Elevated serum CYFRA 21-1 level as a diagnostic marker for thymic carcinoma

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Abstract

**Background:** No useful tumor markers have been identified for the diagnosis of thymic carcinomas. Serum cytokeratin 19 fragment, measured using the CYFRA 21-1 immunoassay, is used as a tumor marker for squamous cell carcinomas in various malignant tumors. Here, we evaluated the value of CYFRA 21-1 in diagnosing thymic carcinoma.

**Methods:** We retrospectively reviewed 94 patients with pathological diagnoses of thymic carcinoma or thymoma (32 and 62 patients, respectively) who were referred to our departments between January 2000 and March 2019. Primary outcomes included tumor marker levels and their diagnostic accuracy.

**Results:** Patients with thymic carcinoma were significantly more likely to be male (thymic carcinoma, 68.8%; thymoma, 40.3%; \( p = 0.02 \)), have an advanced TNM stage (\( p < 0.01 \)), and a significantly higher CYFRA 21-1 level than those with thymoma (thymic carcinoma: median = 4.2 ng/ml; interquartile range [IQR] = 2.1–6.1 ng/ml vs. thymoma: median = 1.2 ng/ml; IQR = 0.9–1.7 ng/ml; \( p < 0.01 \)). Receiver operating characteristic curves demonstrated that the area under the curve for CYFRA 21-1 to distinguish thymic carcinoma from thymoma was 0.86 (95% confidence interval [CI]: 0.74–0.93; cutoff = 2.7 ng/ml; sensitivity = 68.8%; specificity = 95.2%). Multivariable analysis demonstrated that CYFRA 21-1 (odds ratio = 25.6; 95% CI: 4.6–141.6; \( p < 0.01 \)) was an independent predictor for thymic carcinoma after adjusting for TNM stage.

**Conclusions:** Serum CYFRA 21-1 level may help in diagnosing thymic carcinoma.

**KEYWORDS:** CYFRA 21-1, mediastinal tumor, thymic carcinoma, thymoma, tumor marker

**INTRODUCTION**

Thymic carcinoma and thymoma are primary mediastinal tumors derived from thymic epithelium, and are included in the subtypes of thymic epithelial tumors (TETs).1 TETs account for approximately 50% of anterior mediastinal masses, followed by lymphomas (25%), and other tumors.1 Thymic carcinoma is more aggressive than thymoma, and patients with thymic carcinoma have higher frequencies of lymph node metastasis,2 distant metastasis,2 and invasion into neighboring organs.3,4 Complete surgical resection is an important prognostic factor for patients with both thymoma and thymic carcinoma;2,5 however, in cases of advanced disease, it can be challenging to resect tumors because of anatomical limitations of the mediastinum, and neoadjuvant chemotherapy is likely to increase the likelihood of complete resection in such cases.8 If the tumor is likely to be a thymic carcinoma rather than a thymoma, the possibility of invasion to the surrounding organ is higher, and needle or surgical biopsy can be considered rather than upfront surgery. Therefore, distinguishing thymic carcinoma from thymoma, preoperatively, is important to select an optimal treatment strategy.
Recommended tests for assessing mediastinal tumors include chest imaging and blood chemistry studies, although no serum tumor markers have proven to be useful for the diagnosis of TETs. When a tumor is suspected of being a lymphoma or any other tumor for which surgical resection is not appropriate, biopsy is sometimes required to obtain a histological diagnosis; however, biopsy has a potential risk of pleural implantation if the tumor turns out to be a TET. Therefore, alternative diagnostic tools are required.

Serum cytokeratin 19 (CK19) fragment, measured using CYFRA 21-1 immunoassay, is widely used as a tumor marker of squamous cell carcinomas in various malignant tumors. As squamous cell carcinoma is the most common subtype of thymic carcinoma, we hypothesized that CYFRA 21-1 may be a tumor marker for thymic carcinoma. In this study, we evaluated the diagnostic value of CYFRA 21-1 in distinguishing thymic carcinoma from thymoma in comparison with other tumor markers, including squamous cell carcinoma-related antigen (SCC) and carcinoembryonic antigen (CEA).

METHODS

Study design

This study was approved by the Institutional Review Board of the Hokkaido University Hospital (approval number: C-T2021-0007). Considering the retrospective nature of the current study, informed consent was obtained in the form of an opt-out clause on our website, and patients who rejected this option were excluded. The inclusion criteria were as follows: patients who were referred to our departments between January 2000 and March 2019 and were pathologically diagnosed with TETs. Among the 119 patients, two patients with thymic neuroendocrine tumors, 18 patients (four with thymic carcinoma and 14 with thymoma) with missing tumor marker data, and five patients (one with thymic carcinoma and four with thymoma) whose tumor-node-metastasis (TNM) staging classification by the Union for International Cancer Control/American Joint Committee on Cancer could not be restaged, were excluded from the analysis. Finally, 94 patients (32 with thymic carcinoma and 62 with thymoma) were enrolled in this study (Figure 1).

