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ORIGINAL ARTICLE – COLORECTAL CANCER

Feasibility of Pooled One-Step Nucleic Acid Amplification for Molecular Staging of Pathologically Node-Negative Colon Cancer: A Prospective Multicenter Study

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ABSTRACT

Background. Although conventional one-step nucleic acid amplification (OSNA) is a useful molecular-staging method, its complexity hinders its use in clinical practice. A pooled approach for OSNA (pOSNA) has been evaluated for its feasibility in pathologically node-negative colon cancer (pNNCC) for molecular staging of lymph node metastasis in clinical practice.

Methods. Subjects were patients diagnosed with clinical stage II–IIIA colon cancer between January 2017 and September 2018. pOSNA involved harvesting pericolic lymph nodes from fresh surgical specimens, cutting them in half, placing 50% of the nodes in a single test tube, and performing the OSNA assay. The remaining halved pericolic, intermediate, and main lymph nodes were submitted for histopathologic examination, with metastasis determined by hematoxylin and eosin staining of a cut surface of each node.

Results. Of the 98 enrolled patients, 92 formed the analysis set. The mean number of harvested lymph nodes per case was 24.3 (range 5–66) and the mean number of lymph nodes used for pOSNA analysis was 6.9 (range 1–35). The concordance rate, sensitivity, and specificity between methods were 89.1%, 84.6% (95% confidence interval [CI] 0.80–0.91), and 90.9% (95% CI 0.88–0.94), respectively. The pOSNA upstaging rate for node-negative patients was 9.1% (6/66), and pOSNA returned false-negative results in 15.4% of node-positive cases (4/26).

Conclusions. pOSNA demonstrated an upstaging rate for pNNCC equivalent to that in previous studies, suggesting its feasibility for molecular staging of pNNCC in clinical practice.

The utility of conventional one-step nucleic acid amplification (OSNA) as a molecular-staging method has been demonstrated for various cancers.[1–5] Micrometastases (MMs), isolated tumor cells, and occult metastasis have been reported in 4.2%, 19.3%, and 5% of patients with stage I and II colon cancer (CC), respectively, and attracted interest as prognostic factors.[6–8]

To date, the accurate detection of small metastatic foci in lymph nodes has depended on the skill and experience of authorized pathologists. Another issue for detection is that only part of the lymph node is examined, occasionally resulting in undetected MMs. The OSNA assay is capable of performing standard evaluations without being influenced by operator skill or experience, and methods that use OSNA have recently garnered interest for detecting MMs.[1,4,9,10] Itabashi et al.[11] reported significantly worse 3-year disease-free survival among stage II CC patients for whom metastasis was confirmed by OSNA and relative to

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stage II patients with an OSNA-negative result. The OSNA assay reportedly shows high sensitivity and few false negatives because it measures *cytokeratin 19* (*CK19*) messenger RNA (mRNA) in a solubilized sample of the whole lymph cell, resulting in a concordance rate with pathological findings ranging from 85.9% to 98.2%.[1,3,5,9]

Previous reports have detailed three major methods to compare lymph node status between pathological examination and OSNA assay. The first method involves dividing lymph nodes in half and sending each 50% portion for pathology and OSNA (half-division method).[1,2,12] The second method involves dividing lymph nodes into four equal sections and sending two of these sections (50%) for pathology and OSNA (four-sections method).[12–14] In the third method, only 1 mm from the center of lymph nodes are sent for pathological examination and the rest are used for OSNA measurement (center-cut method).[2,15] The latter two methods described above are thought to be technically difficult for evaluating small lymph nodes. In contrast, dividing in half and sending each 50% portion for pathology and OSNA is the simplest method.

Recently, reports have indicated that metastatic lymph nodes could have small size metastasis of colorectal cancer. Yamaoka et al. reported a lymph node size of 7.16 ± 6.00 mm and 3.77 ± 2.31 mm for metastatic and non-metastatic lymph nodes in right CC, respectively, and 5.75 ± 4.56 mm and 3.01 ± 1.78 mm for metastatic and non-metastatic lymph nodes in left CC, respectively.[16] Furthermore, Brown et al. compared the size and pathology of MRI images of 437 lymph nodes and reported that the metastasis rate was 15.3% (25/55), even for lymph nodes smaller than 5 mm.[17] Therefore, preoperative lymph node metastasis diagnosis was difficult using size criteria. The main target of this study was pathological stage II patients with node-negative disease; however, there are limits to preoperative diagnosis, therefore the inclusion criterion was set as cancer cStage II–IIIA.

