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The diagnostic value of one step nucleic acid amplification (OSNA) in differentiating lymph node metastasis of tumors: a systematic review and meta-analysis

Short title: Value of OSNA assay in tumor patients with nodal metastases

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The diagnostic value of one step nucleic acid amplification (OSNA) in differentiating lymph node metastasis of tumors: a systematic review and meta-analysis

Keywords: OSNA; sensitivity; specificity; meta-analysis

Abbreviations: OSNA: one step nucleic acid amplification; AUC: area under the curve; CK-19: cytokeratin 19; Tp: true positive; Fp: false positive; Fn: false negative; Tn: true negative; QUADAS-2: the QUality Assessment of Diagnostic Accuracy Studies; PLR: positive likelihood ratio; NLR: negative likelihood ratio; DOR: diagnostic odds ratio; CI: Confidence interval; OSCC: oral squamous cell carcinoma

Abstract

Background: The aim of this study was clarify the diagnostic accuracy of one step nucleic acid amplification (OSNA) for differentiating metastatic lymph nodes from non-metastatic ones in patients with tumors (not including breast cancer).

Methods: A systematic literature search for original diagnostic studies was performed in PubMed. Findings were pooled by using combined effect models and hierarchic summary receiver operating characteristic curve models. Meta-regression analysis and threshold effect evaluating were performed to explore the sources of heterogeneity affected classification accuracy.

Results: 19 studies (803 positive and 4594 negative lymph nodes) were analyzed, including 4 different tumor types (head and neck cancers, gastrointestinal cancers, lung cancer and gynecological malignancies). In the studies of head and neck cancers the pooled sensitivity, specificity and area under the curve (AUC) of the OSNA method were 0.85(0.79-0.89), 0.96(0.92-0.98) and 0.91(0.88-0.93), respectively. Similarly, the corresponding values in the studies of gastrointestinal cancers were 0.90(0.85-0.94), 0.96(0.94-0.98) and 0.97(0.96-0.99), respectively. Because of limited number of studies, the other two tumor types were inestimable in the subsequent meta-analyses.

Conclusions: Pooled data suggest that the OSNA assay has a high diagnostic accuracy for the detection of lymph node metastases. For wide spread implementation, additional studies on other different types of tumors are warranted.

Introduction

The nodal status of lymph nodes remains a significant prognostic factor in patients with different types of tumors. The presence of lymph node metastasis increases the risk of loco-regional relapse and reduces the survival of patients with these tumors. Thus, due to the importance of assessing the potential lymph node involvement prior to surgery and intraoperative discovery, several different strategies have been developed.

Some studies have reported that frozen sections frequently result in discordant results between intraoperative analyses and definitive histological findings. The sensitivities of examination of frozen sections for finding nodal metastases ranged from 70% to 90% in breast cancer. Similarly, the reported sensitivities were from 60% to 70% for HNSCC [1-4]. This was mainly a result of the failure to detect micrometastases, or small volume nodes. Even though immunohistochemistry and step sectioning have increased the detection rate for micrometastasis to 20-30% in colorectal cancer, they were not suitable for intraoperative examination because they were burdensome and time-consuming [5-6]. Similarly, molecular techniques, such as the reverse-transcription polymerase chain reaction, have been attempted in different types of cancer and reported to be accurate compared with conventional pathological analyses. However, this method has not applicable for intraoperative use because of its complexity and unsatisfying time consumption [7-8]. Therefore, a quick, highly sensitive and specific intraoperative diagnostic technique is necessary.

During the last few years, a new molecular technique called one step nucleic acid

amplification (OSNA), which is a rapid and semi-quantitative intraoperative procedure for quantifying the number of cytokeratin 19 (CK-19) mRNA copies in lymph nodes, has been employed to assess lymph node progression of tumors. OSNA was first described by Tsujimoto and colleagues in breast cancer patients and has been reported to be effective for detecting nodal metastases in several published studies [9-13]. The validity of the OSNA assay for detecting lymph node metastasis has also been widely reported in patients with other types of tumors such as colorectal cancer, HNSCC, lung cancer, gastric cancer and thyroid cancer. However, the clinical efficacy has not been evaluated. The aim of this study is to clarify the diagnostic accuracy of the OSNA method for differentiating metastatic lymph nodes from non-metastatic ones in patients with several included tumors in comparison with final pathological results.

Methods

Literature Search

A comprehensive literature search of studies was searched using PubMed database by two reviewers (X.C and X.B) to identify the diagnostic performance of the OSNA method in detecting lymph node involvement. The following search terms were used: (Neoplasms [Mesh] OR cancer OR carcinoma OR tumor OR neoplasm OR lymphatic metastasis [MeSH] OR lymph node metastasis) AND (OSNA OR one-step nucleic acid amplification). The publication date had an upper limitation of October 2017.

Eligible criteria for study selection

Studies were included if they fulfilled following criteria: (1) the study evaluated the clinical performance of the OSNA assay in patients with nodal metastases (excluding breast cancer); (2) sufficient information was presented to calculate values of true positive (Tp), false positive (Fp), false negative (Fn) and true negative (Tn) for per-node statistics; (3) for quality assurance, from the QUality Assessment of Diagnostic Accuracy Studies (QUADAS-2) checklist, we chose the study which the total score was greater than or equal to 9 points from the 14 questions.

Data collection

Reviewers who performed the database search also extracted the relevant data independently, and disagreements resolved by discussion. The following characteristics of each study including author, tumor type, publication year, nation, values of patient and lymph node, cut-off values of CK-19, results of Tp, Fp, Fn as well as Tn (either found or calculated from data in original published studies), discordant lymph node results were also extracted.

Statistical analysis

All analyses were performed using Stata 12.0 (StataCorp), Meta-DiSc version 1.4, and SPSS 16.0. All statistical tests were two-sided and statistical significance was defined as a *P* value less than 0.05.

