Original Article

A novel diagnostic criterion for lymph node metastasis in cervical cancer using multi-detector computed tomography

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ABSTRACT

Objectives: The sensitivity of the current 10 mm cut-off diameter that is used to diagnose lymph node (LN) metastasis is too low. This is the first study to develop a new criterion to diagnose LN metastasis in a region-by-region manner using multi-detector computed tomography (MDCT).

Methods: 1) The short-axis diameter of the LNs in MDCT images from 1-mm slices obtained immediately prior to surgery was compared with the pathological diagnosis in 78 uterine cervical cancer patients undergoing primary surgery. For the region-by-region analysis, we divided para-aortic and pelvic spaces into 13 regions. 2) In 28 cases in which patients received neoadjuvant chemotherapy (NAC) followed by surgery, we compared MDCT images before and after NAC.

Results: 1) The optimal cut-off in the region-by-region analysis was 5 mm, yielding 71% sensitivity and 79% specificity. 2) NAC significantly decreased LN size ($p<0.0001$). NAC decreased the number of swollen LN regions (>5 mm) from 51% (81/158) to 26% (41/158).

Conclusions: The new criterion developed using MDCT could be effective for accurately assessing LN status. It also facilitates the assessment of NAC efficacy regarding the eradication of LN metastases.

INTRODUCTION

Cervical cancer is the second most common gynecologic malignancy in the world [1]. In addition to surgery and radiotherapy, neoadjuvant chemotherapy (NAC) followed by surgery has been used to treat locally advanced cervical cancer [2]. Because the prognosis of patients with cervical cancer depends largely on lymph node (LN) metastasis [3–5], it is important to assess the LN status accurately before starting treatment.

Based on studies comparing the size of the LNs in images and pathological diagnoses, a 10 mm short-axis diameter became the currently accepted cut-off in both clinical practice and clinical trials [6–8]. However, the sensitivity of this conventional criterion has been reported to be as low as 25% [9]. Furthermore, previous studies have generally predicted the presence of LN metastases in the patient without describing the location. In the present study, we conducted a detailed, region-by-region analysis to generate a new criterion that could identify region-specific, swollen LNs. The use of our criterion could lead to the improvement of individualized treatments.

In conducting the detailed analysis of LN status, the width of the slices, 7 mm in conventional CT and 5 mm in MRI, was considered to be a key limitation [7]. We therefore used a multi-detector CT (MDCT) scanner, which allowed one mm slice images [10, 11].

Using MDCT, we successfully generated a new criterion to diagnose LN metastasis. Using the new criterion, we were able to examine the efficacy of NAC in eradicating LN metastases in a region-by-region manner for the first time.

MATERIALS AND METHODS

Assessment of MDCT images

Patients

Patients with stage IA2 to IIB cervical cancer who were being treated in our department were retrospectively analyzed. Staging was performed according to the Classification of International Federation of Gynecology and Obstetrics (FIGO, 1994) [12]. With the written consent of each patient, and under the approval of the ethics committee, we initiated the MDCT study in January 2007: 106 patients underwent MDCT examination within two weeks before surgery; 78 patients underwent primary surgery; and the remaining 28 patients received NAC followed by surgery. In Table 1, we show the number of patients arranged according to their cancer stage.

Lymphadenectomy was performed during all of the surgeries, and we resected LNs separately in a region-by-region manner and made pathological diagnoses for each sample.

NAC regimen and indication

Because squamous cell carcinoma (SCC) was more sensitive than adenocarcinoma to chemotherapy in our preliminary analysis, we administered NAC primarily in stage IB2/IIA/IIB SCC cases (Table 1). An intravenous infusion of irinotecan (CPT-11) (60 mg/m^2

on days one and eight) and nedaplatin (NDP) (80 mg/m^2 on day one) was administered. Cycles were repeated every 21 days. In total, we conducted two cycles of NAC.

Surgical procedures and the definition of LN location

Pelvic lymphadenectomy was conducted in association with type III radical hysterectomy. The surgical procedure has been reported previously [13].

Para-aortic lymphadenectomy was performed when a metastatic pelvic LN was found after the intraoperative pathological diagnosis of frozen sections or when the surgeon found it necessary. LNs and their surrounding tissue were removed from the bifurcation of the common iliac artery to either the inferior mesenteric artery (IMA) or the level of the renal vein.

MDCT protocol

The MDCT examination was performed using one of three 64-detector-row CT scanners (Aquilion, Toshiba Medical Systems, Otawara, Japan) or a 16-detector-row CT scanner (Aquilion, Toshiba Medical Systems, Otawara, Japan). Patients were placed in the supine position, and the whole abdomen and pelvis were scanned. Image data were acquired with a 7 mm slice thickness, using a 64 × 0.5 mm (64-detector-row CT) or 16 × 1.0 mm (16-detector-row CT) beam collimation, a 500 msec rotation time, and a 120 kVp and 27.5

mm (64-detector-row CT) or 14.9 mm (16-detector-row CT) table feed per rotation. The acquired images were used to generate images with 1 mm slice thickness. All dual-phase contrast-enhanced CT images with a single breath-hold were obtained at 90 seconds following the intravenous injection of 100 ml of 300 or 350 mg/ml non-ionic contrast medium (Iomeron®350, Eisai, Tokyo, Japan; Iopamiron®300, Bayer HealthCare, Osaka, Japan; or Omnipaque®300, Daiichi-Sankyo, Tokyo, Japan) at a rate of 2.5 ml/sec using an automatic injector.

Because our MDCT procedure is the same as previously reported [14], the estimated radiation dose to the whole abdomen was approximately 10mSv. The noise of the images with 1mm thickness was greater than those with 7mm thickness. However, there was no difficulty in evaluating length of LNs, especially in MDCT images with contrast enhancement.

Evaluation of LNs by MDCT

The evaluation of LNs using the MDCT images of 1 mm-thick slices was performed independently by a radiologist (AK) who specializes in the field of gynecology and by a gynecologic oncologist (KY). The size of each LN, measured independently by AK and KY to the first decimal place, was determined by consensus of them.

