Title:

Thyroid metastasis of pulmonary adenocarcinoma with *EGFR* G719A mutation: Genetic confirmation with liquid-based cytology specimens

Running head:

Thyroid metastasis of lung cancer

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Conflict of Interest:

None declared

Disclosure of grants:

None declared

Abbreviations:

None declared

Keywords:

1) Adenocarcinoma of lung, 2) Thyroid metastasis, 3) *EGFR* mutation, 4) Fine needle aspiration, 5) Liquid-based cytology

Introduction

Lung cancer is one of the most common cancers worldwide, and frequently metastasizes to other organs such as the lymph nodes, brain, and bone; metastasis of lung cancer to the thyroid, however, is rare [1]. Primary thyroid cancer, in contrast, is less common, yet the lung is one of its most common sites of distant metastasis [2]. In any patient with both lung and thyroid tumors, therefore, we must obtain conclusive, ideally genetic, evidence, if we are to reach a conclusive diagnosis, especially when the lung cancer is adenocarcinoma, whose pathological features partly overlap with those of papillary thyroid carcinoma (PTC), the most common form of thyroid cancer. Here, we report an elderly woman with pulmonary adenocarcinoma with thyroid metastasis, which we confirmed by genetic analysis using liquid-based cytology (LBC) material from both lung and thyroid tumors.

Case report

A 78-year-old woman was admitted to our hospital for evaluation of a thyroid tumor in 2019 (Figure 1A). She had developed a 2.0 cm lung tumor five years previously in 2014 and had undergone fine-needle aspiration (FNA) for biopsy and LBC (ThinPrep, Hologic, Marlboro, MA, USA). Biopsy results revealed malignant cells with large nuclei and distinct nucleoli forming tubular/papillary structures and invading into the surrounding

53 stroma (Figure 1B). Genetic analysis of the biopsy specimen further revealed that the
54 tumor harbored an *EGFR* (epidermal growth factor receptor) G719A mutation; thus,
55 pulmonary adenocarcinoma with *EGFR* G719A mutation was diagnosed. At that time, a
56 3.0 cm thyroid tumor was also observed and was suspected to be metastasis of the lung
57 cancer; other metastatic lesions (hilar lymph node, lung, brain, and pelvic bone) were also
58 observed. The patient underwent chemoradiotherapy for these lesions; however, it was
59 not effective. She underwent a second FNA of the lung cancer in 2016 to determine
60 whether the cancer acquired an *EGFR* T790M mutation, which is related to drug
61 resistance. The LBC specimen contained malignant cells with large nuclei and central
62 nucleoli forming papillary structures, as had the first biopsy/cytology specimens (Figure
63 1C). A genetic study of the residual samples in the LBC vial by real-time PCR (Cobas
64 EGFR Mutation test, Roche Diagnostics, Switzerland) revealed that the tumor also
65 harbored *EGFR* G719X but not T790M mutations. The patient continued chemotherapy
66 with a different regimen, but the lung and brain lesions were exacerbated, while the
67 thyroid lesion remained almost the same size. Thus the patient underwent a third FNA in
68 2019 to determine whether the thyroid tumor was primary or metastatic.

69 LBC of the FNA sample from the thyroid tumor revealed atypical cells classified
70 as Malignant and featuring a papillary structure somewhat similar to those seen in PTC,

together with intratumoral calcification in imaging. The cytological features, however, were not consistent with PTC because the cells did not exhibit the characteristic irregular contours of the nuclei with nuclear grooves and/or intranuclear pseudo-inclusions. Instead, the nuclei were slightly eccentric and had a distinct central nucleolus, which is characteristic of adenocarcinoma, including that of pulmonary origin (Figure 1D). Although cytomorphology suggested that the thyroid tumor was metastatic, these findings alone were not sufficient to yield a final diagnosis. Genetic analysis of the LBC residue for *EGFR* by real-time PCR detected the *EGFR* G719X mutation (Figure 2). Therefore, we identified this case as pulmonary adenocarcinoma with thyroid metastasis. No thyroidectomy was performed.

Discussion

Because FNA specimens contain higher-quality nucleic acids compared to formalin-fixed paraffin-embedded specimens, cytological materials collected by FNA yield excellent results and are commonly used in genetic analyses, including examinations for *EGFR* in lung cancer [3,4]. Thyroid tumors are also typically evaluated by FNA cytology; as a result, FNA samples are often the only materials available for genetic analysis, including testing for *EGFR* mutations [5]. Here, we used LBC samples obtained through FNA from a thyroid

89 tumor to detect *EGFR* G719X, which was identical to the mutation harbored by the
90 patient's lung cancer.

91 Metastasis of lung cancer to the thyroid is rare [1]. In our local pathological archives,
92 there are only three cases of thyroid metastasis of lung cancer, as compared to about 3000
93 reports of primary thyroid carcinoma (not shown). Just 28 English-language papers on
94 this topic are archived in PubMed, and the diagnosis was confirmed by genetic analysis
95 in only two of these reports [6,7]. Albany et al. achieved a final diagnosis by detecting the
96 *EGFR* L858R mutation in biopsies from the lung and thyroid tumors [6], while Bellevicine
97 et al. confirmed a diagnosis through next-generation sequencing demonstrating that both
98 the lung and thyroid tumors harbored the KRAS G12C mutation as well as through
99 cytomorphological and immunocytochemical analysis [7].

