

Long-term Tracing of Carbon Nanoparticles for Axillary Lymph Node Dissection in Patients with Fusion Lymph Nodes before Neoadjuvant Chemotherapy

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Research article

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Abstract

Background: The regression model of positive nodes in breast cancer after neoadjuvant chemotherapy (NAC) remains controversial. This study aimed to investigate this regression model by injecting and tracing carbon nanoparticles (CNs) into the fusion node prior to NAC in patients with breast cancer.

Methods: Guided by ultrasound, 0.3 mL of CNs suspension was injected in a fusion node prior to NAC in 110 patients with local advanced breast cancer. Patients underwent breast surgery and total axillary lymph node dissection following 2-6 cycles of NAC. The distribution by intercostobrachial nerves (ICBN) of positive nodes, black-stained nodes and lymphovascular invasion was investigated by response to NAC.

Results: When patients were ranked by response to NAC (from sensitive to resistance), the number of positive nodes increased, as did the proportion of lymphovascular invasion, the number of black-stained nodes decreased. A significantly negative relationship was found between the number of positive nodes and the number of black-stained nodes ($p < 0.001$). The positive nodes in patients with sensitive consequence followed the rule from under the ICBN to above the ICBN. However, there was counter-example (skip metastasis) in the patients with resistance result.

Conclusion: The regression model of positive nodes follows the rule from upper to under, inner to outer in the patients with sensitive consequence to NAC. Long-term staining and tracing by CNs might provide an acceptable and feasible technique to investigate the regression model of positive nodes, and would be a potential method for NAC-treated patients by using of ICBN.

Trial registration: NCT 03355261. Retrospectively registered on November 28, 2017.

Background

Over the past 20 years, an increasing number of patients with early-stage breast cancer and negative sentinel lymph nodes (SLNs) have been able to avoid the risk of arm lymphedema caused by axillary lymph node dissection (ALND) [1]. SLN biopsy (SLNB) after neoadjuvant chemotherapy (NAC) is also a feasible and accurate tool in patients with operable T1–3, N1, and clinically node-negative breast cancer after therapy [2]. Some studies, however, regard the false negative rate (FNR) of 14% for SLNB after NAC as unacceptable when compared with the <5% rate produced by the pioneers of SLNB [3]. In fact, about 9% of patients with positive nodes and T0–2 breast cancer have residual positive nodes in the Level III region after NAC [4]. Therefore, it is essential to explore the regression model post-NAC in patients who are node-positive prior to NAC.

Studies from developed countries suggest that SLNB is a reliable method for lymph node evaluation in NAC-treated patients with breast cancer [5, 6]. However, they did not distinguish or exclude patients who were suspected or proven to harbor axillary metastases before receiving NAC. We speculated that the number of patients with N3 nodes in these studies was small. The proportion of patients in China with

advanced stage breast cancer is higher than that in more developed countries [7]. Therefore, Chinese surgeons are interested in the feasibility of SLNB for patients with local advanced breast cancer who are treated with NAC. Recent studies suggest that SLNB is technically feasible and accurate enough for axillary staging in initially clinically node-negative breast cancer patients after NAC [8]. Additionally, SLNB is feasible for patients whose axillary lymph nodal status is N1 before NAC; however, SLNB cannot be used as a reliable indicator of non-SLN status in N2–3 patients [9]. It seems that the more severity of positive nodes, the more FNR of SLNB after NAC, because extensive lymphatic invasion would affect the tracer. Multivariate analysis showed that residual breast tumor size after NAC ≥ 5 mm and lymphovascular invasion remained independent predictors for involved ALND [10]. There have been some approaches that have increased the accuracy of the procedure; for instance, the placement of clips at the time of diagnosis of node-positive disease with removal of the clipped nodes during SLNB reduces the FNR after NAC [11].

A new method is the use of carbon nanoparticle (CNs) suspensions for SLNB [12]. These suspensions contain nanosized polymeric carbon granules with an average diameter of 150 nm, which ensures the passing of CNs through lymphatic vessels (diameter 120–500 nm) rather than blood capillaries (diameter 20–50 nm), because of their special molecular characteristics, CNs are retained in the lymphatic system for a long time, which could be named fondness of lymphatic [13]. In the present study, a CNs suspension was injected into the fusion nodes guided by ultrasound in patients with local advanced breast cancer before NAC. We established hypothesis that if the tumor was sensitive to NAC, the extensive lymphovascular invasion would regress and the CNs would spread widely in axillary nodes. On the other hand, the previous study has proved that ALND inferior to the intercostobrachial nerves (ICBN) could reduce the occurrence rate for postoperative arm lymphedema in the patients with early-stage breast cancer. Therefore, total ALND and breast surgery were then performed after NAC. The distribution by ICBN in axillary of CNs traced nodes was investigated to establish the regression model of positive nodes in relation to NAC.

Methods

Between July 2014 and July 2018, a total of 110 patients with local advanced breast cancer treated at the Shengjing Hospital of China Medical University were recruited to the study. The inclusion criteria were: (1) invasive ductal carcinoma diagnosed by biopsy; (2) clinically positive nodes diagnosed by contrast-enhanced computed tomography (CECT), with the number of strengthened nodes at Level I ≥ 1 , with the longest diameter of the strengthened node ≥ 2 cm; (3) the NAC regimen followed the National Comprehensive Cancer Network guideline [14]; and (4) no prior history of breast cancer or other malignancies. All the patients provided written consent for CNs injection guided by ultrasound into the positive nodes at Level I prior to NAC. Each patient underwent 2 to 6 cycles of NAC, and the response was assessed every 2 cycles. The breast tumor and lymph nodes were assessed via magnetic resonance imaging and CECT, respectively. According to the RECIST 1.1, patients were allocated to the following 4 groups based on their response to NAC: complete remission (CR), partial remission (PR), stable disease (SD), and progressive disease (PD) [15]. Altogether, 32, 61, 12, and 5 patients were allocated to the CR, PR,