Region-by-region analysis

Most previous studies have been conducted in patient-by-patient manner. In this study, we aimed to analyze more closely. Because it is unfeasible to compare each LN detected in MDCT images with LN detected in operation in node-by-node manner, we employed a region-by-region analysis. In a previous study, the pelvic and para-aortic spaces were divided into five regions prior to conducting a region-by-region analysis [9]. In the present study, we divided them into 13 regions (Supplementary Figure 1) to conduct a more detailed analysis. We determined the size of the largest LN in each region, termed the regional LN (Reg-LN), and compared it with the pathological diagnosis of LN metastasis in each region. We should note that even if LN metastasis existed in a region, the Reg-LN might not be the involved LN. Receiver operating characteristic (ROC) curve were drawn to determine the optimal cut-off size. For the development of the new criterion, we analyzed only the primary operation cases and excluded the NAC cases.

Estimate of the efficacy of NAC in eradicating LN metastases using MDCT images

In patients who received NAC, we performed the MDCT examination before and after NAC and compared the results. Using the new criterion for determining the swollen Reg-LN, we estimated the efficacy of NAC in eradicating LN metastasis.

Statistical methods

We used t-tests to compare the parametric values between two groups and one-way ANOVA for three groups. Paired t-tests were used for the paired analysis. To analyze the distribution in a 2x2 table, Fisher's exact test was employed. To analyze the distribution in a larger table, the chi-square test was used. We considered a value of $p<0.05$ to be a significant difference.

RESULTS

Detection of LNs in 1 mm slice images from MDCT.

A total of 4,765 LNs were pathologically identified within 980 regions from 106 patients, whereas the total number of visible LNs in the MDCT images was 800. Thus, the detection rate of LNs by MDCT was 17% (800/4,765). The most difficult LN to detect was the presacral LN (6%), whereas the easiest was the left common iliac LN (46%) (Figure 1a). Only seven of the 800 visible LNs in the MDCT images were over 10 mm in diameter.

Among 980 regions from 106 patients, 563 (57%) regions showed one or more visible LNs in the MDCT images. Metastases were identified pathologically in 133 LNs within 80 regions from 33 patients. Sixty of these 80 (75%) regions showed one or more visible LNs in the MDCT images (Figure 1b). The detection rate was significantly higher in the metastatic regions than in the non-metastatic regions (p=0.006) (Figure 1b). In the following analyses, the largest LN in each region was defined as the Reg-LN.

Development of a new criterion to diagnose LN metastasis.

We compared the size of the Reg-LN identified by MDCT with the presence or absence of a pathologically involved LN in 410 regions from 71 patients who underwent primary surgery and in whom the Reg-LN could be detected in the MDCT images. Statistical analysis using the ROC curve is difficult when the number of metastasis-positive cases is less than five. Only the external iliac and obturator regions were positive for metastasis in five or more patients. Thus, we analyzed the Reg-LNs of the obturator region, external iliac region, and the other regions separately. In all three groups, the size of the Reg-LN was significantly larger in the LN regions containing metastases than in the non-metastatic regions; the mean was 7.9 in the metastatic case vs. 4.0 mm in the non-metastatic case in the external iliac ($p<0.0001$), 6.6 vs. 3.9 mm in the obturator ($p<0.0001$), and 5.1 vs. 3.8 mm in the other regions ($p=0.003$) (Figure 2a). The variation in the size of the Reg-LNs in these three groups was not significant in either metastatic or non-metastatic regions (Figure 2a). The optimal cut-off sizes determined by the ROC curves in the external iliac, obturator, and other regions were 4.9, 5.3, and 5.1 mm, respectively (Figure 2b). Based on these results, 5 mm was selected as the cut-off size for all types of LN regions.

We then analyzed the Reg-LNs from all regions. The size of the Reg-LNs was significantly larger in the LN regions containing metastases than in the regions without metastases: mean 6.4 mm vs. 3.9 mm ($p<0.0001$) (Figure 3a). Based on the ROC curve, the optimal cut-off size to diagnose a LN metastasis was 4.7 mm (AUC = 0.80, Figure 3b). In the region-by-region analysis, we decided the closest whole number, 5 mm, as the cut-off for clinical use. When the 5 mm cut-off was used, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value were 70, 79, 27, and 96%, respectively.

Next, we divided all cases into SCC and non-SCC to examine the influence of histological differences on the detection of LN metastasis. In both SCC and non-SCC patients, the size of the Reg-LN was significantly larger in the LN regions with metastases than in the non-metastatic regions: the mean in SCC was 7.1 vs. 4.0 mm and in non-SCC was 6.0 vs. 3.7 mm ($p<0.0001$) (Figure 3a). The size of the Reg-LN in non-metastatic LN regions was larger in SCC compared with non-SCC, but the difference was small (Figure 3a). The optimal cut-off determined by the ROC curve was 5.4 mm in SCC and 4.7 mm in non-SCC cases (Figure 3c). Thus, 5 mm, the closest whole number, was again selected for use in both histological subtypes.

We also evaluated the conventional 10 mm cut-off. In the primary surgery cases, only four of all the visible LNs in the MDCT images were over 10 mm in diameter (Figure 2a). We found that the conventional cut-off of 10 mm was far from the optimal cut-off based on the ROC analyses (Figure 2b, 3b, 3c). In addition, we randomly divided our data (primary surgery cases, region-by-region data) into three groups and conducted ROC analyses independently, thus generating cut-off values of 4.9, 5.0 and 5.3 (95% CI; 4.55-5.58, data not shown). We therefore concluded that the conventional 10 mm cut-off is statistically inappropriate in MDCT images.

Assessment of NAC efficacy on metastatic LNs in cervical cancer.

We analyzed 153 Reg-LNs from 28 patients who underwent NAC followed by surgery using the MDCT images taken just before surgery. As in the primary surgery cases, the size of the Reg-LN was significantly larger in the metastatic regions than in the non-metastatic regions: the mean was 7.4 vs. 3.9 mm ($p<0.0001$) (Figure 4a). The size of the Reg-LN in the metastatic and non-metastatic LN regions was the same within the NAC and primary surgery groups (Figure 4a). Similar to the primary surgery cases (Figure 4b), the optimal cut-off determined by the ROC curve was 4.7 mm in NAC cases (AUC = 0.83, Figure 4c).