However, pathologic diagnosis confirming one-to-one correspondence of the lymph nodes using the OSNA method is highly complicated and remains an obstacle in using the method in clinical practice. Specifically, clinical application of OSNA has been impeded by the need to simplify the halving procedure for harvested lymph nodes and reduce the operating costs of OSNA analytical equipment. Rakislova et al. reported the feasibility of pooled OSNA (pOSNA) with the center-cut method in colorectal cancer.[15]

In this study, pOSNA and the half-division method were combined and used in the diagnosis of pericolic lymph node metastasis, and applied in clinical practice. In this study, we evaluated a pOSNA method that halves harvested pericolic lymph nodes and uses 50% of each lymph node for individual pathologic examination, but pools the remaining lymph node halves together for OSNA analysis. Furthermore, we evaluated the feasibility of pOSNA with the half-division method for molecular staging of pathologically node-negative CC (pNNCC).

METHODS

Patients

This study included 98 patients diagnosed with clinical stage II–IIIA CC at the participating study sites between January 2017 and September 2018. Lymph nodes larger than 5 mm in size, as observed in the CT image, were diagnosed as metastatic nodes, and patients with up to three metastatic nodes were classified as clinical stage IIIA. Consent to participate in this study was obtained in writing and provided by patients voluntarily after receiving a thorough explanation of the study and a full understanding of what participation in the study entailed.

Patients unable to provide consent and/or those who received chemotherapy or other treatments before surgery, diagnosed with active double cancer (simultaneous double cancer or metachronous cancer with a disease-free interval of <5 years), familial adenomatous polyposis, or otherwise deemed unsuitable by the principal investigator were excluded from the study. Importantly, the definition of active double cancer did not include carcinoma *in situ* deemed cured by topical treatment or lesions equivalent to intramucosal carcinoma.

Study Design

This was a non-randomized, multicenter, prospective study. Routine surgery involving lymph node dissection was performed for CC in each facility, and we compared the pOSNA results with the pathological findings. The metastasis rate of T2 CC to the main and intermediate nodes is low, and therefore the MM is mostly (96.3%) confined to pericolic nodes in node-negative colorectal cancer.[18] Considering this, only the pericolic lymph node was targeted in this study. After verifying that this upstaging rate was equivalent to that for pNNCC patients in previous studies, we calculated the concordance rate (number of pOSNA results matching pathologic diagnosis/total number of pOSNA results), sensitivity (number of pOSNA-positive cases and cases with positive pathological finding/number of cases with positive pathologic findings), and specificity (number of pOSNA-negative cases and cases with negative pathological findings/number of cases with negative pathological findings) of the pathological diagnoses.

Lymph Node Processing and Examination

After excision, specimens were stored at room temperature. Immediately after surgery, the surgeons harvested the regional lymph nodes from the resected specimens; classified them into pericolic, intermediate, and main lymph nodes; and submitted them for pathologic examination (Fig. 1). Only pericolic lymph nodes were examined by pOSNA.[19] The pericolic lymph nodes were halved, placed together into a test tube, and subjected to pOSNA. The weight limitation for the lymph nodes per tube was ≤600 mg, with those exceeding this limit placed in another tube for measurement. Small pericolic lymph nodes (≤4 mm) and other lymph nodes were fixed in 10% buffered formalin and embedded in paraffin. The sections were then stained with hematoxylin and eosin for pathological evaluation of nodal metastasis.

OSNA^{TM} Assay

Harvested pericolic lymph nodes were immersed in a test tube together with a specific solubilizing agent (Lynorhag; Sysmex, Kobe, Japan), homogenized, and centrifuged before removing only the solubilized lymph node fluid. A sample of the solubilized lymph node fluid diluted in the Lynorhag solution was then assayed using a dedicated analyzer (RD-100i; Sysmex). The OSNA Assay is an automated, high-sensitive assay for the detection of CK19 mRNA that uses the reverse transcription loop-mediated isothermal amplification method (RT-LAMP) to amplify *CK19* mRNA from lymph node tissue.

