previous reports37,48,49 and suggests that mutation in p53 genes have a tumor promotion role in breast cancer patients,39 which is supported by previous reports.37 Ki-67 is a nuclear protein that is commonly used for the detection and quantification of proliferating cells because an increase in its expression is associated with cell growth.27 Consistent with the observations of others,6,37,50-53 our observation that Ki-67 positive led breast carcinoma progress to higher histological grade in breast patients implicated that Ki-67 high expression promoted tumor growth in breast cancer patients.50 Our observation that both PR positive and ER positive connected with a slower histological progress in breast cancer, which was in agreement with the observations by Shapochka et al.,37 suggests that PR and ER might have an inhibitive role in breast cancers. Both PR and ER facilitate cell growth through nuclear pathways and non-nuclear pathways.54 ER expression was found to be positively correlated with Her-2 expression in Her-2 non-overexpressing breast cancers.55 The connection of ER positive and a slower histological progress in breast cancer observed in this study might not be due to an ER-mediated inhibitive effect, but instead may be a result of negative associations between ER and Her-2 positive in breast cancers caused by the down-regulation of ER,57 higher ER protein turnover, and lower ER protein expression mediated by Her-2 overexpression through the PI3K/Akt signaling pathway.32,58 The connection of PR positive and a slower histological progress in breast cancer observed in this study might also be a result of a negative association between PR and Her-2 positive in breast cancers caused by the loss of PR protein due to Her-2 overexpression through the PI3K/Akt signaling pathway.59

**Relationship of Her-2, PR, ER, Ki-67 and p53 in signaling transduction pathways**

In this study, it was found that Her-2 overexpression was negatively connected with PR and ER expression and positively connected with Ki-67 positive and p53 positive, which was consistent with previous reports30,32,37,44,46,49,53,59,60 This suggests that there might be some relationship among Her-2 and these genes in signal transduction pathways. Through MAPK and PI3K signaling pathways, Her-2 prevents apoptosis and promotes cell proliferation.9 Both ER and PR, after activation by ligand binding, act on the transcription of their target genes in the nuclei, or through non-genomic pathways.54 Her-2, ER and PR may mediate their effects on breast cancer through crosstalk among Her-2, ER and PR pathways.54 Our observed negative connection among Her-2 overexpression, ER and PR expression might be the result of downregulation of ER,57 higher ER protein turnover, lower ER protein expression mediated by Her-2 overexpression through the PI3K/Akt signaling pathway,32,58 and loss of PR protein mediated by Her-2 overexpression through the PI3K/Akt signaling pathway.59 P53 is a transcription factor that is encoded by gene Tp53. Mutation in the p53 genes causes the formation of stable proteins that can be analyzed by immunohistochemistry.15,16 The detection of mutations in p53 genes in germline30 suggests that they may be genetically inherited. Both the p53 gene and the Her-2 gene are located in chromosome 17.30 It is not clear why p53 mutations are associated with Her-2 gene amplification.39,40 Ki-67 is a nuclear protein that is commonly used for the detection and quantification of proliferating cells.28 In agreement with the observations by others,35,50,51 it was found that Ki-67 positively connected with Her-2 overexpression in this study, suggesting that Her-2 overexpression might up-regulate the expression of Ki-67.
Her-2, PR, ER, Ki-67 and p53 as breast cancer biomarkers

Our finding that Her-2 overexpression is connected with a higher incidence rate of infiltrating ductal carcinoma, a lower incidence rate of ductal carcinoma in situ, bigger diameters of the primary tumors, a higher incidence rate of axillary lymph node metastasis, a higher histological grading, and a later pTNM staging in breast cancer patients further confirmed the observation that Her-2 positive breast cancer patients had a lower survival rate than patients without Her-2 overexpression, and supports the use of Her-2 as a predictive and prognostic biomarker of breast cancer.

In agreement with the observation by Kobayashi et al., our finding that p53 positive is connected with the progression of carcinoma to a higher histological grade and Her-2 overexpression in breast cancer patients suggests that p53 positive could be used as a prognostic biomarker for breast cancer in addition to being used as a diagnostic biomarker. Our findings that Ki-67 positive is connected with the progression of carcinoma to a higher histological grade as well as Her-2 overexpression in breast cancer patients are consistent with the previous observations and increase the reliability of this emerging biomarker as a predictor of breast cancer prognosis outcomes. Our observation that both PR positive and ER positive are connected with a slower histological progress and are negatively connected with Her-2 overexpression in breast cancer supports that PR positive and ER positive are good biomarkers for predicting better prognosis and response to endocrine therapy than patients that lacked these receptors.

It is concluded that Her-2 overexpression is related with invasive breast cancer. Her-2 overexpression is positively connected with p53 and Ki-67 expression, supporting that in addition to Her-2 and p53, Ki-67 is a prognosis biomarker for breast cancer.

Patients and methods

Patients

A cohort of 260 female breast cancer patients of northern China was included in the study. 160 of the patients were surgically and pathologically diagnosed to be primary breast cancer patients, and histochemically confirmed to be Her-2 positive; these patients had a mean age of 52.5 y (range 26–79, median 49 years). 100 cases were Her-2 negative patients, with a mean age of 53 y (range 28–78, median 50 years) (Table 1).

Methods

This was a comparative study retrieving the clinical records of 260 breast cancer patients for information about pathologic type, histological grade, vascular cancer embolus, axillary lymph node status, clinical tumor stage, Her-2 expression, p53 expression, ER expression, PR expression and Ki-67 expression. The patients were further grouped according to the TNM cancer staging system by the American Joint Committee on Cancer (AJCC) and the ASCO-CAP guideline for the pathological diagnosis of breast cancer. CerbB-2 was tested with immunochemistry and FISH. Her-2 (+++) was designated as Her-2 positive while Her-2 (+) was designated as Her-2 positive only when there was amplification of Her-2 gene detected with FISH. Estrogen receptor (ER), progesterone receptor (PR), p53 and Ki-67 were tested with immunochemistry. The percentage of ER positive cells or PR positive cells greater than 1% was designated as ER positive or PR positive. The percentage of Ki-67 positive cells less than 14% was designated as low expression, while greater than or equal to 14% was medium/high expression. For p53, cells were p53 positive if there were brown granules in the nuclei. Ten microscopic fields (x400) were examined in each section, and the percentage of p53 positive cells/C20 was designated as negative while > 5% as positive.

Data analysis

Statistical analysis was performed using SPSS 14.0 software. T-test was performed for normal distribution measurement data, and presented as mean ± SE. Rank sum test was performed for abnormal distribution measurement data, and presented as median (maximum, minimum). X2 test (Chi-square test) or Fisher’s exact probability analysis was performed for enumeration data and presented as the number of cases n (%). Rank sum test was performed for rank variable data and presented as the number of case n (%). p < 0.05 was considered statistically significant.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.
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