SD, and PD groups, respectively (Figure 1). Participants without disease regression had the right to choose to terminate NAC for surgery or opt for a second-line NAC regimen. Twelve (10.9%) patients opted for second-line NAC and got the terminal consequence of PR (7), SD (3) or PD (2). Thirty-eight (34.5%) patients opted to terminate NAC to carry out surgery, and got consequence of CR (12 patients with 4–5 cycles), PR (13 patients with 3–5 cycles), SD (9 patients with 2–4 cycles) or PD (4 patients with 4 cycles). All the patients provided written consent for total ALND, regardless of the type of breast surgery received (breast conserving surgery, breast reconstruction surgery, or mastectomy). All the operations were performed by the same surgical team. The study was approved by the Ethics Committee of Shengjing Hospital Ethics Committee of China Medical University (2016PS55J). The clinical parameters analyzed were number of positive nodes, number of black-stained nodes, axillary lymph node distribution by ICBN, and other conventional indicators.

Black-stained by CNs before NAC

A total of 0.3 mL of CNs suspension (China Food and Drug Administration approval H20041829; Lai Mei Pharmaceutical Co, Chongqing, People's Republic of China) was injected, guided by ultrasound, into the nodal cortex of 1 fusion node prior to NAC (Figure 2). Fusion nodes were easily detected by using ultrasonography. The suspension was injected on the nodal surface when it was difficult to distinguish between the medulla and cortex of the fusion node.

Grouping of Nodes for each Patient

Lymph nodes in the axillary space are divided into 2 groups by ICBN (under ICBN and above ICBN) according to the previous studies [16]. The ALND for each patient included two steps: the first step was performed in the area under the ICBN, the ICBN was exposed completely in the axillary space, and the nodes adjoining the ICBN belong to the lower nodes, and the long thoracic and thoracodorsal nerves were found and preserved during the step; the second step was performed in the area above the ICBN which involved a part of Level I, Level II and Level III [17]. Considering of metastasis, black-stained and distribution by ICBN, there were 8 possibilities for node status, judged by histological examination: positive and black-stained under ICBN, positive and not black-stained under ICBN, negative and black-stained under ICBN, negative and not black-stained under ICBN, positive and black-stained above ICBN, positive and not black-stained above ICBN, negative and black-stained above ICBN, and negative and not black-stained above ICBN. The relationship between node status and nodal location was analyzed.

Statistical analysis

The data are represented as the mean values or frequency depending on the data type. All statistical analyses were performed using SPSS software (version 13.0 for Windows; SPSS Inc., Chicago, IL). The

inter-group comparisons were performed using the χ^2 test or t test, as appropriate. Differences were considered statistically significant at P-values of <0.05 .

Results

The characteristics of CR, PR, SD, and PD groups are listed in Table 1. There was no significant difference in age, menopausal status, family history, quadrant distribution, tumor size, histological grade, progesterone receptor status, epidermal growth factor receptor status, Ki67 labeling index, NAC regimen, surgery, or the number of axillary nodes above ICBN among the groups ($p > 0.05$) (Table 1). There were significant differences in lymphovascular invasion, estrogen receptor status, clinicopathological subtype, number of cycles of NAC, pathological complete remission, Miller Payne, axillary lymph node status, number of axillary nodes, number of axillary nodes under ICBN, number of positive nodes, number of positive nodes under ICBN, number of positive nodes above ICBN, number of black-stained nodes, number of black-stained nodes under ICBN, number of black-stained nodes above ICBN, number of no black-stained nodes, number of positive and black-stained nodes, number of negative and black-stained nodes, number of positive and no black-stained nodes, number of negative and no black-stained nodes ($p < 0.05$) (Table 1). The nodes under the ICBN in surgery from the patient who achieved PR after 6 cycles of NAC are shown in Figure 3A and all the nodes are shown in Figure 3B. It is interesting to note that most nodes were black-stained. Two nodes were confirmed positive by histology: the first was traced by CNs prior to NAC; the other was not black-stained and near the tracing node (Figure 3B). Another patient had SD after 2 cycles of NAC and opted for surgery. We found that most of this patient's nodes were metastatic; only 1 node was black-stained, and was traced by CNs prior to NAC (Figure 3C).

When patients were ranked by response to NAC (CR, PR, SD, PD), the number of positive nodes increased, as did the proportion of lymphatic invasion, the number of black-stained nodes decreased (Figure 4A, Table 1). There were significant differences among the groups in the number of total lymph nodes, black-stained nodes and positive nodes ($p < 0.001$) (Figure 4B-D). A significantly negative relationship was found between the number of positive nodes and the number of black-stained nodes ($p < 0.001$) (Figure 4E). Almost the nodes under the ICBN were black-stained from the patients with CR and PR result (Figure 5A). We also found the rule in the patients with CR and PR consequence that when the nodes under the ICBN were negative, the nodes above the ICBN were negative, and when the nodes above the ICBN were positive, the nodes under the ICBN were positive (Figure 5A and B). However, we found the two counter-examples in the patients with SD and PD consequence that the nodes under the ICBN were negative and the nodes above the ICBN were positive (Figure 5A and B). The lymphovascular invasion had been detected in all the patients with SD and PD results (Figure 5A and B).