Next, we examined the size change of LNs after NAC. For this analysis, node-by-node comparison was performed. First, we compared the size of all visible 328 LNs individually between before and after NAC. NAC significantly decreased the size of LNs from 5.1 to 3.5 mm ($p<0.0001$) (Figure 5a): this is the reduction in the average size of all visible LNs. For Reg-LNs, we found 158 Reg-LNs in MDCT images before NAC. The size of these 158 Reg-LNs was also significantly decreased by NAC from 5.9 to 4.3 mm

($p<0.0001$) (Figure 5b). Using the cut-off of 5 mm, 80 out of 158 Reg-LNs (51%) were 5 mm or greater in diameter before NAC. After NAC, the number of Reg-LNs that were 5 mm or greater was significantly decreased to 41 ($p<0.0001$, 26%) (Figure 5c). Of these 41 regions, 13 (32%) had LN metastases based on their pathology. Among 78 regions that were less than 5 mm in diameter before NAC, a LN metastasis was identified in only 1 region (1%) after NAC.

In addition to the analysis of MDCT images, we reviewed the medical records of cervical cancer cases that were treated in our hospital between 1999 and 2011. In accordance with the MDCT analysis, we found that NAC decreased the frequency of LN metastasis (Supplementary text, Supplementary Figure 2b).

DISCUSSION

The main objective of this study was to generate a new and more reliable criterion to diagnose LN metastasis pre-operatively. We also evaluated the efficacy of NAC in eradicating LN metastasis using the newly developed criterion because NAC has the potential to eradicate LN and distant metastases [2, 15, 16].

CT and MRI have been widely used to diagnose LN metastasis [17, 18]. In conventional CT or MRI images of 5-7 mm slices, 10 mm is used as the conventional cut-off for the short-axis diameter in the diagnosis of swollen LNs as potential metastases. However, only seven of 800 visible LNs in the MDCT images were over 10 mm in diameter in this study (data not shown). Therefore, the conventional cut-off of 10 mm is not effective in clinical practice. The conventional cut-off is used because of the limitations of conventional CT, which cannot reproducibly measure an object smaller than 10 mm in diameter in 5-7 mm slices [7].

MDCT is a new form of CT technology and that has an increased speed of CT image acquisition [18]. In addition, MDCT provides more information than CT without increasing cost, examination time, or exposure dose depending on how it is protocoled. Although there are reports demonstrating the use of MDCT to diagnose LN metastasis in gastric, oesophageal, breast and pancreatic cancer [10, 11, 20-22], to the best of our knowledge, this is the first report on the use of MDCT in a gynecological cancer.

In previous studies that examined the diagnostic rate for LN metastasis, patient-by-patient analysis was used to compare the size of the largest LN in a patient with the pathological diagnosis of the LN metastasis, without describing the location of LN metastasis. However, to consider individually the extent of the lymphadenectomy and field of radiotherapy, a diagnosis of not only the presence of a LN metastasis but also the location(s) of the LN metastasis is essential. Accordingly, we determined that the cut-off should be ascertained using region-by-region analysis.

Our findings suggested that 5 mm was the most appropriate cut-off for clinical practice. In addition, a cut-off of 5 mm using MDCT was reproducible regardless of the location or pathological subtype of the tumor (Figures 3b, 3c, 4c). The ROC analyses clearly showed that a cut-off of 10mm is not appropriate.

Using historical analysis of stage IB2/IIA cases, we found that the frequency of LN metastasis in NAC cases was significantly lower than in primary surgery cases (Supplementary Figure 2b). This result is consistent with previous reports that the frequency of LN metastasis is lower in patients who undergo NAC [15, 16]. In our analysis, approximately half of the Reg-LNs that were 5 mm or larger shrank to less than 5 mm after NAC (Figure 5c). Although shrinkage of LNs does not necessary mean anti-tumor effect against involved LNs, this study shows a substantial number of involved LNs might have been cured by NAC. Ours is the first study to report the precise change in LN size due to NAC; however, further studies are required to confirm whether NAC is effective against the LN metastasis of cervical cancer.

In this study, using 1mm slice images, we tried evaluating LN size as closely as possible in region-by-region manner, but there are still some limitations. The largest LN in an involved region may not always be the involved LN. To compare the preoperative image with the pathological diagnosis in node-by-node manner, data accumulated from image-guided surgery, currently used for gastric or liver cancer [23, 24] are necessary. Although the detection rate of at least one LN in a region was increased when involved LNs exist, the detection rate was still 75 %, not 100% (Figure 1b). Among operable cervical caner patients, PPV of 5 mm cut-off was only 27% although PPV is thought to be higher in advanced cases where operation is impossible and radiotherapy is indicated.

Regardless, the results of our study should have an enormous impact on clinical practice. We consider LNs of 5 mm or larger to be potentially metastatic. We hope this result would be validated in a prospective study with a large number of cases and node-by-node surgery with imaging analysis.

In conclusion, we suggested an optimal cut-off of 5 mm in MDCT images for the diagnosis of metastatic LNs within an LN region. This novel diagnostic criterion would improve the individualized treatment of cervical cancer patients. In the future, clinical trials should be performed to investigate whether this strategy could improve the prognosis of cervical cancer patients.

Conflict of Interest Statement: All authors declare that they have no conflicts of interest.

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FIGURE LEGENDS

Figure 1. Detection of LNs in MDCT images. a) The number of detected LNs by MDCT is indicated by a black box. Pathologically detected LNs are indicated by the sum of the detected and undetected LNs. The distribution was statistically significant ($p<0.0001$, chi-square test). PAN: para-aortic LN region; IMA: inferior mesenteric artery; Bif: aortic bifurcation; L: left; R: right. b) The detection rate of Reg-LNs was significantly higher in the involved regions (pN1) than in the uninvolved regions (pN0) ($p=0.006$).

Figure 2. Region-by-region analysis of LNs. a) Each dot indicates the size of the Reg-LN. Red dots indicate size of involved Reg-LNs and tiny black dots indicate size of uninvolved Reg-LNs. Black bar indicates the mean size. Ext: external iliac region; Obt: obturator region; Others: all other regions. One-way ANOVA was used for the statistical analysis. b) ROC curves based on the size of the Reg-LN and the presence of the LN metastasis. The gray arrowhead indicates the optimal cut-off based on the ROC curves. Note that the 10mm cut-off indicated by the black arrowhead is away from the optimal cut-off based on the ROC analysis.