For each study, diagnostic parameters for the OSNA assay were calculated by the following formulas: sensitivity = Tp/(Tp+Fn), specificity = Tn/(Tn+Fp), positive likelihood ratio (PLR) = sensitivity/(1-specificity), negative likelihood ratio (NLR) = (1-sensitivity)/specificity and diagnostic odds ratio (DOR) = Tp*Tn/Fn*Fp, along

with their 95% confidence intervals (CIs). The bivariate model was adjusted to obtain the area under the curve (AUC). To realize the potential factors that influenced accuracy estimates, we tried to explore the source of heterogeneity among included studies when the quantified I^2 value was greater than 50% [14]. Because of the threshold effect was an important source of heterogeneity, we assessed the Spearman's correlation coefficient, and negative correlation ($p< 0.05$) suggested existence of the threshold effect. If no threshold effect existed but significant heterogeneity, further meta-regression analysis was to explore other sources of heterogeneity in these included studies.

Furthermore, publication bias was assessed directly by using Deeks' funnel plot symmetry tests [15].

Results

Study selection

PubMed identified in 384 potentially relevant studies after the comprehensive computerised searches performed. 268 studies were initially excluded after screening the titles and abstracts. From the remaining studies, 97 were excluded after reviewing the full article, consisting of 76 articles were to evaluate nodal involvement in breast cancer, 3 were published in non-English, 5 were review articles, and 13 articles did not obtain sufficient information or per-patient analysis. Finally, 19 studies fulfilled the eligible criteria and were considered. The detailed procedure of study selection process was presented in Fig 1.

Study characteristics

This systematic review included a total of 803 positive and 4594 negative lymph nodes from 19 studies, including 4 different tumor types (Group A, head and neck cancers [16-21]; Group B, gastrointestinal cancers [22-30]; Group C, lung cancer [31,32]; Group D, gynecological malignancies [33,34]) (#1-19 in Table 1). Additionally, information about tumor type, publication year, nation of author, number of patient and node of each study, and discordant lymph node results were also presented. Because of limited number of studies, Group C and D were not able to pool in the subsequent analysis. Finally, 15 published studies (Group A and B) were considered to the following meta-analyses.

Assessment of study quality

For quality assurance, from the QUADAS-2 checklist, all these 15 studies, which the number of the answer “no bias” for the 14 questions were greater than or equal to 9, were included (presented in Table 2).

Diagnostic performance for the OSNA method

Fig 2 showed the forest plots of the sensitivity and 1- specificity for Group A and B. On the basis of the combined effect model, the corresponding sensitivity and specificity for Group A were 0.85(0.79-0.89) and 0.96(0.92-0.98), for Group B the sensitivity was 0.90(0.85-0.94) and the specificity was 0.96(0.94-0.98). The AUCs of OSNA assay were 0.91(0.88-0.93) (Group A) and 0.97(0.96-0.99) (Group B) (Fig 3).

In addition, the pooled PLR, NLR and DOR for Group A and B were 20.6(10.3-41.0), 0.16(0.11-0.22), 130.5(62.3-273.3) and 23.4(13.7-40.0), 0.10(0.06-0.16), 235.2(99.9-554.1), respectively (in Table 3).

Assessment of publication bias

The Deeks' funnel plot presented that studies were distributed on the asymmetrical funnel plots of DOR against 1/(effective sample size (ESS))$^{1/2}$. The result of the Deeks' tests showed no evidence of the existence of significant publication bias (Group A: bias =0.41, *P*=0.701; Group B: bias =0.19, *P*=0.858) (Fig 4).

Results of heterogeneity analysis

The combined effect model indicated that obvious significant between-study heterogeneity among the studies (Group A: I^2 =73.8% in specificity, *P*<0.05; Group B: I^2 =89.7% in specificity, *P*<0.05), thus, the following threshold effect evaluating and meta-regression analysis were necessary for exploring the sources of heterogeneity.

The threshold effect did not exist (spearman correlation coefficient in Group A = 0.486, *P* = 0.329; in Group B =-0.550, *P* = 0.125).

Single-factor meta-regression analysis by applying tumor type, nation, number of lymph nodes and the percentage of discordant lymph node results, was performed. Table 4 showed that none of these single factors were the sources of heterogeneity in Group A (all *P* value >0.05), however, in Group B, the percentage of discordant node results (≤10% or >10%) can be viewed as sources of heterogeneity (*P* <0.05).

Posttest probability of lymph node metastases using the OSNA assay

To determine the potential utility of our results for decision making in clinical practice, for Group A, when we defined the pretest probability as 25%, 36%, and 75%, the corresponding positive posttest probability (PPP) and negative posttest probability

(NPP) were 87%, 92%, 98% and 5%, 8%, 32%. Similarly, for Group B, when the pretest probability defined as 25%, 39%, and 75%, the corresponding PPP and NPP were 89%, 94%, 99% and 3%, 6%, 23% (Fig 5).

Discussion

To our knowledge, this is the first systematic review and meta-analysis to evaluate the performance of the OSNA assay in different types of cancer (not including breast cancer) with lymph node metastases. In this meta-analysis we included 6 studies in patients with head and neck cancers and 9 studies in gastrointestinal cancers. In the studies of head and neck cancers the pooled sensitivity, specificity and AUC of OSNA assay were 0.85(0.79-0.89), 0.96(0.92-0.98) and 0.91(0.88-0.93), respectively. Similarly, the corresponding values in the studies of gastrointestinal cancers were 0.90(0.85-0.94), 0.96(0.94-0.98) and 0.97(0.96-0.99), respectively. All these diagnostic values were showed that the OSNA assay is useful to distinguish metastatic lymph nodes from non-metastatic ones.