Discussion

In our study, the distribution of lymphovascular invasion was significantly different between the CR, PR, SD and PD groups (Figure 4A, Table 1). Furthermore, lymphovascular invasion could be detected in the biopsy samples of all patients with SD or PD. Previous research demonstrated that the degree of

lymphovascular invasion is an important factor in the prediction of NAC efficacy in breast cancer [18]. For patients who achieved CR or PR after NAC, most of their previously positive nodes had become negative, and the cancer cells that had invaded the lymphatic system died, causing a lymphatic flow of CNs from the CNs-injected node to other axillary lymph nodes. For patients who had SD or PD after NAC, most of the positive nodes remained positive and the patients still had lymphovascular invasion; therefore, the CNs remained in the CNs-injected node only or in a few adjacent nodes. That's why the tumors were sensitive to NAC (CR or PR), there were many black-stained nodes, and if the patients were resistant to NAC (SD or PD), there were fewer than three black-stained nodes (Figure 4C and D). Moreover, when classifying by response (CR, PR, SD, and PD), the number of positive nodes increased and the number of black-stained nodes decreased as response to NAC decreased, and there was significant negative correlation between the black-stained and positive nodes (Figure 4E). This hypothesis may perfectly explain the findings of our study. According to previous study, lymphatic fondness of CNs were benefit from not only its special molecular structure but also phagocytosed by macrophage [19]. Furthermore, the long-term staining and tracing of CNs provide a feasible technique to explore the relationship between lymphovascular invasion and the regression of positive nodes.

Additionally, the data from our study show that the regression model of positive nodes follows the rule from above the ICBN to under the ICBN in the patients with CR and PR consequence: when nodes above the ICBN are positive, the nodes under the ICBN are positive; and when the nodes under the ICBN are negative, the nodes above the ICBN are negative. The findings also demonstrated that positive node regression progresses stepwise from the upper to lower area and from the inner to outer area (Figure 5A and B). It is very interesting that the regression rule of positive nodes in the patients with sensitive result was in contrast to metastatic rule of nodes which was from Level I to Level III or from under the ICBN to above the ICBN [16, 20]. However, the rule could not apply to the patients with SD and PD result, there are two counter-examples in the patients with SD and PD consequence that the nodes under the ICBN were negative and the nodes above the ICBN were positive (Figure 5A and B). Certainly, technically confounding factors could affect the situation, that the fusion node with CNs-injected previous NAC would be above or adjacent to the ICBN. According to previous study, 98.2 percent of SLNs could be found in the medial part of the axillary, alongside the lateral thoracic vein (LTV) [21]. Therefore, the positive nodes often appeared in the area alongside the LTV, due to the ICBN was perpendicular to the LTV, there was a little part of positive nodes above the ICBN. Anyway, for ALND, lymph nodes need to be extracted from under the ICBN to above the ICBN, step by step, in the patients who were sensitive to NAC.

Conclusions

Owing to the limitations of our study, there might be additional hypotheses that interpret the regression model after NAC in patients with fusion nodes. Our hypothesis was helpful to explore the regression model of positive node during NAC. In fact, surgeons need an appropriate space range of ALND to prevent lymphedema for the patients with N2 or N3 before NAC. Long-term tracing by CNs before NAC may act as a marker to help surgeons judge the response of the fusion nodes after NAC. In combination

with ALND under the ICBN, CNs long-term tracing would be a potential method to treat the axillary node in the patients with NAC.

Abbreviations

SLNs: Sentinel lymph nodes; ALND: Axillary lymph nodes dissection; SLNB: Sentinel lymph node biopsy; NAC: Neoadjuvant Chemotherapy; FNR: False Negative Rate; CNs: Carbon Nanoparticles; ICBN: Intercostobrachial Nerves; CECT: Contrast-enhanced Computed Tomography; CR: complete remission; PR: partial remission; SD: stable disease; PD: progressive disease; LTV: lateral thoracic vein

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of Shengjing Hospital of China Medical University. The consent to participate from the patients was obtained.

Consent for publish

Yes.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

Li J and Chen B contributed equally to this work. Li J and Chen B designed the research; Li J, Jia S, Wang Y, Zhang Y and Kong L performed the research; Li J, Qi J, Cao Y, Liu Y and Zhang Y analyzed the data;

and Li J and Chen B wrote the paper.

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Table 1

Table 1. Patients Characteristics

Parameters	CR (n=32)	PR (n=61)	SD (n=12)	PD (n=5)	χ^2 or F	P
Age(years)	43.75±9.92	47.92±6.94	47.58±8.14	41.40±9.84	2.539	0.060
Menopause					0.275	0.843
Postmenopausal	13(40.6%)	23(37.75)	5(41.7%)	1(20.0%)		
Premenopausal	19(59.45)	38(62.3%)	7(58.3%)	4(80.0%)		
Family History					2.569	0.058
No	24(75.0%)	56(91.8%)	12(100.0%)	4(80.0%)		
Yes	8(25.0%)	5(8.2%)	0(0%)	1(20.0%)		
Quadrant					1.098	0.354
Areolar	3(9.4%)	6(9.8%)	0(0.0%)	0(0.0%)		
Inner upper	1(3.1%)	5(8.2%)	3(25.0%)	2(40.0%)		
Inner lower	0(0.0%)	3(4.9%)	2(16.7%)	0(0.0%)		
Outer lower	6(18.8%)	16(26.2%)	3(25.0%)	1(20.0%)		
Outer upper	22(68.8%)	31(50.8%)	4(33.3%)	2(40.0%)		
Diameter (cm)	2.23±0.919	2.93±1.40	2.45±0.87	3.04±1.80	2.549	0.060
Histological Grade					1.688	0.174
I	3(9.4%)	1(1.6%)	1(8.3%)	0(0.0%)		
II	17(53.1%)	32(52.5%)	3(25.0%)	1(20.0%)		
III	12(37.5%)	28(45.9%)	8(66.7%)	4(80.0%)		
Lymphovascular Invasion					4.178	0.008
Negative	15(46.9%)	18(29.5%)	0(0.0%)	0(0.0%)		
Positive	17(53.1%)	43(70.5%)	12(100.0%)	5(100.0%)		
ER (≥10%)					2.849	0.041
Negative	21(65.6%)	25(41.0%)	4(33.3%)	4(80.0%)		
Positive	11(34.4%)	36(59.0%)	8(66.7%)	1(20.0%)		
PR (≥10%)					0.940	0.424
Negative	24(75.0%)	42(68.9%)	6(50.0%)	4(80.0%)		
Positive	8(25.0%)	19(31.1%)	6(50.0%)	1(20.0%)		