Figure 3. Region-by-region analysis of all Reg-LNs among different histological subtypes. a) Each dot indicates the size of the Reg-LN. Red dots indicate size of involved Reg-LNs and

tiny black dots indicate size of uninvolved Reg-LNs. Black bar indicates the mean size. To detect the association of all Reg-LNs with the different histological subtypes present in the three groups, one-way ANOVA was performed. b) ROC curves are based on the size of the Reg-LN and the presence of the LN metastases. c) ROC curve analyses were conducted by dividing the cases into different histological subtypes. The gray arrowhead indicates the optimal cut-off based on the ROC curves. Note that the 10mm cut-off indicated by the black arrowhead is away from the optimal cut-off based on the ROC analysis.

Figure 4. Region-by-region analysis for primary surgery cases and NAC cases, and the effect of NAC based on the analysis of MDCT images. a) Each dot indicates the size of the Reg-LN. Primary: primary surgery; pN0: no LN metastasis; pN1: LN metastasis; NS: not statistically significant. b) ROC curves are based on the size of the Reg-LN and the presence of the LN metastasis in all primary surgery cases. The gray arrowhead indicates the optimal cut-off based on the ROC curves. c) ROC curve representing only the NAC cases. The cut-off in the NAC cases was 4.7 mm.

Figure 5. Effect of NAC according to MDCT images. a) Changes in all LN sizes that were visible before NAC. They shrank significantly after NAC ($p<0.0001$). b) Changes in all Reg-LN sizes by NAC. They also shrank significantly ($p<0.0001$). c) NAC's effect of

decreasing the proportion of regions with a LN larger than 5 mm (black bar). Red boxes indicate regions with LN metastasis. NAC decreased the number of regions with LNs larger than 5 mm from 80 to 41. In the 41 regions that had Reg-LNs larger than 5mm after NAC, we found 13 (32%) involved regions; in the 117 regions that had Reg-LNs smaller than 5 mm after NAC, we found 6 (5%) involved regions.

Supplementary Figure 1. We divided para-aortic and pelvic lymph nodes into 13 regions to compare pre-operative images with the pathological diagnosis. a) para-aortic lymph nodes, from IMA to renal vein; b) para-aortic lymph nodes, from bifurcation to IMA; c) right common iliac lymph nodes; d) left common iliac lymph nodes; e) presacral lymph nodes; f) right external iliac lymph nodes; g) right internal iliac lymph nodes; h) left internal iliac lymph nodes; i) left external iliac lymph nodes; j) right superficial inguinal lymph nodes; k) lymph nodes in the right obturator fossa (obturator, carinal, uterine artery, and deep inguinal lymph nodes); l) lymph nodes in the left obturator fossa; m) left superficial inguinal lymph nodes.

Supplementary Figure 2. a) Changes in the treatment modality for SCC cases in our hospital. For stage IB2/IIA SCC cases, we predominantly performed the primary operation before 2006 and the NAC after 2007. b) NAC decreased the frequency of LN metastasis in stage IB2/IIA SCC patients. c) Among IB2/IIA cases, NAC-treated cases showed a tendency toward better survival compared with the primary operation cases, although this result was not statistically significant.

TABLE

Table 1. Number of patients multi-detector CT image was analyzed.

Stage	SCC	Non-SCC	Total
IA	4	3	7
IB1	32	20	52
IB2	10 (7)	4 (1)	14 (8)
IIA	4	1	5
IIB	23 (19)	5 (1)	28 (20)
Total	73 (26)	33 (2)	106 (28)

( ); NAC cases.