The pOSNA cut-off value for CC metastasis-positive/-negative lymph nodes was 250 copies/μL.[9,10] If the copy number was not 0 but <250, it was defined as a weak response. The pOSNA method analyzes solubilized lymph node fluid after 10- and 100-fold dilutions. If the 100-fold diluted sample produced an opposite result to that of the 10-fold diluted sample, the sample was determined as a positive result with strong reaction inhibition and marked with a '+I'.[20]

Ethics

This study was conducted with the approval of the Institutional Review Boards at Tokyo Women's Medical University, Sapporo Medical University, and Hyogo College of Medicine.

Statistical Analysis

Previous studies reported an upstaging rate of 3.2–25.2% for early-stage CC.[1,2,12–14] The upstaging rate obtained in this study was compared with previously published data using the Chi-square test, with a p-value <0.05 considered statistically significant. Statistical analysis was performed using JMP Pro software (version 14.0.0; SAS Institute Inc., Cary, NC, USA).

RESULTS

We enrolled 98 patients between January 2017 and September 2018 (Table 1). Ninety-two patients were subsequently used for analysis after excluding five patients with metastatic lymph nodes located just below the primary tumor, that could not be harvested or halved, as well as one diagnosed as stage IV during surgery. After pathologic examination, 17, 49, and 26 patients were classified as stage I, II, and III, respectively. The mean number of harvested lymph nodes was 24.3 (range 5–66) and the mean number of lymph nodes used for pOSNA analysis

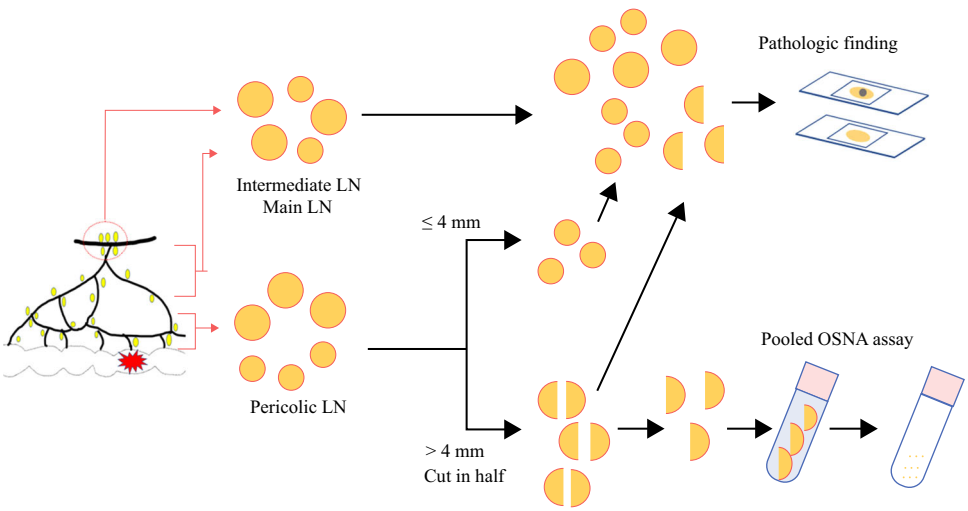

FIG. 1 Lymph node harvesting, pathologic diagnosis, and pooled OSNA analysis. *OSNA* one-step nucleic acid amplification, *LN* lymph node