Her2(FISH)					2.281	0.083
Negative	22(68.8%)	44(72.1%)	12(100.0%)	5(100.0%)		
Positive	10(31.3%)	17(27.9%)	0(0.0%)	0(0.0%)		
Ki67 (≥20%)					0.633	0.595
Negative	8(25.0%)	15(24.6%)	2(16.7%)	0(0.0%)		
Positive	24(75.0%)	46(75.4%)	10(83.3%)	5(100.0%)		
P53	54.31±22.58	64.80±23.53	65.42±12.70	60.00±26.46	1.657	0.181
Clinicopathological Subtypes					3.960	0.010
Luminal A	5(15.6%)	7(11.5%)	5(41.7%)	0(0.0%)		
Luminal B Low-PR	2(6.3%)	8(13.1%)	3(25.0%)	0(0.0%)		
Luminal B Ki67+	2(6.3%)	13(21.3%)	1(8.3%)	1(25.0%)		
Luminal B Her2+	2(6.3%)	8(13.1%)	0(0.0%)	0(0.0%)		
Her2 Enriched	8(25.0%)	11(18.0%)	0(0.0%)	0(0.0%)		
TNBC	13(40.6%)	14(23.0%)	3(25.0%)	4(75.0%)		
Chemotherapy					0.430	0.732
TAC or TEC or ET	13(40.6%)	34(55.7%)	9(75.0%)	2(40.0%)		
TP	9(28.1%)	1(1.6%)	0(0.0%)	1(20.0%)		
TCH	10(31.3%)	19(31.1%)	0(0.0%)	0(0.0%)		
TEC-GP	0(0.0%)	7(11.5%)	3(25.0%)	2(40.0%)		
Cycle	5.44±0.801	5.69±0.672	3.50±1.732	4.40±0.894	22.395	0.000
Surgery					1.909	0.133
Mastectomy	24(75.0%)	56(91.8%)	11(91.7%)	5(100.0%)		
Tumorectomy	3(9.4%)	2(3.3%)	0(0.0%)	0(0.0%)		
Breast Reconstruction	5(15.6%)	3(4.9%)	1(8.3%)	0(0.0%)		
PCR					36.618	0.000
Negative	13(40.6%)	61(100.0%)	12(100.0%)	5(100.0%)		
Positive	19(59.4%)	0(0.0%)	0(0.0%)	0(0.0%)		
Miller Payne					52.058	0.000
I	0(0.0%)	0(0.0%)	6(50.0%)	3(60.0%)		
II	0(0.0%)	8(13.1%)	4(33.3%)	2(40.0%)		

III	7(21.9%)	25(41.0%)	2(16.7%)	0(0.0%)		
IV	6(18.8%)	28(45.9%)	0(0.0%)	0(0.0%)		
V	19(59.4%)	0(0.0%)	0(0.0%)	0(0.0%)		
Axillary Node Status					3.237	0.025
Negative	13(40.6%)	20(32.8%)	0(0.0%)	0(0.0%)		
Positive	19(59.4%)	41(67.2%)	12(100.0%)	5(100.0%)		
Number of Axillary Nodes					6.029	0.001
Mean	25.22±3.643	25.08±3.796	28.42±3.655	30.80±3.033		
Range	19~33	19~35	23~36	28~35		
Number of Axillary Nodes under ICBN					10.500	0.000
Mean	11.69±1.615	11.66±1.750	14.33±1.497	13.80±2.168		
Range	10~16	10~16	11~17	11~16		
Number of Axillary Nodes above ICBN					1.512	0.216
Mean	13.53±3.619	13.43±3.922	14.08±2.937	17.00±2.121		
Range	8~22	8~24	11~19	14~20		
Number of Positive Nodes					88.499	0.000
Mean	0.88±0.907	2.93±2.903	17.50±6.571	16.60±8.989		
Range	0~3	0~9	7~28	7~29		
Number of Positive Nodes under ICBN					48.881	0.000
Mean	0.88±0.907	2.74±2.588	11.08±4.719	9.00±5.477		
Range	0~3	0~9	0~16	0~14		
Number of Positive Nodes above ICBN					67.205	0.000
Mean	0.00±0.000	0.20±0.771	6.42±3.872	7.60±5.814		
Range	0~0	0~4	2~15	1~17		
Number of Black-Stained Nodes					105.708	0.000
Mean	18.47±3.742	15.61±3.353	1.67±0.985	1.80±0.837		

Range	12~26	10~25	1~4	1~3		
Number of Black-Stained Nodes					210.640	0.000
under ICBN						
Mean	11.69±1.615	11.66±1.750	1.17±0.577	1.00±0.00		
Range	10~16	10~16	1~3	1~1		
Number of Black-Stained Nodes					16.113	0.000
above ICBN						
Mean	6.78±3.748	3.95±2.941	0.50±0.674	0.80±0.837		
Range	1~16	0~13	0~2	0~2		
Number of No Black-Stained					89.434	0.000
Nodes						
Mean	6.75±4.684	9.48±4.511	26.75±3.817	29.00±2.739		
Range	1~19	2~22	19~34	27~32		
Number of Positive and Black-					6.016	0.001
Stained Nodes						
Mean	0.88±0.907	2.80±2.695	1.67±0.985	1.80±0.837		
Range	0~3	0~9	1~4	1~3		
Number of Negative and Black-					82.974	0.000
Stained Nodes						
Mean	17.59±3.582	12.80±4.254	0.00±0.000	0.00±0.000		
Range	11~25	5~24	0~0	0~0		
Number of Positive and No					134.526	0.000
Black-Stained Nodes						
Mean	0.00±0.000	0.13±0.532	15.83±7.297	14.80±8.643		
Range	0~0	0~3	3~27	6~27		
Number of Negative and No					4.417	0.006
Black-Stained Nodes						
Mean	6.75±4.684	9.34±4.475	10.92±6.882	14.20±9.808		
Range	1~19	2~22	0~25	0~26		