DOR values combine sensitivity and specificity and represent the ratio of the odds of positivity in metastatic lymph nodes relative to that of non-metastatic. A higher DOR value indicates better discrimination performance of the OSNA assay. In this meta-analysis, the DOR values in head and neck cancers and gastrointestinal cancers were 130.5 and 235.2, indicating that the differential ability of the OSNA assay is high. Likelihood ratio (LR) is another measure of diagnostic accuracy. For a test to be highly useful, it should have an LR > 10 or < 0.10. Based on the LR values, the OSNA assay is considered to be higher value for evaluating lymph node

involvement. To better understand the OSNA assay's clinical utility, we used a Fagan's nomogram to estimate a patient's possibility of having lymph nodal metastases. For a head and neck cancer patient, if the pretest probability was defined as 25%, the posttest probability of nodal metastasis with a positive OSNA assay result was 87%, while a negative result reduced the probability to 5%. If the pretest probability was defined as 75%, a positive or negative result changed the posttest probability to 98% or 32%. As the pretest probability increased, the OSNA assay was more likely to confirm rather than exclude lymph nodal metastasis. In contrast, as the pretest probability decreased, the OSNA assay was suitable for metastasis exclusion rather than confirmation. In patients with gastrointestinal cancer, the OSNA assay has been reached similar results. Thus, a specific pretest probability, which achieved the same effect for metastasis confirmation and exclusion, exists and can be viewed as the cutoff pretest probability for the OSNA test to assess lymph node involvement. As Fig 5 shows, the cutoff pretest probabilities were 36% in patients with head and neck cancer and 39% in patients with gastrointestinal cancer.

As Table 1 reveals, some discordant results between the OSNA assay and conventional pathological diagnoses were found in our included studies. Low or no expression of CK-19 mRNA in different types of tumor cell was one of the most important causes of these discrepant results. Consequently, many published studies have been evaluated the performance of other mRNA markers using a mixture of histopathologically positive and negative lymph nodes. A study by Yamamoto et al. examined 98 candidate mRNA genetic markers which were from a genome-wide

database by comparing an expression frequency in colon cancer [35]. After four sequencing phases, CK-19, CEA and CK-20 mRNAs were evaluated using OSNA assay. The expression of CK-19 mRNA was observed in all pathologically positive lymph nodes, however, CEA and CK-20 mRNAs were not found in metastatic nodes. Similarly, the expression frequency of CK-19 is significantly higher than other candidate mRNA markers in different tumor tissues such as gastric, HNSCC and oral squamous cell carcinoma (OSCC), and these studies determined CK-19 to be the best marker for the OSNA assay [20,24,36-37]. However, a few studies described that CK-19 was not a usefully expressed cytokeratin in OSCC because its expression was low in all OSCC (65%), especially in early OSCC (56%) [38]. Therefore, they questioned the applicability of CK-19-based OSNA assay in head and neck cancers. The study of Masai et al. summarizes the CK-19 expression in different histological types of lung cancer. Most subtypes observed had a high prevalence of CK-19 expression, but few thoracic tumors indicated lowly positive rates for expression of CK-19 mRNA, such as 54.8% of pleomorphic carcinoma, 54.5% of large-cell carcinoma, 34.0% of carcinoid tumor and 31.8% of small cell carcinoma [39]. Thus, CK-19 was selected as a useful mRNA marker for the OSNA assay; however, further trials and tumor types are necessary to evaluate the efficacy of CK-19 mRNA or other biomarkers.

In patients with breast cancer, previous studies have used a cutoff CK-19 value of 250 copies/μl for assessing lymph node metastasis. A CK-19 mRNA copy number <250/μl was viewed as negative result and a copy number ≥250/μl was regarded as

positive. To explore the cutoff value of the OSNA assay between metastatic and non-metastatic lymph nodes in other tumor types, several studies evaluated the diagnostic performance of the serial cutoff values of the OSNA method [24,33]. Moreover, 250 copies/μl appeared to be an optimal cutoff value that distinguished between positive and negative lymph nodes. However, Goda et al. reported that the optimal cutoff of CK-19 mRNA in detecting lymph node metastasis was 300 copies/μl in HNSCC [16]. The study of Matsuzuka et al. concluded that the optimum cutoff point in HNSCC patients was 131 copies/μl, which was the highest diagnostic performance [17]. Furthermore, the optimal cutoff value for the number of CK-19 mRNA copies was 93 in thyroid cancer [19]. Therefore, with all that said, further trials are necessary to verify the best cutoff CK-19 value in each tumor type, especially in head and neck cancers.

Some problems of this systematic review require further explanation. First, because of limited number of studies, the pooled diagnostic performance of the OSNA assay in patients with lung cancer or gynecological malignancy was inestimable. Even though their specificities were high (all >90%), the sensitivities for the detection of nodal metastases in the OSNA method were widely ranged (80%, 100% in lung cancer; 50%, 82% in gynecological malignancies) [31-34]. Further large-scale, highly-quality trials are needed to evaluate the efficacy of the OSNA assay in patients with these tumor types. Second, as Fig 2 revealed, there are obvious significant between-study heterogeneities among studies of head and neck cancer and gastrointestinal cancer. Table 2 shows that the discordant node results can be viewed

as sources of heterogeneity in patients with gastrointestinal cancer, and if the study of Vogelaar et al (19.7%) is removed, we could observe an increase in sensitivity and specificity by using the OSNA method. However, neither threshold effects nor evaluated covariates were the sources of heterogeneity in the studies of head and neck cancers.

In conclusion, pooled data suggest that the OSNA assay has a high diagnostic accuracy for the detection of lymph node metastases. For wide spread implementation, additional studies on other different types of tumor are warranted.

References

[1] Van Diest PJ, Torrenga H, Borgstein PJ, et al. Reliability of intraoperative frozen section and imprint cytological investigation of sentinel lymph nodes in breast cancer. Histopathology 2013; 35: 14–18.

[2] Tanis PJ, Boom RP, Koops HS, et al. Frozen section investigation of the sentinel node in malignant melanoma and breast cancer. Ann Surg Oncol 2001; 8: 222–226.

[3] Civantos FJ, Gomez C, Duque C, Pedroso F, Goodwin WJ, Weed DT, et al. Sentinel node biopsy in oral cavity cancer: correlation with PET scan and immunohistochemistry. Head Neck 2003; 25: 1–9.

[4] Terada A, Hasegawa Y, Yatabe Y, Hanai N, Ozawa T, Hirakawa H, et al. Follow-up after intraoperative sentinel node biopsy of N0 neck oral cancer patients. Eur Arch Otorhinolaryngol 2001; 268: 429–435.