TABLE 1 Patient information

Age (year)	67.5 (Ave.36–94)		
	N=92 %		
Sex (M / F)	44/48		48%: 52%
Tumor site	Cecum	7	8%
	Ascending colon	15	16%
	Transverse colon	13	14%
	Descending colon	3	3%
	Sigmoid colon	54	59%
Maximum tumor diameter (mm)			43.2 (10–90)
Lymph node metastasis	N0	66	72%
	N1 N1a	13	14%
	N1b	7	8%
	N1c	0	0%
	N2a	5	5%
	N2b	1	1%
Depth invasion	T1b	1	1%
	T2	19	21%
	T3	64	70%
	T4a	7	8%
	T4b	1	1%
Histological type	Tub1	30	33%
	Tub2	56	61%
	Tub	1	1%
	Por	2	2%
	Muc	3	3%
Lymphatic invasion	Negative	52	57%
	Positive	40	43%
Venous invasion	Negative	40	44%
	Positive	52	56%
Perineural invasion	Negative	70	76%
	Positive	22	24%
Pathological stage	I	17	19%
	IIa	47	51%
	IIb	1	1%
	IIc	1	1%
	III	26	28%

Muc, mucinous adenocarcinoma; Por, poorly differentiated carcinoma; Tub, tubular carcinoma; Tub1, well-differentiated adenocarcinoma; Tub2, moderately differentiated adenocarcinoma.

was 6.9 (range 1–35). In our actual pathological investigations, metastasis to the intermediate and main nodes was found in no cases.

Relationships Between Pooled One-Step Nucleic Acid Amplification (pOSNA) Status and Pathologic Examination

Six of the 66 cases with no pathological lymph node metastasis were deemed positive by pOSNA, and the upstaging rate for early-stage CC patients was 9.1% (6/66). The concordance rate, sensitivity, and specificity between methods were 89.1%, 84.6% (95% confidence interval [CI] 0.795–0.905) and 90.9% (95% CI 0.882–0.939), respectively (Table 2). Six (9.1%) of 66 cases with pathologically negative lymph node metastasis were positive according to pOSNA (Table 3), with the mean number of lymph nodes removed in these six cases 26.7 (range 8–50). Four of these six cases had stage II cancer, but no characteristic findings were identified in terms of tumor location, size, or vascular infiltration. However, four (15.4%) of 26 pathologically positive metastasis cases were negative according to pOSNA (Table 4), with three of the four cases having one lymph node metastasis with either small lymph nodes or a small proportion of cancer in the lymph nodes (Fig. 2). In all cases, positive CK19 staining was observed in the primary tumors, but the responses of the pOSNA method were weak.

DISCUSSION

In previous studies using cOSNA, 50% of each lymph node was submitted for pathologic examination, followed by evaluation of each remaining half by OSNA. The obstacles for clinical applications of cOSNA include a need to simplify the procedure for halving the dissected lymph nodes and reducing the operating costs associated with the equipment used for OSNA analysis. We evaluated a pOSNA method in which the harvested pericolic lymph nodes are halved, 50% of each lymph node is submitted for pathologic examination, and the remaining lymph node halves are pooled together in a test tube for OSNA analysis.

TABLE 2 Relationship between pOSNA status and pathologic examination

	pOSNA status	
	Negative (n)	Positive (n)
Pathologic examination		
Negative	60	6
Positive	4	22

The concordance rate, sensitivity, and specificity between methods were 89.1%, 84.6%, and 90.9%, respectively.

pOSNA, pooled one-step nucleic acid amplification.

TABLE 3 Pathological node-negative cases with pOSNA-positive status

Case	Age	Sex	Tumor location	Maximum tumor diameter (mm)	Histological type	pStage	Invasion depth	Pathological metastases	Lymphatic invasion	Venous invasion	No. of harvested LNs	No. of LNs used for pOSNA analysis	Copy no.
4-1	88	Female	S	25	Tub2	I	T2	N0	Negative	Positive	8	3	460
4-2	39	Male	T	30	Tub1	I	T2	N0	Negative	Negative	50	7	6300
4-3	65	Male	S	90	Muc	II	T3	N0	Negative	Negative	25	8	460
4-4	47	Male	D	39	Tub2	II	T3	N0	Positive	Positive	20	10	5000
4-5	63	Male	A	30	Tub2	II	T3	N0	Negative	Positive	26	1	+I[a]
4-6	68	Male	C	50	Tub1	II	T3	N0	Negative	Negative	31	12	1200

A ascending colon, *C* cecum, *CK19* cytokeratin, *D* descending colon, *LN* lymph node, *Muc* mucinous adenocarcinoma, *pOSNA* pooled one-step nucleic acid amplification, *S* sigmoid colon, *T* transverse colon, *Tub1* well-differentiated adenocarcinoma, *Tub2* moderately differentiated adenocarcinoma.