Illustration: CR = complete remission; PR = partial remission; SD = stable disease; PD = progression disease; ER = estrogen receptor; PR = progesterone receptor; Her2 = epidermal growth factor receptor; FISH = fluorescence in situ hybridization; TNBC = triple negative breast cancer; PCR = pathological complete remission; TAC = docetaxel + doxorubicin + cyclophosphamide; TEC = docetaxel + epirubicin + cyclophosphamide; ET = epirubicin + docetaxel; TP = docetaxel + cisplatin; TC = docetaxel + cyclophosphamide; TCH = docetaxel + cyclophosphamide + Herceptin; TEC-GP = docetaxel + epirubicin + cyclophosphamide follow gemcitabine and cisplatin; PCR = pathological complete remission; ICBN = intercostal brachial nerve.

Figures

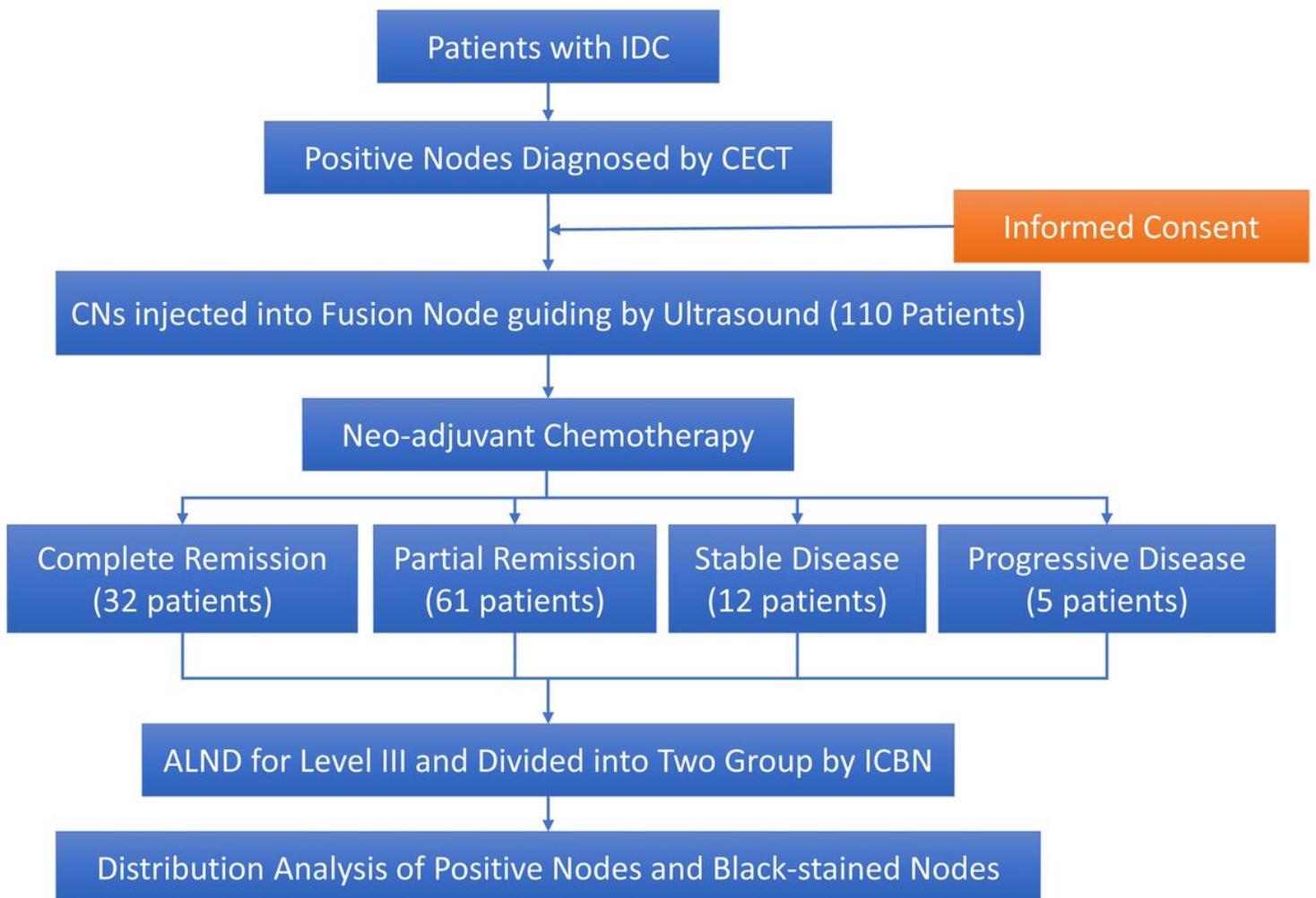


Figure 1

Study Protocol Flow Chart. IDC means invasive ductal carcinoma; CECT means contrast enhanced computer tomography; CNs means carbon nanoparticles; ALND means axillary lymph node dissection;

ICBN means intercostal brachial nerve. Each patient's nodes were divided into two groups (nodes above ICBN and nodes under ICBN) by ICBN.