100 Unlike these previous studies, we used residual samples from the LBC vial for
101 EGFR testing after we had prepared Papanicolaou and Giemsa specimens. This method,
102 as already demonstrated by previous studies [3], enabled us to keep the stained specimens
103 for further review if necessary. Our case would underline the usefulness of it to reconcile
104 diagnostics and molecular analysis, which is routinely required nowadays.

105 When genetic studies are not available, morphological and immunocytochemical
106 analysis remain the standard strategies for determining whether an FNA sample from a

107 thyroid lesion represents lung or thyroid cancer. We must recall, however, that pulmonary
108 adenocarcinoma can vary histologically and that its cytomorphology is rarely similar to
109 that of PTC even when it exhibits its characteristic nuclear features (e.g., nuclear grooves,
110 intranuclear pseudo-inclusions, ground glass nuclei) [8,9]. Immunocytochemical analysis,
111 especially the combination of PAX-8, thyroglobulin, and TTF-1, can assist in reaching a
112 correct diagnosis even in challenging cases [7]; pulmonary adenocarcinoma is generally
113 PAX-8(-), thyroglobulin(-), and TTF-1(+), while PTC is PAX-8(+), thyroglobulin(+), and
114 TTF-1(+). In some cases, immunocytochemistry for PAX-8 or thyroglobulin alone might
115 be sufficient to achieve a correct diagnosis.

116 Molecular analysis may be therapeutically relevant, however, and enabling
117 molecular analysis is one of the advantages offered by genetic testing with residual LBC
118 samples as well as preserved routine specimens. As molecular target therapies continue
119 to advance, this advantage will be magnified and will eventually exceed its drawbacks,
120 which currently include higher cost and limited availability.

121 EGFR mutation is the most common and targetable mutation in lung cancer. The
122 most frequent patterns are exon 19 deletion and L858R, but other rare mutations such as
123 G719X are known. Lung cancer with G719X mutation is reported to be sensitive for the
124 second and third-generation tyrosine kinase inhibitors, e.g., Afatinib [10]. To reiterate, our

125 case is an example that highlights the diagnostic and therapeutic significance of genetic
126 testing.

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128 **Author Contributions**

129 Drafting the manuscript and figures; YY, HS, MF, and SM. Acquisition and analysis of
130 data; HS. Correction and approval of manuscript; All authors.

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Figure legends:

Figure 1:

CT imaging of the thyroid tumor, and histological and cytological findings of the lung and thyroid tumors

a) CT imaging showed a thyroid tumor with calcification in the left lobe (dotted circle).

b) HE specimen of lung cancer. Atypical cells with large nuclei and distinct nucleoli form an irregular tubular structure.

c) Papanicolaou-stained liquid-based cytology (LBC) specimen obtained through fine-needle aspiration (FNA) from the lung tumor. Atypical epithelial cells with slightly eccentric large nuclei and distinct nucleoli aggregates.

d) Papanicolaou-stained LBC specimen obtained through FNA from the thyroid tumor. Atypical epithelial cells with slightly eccentric large nuclei and distinct nucleoli form irregular papillary structures similar to those seen in the patient's pulmonary adenocarcinoma. No nuclear grooves or intracytoplasmic pseudo-inclusions were observed.

Figure 2:

Real-time PCR for *EGFR* mutation testing (Cobas EGFR Mutation test, Roche Diagnostics, Switzerland). MMX1, 2, and 3 show that only the amplicons specific to

EGFR G719X (MMX3) were efficiently amplified.

