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BRIEF REPORT

Two rare cases of endosalpingiosis in the axillary sentinel lymph nodes: evaluation of immunohistochemical staining and one-step nucleic acid amplification (OSNA) assay in patients with breast cancer

Sho Shiino[1,2] · Masayuki Yoshida[3] · Kenjiro Jimbo[1] · Sota Asaga[1] · Shin Takayama[1] · Akiko Maeshima[3] · Hitoshi Tsuda[4] · Takayuki Kinoshita[1] · Nobuyoshi Hiraoka[3]

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Abstract

Benign inclusions, such as endosalpingiosis, in an axillary sentinel lymph node (SLN) can be misdiagnosed as metastatic breast carcinoma. However, endosalpingiosis is rare in lymph nodes above the diaphragm. Among 792 patients with breast carcinoma who underwent sentinel lymph node biopsy at our center, 2 patients have experienced benign glandular inclusions in 3 SLNs, and all of these glandular inclusions were lined with columnar and ciliated epithelial cells. Immunohistochemistry revealed that the epithelial cells were positive for Müllerian markers (e.g., PAX8 and WT-1) and negative for mammary markers (e.g., mammaglobin, GCDFP-15, and GATA3), which confirm the diagnosis of endosalpingiosis. The epithelial cells were positive for CK19 but the one-step nucleic acid amplification assay revealed negative results for the axillary SLNs. Although endosalpingiosis is rare in axillary SLNs, care is needed to identify these rare cases and avoid unnecessary axillary lymph node dissection, overstaging, and overtreatment.

Keywords Endosalpingiosis · Benign glandular inclusion · Axillary sentinel lymph node · Immunohistochemistry · OSNA assay

Introduction

Sentinel lymph node (SLN) biopsy is a common procedure to determine if breast cancer has spread to the axillary lymph nodes (ALNs) and to decide if axillary lymph node dissection (ALND) should be performed intraoperatively [1]. Although a false-positive SLN diagnosis in breast cancer is uncommon, this result can lead to unnecessary ALND and drug therapy. Furthermore, benign inclusions can be found in the ALNs and result in a diagnosis of metastatic carcinoma, which can lead to unnecessary ALND [2]. Endosalpingiosis is a non-neoplastic Müllerian lesion that is common in the pelvic and intraabdominal lymph nodes, although it is rare above the diaphragm. However, it is difficult for even skilled pathologists to differentiate between endosalpingiosis and metastatic carcinoma using intraoperatively frozen sections. Nevertheless, some authors have recently reported endosalpingiosis in ALNs, and that immunohistochemical staining can be effectively used to identify Müllerian differentiation [2–4].

Molecular diagnoses have recently been implemented for intraoperative SLN examinations, and the one-step nucleic acid amplification (OSNA) assay is an automated rapid test that is used to detect cytokeratin 19 (CK19) mRNA expression [5]. The OSNA assay is more sensitive for detecting CK19-positive carcinoma cells, compared with conventional histology [5]. However, benign epithelial cells could be diagnosed as positive and lead to unnecessary ALND, as these cells can also be positive for CK19. We report our experience with two cases of benign glandular inclusions in the axillary SLNs, and we discuss the diagnostic utility of immunohistochemistry and the potential pitfalls of the OSNA assay in cases of endosalpingiosis.

✉ Masayuki Yoshida
masayosh@ncc.go.jp

[1] Department of Breast Surgery, National Cancer Center Hospital, Tokyo 1040045, Japan

[2] Graduate School of Medicine, Keio University School of Medicine, Tokyo 1608582, Japan

[3] Department of Pathology and Clinical Laboratories, National Cancer Center Hospital, Tsukiji 5-1-1, Chuo-ku, Tokyo 104-0045, Japan

[4] Department of Basic Pathology, National Defense Medical College, Tokorozawa, Saitama 3598513, Japan