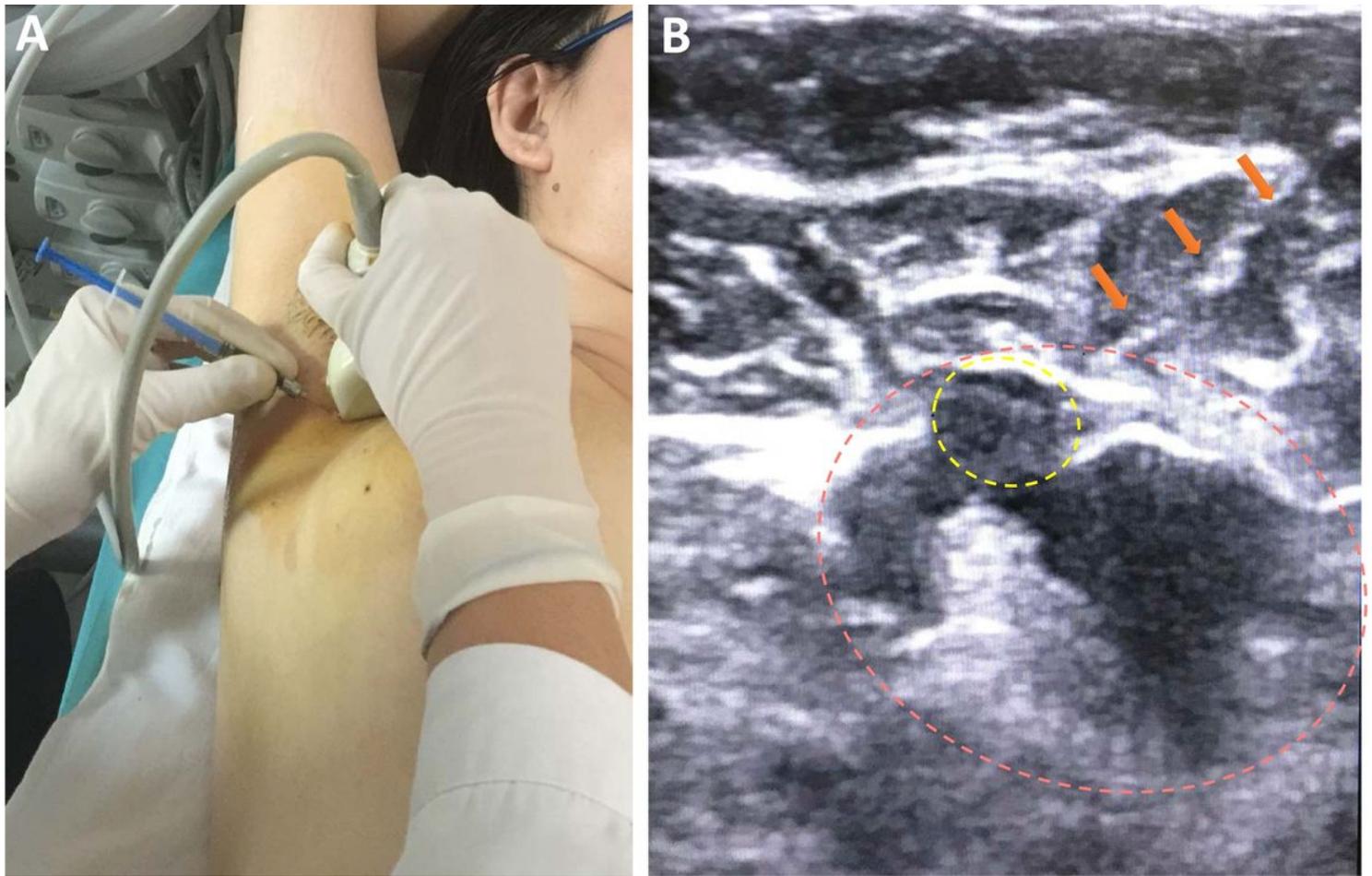


Figure 2

Positive Node was CNs-stained Guiding by Ultrasound. The photo A shows that the patient was accepted the nano-carbon injection guiding by the ultrasound. The Photo B shows that the ultrasound image: the orange circle is positive node; the orange arrows point to the needle; and the yellow circle is the membrane of the positive node for injection.