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Figure 1

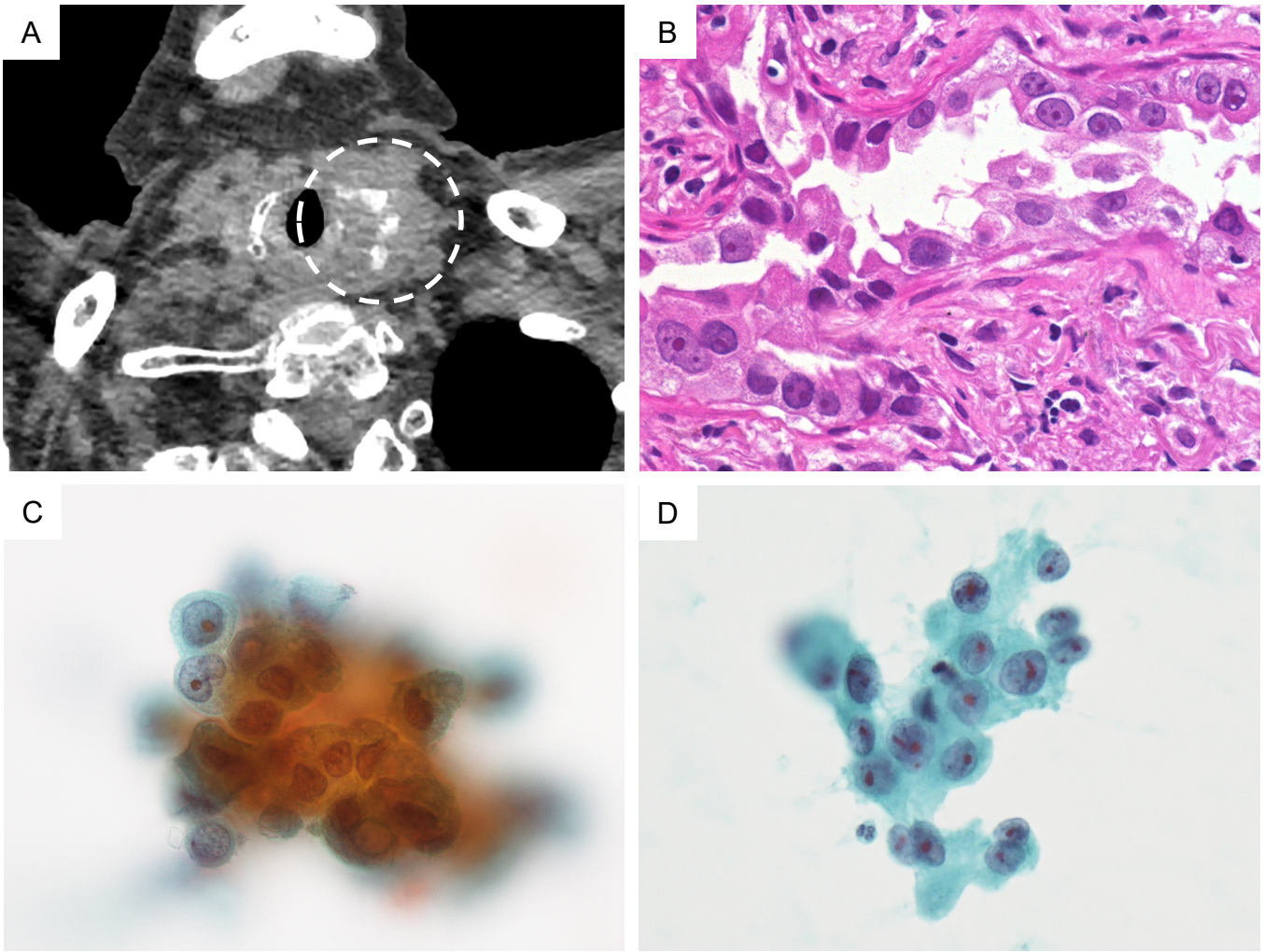
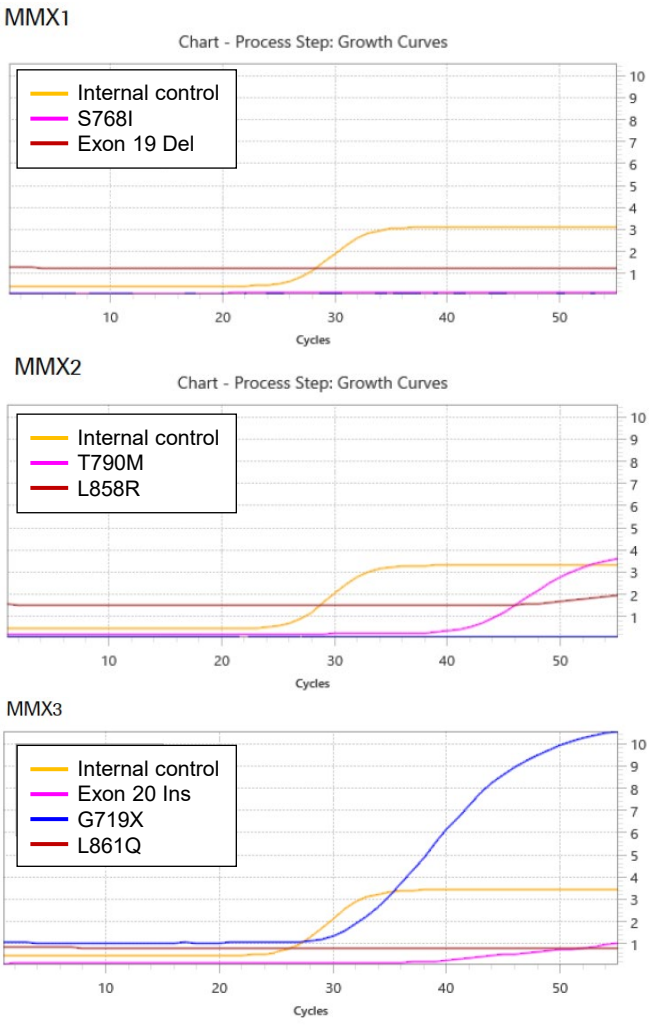


Figure 2


MMX1
Chart - Process Step: Growth Curves
Internal control
S768I
Exon 19 Del
Cycles
MMX2
Chart - Process Step: Growth Curves
Internal control
T790M
L858R
Cycles
MMX3
Internal control
Exon 20 Ins
G719X
L861Q
Cycles