Table 1 The results from histology and the OSNA assay for the two patients

Case	1	2	
Lymph nodes	No. 1	No. 1	No. 2
Frozen section diagnosis	Negative	Glandular inclusion	Glandular inclusion
OSNA assay result	Negative	Negative	Negative
Paraffin section diagnosis	Endosalpingiosis	Endosalpingiosis	Negative

Methods

We retrospectively examined the database of our Department of Pathology (National Cancer Center Hospital, Tokyo) and identified 2 patients with breast cancer and benign glandular inclusions in axillary SLNs who were treated in 2011 and 2012. At our center, the SLNs had been intraoperatively evaluated using frozen histological sections and the OSNA assay. Excised SLNs were cut into 2-mm slices along the short axis and were alternately used for the OSNA assay and histological examination. We also confirmed the diagnosis using permanent paraffin sections of the SLNs. Immunohistochemistry was used to test the glandular inclusions using the following markers: PAX8, WT-1, mammaglobin, GCDFP-15, CK19, and GATA3. Incident rate with the 95% confidence interval (CI) of the proportions was calculated using the Clopper-Pearson exact method by SPSS Statistics Version 24 (IBM SPSS, Armonk, NY, USA).

Cases

Case 1

In 2011, a 48-year-old woman underwent partial resection and SLN biopsy for invasive ductal carcinoma of her right breast. Microscopic examination revealed an invasive ductal carcinoma with a diameter of 1.3 cm but without lymphovascular invasion. The tumor was histological grade 3, > 90% positive for estrogen receptor (ER) and progesterone receptor (PgR), had a human epidermal growth factor receptor 2 (HER2) score of 3+, and had a Ki-67 labeling index of 35.4%. Three SLNs (nos. 1–3) were detected intraoperatively and diagnosed as negative using the frozen sections and the OSNA assay; thus, ALND was omitted. However, examination of the permanent paraffin section revealed glandular lesions lined with ciliated columnar epithelial cells in the third SLN (Fig. 1a, 0.3 × 0.2 mm). These cells did not have atypical cellular and structural characteristics and exhibited characteristic features of endosalpingiosis (Fig. 1b). The cells were strongly positive for WT-1 and PAX8 (Müllerian markers) (Fig. 1c, d) and were negative for mammaglobin, GCDFP-15, and GATA3 (sensitive breast epithelial markers) (Fig. 1e–g). In addition, the glandular cells were positive for CK19 (Fig. 1h). Therefore, the patient was diagnosed with endosalpingiosis. This patient received adjuvant chemotherapy using doxorubicin and cyclophosphamide, as well as hormonal therapy using tamoxifen, and had no evidence of recurrence for approximately 5 years.

Case 2

In 2012, a 52-year-old woman underwent partial resection and SLN biopsy for non-invasive ductal carcinoma of her right breast. Microscopic examination revealed a non-invasive ductal carcinoma with a diameter of 2.5 cm but without lymphovascular invasion. The tumor had an intermediate nuclear grade, > 90% positivity for ER, 10% positivity for PgR, and a HER2 score of 1+. Four SLNs (nos. 1–4) were detected intraoperatively, and glandular lesions were identified in the first and second SLNs using the frozen sections (Fig. 2a, 0.1 × 0.1 mm; Fig. 2b, 0.2 × 0.1 mm). The glandular lesions did not have atypical features and were considered benign inclusions. In addition, as the OSNA assay results were negative, ALND was omitted. We could also identify a glandular lesion in the paraffin section of the first SLN (Fig. 2c, 0.1 × 0.1 mm). Immunohistochemistry revealed that the glandular lesion was strongly positive for WT-1 and PAX8 (Fig. 2d, e), as well as CK19 (Fig. 2h). However, the lesion was negative for mammaglobin and GATA3 (Fig. 2f, g). Therefore, the patient was diagnosed with endosalpingiosis, received postoperative radiation therapy without drug therapy to preserve her breast, and had no evidence of recurrence for approximately 3 years. The results from histology and the OSNA assay for the two patients are summarized in Table 1.