Primary outcomes of this study were the levels of each tumor marker and their diagnostic accuracy. Secondary outcomes included the levels of each tumor marker according to the status of the tumor and changes in each tumor marker level after treatment. When the primary outcomes proved to be significant, the secondary outcomes were evaluated.

Data collection and definitions

Background information (age, sex, history of smoking, history of myasthenia gravis, and history of malignancy), clinical information (maximum diameter of the tumor and presence of disseminated tumors on computed tomography [CT]), and pathological information (histology, Masaoka stage, and TNM classification) of the patients were collected from the medical records of the Hokkaido University Hospital. Restaging of thymoma and thymic carcinoma was performed based on the eighth edition TNM stage of the Union for International Cancer Control/American Joint Committee on Cancer. The histological type was based on the World Health Organization (WHO) classification criteria, and Masaoka stage was based on the criteria proposed by Masaoka et al. The presence of myasthenia gravis, history of smoking, and history of other malignancies were evaluated from the data obtained before treatment. Maximum tumor diameter was measured using axial CT imaging performed within 3 months prior to the treatment. Serum tumor markers, including CYFRA 21-1, SCC, and CEA, were measured prior to the treatment and used for subsequent analysis. Before 2010, CYFRA 21-1 levels were measured using radioimmunoassay. However, the method changed to electrochemiluminescence immunoassay (ECLIA) in 2010, and to chemiluminescent immunoassay (CLIA) after 2012. The correlation formula between radioimmunoassay and ECLIA, and that between ECLIA and CLIA were used to correct the differences between the measurement methods (see Supporting information, Figures S1 and S2). In this study, CYFRA 21-1 levels measured by using or corrected for CLIA were used for subsequent analysis.

Statistical analysis

Statistical analyses were performed using JMP 15 (SAS Institute Inc.). Continuous data are presented as median and
interquartile range (IQR). Categorical data are presented as numbers and proportions. The Mann–Whitney U test was used to compare the two groups. Frequencies were compared using Fisher’s exact test for categorical variables. The analyses were performed in the following order. First, the differences in the levels of tumor markers between the two groups were evaluated using the Mann–Whitney U test. Second, the area under the receiver operating characteristic (ROC) curve (AUC) was used as an indicator of diagnostic accuracy. The cutoff value for the tumor marker to predict the diagnosis of thymic carcinoma was determined using the Youden index on the ROC curve, and the cutoff value was used in the subsequent univariable and multivariable analyses. Patients with thymic carcinoma were treated as positive, and those with thymoma were treated as negative. Then, to evaluate the significance of the tumor marker as an independent diagnostic factor for thymic carcinoma, a logistic regression model was used in the univariable and multivariable analyses. Tumor marker levels were included in the analyses as a categorical variable divided by the optimal cutoff in the ROC curves because, generally, tumor marker levels are not linearly correlated with disease probability. Variables were selected based on their confounding and potential effect on tumor marker levels as well as multicollinearity (variance inflation factor < 10). A history of smoking, previous malignancy, maximum tumor diameter divided by the median, and the TNM stage (IV/I–III) were included. Baseline variables that were significantly different between the two groups were also included in the models, and the simultaneous entry was used in the multivariable analysis. Finally, tumor marker levels according to the tumor status were evaluated. Comparisons between more than two groups were performed using the Kruskal–Wallis test. Wilcoxon signed-rank test was performed to compare the pre- and postoperative serum tumor marker levels. All analyses were two-tailed, and statistical significance was set at \( p < 0.05 \). When performing multiple statistical tests, Bonferroni correction was used to adjust the optimal cutoff value of the \( p \)-value. All analyses were confirmed by biostatisticians (KO and YMI).

**Immunohistochemical evaluation**

The immunohistochemical expression of CK19 was evaluated in two representative cases. Formalin-fixed paraffin-embedded tissue sections were subject to immunohistochemical analysis using an antibody against CK19 (b170, Novocastra).