Figure 1

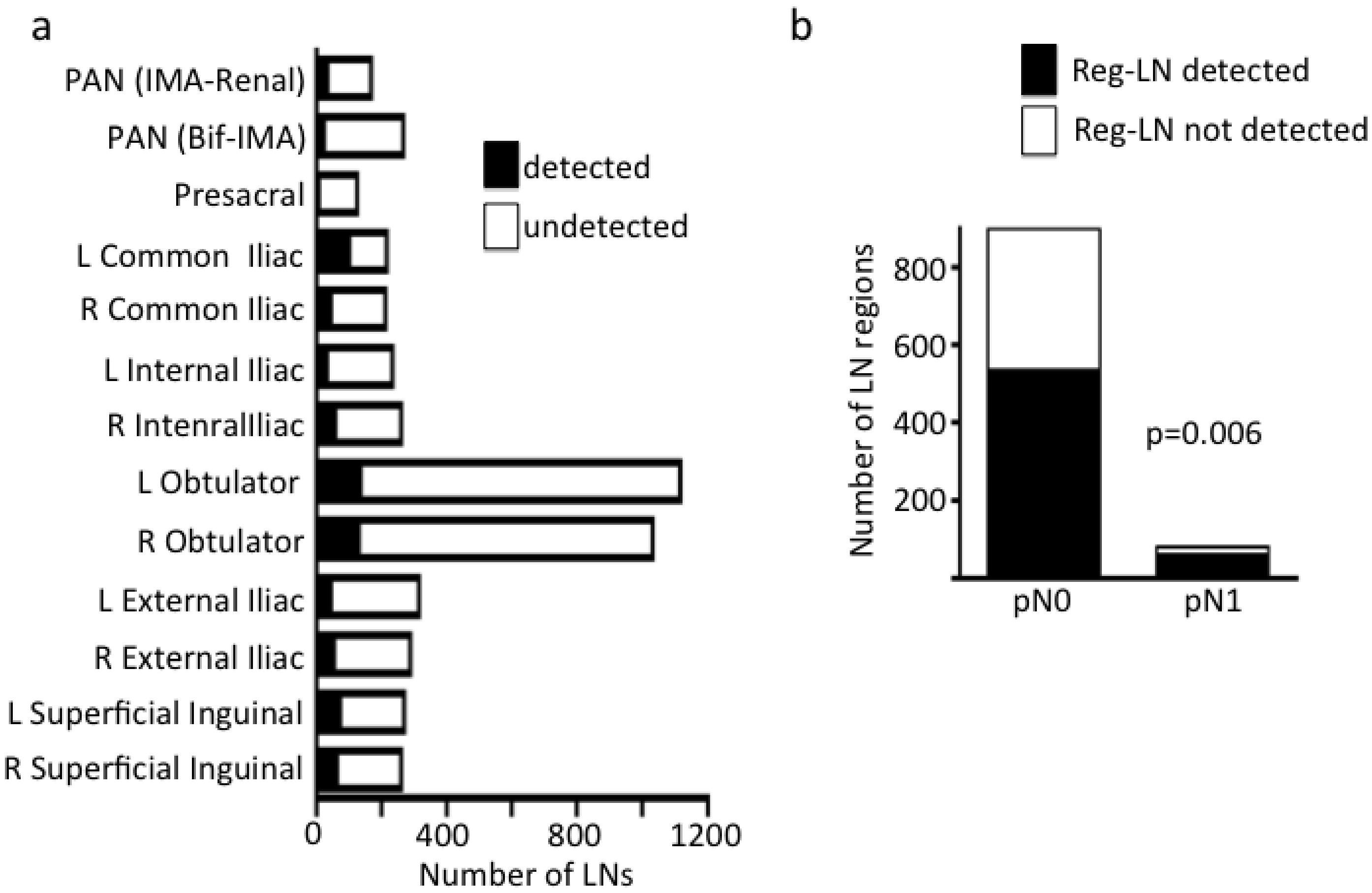

a
PAN (IMA-Renal)
PAN (Bif-IMA)
Presacral
L Common Iliac
R Common Iliac
L Internal Iliac
R IntenralIliac
L Obtulator
R Obtulator
L External Iliac
R External Iliac
L Superficial Inguinal
R Superficial Inguinal
detected
undetected
0
400
800
1200
Number of LNs
b
Reg-LN detected
Reg-LN not detected
Number of LN regions
200
400
600
800
p=0.006
pN0
pN1