Discussion

A histologically benign cystic epithelial lesion within an axillary SLN may be confused with metastatic carcinoma, especially when the diagnosis is based on a frozen section. Thus, although epithelial inclusions within an ALN are rare, this confusion could result in a false-positive diagnosis during the intraoperative evaluation [6]. Fellegara et al. reported that recognizing the cytoarchitectural features of the inclusions can improve the differential diagnosis and reduce the likelihood of overtreatment [7]. In case 2, the ALND was omitted intraoperatively because the glandular lesions within the two SLNs were small and had no atypia. Thus, if a patient has benign glandular lesions without cytological atypia in an axillary SLN, it may be appropriate to

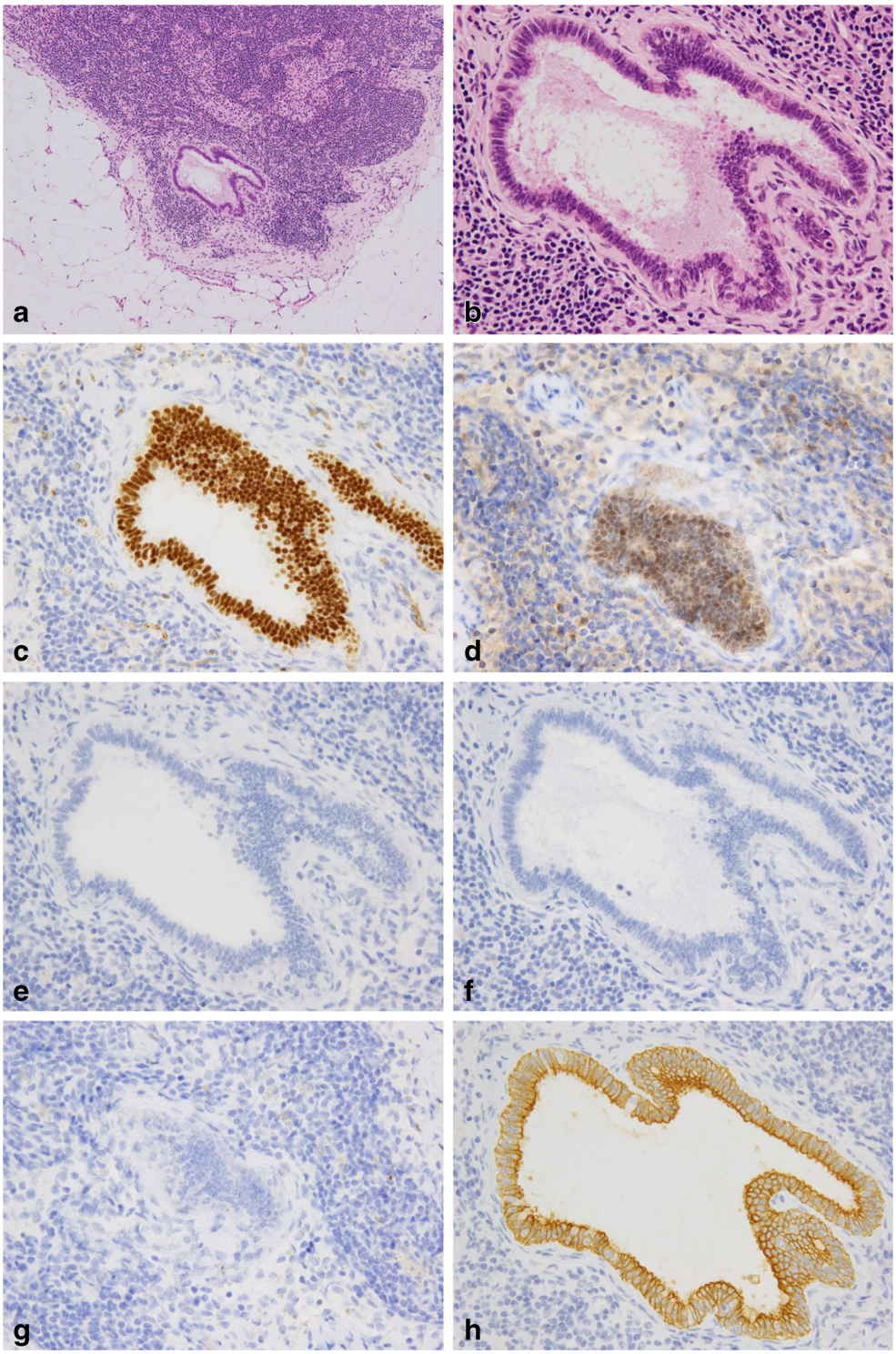

Fig. 1 Endosalpingiosis within the axillary sentinel lymph node in case 1. **a** A glandular lesion was identified within the lymph node (hematoxylin and eosin staining). **b** The glandular lesion was lined with a single layer of columnar cells (hematoxylin and eosin staining). The epithelial cells were positive for WT-1 (**c**), PAX8 (**d**), and CK19 (**h**), and negative for mammaglobin (**e**), GCDFP-15 (**f**), and GATA3 (**g**)

defer the ALND until a pathological diagnosis can be confirmed using immunohistochemical analysis of the permanent section.

Benign inclusions in ALNs consist of both epithelial inclusions and non-epithelial inclusions [8], and the differential diagnosis is summarized in Table 2. Epithelial inclusions include mammary-type glandular inclusions, Mullerian-type glandular inclusions, and squamous inclusions. Meanwhile, non-epithelial inclusions include nodal nevi. Endosalpingiosis in the lymph nodes below the diaphragm is relatively common and has been detected in up to 41% of excised pelvic and para-aortic lymph node specimens from women [9]. In contrast, endosalpingiosis above the diaphragm is less common [10] and the rate of this inclusion within axillary SLNs is unclear. Based on 792 patients with breast cancer who underwent SLN biopsy between 2011

