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One-step nucleic acid amplification (OSNA) for the detection of sentinel lymph node metastasis in endometrial cancer☆

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HIGHLIGHTS

- We examined the utility of the OSNA method in the detection of metastases in SLNs in EC.
- The OSNA assay shows high sensitivity and specificity in detecting SLN metastases in EC.
- The OSNA assay is a novel tool for the molecular detection of SLN metastasis in patients with EC.
- These results should be validated in a larger series of patients.

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ABSTRACT

Objective. To evaluate the efficacy of one-step nucleic acid amplification (OSNA) for the diagnosis of sentinel lymph node (SLN) metastasis compared with histopathological examination in patients with endometrial carcinoma.

Methods. A total of 94 SLNs from 34 patients with endometrial carcinoma were enrolled. The central 1-mm portion of each node was subjected to semi-serial sectioning, sliced at 200-μm intervals and examined by hematoxylin and eosin and cytokeratin 19 (CK19) immunohistochemical staining, and the remaining tissue was analysed by OSNA using CK19 mRNA. The accuracy of the OSNA assay was evaluated based on histopathological diagnosis.

Results. Histologically, 89 SLNs were determined to be metastasis negative, and the remaining five SLNs were metastasis positive. Using the breast cancer cutoff value for detecting lymph node metastasis (OSNA criteria for breast cancer, >250 copies/μl) the sensitivity of the OSNA assay was 100%; specificity was 87.6%; diagnostic accuracy was 88.3%. Discordant results were recorded for 11 of 94 SLNs. In all 11 cases, a positive result was given by the OSNA assay but not by histopathological examination. In two SLNs from the same patient, histopathological examination revealed the presence of benign epithelial inclusions that were CK19 positive; both SLNs yielded a positive result in the OSNA assay (true–false positive). All remaining nine histologically-negative/OSNA-positive SLNs were classified as micrometastasis (+) by the OSNA assay.

Conclusion. The OSNA assay shows high sensitivity and specificity, which suggests its utility as a novel tool for the molecular detection of SLN metastasis in patients with endometrial carcinoma.

1. Introduction

The prognostic value of lymphadenectomy for patients with early-stage endometrial carcinoma (EC) is a subject of debate. Recently, two trials and a meta-analysis have reported that pelvic lymphadenectomy has no impact on overall or disease-free survival in patients with early-stage EC but did increase comorbidity [1]. Mapping lymphatic spread via sentinel lymph node (SLN) sampling has been well established in many solid tumours and this approach has become a

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standard of care in patient with breast carcinoma and cutaneous melanoma. In EC, SLN mapping and ultrastaging of SLNs has been proposed as a method to potentially reduce surgical staging morbidity while maintaining prognostic information of lymph node status [2]. Two main reasons support the use of SLN biopsy in patients with early stage endometrial cancer. First, SLN reduces morbidity compared to pelvic lymph node dissection. Second, ultrastaging of SLNs in addition to conventional histology increases the likelihood of identifying micrometastases. With serial sectioning and immunohistochemistry (IHC), SLN micrometastases have been detected in up to 25% of endometrial cancer patients [3]. However, serial sectioning combined with IHC is time consuming and expensive, even limited to SLNs [4,5].

To overcome these problems, several authors have investigated molecular-based metastasis detection systems using molecular techniques. The one-step nucleic acid amplification (OSNA) assay is a molecular-based metastasis detection system that adopts reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay, using CK19 as a single marker [6]. CK19 is a low-molecular-weight member of the intermediate family of proteins that are commonly expressed in normal epithelial tissues. In recent years, alterations in CK19 expression have been demonstrated in several human cancers, and CK19 is considered a promising marker with high sensitivity for the detection of lymph node metastases from various cancers, including endometrial carcinoma [7]. A recent study has demonstrated that an OSNA assay using CK19 mRNA was applicable for detecting LN metastases in EC [8].

In the present study, we evaluate the usefulness of the OSNA method measuring CK19 mRNA copy numbers in the detection of SLN metastases in EC in comparison with serial sectioning of SLNs followed by IHC.

2. Materials and methods

2.1. Patient selection

This study involved 34 patients with endometrial carcinoma. All patients underwent surgery between March 2014 and December 2015 at the University Hospital La Paz, Madrid, Spain. Data on age, histological type of tumour, histological grade, presence or absence of lymphovascular invasion, and result of the pelvic or para-aortic lymphadenectomy (when performed) were collected for all patients. Histological type was considered according to the 2014 World Health Organization classification of tumours of the endometrium [9].