[a] *CK19*-positive reactions, but quantitative results cannot be presented due to reaction inhibition.

In this study, the upstaging rate for early-stage CC patients was 9.1% (6/66). The upstaging rates of our study were slightly lower than those reported previously;[1,12–14] however, one study showed significant differences from our series (Table 5).[2] In that study, the center-cut method was submitted for histopathologic examination and OSNA analysis. In the present study, the half-division method. Therefore, a different specimen volume was used for OSNA analysis in the previous study when compared with that in the present study. We speculate that this difference in specimen harvesting from removed lymph nodes was the reason for the significantly different upstaging rate between studies. It must also be considered that different methods of pathological evaluation, such as immunohistochemistry staining, are used. However, it cannot be ruled out that the small number of cases used in our study and the median number of lymph nodes measured by pOSNA (6.9) were slightly lower than in other reports. We speculate that the reliability of pOSNA improves with the number of lymph nodes measured using this procedure. These results revealed that the pOSNA with the half-division method might be useful as a clinical molecular-staging method.

Following identification of metastasis in the halved lymph nodes, we determined the OSNA-negative results for four patients diagnosed with stage III cancer were false negatives. Possible reasons for this might be the presence of a small metastasis at a single location that escaped detection, and/or low *CK19* mRNA levels. The cut-off point was set to 250 based on the results from previous investigations.[9] A large number of patients is necessary for investigating the relationship between OSNA data and pathological findings in false-negative cases. Furthermore, appropriate cut-off points need to be investigated, including the clinical implications of detecting micro lymph node metastasis using the OSNA method.

In the case 5-4, pOSNA was performed using seven lymph nodes and an OSNA-negative result was obtained, despite finding metastasis in four of these lymph nodes and also confirming *CK19* expression in the metastatic lymph nodes. We do not believe that this false-negative result was caused by low or localized *CK19* expression. Horimoto et al.[21] reported that protein and DNA in a specimen can inhibit the reaction during OSNA analysis and that this inhibition is stronger for highly malignant tumors. We re-examined the pathology of patients who were positive in pathological examinations and pOSNA-negative, and checked the proportion of metastasis in the lymph nodes as well as the expression of *CK19*. However, we did not re-examine the pathology of patients who were negative in pathological examinations and pOSNA-positive. In the present study, the sample potentially contained large amounts of specimen-derived protein and DNA due to the

TABLE 4 Pathological node-positive cases with pOSNA-negative status

Case	Age	Sex	Tumor location	Maximum tumor diameter (mm)	Histological type	Invasion depth	Lymphatic invasion	Venous invasion	Perineural invasion	CK19 status of the main tumor	No. of harvested LNs	No. of LNs used for pOSNA analysis	No. of pMLNs	Location of pMLNs	Maximum pMLN diameter (mm)	Cancer degree in pMLNs (%)	Degree of pOSNA response
5-1	62	Female	S	36	Tub2	T3	Positive	Negative	PN1a	Positive	12	5	1	LN with	OSNA	6.0	<5%
		Weak															
5-2	85	Male	C	40	Tub2	T2	Negative	Positive	PN0	Positive	18	6	1	LN with	OSNA	7.6	<5%
		Weak															
5-3	80	Male	S	35	Tub1	T4a	Positive	Positive	PN0	Positive	14	10	1	LN with	OSNA	2.9	80%
		Weak															
5-4	74	Female	A	33	Tub1	T3	Positive	Negative	PN1a	Positive	27	7	4	LN with	OSNA	4.7/ 3.5/ 3.5 /2.0	70%
		Weak															

A ascending colon, *C* cecum, *CK19* cytokeratin, *LN* lymph node, *pMLN* pooled metastatic lymph node, *pOSNA* pooled one-step nucleic acid amplification, *S* sigmoid colon, *Tub1* well-differentiated adenocarcinoma, *Tub2* moderately differentiated adenocarcinoma

addition of seven lymph nodes to a single test tube. Accordingly, in this case, the OSNA false-negative result was potentially caused by strong reaction inhibition, which suggests that the use of OSNA analysis in clinical practice requires critical attention to the number of specimens used in the analytical sample.