[5] Lockett MA, Metcalf JS, Baron PL, O'Brien PH, Elliott BM, Robison JG, et al. Efficacy of reverse transcriptase-polymerase chain reaction screening for micrometastatic disease in axillary lymph nodes of breast cancer patients. Am Surg 1998; 64: 539–543.

[6] Viale G, Bosari S, Mazzarol G, Galimberti V, Luini A, Veronesi P, et al. Intraoperative examination of axillary sentinel lymph nodes in breast carcinoma patients. Cancer 1999; 85: 2433–2438.

[7] Saintigny P, Coulon S, Kambouchner M, Ricci S, Martinot E, Danel C, et al. Real-time RT-PCR detection of CK19, CK7 and MUC1 mRNA for diagnosis of

lymph node micrometastases in non-small cell lung carcinoma. Int J Cancer 2005; 115: 777–782.

[8] Xi L, Coello MC, Litle VR, Raja S, Gooding WE, Yousem SA, et al. A combination of molecular markers accurately detects lymph node metastasis in non-small cell lung cancer patients. Clin Cancer Res 2006; 12: 2484–2491.

[9] Tsujimoto M, Nakabayashi K, Yoshidome K, et al. One-step nucleic acid amplification for intraoperative detection of lymph node metastasis in breast cancer patients. Clin Cancer Res 2007; 13 (16):4807-4816.

[10] Osako T, Tsuda H, Horii R, Iwase T, Yamauchi H, Yagata H, Tsugawa K, Suzuki K, Kinoshita T, Akiyama F, Nakamura S. Molecular detection of lymph node metastasis in breast cancer patients treated with preoperative systemic chemotherapy: a prospective multicentre trial using the one-step nucleic acid amplification assay. Br J Cancer 2013; 109(6):1693-1698.

[11] Li D, Xu X, Chen J, Chen J, Yang B, Yang W, Xu W, Wu J, Shi D. Utility of one-step nucleic acid amplification (OSNA) assay in detecting breast cancer metastases of sentinel lymph nodes in a Chinese population. Breast Cancer 2015 22(2):135-140.

[12] Shi F, Zhang Q, Liang Z, Zhang M, Liu X. One-step nucleic acid amplification assay is an accurate technique for sentinel lymph node biopsy of breast cancer patients: a meta-analysis. Br J Cancer 2017; 117(8):1185-1191.

[13] Tiernan JP, Verghese ET, Nair A, Pathak S, Kim B, White J, Thygesen H, Horgan K, Hanby AM. Systematic review and meta-analysis of cytokeratin 19-based

one-step nucleic acid amplification versus histopathology for sentinel lymph node assessment in breast cancer. Br J Surg 2014; 101(4):298-306.

[14] Goda H, Nakashiro K, Oka R, Tanaka H et al. One-step nucleic acid amplification for detecting lymph node metastasis of head and neck squamous cell carcinoma. Oral Oncol 2012; 48(10):958-963.

[15] Matsuzuka T, Takahashi K, Kawakita D, Kohno N, et al. Intraoperative molecular assessment for lymph node metastasis in head and neck squamous cell carcinoma using one-step nucleic acid amplification (OSNA) assay. Ann Surg Oncol 2012; 19(12):3865-3870.

[16] Kaczka K, Fendler W, Borowiec M, Młynarski W, Pomorski L. First one-step nucleic acid amplification testing in papillary thyroid cancer lymph nodes – a comparison with histopathology and real-time PCR. Endokrynol Pol 2014; 65(6):422-430.

[17] González O, Iglesias C, Zafon C, Castellví J, et al. Detection of thyroid papillary carcinoma lymph node metastases using one step nucleic acid amplification (OSNA): preliminary results. J Invest Surg 2015; 28(3):153-159.

[18] Suzuki M, Matsuzuka T, Hashimoto Y, Ikeda M, Saijo S, Omori K. Diagnostic potential of one-step nucleic acid amplification assay in patients with head and neck squamous cell carcinoma based on CK19 expression in a primary lesion. Head Neck doi: 10.1002/hed.23979.

[19] del Carmen S, Gatius S, Franch-Arcas G, Baena JA, Gonzalez O, et al. Concordance study between one-step nucleic acid amplification and morphologic

techniques to detect lymph node metastasis in papillary carcinoma of the thyroid. Hum Pathol 2016; 48:132-141.

[20] Croner RS, Schellerer V, Demund H, et al. One step nucleic acid amplification (OSNA) - a new method for lymph node staging in colorectal carcinomas. J Transl Med 2010; 8:83.

[21] Yamamoto H, Sekimoto M, Oya M, et al. OSNA-based novel molecular testing for lymph node metastases in colorectal cancer patients: results from a multicenter clinical performance study in Japan. Ann Surg Oncol 2011; 18(7):1891-1898.

[22] Yaguchi Y, Sugasawa H, Tsujimoto H, et al. One-step nucleic acid amplification (OSNA) for the application of sentinel node concept in gastric cancer. Ann Surg Oncol 2011; 18(8):2289-2296.

[23] Güller U, Zettl A, Worni M, Langer I, et al. Molecular investigation of lymph nodes in colon cancer patients using one-step nucleic acid amplification (OSNA):a new road to better staging? Cancer 2012; 118(24):6039-6045.

[24] Kumagai K, Yamamoto N, Miyashiro I, et al. Multicenter study evaluating the clinical performance of the OSNA assay for the molecular detection of lymph node metastases in gastric cancer patients. Gastric Cancer 2014; 17(2):273-280.

[25] Vogelaar FJ, Reimers MS, van der Linden RL, van der Linden JC, Smit VT, Lips DJ, van de Velde CJ, Bosscha K. The Diagnostic Value of One-Step Nucleic acid Amplification (OSNA) for Sentinel Lymph Nodes in Colon Cancer Patients. Ann Surg Oncol 2014; 21(12):3924-3930.

[26] Yamamoto H, Tomita N, Inomata M, Furuhata T, et al. OSNA-Assisted Molecular Staging in Colorectal Cancer: A Prospective Multicenter Trial in Japan. Ann Surg Oncol 2016; 23(2):391-396.