2.2. Study design

The objective of the current investigation was to compare the performance of one-step nucleic acid amplification (OSNA) with the performance of intensive histopathological analysis (multilevel sectioning and immunohistochemistry, IHC) in the detection of endometrial cancer SLN metastases. An intensive histological workup (H&E plus IHC), as described below, was considered the gold standard for assessing the sensitivity and specificity of OSNA.

The study was approved by the Ethics Committee of the University Hospital La Paz, Madrid, Spain (code HULP: PI-1460).

2.3. Sentinel lymph node procedure

In most patients (n = 27; 79.4%) a combination of radioisotope tracer and methylene blue/green indocyanine was used to identify the SLNs. On the day before surgery, a total dose of 4 ml containing 148 MBq (4 mCI) 99mTc-albumin nanocolloid was injected intracervically at 3 and 9 o'clock positions, both submucosally and deep into the cervical stroma with a 20-gauge needle. A combined lymphoscintigraphy and SPECT-TC study, including the pelvic and para-aortic region, was performed to locate the SLN drainage. On the day of the surgery, methylene blue or indocyanine green was injected intracervically in the same way as the radiotracer, after induction of anesthesia before beginning the surgery. In four patients, SLN mapping was performed alone after indocyanine green injection, and in three patients SLN detection was performed after 99mTc-sulfur colloid injection alone. The SLNs were identified intraoperatively guided both by a γ-probe and visual inspection of the blue or green dye. The number of SLNs, as well as their anatomical locations was noted.

2.4. Sentinel lymph node workup

Sentinel lymph nodes were dissected away from the surrounding fat, measured and weighed. In nodes weighing >50 mg (the cutoff for validity using the OSNA method) and measuring >3 mm along the largest dimension, a central longitudinal 1-mm slice was immediately taken from each node using a fresh scalpel. The remaining tissue was shock-frozen in liquid nitrogen and stored at −80 °C until the OSNA assay was performed. The central tissue slice was then fixed in formalin and embedded in paraffin for histopathological analysis. Initially, a 4-μm paraffin section was obtained from each central slice and investigated by standard histology (one section of hematoxylin–eosin staining) and diagnosed as metastasis or non-metastasis. All metastasis-negative lymph node sections were then sliced at 200-μm intervals for a total of four slides per block. At each level, two sections of 4-μm were taken: one section was examined with H&E and the other section was processed using the immunohistochemical method for cytokeratin 19 (mouse monoclonal, clone RCK 108, prediluted, Dako, Glostrup, Denmark) (Fig. 1). CK19 was chosen as the target marker because is both highly specific and sensitive for the detection of endometrial cancer lymph node metastases [7]. The basis for lymph node assessment was the AJCC criteria known from breast cancer [10]. The size of node metastases was estimated with an eyepiece micrometer. Macrometastases measured >2 mm. Micrometastases were defined as a focus of metastatic tumour cells measuring >0.2 mm and 2 mm or less. Isolated tumour cells (ITC) were defined as microscopic clusters and single cells measuring 0.2 mm or less.

2.5. OSNA assay

OSNA was performed as described previously [6]. Briefly, the lymph node tissue was homogenized in 4 ml of lysis buffer (Lynorhag, Sysmex, Kobe, Japan) for 90 s and centrifuged for 1 min at 10,000 ×g at room temperature. CK19 mRNA was then amplified by reverse-transcription loop-mediated amplification with a ready-to-use reagent kit (Lynoamp, Sysmex, Kobe, Japan) in an RD-100i apparatus (Sysmex, Kobe, Japan) according to the manufacturer's instructions. Amplification products were detected by an increase in magnesium pyrophosphate concentration, a by-product of the amplification reaction. A standard positive control sample containing 5000 copies/μl of CK19 mRNA and a negative control sample containing 0 copy/μl were used for calibration in each study. The results of the assay were expressed as the level of CK19 mRNA. Previous studies classified OSNA levels in breast cancer as follows: a CK19 mRNA level <250 copies/μl was designated as a negative (−) result; a level between 250 and 4999 copies/μl was labelled as micrometastasis (+); and a level ≥5000 copies/μl was considered as macrometastasis (++). When the copy number of CK19 mRNA was over 250 copies/μl lymph node, metastasis was deemed to be positive. This cutoff of 250 copies has been previously validated as an optimal cutoff value for diagnosing lymph node metastasis in endometrial carcinoma [8].

2.6. Statistical analysis

The population characteristics were described by the usual statistics: median with range for continuous variables and numbers with percentages for discrete variables. The diagnostic value of the OSNA assay was evaluated by calculating sensitivity, specificity, and diagnostic accuracy using the OSNA assay as the diagnostic test value, and the histological