**RESULTS**

**Baseline characteristics**

The characteristics of 94 patients, including 32 patients with thymic carcinoma and 62 with thymoma, are summarized in Table 1. The thymic carcinoma group included 21 squamous cell carcinomas, two adenocarcinomas, a basaloid carcinoma, a mucoepidermoid carcinoma, an undifferentiated carcinoma, and six carcinomas of an unspecified histological type. The thymoma group included nine type A, 22 type AB, 9 type B1, 15 type B2, 7 type B3 according to the WHO classification. Baseline age was not significantly different between the patients with thymic carcinoma and those with thymoma (\( p = 0.16 \)). Frequencies of history of smoking (\( p = 0.16 \)), myasthenia gravis (\( p = 0.09 \)), and other malignancies (\( p = 0.49 \)) were also not significantly different between the two groups. In total, nine patients (four with thymic carcinoma and five with thymoma) had a history of malignancies. Among the four thymic carcinoma patients, two had a history of thyroid cancer and two had a history of prostate cancer. The five thymoma patients included two with a history of gastric cancer; one, lung and prostate cancer; one, renal cancer, and one, skin squamous cell carcinoma. All previous

### Table 1 Baseline patient characteristics

|                      | All patients (n = 94) | Thymic carcinoma (n = 32) | Thymoma (n = 62) | \( p \)-value |
|----------------------|----------------------|--------------------------|-----------------|--------------|
| Age, years           | 62 (49–69)           | 64 (54–69)               | 57.5 (47–69)    | 0.15*        |
| Sex                  |                      |                          |                 |              |
| Male                 | 47 (50.0%)           | 22 (68.8%)               | 25 (40.3%)      | 0.02*        |
| Female               | 47 (50.0%)           | 10 (31.3%)               | 37 (59.7%)      |              |
| History of smoking   |                      |                          |                 | 0.16         |
| Yes                  | 58 (61.7%)           | 23 (71.9%)               | 35 (56.5%)      |              |
| No                   | 31 (33.0%)           | 7 (21.9%)                | 24 (38.7%)      |              |
| Missing data         | 5 (5.3%)             | 2 (6.3%)                 | 3 (4.8%)        |              |
| Myasthenia gravis    |                      |                          |                 | 0.09*        |
| Yes                  | 6 (6.4%)             | 0 (0%)                   | 6 (9.7%)        |              |
| No                   | 88 (93.6%)           | 32 (100%)                | 56 (90.3%)      |              |
| History of malignancy|                      |                          |                 | 0.49         |
| Yes                  | 9 (9.6%)             | 4 (12.5%)                | 5 (8.1%)        |              |
| No                   | 85 (90.4%)           | 28 (87.5%)               | 57 (91.9%)      |              |
| Maximum diameter, mm | 49 (34.0–66.0)       | 51.5 (38.5–65.3)         | 47 (30.5–66.5)  | 0.51*        |
| ≥49 mm               | 43 (45.7%)           | 15 (46.9%)               | 28 (45.2%)      | 0.82*        |
| Missing data         | 3 (3.2%)             | 2 (6.3%)                 | 1 (1.6%)        |              |
| TNM stage\(^c\)      |                      |                          |                 | <0.01        |
| I                    | 57 (60.6%)           | 7 (21.9%)                | 50 (80.6%)      |              |
| II                   | 0 (0%)               | 0 (0%)                   | 0 (0%)          |              |
| III                  | 11 (11.7%)           | 5 (15.6%)                | 6 (9.7%)        |              |
| IV                   | 26 (27.7%)           | 20 (62.5%)               | 6 (9.7%)        |              |
| Masaoka stage        |                      |                          |                 | <0.01        |
| I                    | 20 (21.3%)           | 1 (3.1%)                 | 19 (30.6%)      |              |
| II                   | 37 (39.4%)           | 6 (18.8%)                | 31 (50.0%)      |              |
| III                  | 12 (12.8%)           | 5 (15.6%)                | 6 (9.7%)        |              |
| IV                   | 25 (26.6%)           | 20 (62.5%)               | 6 (9.7%)        |              |

Note: Data are presented as numbers (%) or median values (interquartile ranges).

*Continuous variables were compared using the Mann–Whitney U test.

\(^a\) Frequencies were compared using Fisher’s exact test for categorical variables.

\(^b\) Tumor-node-metastasis (TNM) stage was determined according to the eighth edition of the Union for International Cancer Control/American Joint Committee on Cancer.

<ref>SHIIYA ET AL. 2023. Immunohistochemistry of CK19 in Thymic Carcinoma and Thymoma. Journal of Pathology and Laboratory Medicine. 2935, 2023. Pp. 1-10. DOI: 10.1002/jpl2.2023</ref>
malignancies were controlled without recurrence, as evaluated by positron emission tomography/CT prior to the measurement of tumor markers. Patients with thymic carcinoma were significantly more likely to be male (thymic carcinoma, 68.8%; thymoma, 40.3%; \( p = 0.02 \)). The median maximum diameter of the tumor for all patients was 49 mm.