Figure 2

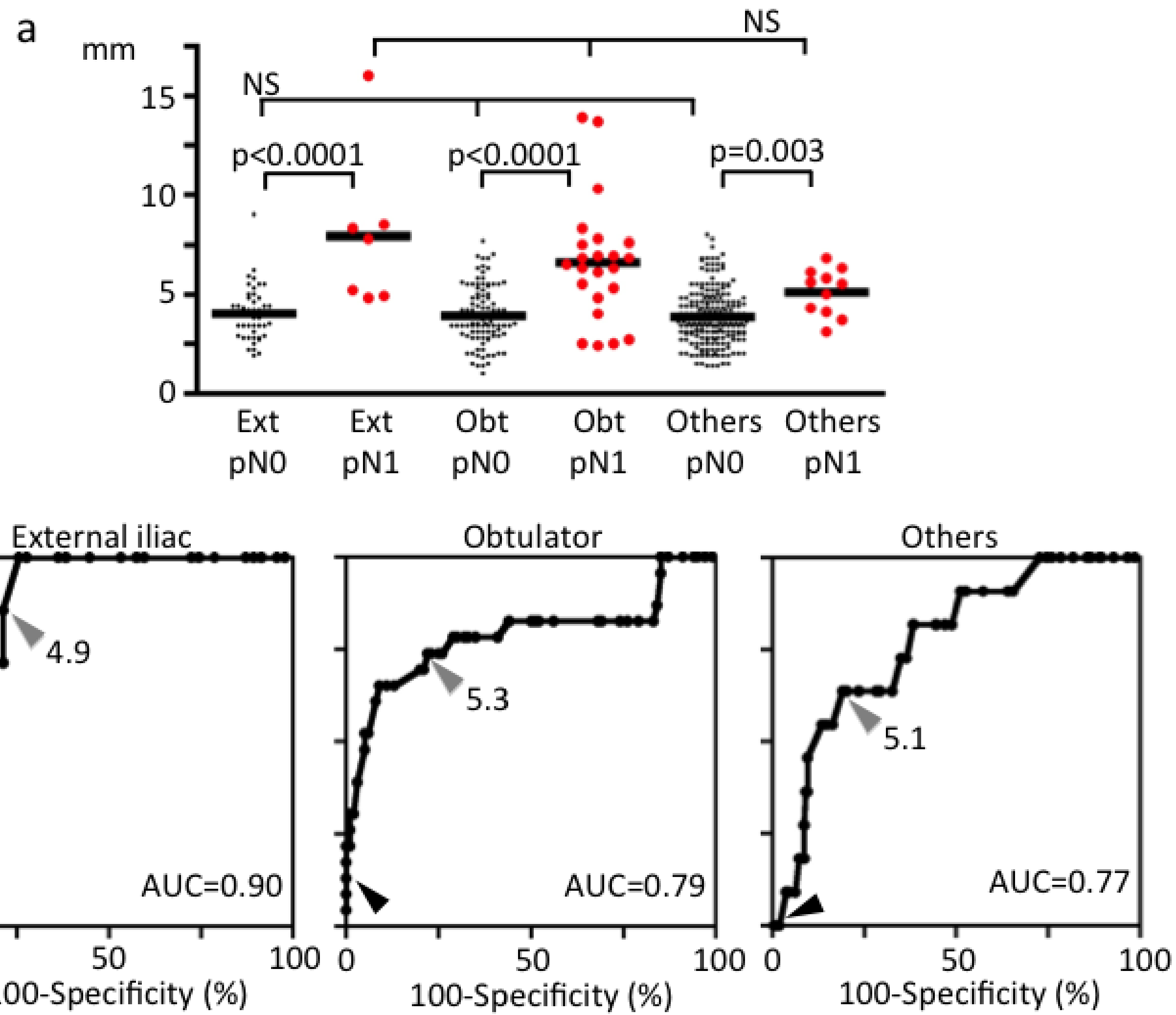
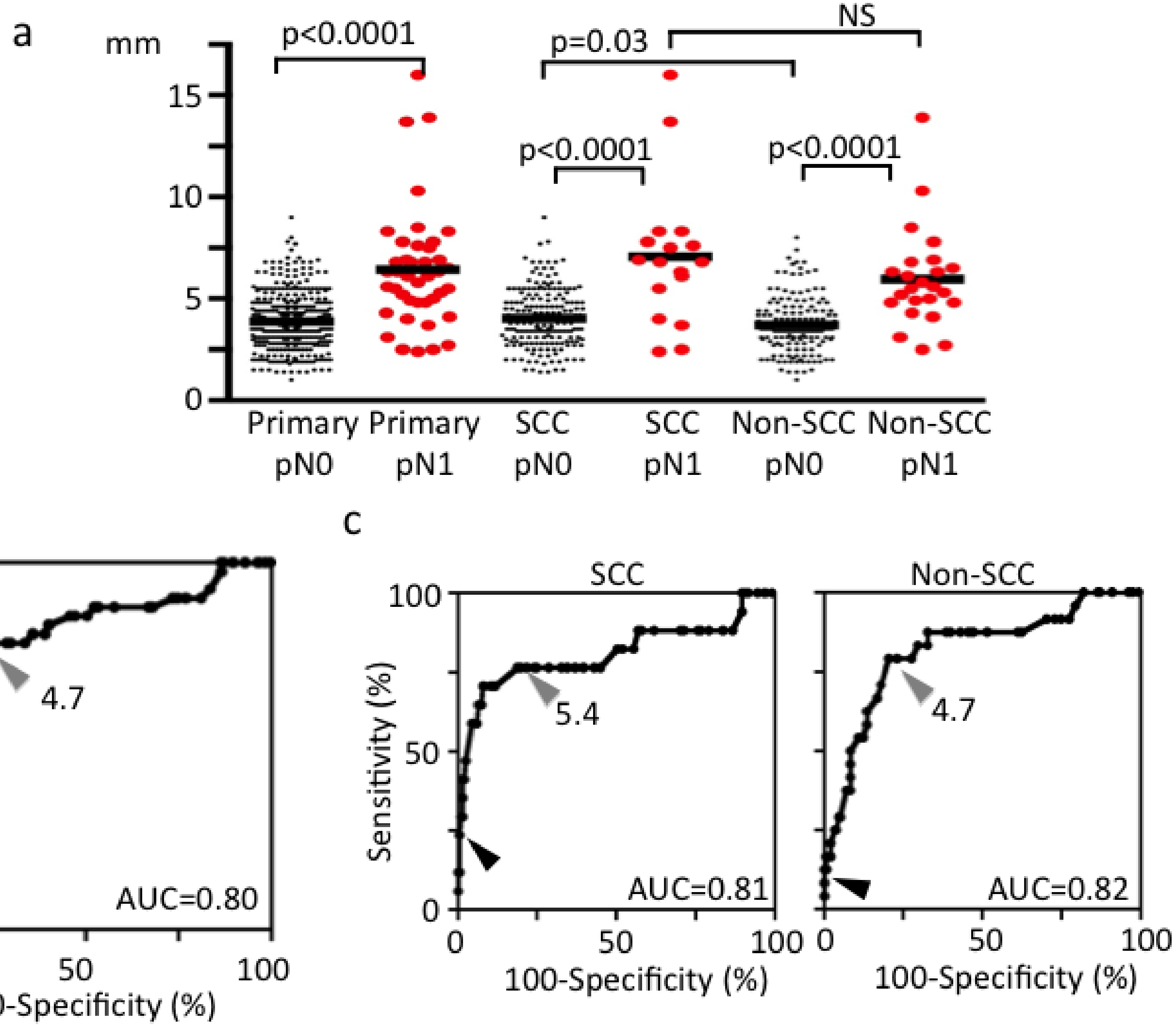
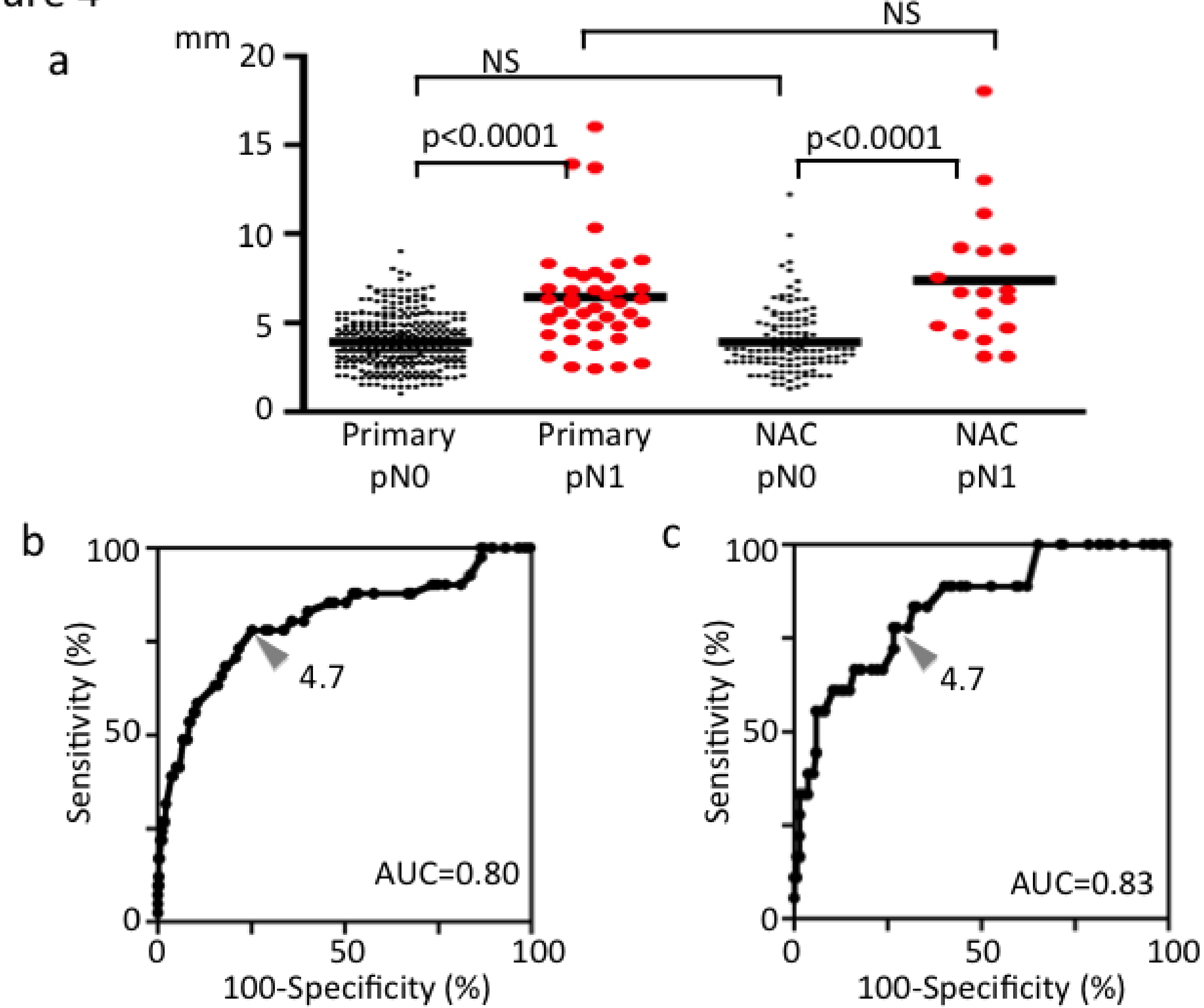

a
mm
15
10
5
0
NS
NS
p<0.0001
p<0.0001
p=0.003
Ext pN0
Ext pN1
Obt pN0
Obt pN1
Others pN0
Others pN1
b
External iliac
Obtulator
Others
Sensitivity (%)
100
50
0
4.9
5.3
5.1
AUC=0.90
AUC=0.79
AUC=0.77
100-Specificity (%)