[27] Yeung TM, Wang LM, Colling R, Kraus R, Cahill R, Hompes R, Mortensen NJ. Intraoperative identification and analysis of lymph nodes at laparoscopic colorectal cancer surgery using fluorescence imaging combined with rapid OSNA pathological assessment. Surg Endosc 2018;32(2):1073-1076.

[28] Colling R, Yeung T, Hompes R, Kraus R, Cahill R, Mortensen N, Wang LM. OSNA testing for lymph node staging in colorectal cancer. J Clin Pathol 2017; 70(7):638-639.

[29] Hayama M, Chida M, Karube Y, et al. One-step nucleic acid amplification for detection of lymph node metastasis in lung cancer. Ann Thorac Cardiovasc Surg 2014; 20(3):181-184.

[30] Nakagawa K, Asamura H, et al. The novel one-step nucleic acid amplification (OSNA) assay for the diagnosis of lymph node metastasis in patients with non-small cell lung cancer (NSCLC): Results of a multicenter prospective study. Lung Cancer 2016; 97:1-7.

[31] Okamoto S, Niikura H, Nakabayashi K, et al. Detection of sentinel lymph node metastases in cervical cancer: assessment of KRT19 mRNA in the one-step nucleic acid amplification (OSNA) method. Gynecol Oncol 2013; 130(3):530-536.

[32] Nagai T, Niikura H, Okamoto S, Nakabayashi K, et al. A new diagnostic method

for rapid detection of lymph node metastases using a one-step nucleic acid amplification (OSNA) assay in endometrial cancer. Ann Surg Oncol 2015; 22(3):980-986.

[33] Yamamoto N, Daito M, Hiyama K, Ding J, Nakabayashi K, Otomo Y, Tsujimoto M, Matsuura N, Kato Y. An Optimal mRNA Marker for OSNA (One-step Nucleic Acid Amplification) Based Lymph Node Metastasis Detection in Colorectal Cancer Patients. Jpn J Clin Oncol 2013; 43(3):264-270.

[34] Tao L, Lefèvre M, Ricci S, et al. Detection of occult carcinomatous diffusion in lymph nodes from head and neck squamous cell carcinoma using real-time RT-PCR detection of cytokeratin 19 mRNA. Br J Cancer 2006; 94:1164–1169.

[35] Zhong LP, Chen WT, Zhang CP, Zhang ZY. Increased CK19 expression correlated with pathologic differentiation grade and prognosis in oral squamous cell carcinoma patients. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104:377–384.

[36] Noorlag R, van Es RJJ, de Bree R, Willems SM. Cytokeratin 19 expression in early oral squamous cell carcinoma and their metastasis: Inadequate biomarker for one-step nucleic acid amplification implementation in sentinel lymph node biopsy procedure. Head Neck 2017; 39(9):1864-1868.

[37] Masai K, Nakagawa K, Yoshida A, Sakurai H, Watanabe S, Asamura H, Tsuta K. Cytokeratin 19 expression in primary thoracic tumors and lymph node metastases. Lung Cancer 2014; 86(3):318-323.

[38] Higgins J, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat

Med 2002; 21:1539–1558.

[39] Deeks JJ. Systematic reviews in health care: systematic reviews of evaluations of diagnostic and screening tests. BMJ 2001; 323:157–162.

Figure Legends

Fig 1 Flow Diagram: selection process of the studies.

Fig 2 Forest plots of sensitivity and specificity with corresponding 95 % CIs (A and B for head and neck cancers; C and D for gastrointestinal cancers). In the studies of head and neck cancers the pooled sensitivity, specificity of OSNA assay were 0.85(0.79-0.89) (A) and 0.96(0.92-0.98) (B). The corresponding values in the studies of gastrointestinal cancers were 0.90(0.85-0.94) (C) and 0.96(0.94-0.98) (D).

Fig 3 SROC curves for the diagnostic performance of OSNA assay for head and neck cancers (A) and gastrointestinal cancers (B).

Fig 4 Asymmetrical funnel plots indicated no publication bias both head and neck cancers (A) and gastrointestinal cancers (B).

Fig 5 Fagan's nomograms were calculated post-test probabilities using different pre-test probabilities of lymph node metastases in three clinical scenarios (A, B and C for head and neck cancers; D, E and F for gastrointestinal cancers).

Table 1 Characteristics of included studies

Group	Study ID No. and author	Tumor type	Year	Nation	No. of patients and lymph nodes	Tp	Fp	Fn	Tn	Discordant node results (%)
A	1 Goda	HNSCC	2012	Japan	65,312	53	10	8	241	18(5.8)
	2 Matsuzuka	HNSCC	2012	Japan	56,175	28	1	6	140	7(4.0)
	3 Kaczka	Thyroid	2014	Poland	32,92	13	3	4	72	7(7.6)
	4 Gonzalez	Thyroid	2014	Spain	5,50	19	3	2	26	5(10)
	5 Suzuki	HNSCC	2015	Japan	21,54	7	1	2	44	3(5.5)
	6 del Carmen	Thyroid	2015	Spain	37,284	84	19	13	168	32(11.3)
B	7 Croner	Colorectal	2010	Germany	184,184	37	5	3	139	8(4.3)
	8 Yamamoto	Colorectal	2011	Japan	85,385	79	7	4	295	11(2.9)
	9 Yaguchi	Gastric	2011	Japan	32,162	40	4	5	113	9(5.6)
	10 Güller	Colon	2012	Switzerland	22,313	51	11	2	249	13(4.2)
	11 Kumagai	Gastric	2014	Japan	61,394	45	14	9	326	23(5.8)
	12 Vogelaar	Colon	2014	The Netherlands	-,127	23	20	5	79	25(19.7)
	13 Yamamoto	Colorectal	2016	Japan	204,1925	125	63	20	1717	83(4.3)
	14 Yeung	Colorectal	2017	UK	16,78	16	1	0	61	1(1.3)
	15 Colling	Colorectal	2017	UK	19,82	13	2	1	66	3(3.7)
C	16 Hayama	Lung	2014	Japan	20,40	4	3	0	33	3(7.5)
	17 Nakagaw	NSCLC	2016	Japan	111,410	47	18	12	333	30(7.3)

	a									
D	18 Okamoto	Cervical	2013	Japan	32,130	3	2	3	122	5(3.8)
	19 Nagai	Endometrial	2015	Japan	35,137	14	1	3	119	4(2.9)

A, head and neck cancers; B, gastrointestinal cancers; C, lung cancer; D, gynecological malignancies; Tp, true positive; Fp, false positive;
Fn, false negative; Tn, true negative.