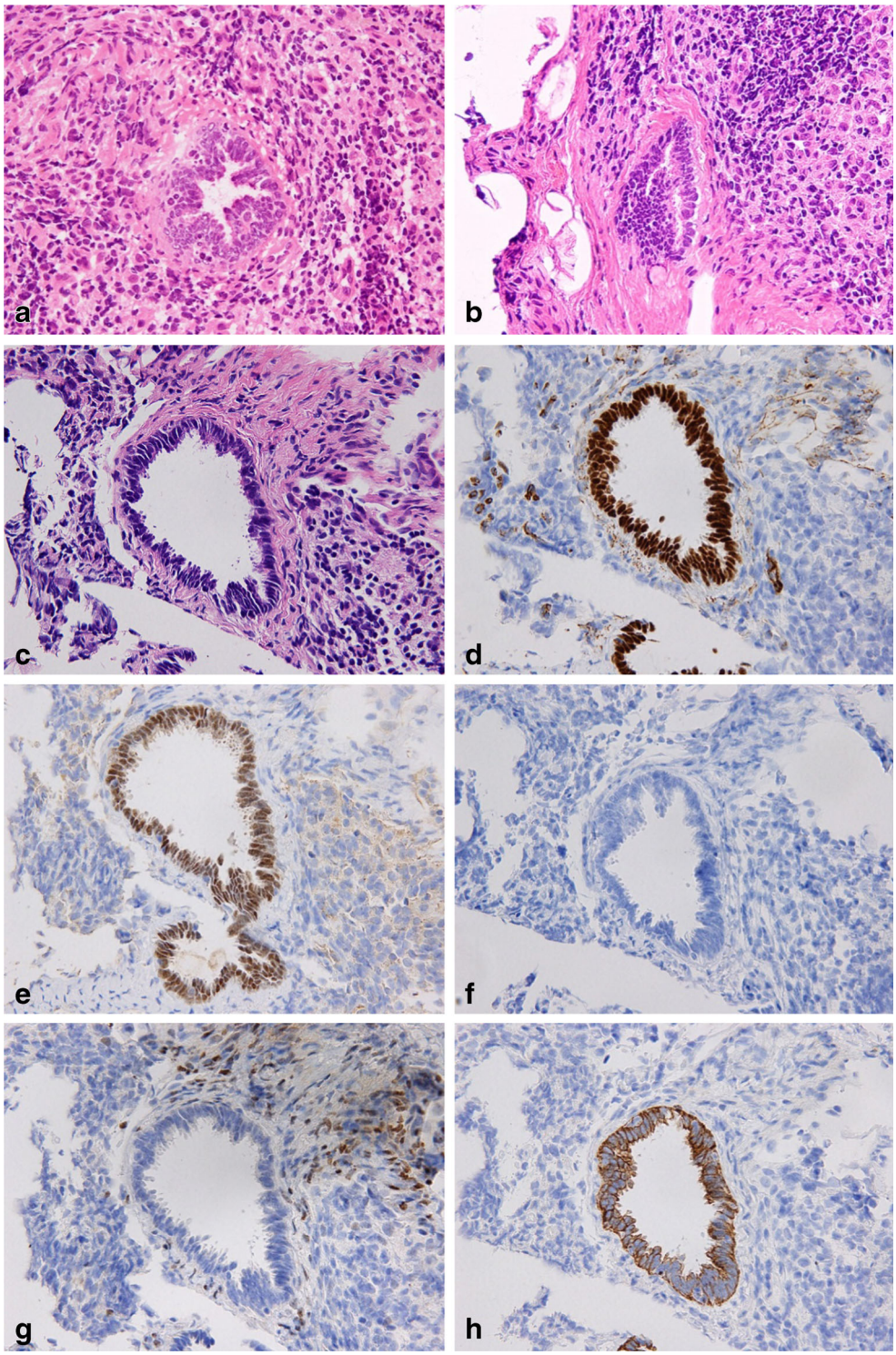

Fig. 2 Endosalpingiosis within the sentinel axillary lymph node in case 2. Glandular lesions were detected in the first (**a**) and second (**b**) sentinel lymph nodes (hematoxylin and eosin staining, frozen section). **c** The glandular lesion in the first sentinel lymph node was lined with a single layer of columnar cells (hematoxylin and eosin staining, permanent section). The epithelial cells were positive for WT-1 (**d**), PAX8 (**e**), and CK19 (**h**), and negative for mammaglobin (**f**) and GATA3 (**g**)

and 2012 at our center, the incidences of endosalpingiosis in axillary SLNs were 0.3% (95% CI, 0–0.9%; 2/792 patients) and 0.1% (95% CI, 0–0.4%; 3/2203 lymph nodes).

Previous reports have described the misdiagnosis of endosalpingiosis in ALNs as metastatic carcinoma and those patients underwent unnecessary ALND [2]. The morphological characteristics of endosalpingiosis are cells with terminal bars and cilia admixed with intercalated "peg" cells that have an ovoid shape and clear cytoplasm [11]. It is difficult to distinguish between endosalpingiosis and metastatic breast carcinoma if these features are not present, and some authors have highlighted the utility of immunohistochemical staining for PAX8 and WT-1 in this context. Corben et al. have reported three cases of endosalpingiosis within axillary SLNs [2], and