Figure 3



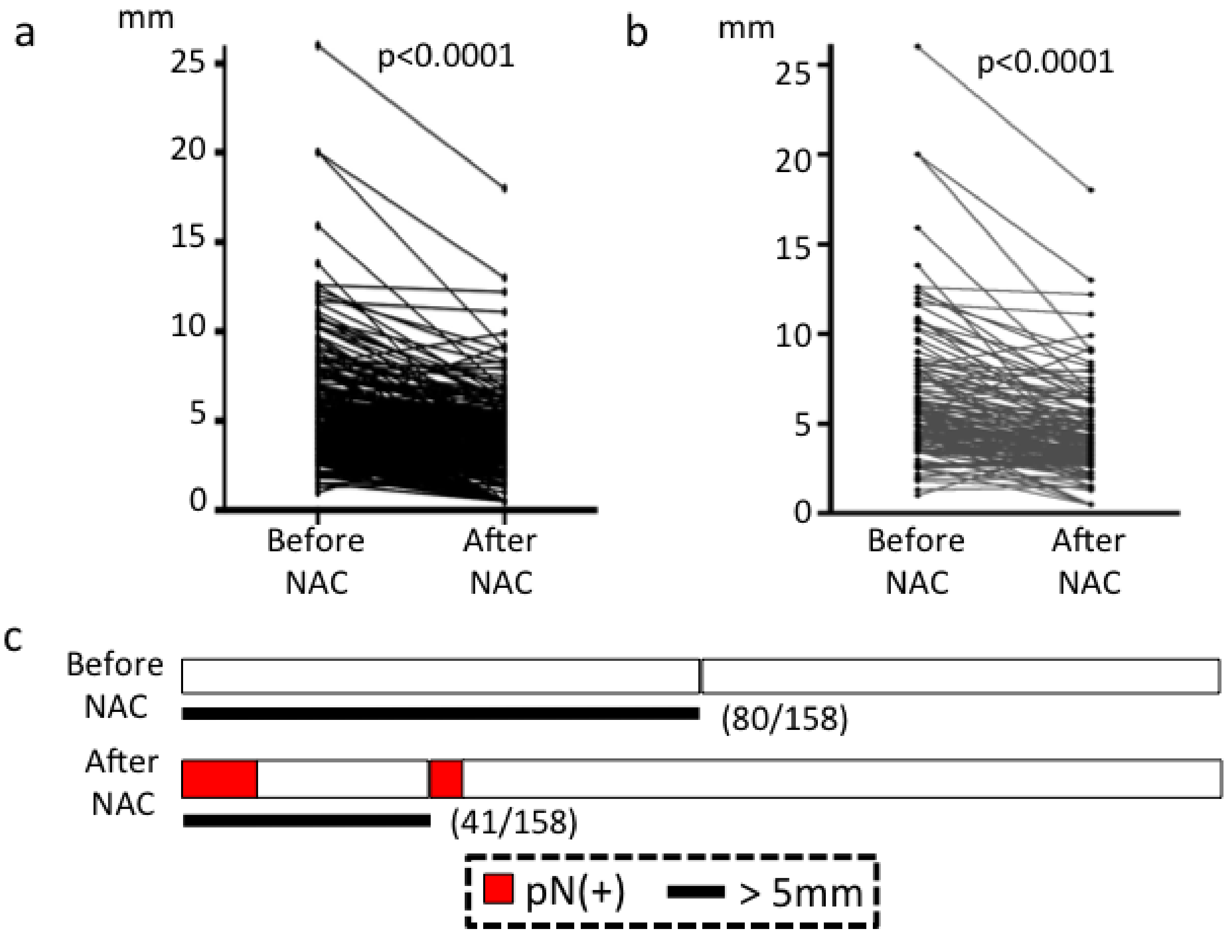
a
mm
p<0.0001
p=0.03
NS
p<0.0001
p<0.0001
15
10
5
0
Primary pN0
Primary pN1
SCC pN0
SCC pN1
Non-SCC pN0
Non-SCC pN1
b
100
50
0
Sensitivity (%)
4.7
AUC=0.80
0
50
100
100-Specificity (%)
c
SCC
Non-SCC
100
50
0
Sensitivity (%)
5.4
AUC=0.81
0
50
100
100-Specificity (%)
4.7
AUC=0.82
0
50
100
100-Specificity (%)

Figure 4



a
mm
20
15
10
5
0
NS
NS
p<0.0001
p<0.0001
Primary pN0
Primary pN1
NAC pN0
NAC pN1
b
100
50
0
Sensitivity (%)
100-Specificity (%)
4.7
AUC=0.80
c
4.7
AUC=0.83

Figure 5


a
mm
25
20
15
10
5
0
p<0.0001
Before NAC
After NAC
b
mm
25
20
15
10
5
0
p<0.0001
Before NAC
After NAC
c
Before NAC
(80/158)
After NAC
(41/158)
pN(+)
> 5mm

Supplementary Text

Historical analysis of the effect of NAC in eradicating LN metastases

MATERIALS AND METHODS

Patients

With the written consent of each patient and the approval of our ethics committee, we retrospectively reviewed the medical records of 296 cervical cancer cases (stage IA, IB, and IIB) who underwent systematic lymphadenectomy at our department between 1999 and 2011. The median number of resected pelvic lymph nodes was 44±38 (mean±2SD) per patient. A total of 199 were diagnosed with SCC and 97 with non-SCC (adenocarcinoma, adenosquamous carcinoma, etc.) (Supplementary Table 1).

NAC regimen and indication

NAC regimens are shown in Supplementary table 2. Between 1999 and 2006, the most frequently used regimen was transarterial infusion of cisplatin (CDDP), (100 mg/m^2), pirarubicin (THP), (25 mg/m^2), and mitomycin-C (MMC), (20 mg/patient) every 28 days. Between 2007 and 2011, intravenous infusion of irinotecan (CPT-11), (60 mg/m^2 on days 1 and 8) and nedaplatin (NDP), (80 mg/m^2 on day 1) every 21 days was most frequently used. In principle, we conducted 2 cycles of NAC.