Previous studies report that mRNA of other tumor-specific factors, along with *CK19*, were recognized in a pathologically node-negative patient,[1,3,9] suggesting that OSNA analysis is potentially more accurate than conventional pathologic methods of identifying metastasis because it solubilizes the entire lymph node and analyzes *CK19* mRNA levels in the resulting sample. The advantages of OSNA include a short analysis time of approximately 30–40 min from start to completion, and the ability to automate the OSNA assay eliminates interlaboratory differences based on pathologist skill and experience. A disadvantage of OSNA is that it is more costly than pathologic examination. We found no significant differences in the sensitivity, specificity, and concordance rate between the two techniques, which required the same amount of time to make a judgment and used the same methods to halve the lymph nodes. With pOSNA, only one measurement is required, at a cost of US$225 (¥24,000). In contrast, cOSNA requires three or more measurements, at a cost of US$670 (¥72,000) or more. The upper limit of the lymph node weight that can be assayed in one tube by the OSNA method is 600 mg. In pOSNA, multiple lymph nodes can be measured in one tube, with the same upper weight limit allowed per tube; however, in cOSNA, only one lymph node can be measured in a single tube. In this study, we only measured pericolic lymph nodes using pOSNA; the guidelines for CC diagnosis and treatment recommend that at least $\geq$12 lymph nodes should be examined.[22] Using cOSNA to measure 12 or more halved lymph nodes would require at least 12 tubes and three or more measurements per tube, whereas assuming that 12 pericolic lymph nodes are measured, pOSNA would require only one or two tubes for each case. The OSNA measuring device can measure up to four tubes at once; however, because pOSNA requires only one measurement per case, this allows the device to take measurements for two to four cases simultaneously depending on the number and weight of the removed lymph nodes. In Japan, a single measurement incurs a cost of 2400 points (¥24,000), with medical insurance covering up to 2400 points. Therefore, assuming that 12 lymph nodes are to be removed, the cost of pOSNA can be fully covered by medical insurance, thereby reducing the financial burden.

The use of the OSNA assay is eligible for reimbursement (for a billable amount of 2400 points [up to four specimens]) under Japan's national health insurance as of

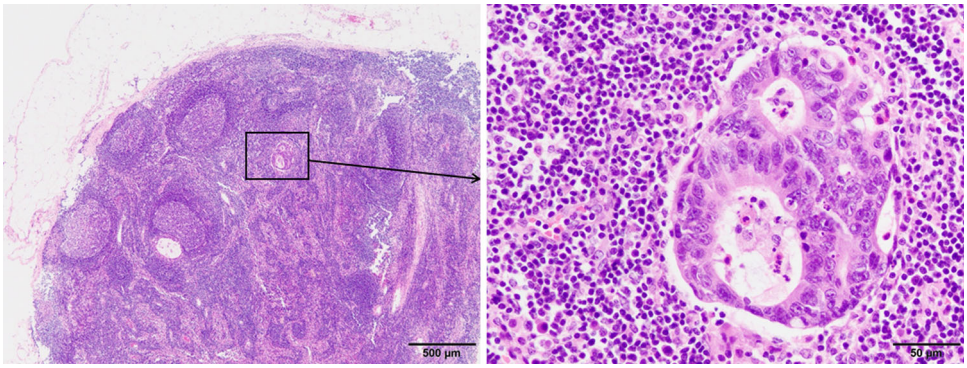

FIG. 2 A pathologically positive case with OSNA-negative status. Magnification of the finding shows a small metastatic cluster in the lymph node according to hematoxylin and eosin staining. *OSNA* one-step nucleic acid amplification

TABLE 5 Differences of lymph node processing methods and upstage rates of previous reports