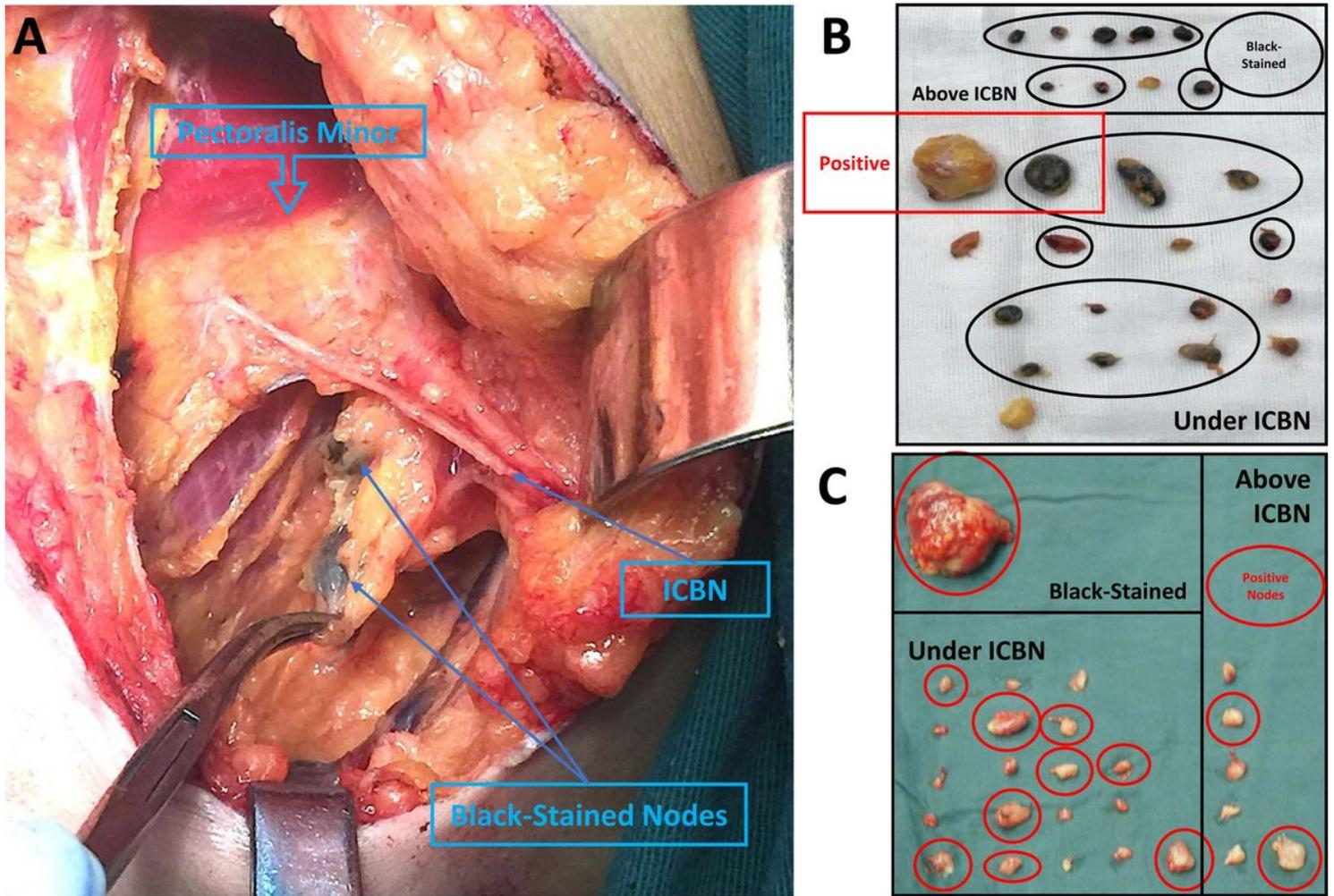


Figure 3

The Distribution by ICBN of Positive Nodes and Black-stained Nodes after Neo-Adjuvant Chemotherapy. A patient with partial remission consequence after neo-adjuvant chemotherapy, who has been accepted the radical mastectomy, the photo A shows that the black-stained nodes are under the ICBN and the photo B shows that there are 17 nodes under the ICBN, 9 nodes above the ICBN, and only 2 nodes are positive: one is black-stained and the other is no black-stained. Another patient with stable disease consequence after neo-adjuvant chemotherapy, who has been accepted the total axillary lymph nodes dissection, whose axillary nodes are showed by photo C. There are 20 nodes under the ICBN, 6 nodes above the ICBN, only one positive node is black-stained and the other 11 positive nodes are no black-stained.