Table 2 Differential diagnosis of axillary node inclusions

Histological diagnosis	Histological findings	Immunohistochemical findings	Differential diagnosis
Müllerian-type glandular inclusions	Endosalpingiosis: bland glands with ciliated cells admixed with intercalated (peg) cells (*) Endometriosis: bland glands associated with endometrial stromal cells (*)	ER+, Pax8+, WT1(+, endosalpingiosis), GATA3–, GCDFP15–, mammaglobin–, S100–	Metastatic breast (atypical tumor cells, always Pax8– and GATA3+, without myoepithelial layer) or Müllerian-type gynecological carcinoma (atypical tumor cells, always Pax8+, and GATA3–)
Mammary-type glandular inclusions	Bland mammary glands with associated myoepithelial layer	Epithelial cells: GATA3+, ER+, GCDFP15+, mammaglobin+, S100 +/−, Pax8–, WT1–myoepithelial cells: p63+, CD10+, αSMA+	Metastatic breast carcinoma (atypical tumor cells without myoepithelial layer)
Squamous inclusions	Bland squamous nest or squamous -lined cysts	p40+, p63+, CK5/6+, GATA3±, ER–, GCDFP15–, mammaglobin–, Pax8–, WT1–, S100–	Metastatic squamous cell carcinoma or metastatic metaplastic breast carcinoma (atypical tumor cells)
Nodal nevi	Bland spindled nevocytes located within the lymph node capsule	SOX10+, S100+, Melan A+, MITF+, cytokeratin–, ER–, GATA3–, GCDFP15–, mammaglobin–, Pax8–, WT1–	Metastatic melanoma (atypical tumor cells with melanocytic marker expression) or metastatic spindle cell carcinoma (atypical tumor cells with cytokeratin expression)

*Without myoepithelial layer

they diagnosed the glandular inclusions as endosalpingiosis based on their morphological characteristics and immunohistochemical expression of PAX 8 and WT-1. Carney et al. have also recognized the importance of testing for PAX8 and WT-1 expression if the morphological features of endosalpingiosis were not present [3]. In the present cases, the glandular inclusions were also positive for these markers, which confirmed the diagnosis of endosalpingiosis. In addition, testing for the expression of mammaglobin, GCDFP-15 [12], and/or GATA3 [13] may help support a diagnosis of metastatic breast carcinoma. However, these markers were not detected in our cases, which exclude the possibility of a breast origin. Additionally, these patients were excluded from endometriosis because no endometrial glands and stroma were present in the glandular inclusions. These patients had no history of endometriosis even though they were premenopausal patients.

The OSNA assay uses automated rapid nucleic acid amplification to detect CK19 mRNA expression [5]. In our hospital, the SLNs are intraoperatively evaluated using both hematoxylin and eosin-stained frozen sections and the OSNA assay, which can provide more accurate diagnoses, compared to only the OSNA assay or histological examination [14]. In the present cases, we confirmed that the epithelial cells of endosalpingiosis were positive for CK19. If the glandular lesions were located in the pieces of the lymph nodes for the OSNA assay, the results might have been positive and we might have unnecessarily performed ALND. However, the inclusions were relatively small in both cases (< 0.3 mm) that would almost be comparable to isolated tumor cells (iTC), and the OSNA assay is designed not to detect iTC. Thus, OSNA assay result would be less likely to be positive in our cases. However, endosalpingiosis of the axillary SLNs can rarely present as an extensive and multiple florid papillary form, which may mimic macrometastasis [15]. In those cases, the ALNs were palpable masses, which suggested macrometastasis. Therefore, the SLNs should generally not be assessed only using the OSNA assay, in order to identify other nodal disorders, including endosalpingiosis.

In conclusion, we report our experience with two cases of endosalpingiosis in axillary SLNs, which we confirmed using immunohistochemistry. Although endosalpingiosis is rare in axillary SLNs, care is needed to identify these rare cases and to avoid unnecessary ALND, overstaging, and overtreatment.

Author contributions SS and MY designed the study researched and analyzed the data; and wrote, edited, and reviewed the manuscript. HT analyzed the data and reviewed the manuscript. KJ, SA, ST, and TK provided clinical details and reviewed the manuscript. AM and NH provided pathological information and reviewed the manuscript. All authors gave final approval for publication.

Compliance with ethical standards

This study was approved by the Ethics Committee of the National Cancer Center Hospital, Tokyo, Japan.

Conflict of interest The authors declare that they have no conflict of interest.

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