Author (year)	Subject (number of patients)	OSNA method	Number of Harvested LN	Median of harvested LN	Dividing method of LN	Pathological staining	Number of measured LN by OSNA	Median of measured LN by OSNA	Upstage rate (pStage I and II)
Guller (2012)	Stage I, II, III (22)	cOSNA	313	30 (16–60)	Four[b]; 3 mm over diameter of LN	HE[e] and IHC[f]	56	13 (6–24)	15.3% (2/13)
Croner (2014)	Stage I, II (103)	cOSNA	N/A	N/A	Center[c]; 6 mm over Half[d]; 4 to 6 mm diameter of LN	HE[e]	1594	14 (1–46)	25.2% (26/103)
Vogelaar (2014)	Stage I, II (128)	cOSNA	N/A[a]	N/A[a]	Four[b]; 10 mm over Half[d]; 10 or less than 10 mm diameter of LN	HE[e] and IHC[f]	317	Mean 15.3 (4–40)	20.2% (20/90)
Yamamoto (2016)	Stage I, II, III (204)	cOSNA	4324	19(3–25)	Half[d]	HE[e]	1925	8 (2–25)	17.6% (13/74)
Rakislova (2017)	Stage I, II, III (188)	cOSNA,			pOSNA	N/A[a]	N/A[a]	Center[c]	HE[e]
					cOSNA1828 pOSNA 1992	cOSNA 13 (10–18) pOSNA 18 (13–25)	55.4%(51/92) 20.8% (16/77)		
Authors series	Stage II, IIIA (92)	pOSNA	2236	22 (5–66)	Half[d]; 4 mm over diameter of LN	HE[e]	636	6.9 (1–35)	9.1% (6/66)

[a]*N/A* not applicable

[b]*Four* Four sections method: dividing lymph nodes into four equal sections and sending two of these sections (50%) for pathology and OSNA measurement.

[c]*Center* Center-cut method: 1 mm from the center of lymph nodes are sent for pathological examination and the rest are used for OSNA

[d]*Half* Half-division method: dividing lymph nodes in half and sending each 50% portion for pathology and OSNA.

[e]*HE* Hematoxylin Eosin staining

[f]*IHC* Immunohistochemistry staining

October 2013. Consequently, the effective use of the OSNA assay in clinical practice requires a reduction in the number of assayed samples by combining specimens. pOSNA can simplify the complexity of cOSNA in clinical practice, employ the measurement limits of current analyzers, and reduce the burden on pathologists. Because

pOSNA assays all lymph nodes, it is incompatible with counting the number of involved lymph nodes typically used for TNM staging, and therefore cannot be used for conventional cancer staging. However, the OSNA assay can potentially be used to infer the size of metastatic foci based on the detected copy numbers.[3,10]

There are notable limitations with this study and the OSNA method. Evaluating the efficacy of OSNA-based methods requires cutting a harvested lymph node in half and comparing the pathological findings with those from OSNA assays; however, this evaluation is limited because lymph nodes directly present under the tumor and small lymph nodes that cannot be cut in half are sometimes metastatic. Another problem with OSNA-based methods is their ineffectiveness in patients with low *CK19* expression. In regard to comparing the findings of the present study with those published previously, because each study has a small number of cases and the background of each study might differ, a more detailed investigation of pOSNA will require a larger patient cohort. Unfortunately, this study lacked correlation with clinical outcomes, such as relapse-free survival, regarding pOSNA status. Indeed, adjuvant therapy of the registered target patients in this study was determined by the attending physician and no data on the prognosis were collected, which limited the assessment of the overall data. Moreover, comparing the prognosis of pathologically node-negative and pOSNA-negative patients with double-negative patients would require the registration of a large number of participants. Additionally, the effectiveness of adjuvant chemotherapy for pathologically node-negative and pOSNA node-positive patients is not clear. Therefore, to overcome this limitation of our study, we have confirmed, by comparison with previous reports, that prognosis was poor if OSNA was positive in pStage II.[11] We are currently conducting prospective clinical trials to further explore this subject.

CONCLUSIONS

The results showed that pOSNA can simplify the process of cutting harvested lymph nodes in half while reducing the equipment-related costs associated with OSNA assays used in clinical practice. Additionally, pOSNA demonstrated an upstaging rate for pNNCC equivalent to that reported in previous studies, suggesting its feasibility for molecular staging of pNNCC in clinical practice. However, the false-negative rate was 15.4% and this is a point to be considered when applying pOSNA in clinical practice.

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