Because squamous cell carcinoma (SCC) was more sensitive than adenocarcinoma to chemotherapy in our preliminary analysis, we administered NAC primarily in SCC cases. As a result, most NAC cases (57/63) were SCC. From 1999 to 2011, for most stage IIB SCC patients, we administered NAC before operation. For stage IB2/IIA SCC patients, until 2006, we administered NAC for only those who had very bulky tumours, and most cases underwent primary operation. In 2007, we changed our policy and have therefore administered NAC for most IB2/IIA SCC cases. As a result, the proportion of stage IB2/IIA patients who received NAC increased significantly from 4/29 (14% through 2006) to 10/17 (59%, 2007 onward) (supplementary Figure 2a). Therefore, the primary operation and NAC were comparable among IB2/IIA cases.

Statistical methods

To analyse the distribution in a 2x2 table, Fisher's exact test was employed. To analyse the distribution in a larger table, the chi-square test was used. We considered $p<0.05$ a significant difference.

RESULTS

Of 296 patients receiving lymphadenectomy from 1999 to 2011, 63 received NAC. Response rate (complete response or partial response) of NAC was 91% (52/57). Because we changed our treatment policy for stage IB2/IIA SCC cases in 2007, we compared the rate of pathologically positive LN metastases in stage IB2/IIA SCC cases between the period of 1999-2006 and the period of 2007-2011. The rate of LN metastasis decreased from 41% (12/29, through 2006) to 24% (4/17, 2007 onward), although this difference was not statistically significant. Dividing all IB2/IIA SCC cases into NAC and primary surgery groups, the LN metastasis rate was significantly lower in NAC than in the primary surgery group (1/14, 7% vs 15/32, 47%) ($p=0.02$) (Supplementary Figure 2b). The overall survival rate of IB2/IIA SCC patients who received NAC was better than primary operation cases (Supplementary Figure 2c).

SUPPLEMENTARY TABLE

Supplementary Table 1: Number of patients receiving lymphadenectomy from 1999 to 2011.

Stage	SCC	Non-SCC	Total
IA	14	7	21
IB1	87	56(1)	141(1)
IB2	28(11)	18(1)	46(12)
IIA	16(2)	4(1)	20(3)
IIB	54(43)	12(4)	66(47)
Total	199(56)	97(7)	296 (63)

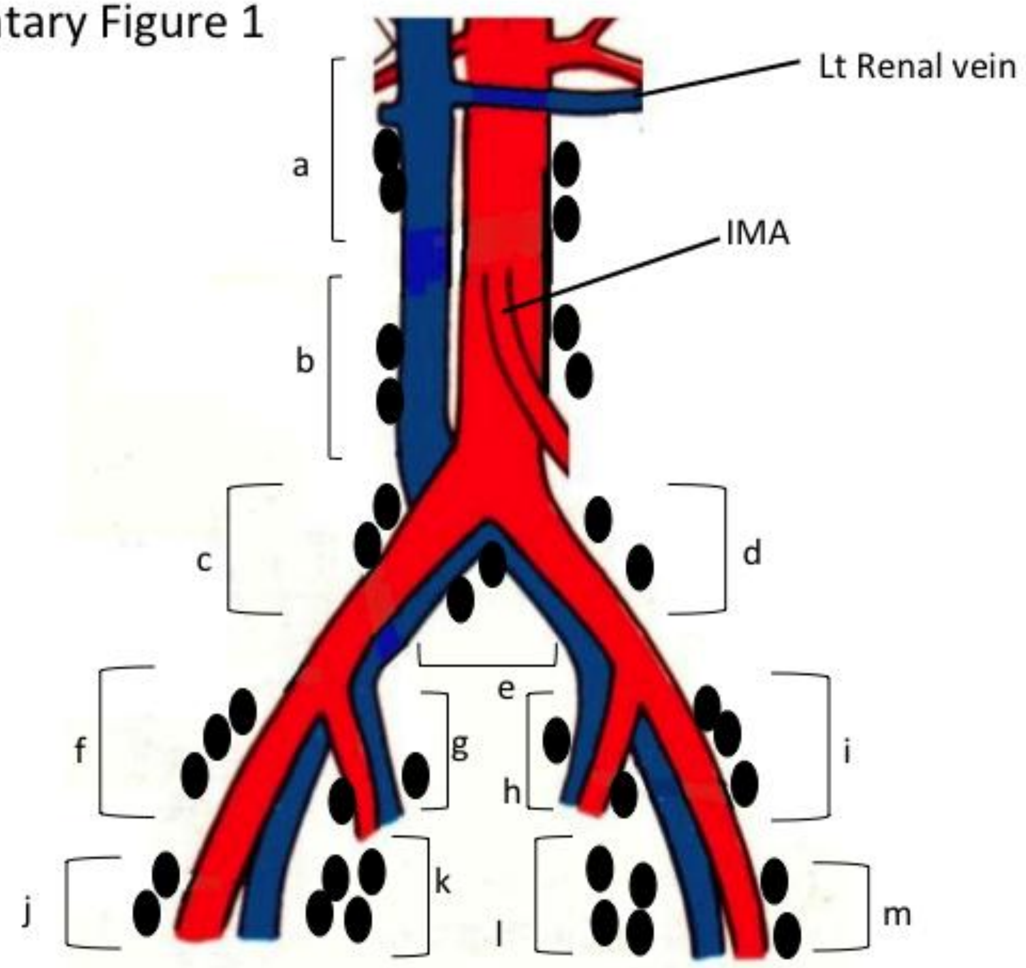
( ); NAC cases.

Supplementary Table 2: NAC regimen. Numbers of patients are shown in the table.

	stage IB2	stage IIA	stage IIB	Total
CPT-11/NDP	9	0	22	31
CDDP/THP/MMC *	2	2	15	19
NDP/Peplomycin/Ifomide	1	1	4	6
CPT-11/CDDP	1	0	1	2
Taxol/Carboplatin	0	0	4	4
Docetaxel/Carboplatin	0	0	1	1

*Transarterial infusion.

Supplementary Figure 1



Supplementary Figure 2