Table2 Results of the evaluation of each study according to QUADAS-2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Score
#1 Goda	+	+	+	+	+	+	+	+	+	?	?	+	+	+	12
#2 Matsuzuka	+	+	+	+	+	+	+	+	+	?	+	+	+	+	13
#3 Kaczka	+	+	+	+	+	+	+	+	?	?	+	+	+	+	12
#4 Gonzalez	+	+	+	+	+	+	+	+	?	?	+	+	+	+	12
#5 Suzuki	+	+	+	+	+	+	+	+	+	?	+	+	+	+	13
#6 del Carmen	+	+	+	+	+	+	+	+	+	?	?	+	+	+	12
#7 Croner	+	+	+	+	+	+	+	+	+	?	?	+	+	+	12
#8 Yamamoto	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
#9 Yaguchi	+	+	+	+	+	+	+	+	?	?	?	+	+	+	11
#10 Güller	+	+	+	+	+	+	+	+	+	?	+	+	+	+	13
#11 Kumagai	+	+	+	+	+	+	+	+	+	+	+	+	+	+	14
#12 Vogelaar	+	+	+	+	+	+	+	+	+	?	?	+	+	+	12
#13 Yamamoto	+	+	+	+	+	+	+	+	+	?	?	+	+	+	12
#14 Yeung	+	+	+	+	+	+	+	+	+	?	?	+	+	+	12
#15 Colling	+	+	+	+	+	+	+	+	?	?	?	+	+	+	11

(+)=no bias; (-)=potential bias; (?)=bias unclear

1, representative spectrum?; 2, selection criteria clearly described?; 3, acceptable reference standard?; 4, time interval between OSNA and pathology?; 5, partial verification avoided?; 6, differential verification avoided?; 7, incorporation avoided?; 8, description execution of OSNA?; 9, description execution of pathology?; 10, pathology results blinded?; 11, OSNA results blinded ?; 12, clinical data available as in practice?; 13, uninterpretable results reported?; 14, withdrawals explained?

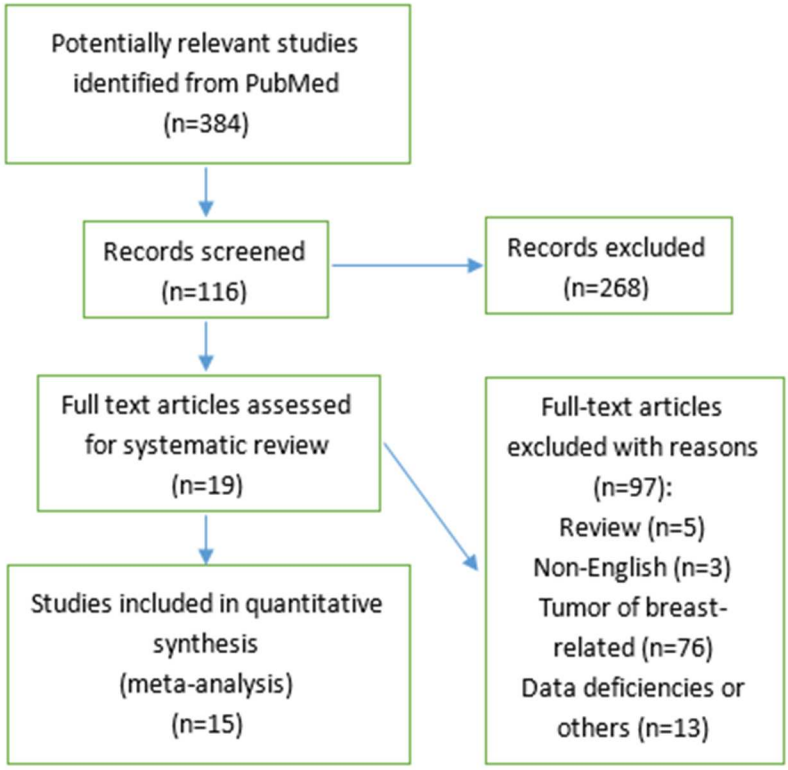
Table 3 Comparison of the diagnostic accuracy of OSNA assay in included tumor types