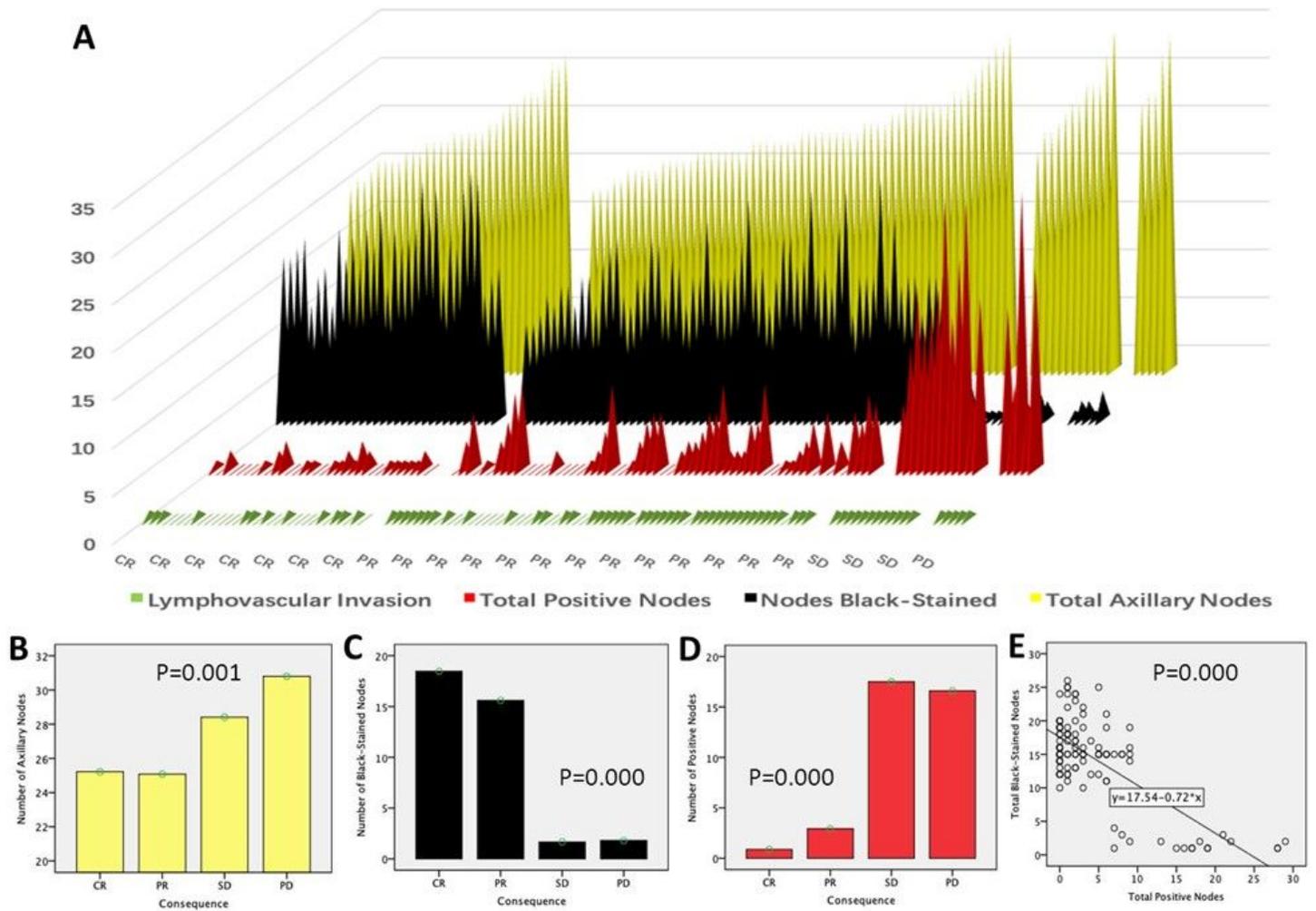


Figure 4

The Relationship between Nodes Number, Positive Nodes number, Black-Stained Nodes Number and Lymphovascular Invasion. CR means complete remission; PR means partial remission; SD means stable disease; PD means progression disease. There are 32 patients with complete remission, 61 patients with partial remission, 12 patients with stable disease and 5 patients with progressive disease. The photo A shows: the yellow bar represents axillary nodes; the black bar represents black-stained nodes; the red bar represents positive nodes; the green bar represents lymphovascular invasion. The photo B, C, D show that the mean of nodes number, black-stained nodes number and positive nodes number from CR, PR, SD and PD group, respectively. The photo E shows that scatter diagram analysis between the positive nodes and the black-stained nodes.

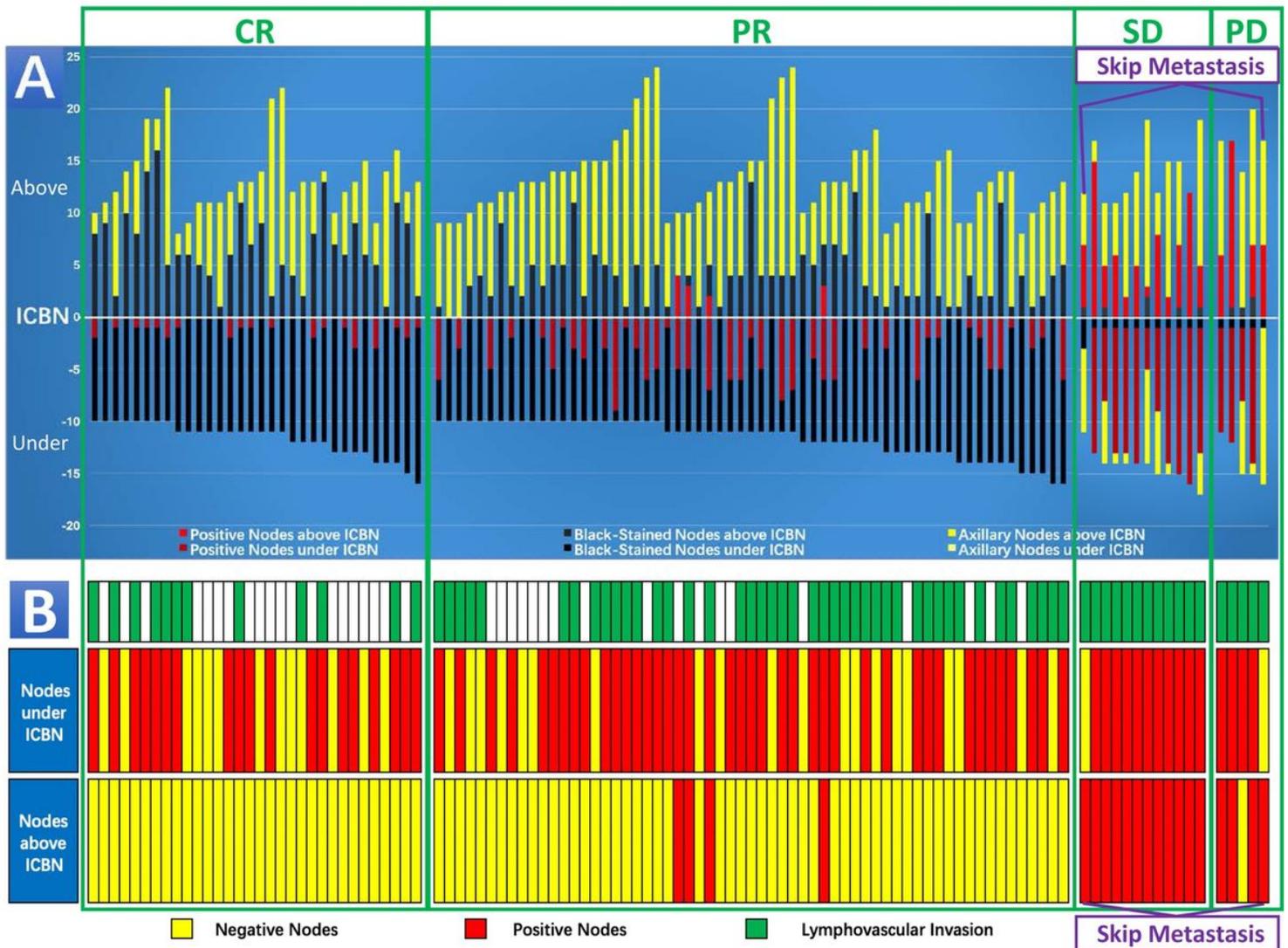


Figure 5

Distribution Analysis of Positive Nodes, Black-stained Nodes and Lymphovascular Invasion. Each patient's nodes were divided into two groups by ICBN from CR, PR, SD, PD group and showed by photo A and B. The yellow bar represents negative nodes; the red bar represents positive nodes; the black bar represents black-stained nodes; the green bar represents lymphovascular invasion. The photo A shows the distribution of nodes: the red bars cover the black bars in the CR and PR groups, while the black bars cover the red bars in the SD and PD groups; and the yellow bars are at the bottom in all groups. Each row of bar represents the total axillary nodes for each patient. The photo B shows the distribution of positive and negative nodes by the ICBN and lymphovascular invasion of each patient from CR, PR, SD, PD group. There are two patients with skip metastasis from SD and PD group.