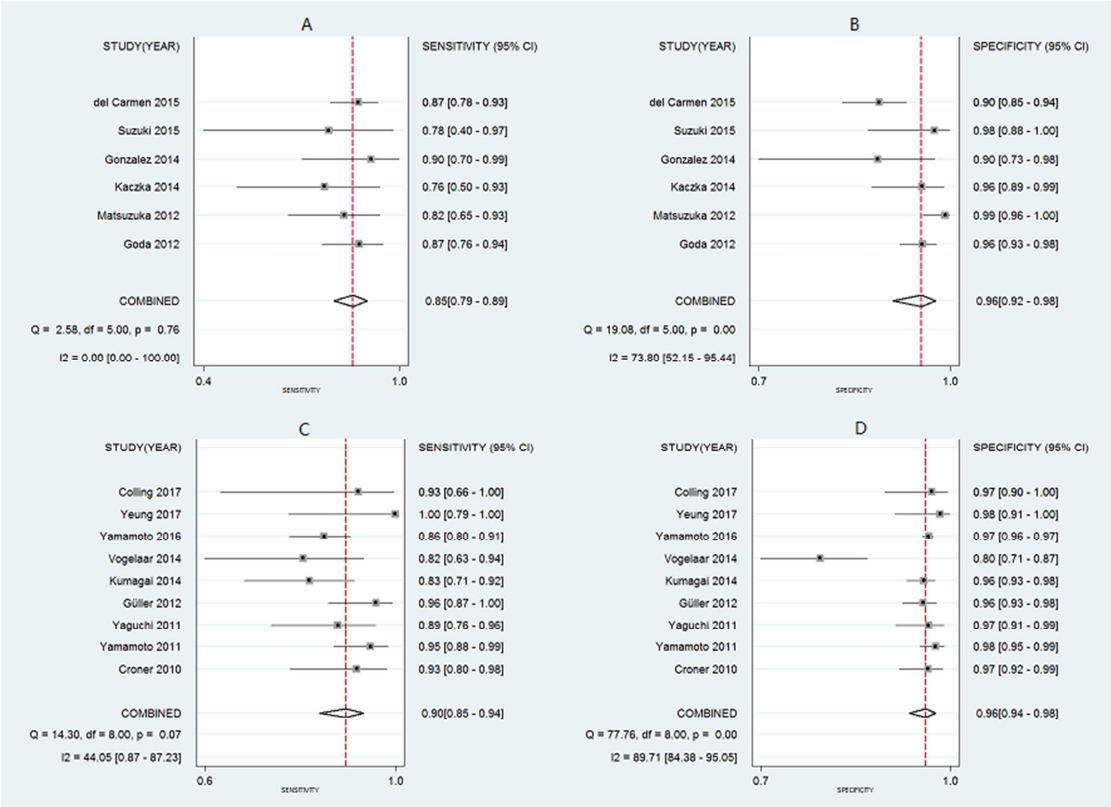
		Sensitivity	Specificity	PLR	NLR	DOR	AUC
Head and neck cancers		0.85(0.79-0.89)	0.96(0.92-0.98)	20.6(10.3-41.0)	0.16(0.11-0.22)	130.5(62.3-273.3)	0.91(0.88-0.93)
Gastrointestinal cancers		0.90(0.85-0.94)	0.96(0.94-0.98)	23.4(13.7-40.0)	0.10(0.06-0.16)	235.2(99.9-554.1)	0.97(0.96-0.99)
Lung cancer	Hayama	1.0	0.92	12.5	-	-	-
	Nakagawa	0.80	0.95	16	0.21	72.5	-
Gynecological malignancies	Okamoto	0.50	0.98	25	0.51	61	-
	Nagai	0.82	0.99	82	0.18	555.3	-

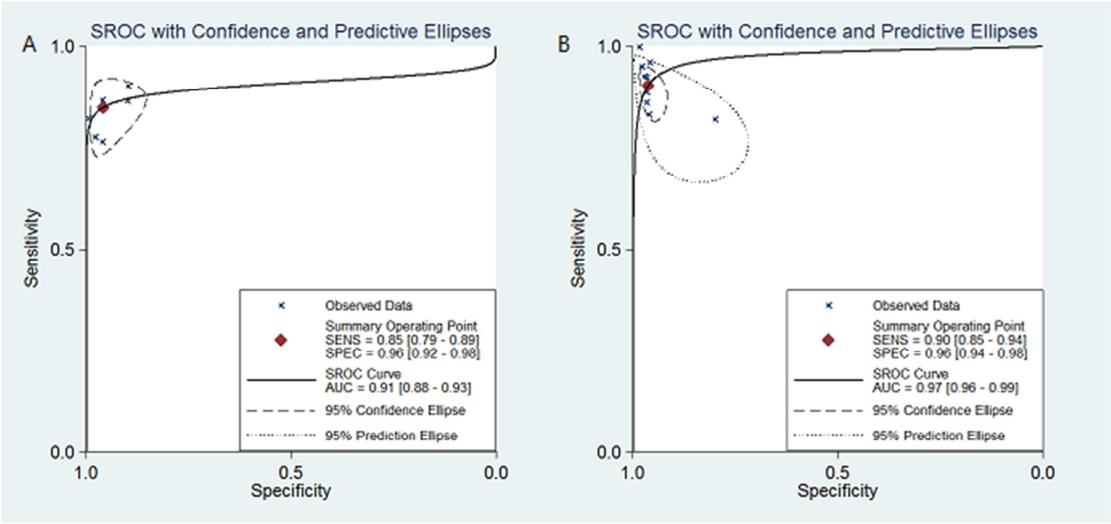
Table 4 Results of meta-regression analysis

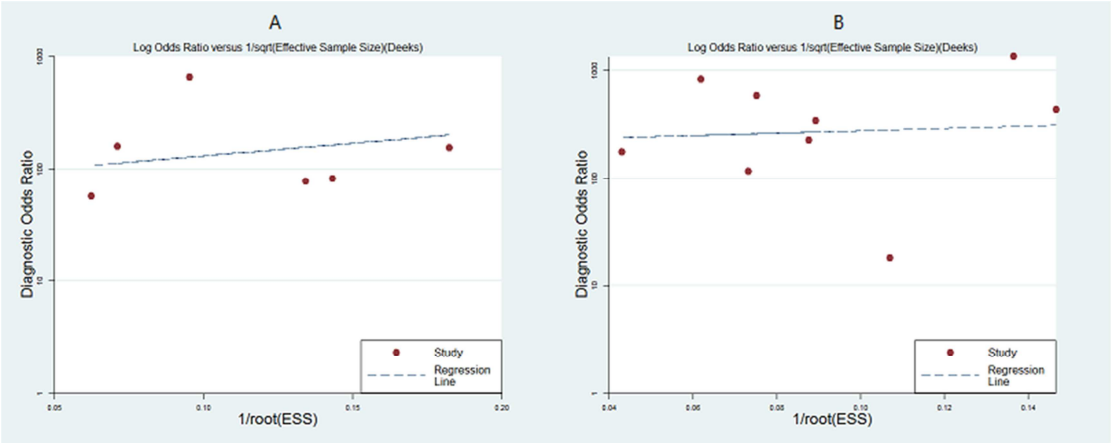
	Group	Coefficient	Standard error	*P* value	Diagnostic odd ratio	95%CI
Tumor type [a]	A	-0.762	0.6991	0.3553	0.47	0.05-4.32
	B	-0.874	1.3077	0.5286	0.42	0.02-10.23
Nation [b]	A	-0.762	0.6991	0.3553	0.47	0.05-4.32
	B	-0.110	1.5792	0.9465	0.90	0.02-42.68
Number of lymph node [c]	A	-0.098	0.6784	0.8939	0.91	0.10-7.85
	B	1.339	1.4158	0.3809	3.81	0.12-121.88
Percentage of discordant results [d]	A	-0.443	0.7016	0.5730	0.64	0.07-5.99
	B	-4.130	0.8295	0.0025	0.02	0.00-0.12

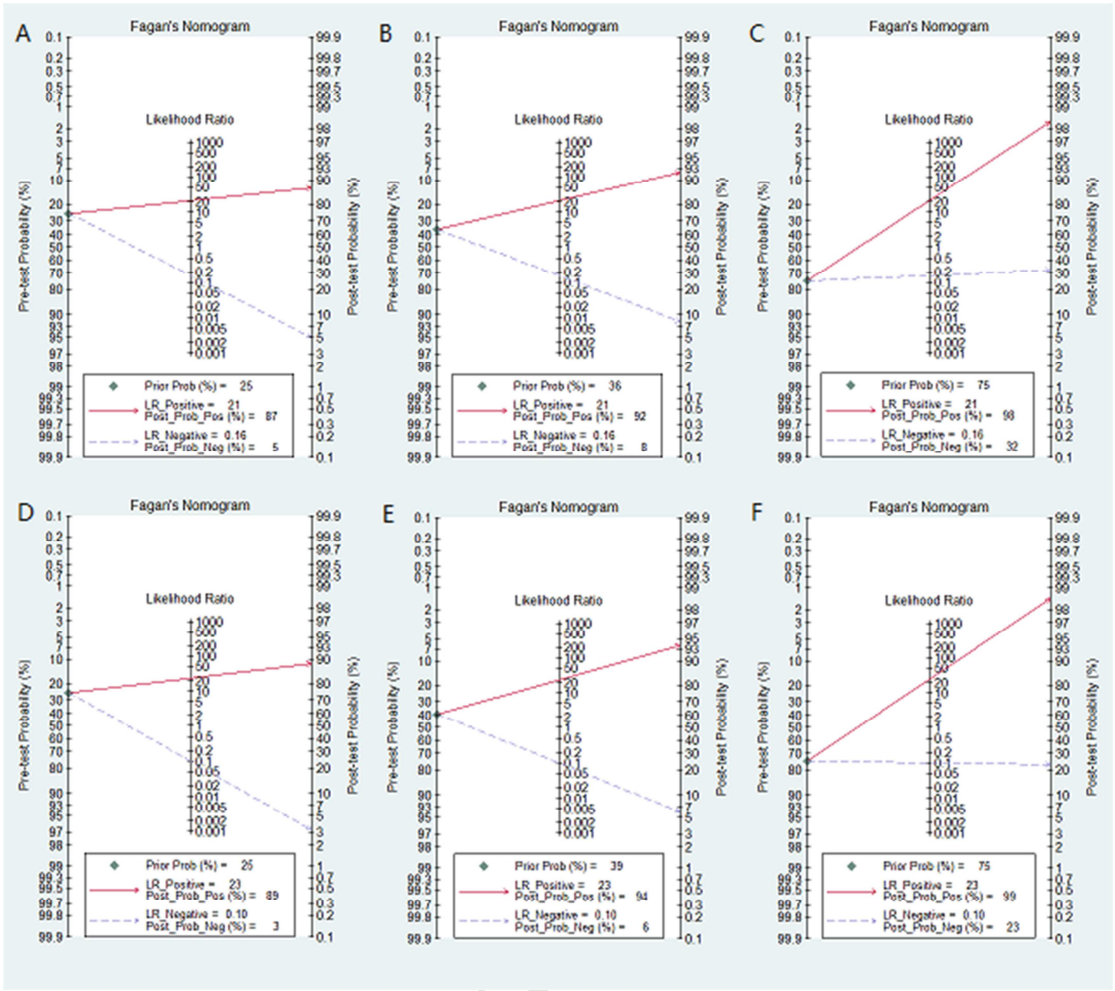
A, head and neck cancers; B, gastrointestinal cancers; CI, confidence interval; [a], thyroid cancer or HNSCC in Group A, colon cancer or gastric cancer in Group B; [b], Japan or Europe; [c], number of lymph nodes <100 or >100; [d], the percentage of discordant lymph node results (≤10% or >10%).










A
Fagan's Nomogram
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 25
LR_Positive = 21
Post_Prob_Pos (%) = 87
LR_Negative = 0.16
Post_Prob_Neg (%) = 5
B
Fagan's Nomogram
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 36
LR_Positive = 21
Post_Prob_Pos (%) = 92
LR_Negative = 0.16
Post_Prob_Neg (%) = 8
C
Fagan's Nomogram
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 75
LR_Positive = 21
Post_Prob_Pos (%) = 98
LR_Negative = 0.16
Post_Prob_Neg (%) = 32
D
Fagan's Nomogram
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 25
LR_Positive = 23
Post_Prob_Pos (%) = 89
LR_Negative = 0.10
Post_Prob_Neg (%) = 3
E
Fagan's Nomogram
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 39
LR_Positive = 23
Post_Prob_Pos (%) = 94
LR_Negative = 0.10
Post_Prob_Neg (%) = 6
F
Fagan's Nomogram
Likelihood Ratio
Pre-test Probability (%)
Post-test Probability (%)
Prior Prob (%) = 75
LR_Positive = 23
Post_Prob_Pos (%) = 99
LR_Negative = 0.10
Post_Prob_Neg (%) = 23

1. The OSNA assay is useful to distinguish metastatic lymph nodes from non-metastatic ones in several tumor types.
2. 250 copies/μl appeared to be an optimal cutoff value that distinguished between positive and negative lymph nodes.
3. CK-19 was selected as a useful mRNA marker for the OSNA assay.