| TABLE 2 Surgical/diagnostic approach of the patients |
|-----------------------------------------------|
| **All patients** (n = 94) | **Thymic carcinoma** (n = 32) | **Thymoma** (n = 62) |
|--------------------------|-----------------------------|-------------------|
| Surgical/diagnostic approach | | |
| Video-assisted thoracic surgery | 43 (45.8%) | 10 (31.3%) | 33 (53.2%) |
| Extended thymectomy | 10 | 1 | 9 |
| Total thymectomy | 6 | 0 | 6 |
| Partial thymectomy | 24 | 6 | 18 |
| Biopsy | 3 | 3 | 0 |
| Median sternotomy | 27 (28.7%) | 8 (25.0%) | 19 (30.6%) |
| Extended thymectomy | 17 | 3 | 14 |
| Total thymectomy | 9 | 4 | 5 |
| Partial thymectomy | 1 | 1 | 0 |
| Thoracotomy | 3 (3.2%) | 0 (0.0%) | 3 (4.8%) |
| Partial thymectomy | 2 | 0 | 2 |
| Extrapleural pneumonectomy | 1 | 0 | 1 |
| Robot-assisted thoracic surgery | 2 (2.1%) | 0 (0.0%) | 2 (3.2%) |
| Total thymectomy | 2 | 0 | 2 |
| Clamshell approach | 1 (1.0%) | 0 (0.0%) | 1 (1.6%) |
| Extended thymectomy | 1 | 0 | 1 |
| Percutaneous biopsy | 18 (19.1%) | 14 (43.8%) | 4 (6.5%) |

Note: Data are presented as numbers (%).

**FIGURE 2** Comparison of serum tumor marker levels between patients with thymic carcinoma and those with thymoma. (a) Comparison of CYFRA 21-1 level between the two groups. The CYFRA 21-1 level in patients with thymic carcinoma was significantly higher than that in patients with thymoma (thymic carcinoma: \(4.2 \, [2.1–6.1] \, \text{ng/ml} \) vs. thymoma: \(1.2 \, [0.9–1.7] \, \text{ng/ml}; \, p < 0.01\) as per the Mann–Whitney U test). (b) Comparison of SCC and (c) CEA levels between the two groups. There were no differences in either the SCC level (thymic carcinoma: \(1.0 \, [0.63–1.4] \, \text{ng/ml} \) vs. thymoma: \(0.8 \, [0.6–1.1] \, \text{ng/ml}; \, p = 0.13\) as per the Mann–Whitney U test) or the CEA level (thymic carcinoma: \(2.4 \, [1.6–4.0] \, \text{ng/ml} \) vs. thymoma: \(2.2 \, [1.4–3.8] \, \text{ng/ml}; \, p = 0.42\) as per the Mann–Whitney U test) between the two groups. Data are presented as median (interquartile range). CEA, carcinoembryonic antigen; SCC, squamous cell carcinoma-related antigen.
The maximum diameter of the tumor was not significantly different between the two groups (thymic carcinoma: median = 51.5 mm; IQR = 38.5–65.3 mm vs. thymoma: median = 47 mm; IQR = 30.5–66.5; p = 0.51). The TNM stage and Masaoka stage were more likely to be advanced in patients with thymic carcinoma (both p < 0.01; Table 1).

The choice of surgical or diagnostic approach for the patients are summarized in Table 2. Seventy-six patients (18 with thymic carcinoma and 58 with thymoma) underwent surgical procedures, whereas percutaneous biopsy was performed in 18 patients (14 with thymic carcinoma and four with thymoma).

### Diagnostic value of tumor markers

Patients with thymic carcinoma showed a significantly higher CYFRA 21-1 level than those with thymoma (thymic carcinoma: median = 4.2 ng/ml; IQR = 2.1–6.1 ng/ml vs. thymoma: median = 1.2 ng/ml; IQR = 0.9–1.7 ng/ml; p < 0.01; Figure 2a); whereas, there were no significant differences in the SCC (thymic carcinoma: median = 1.0 ng/ml; IQR = 0.63–1.4 ng/ml vs. thymoma: median = 0.8 ng/ml; IQR = 0.6–1.1 ng/ml; p = 0.13; Figure 2b) and CEA levels (thymic carcinoma: median = 2.4 ng/ml; IQR = 1.6–4.0 ng/ml vs. thymoma: median = 2.2 ng/ml; IQR = 1.4–3.8 ng/ml; p = 0.42; Figure 2c) between the two groups. CYFRA 21-1 level did not differ significantly between patients with thymic squamous cell carcinoma and those with other thymic carcinomas (squamous cell carcinoma: median = 4.4 ng/ml; IQR = 1.95–11.5 ng/ml vs. non-squamous cell carcinomas: median = 4.2 ng/ml; IQR = 2.4–5.7 ng/ml; p = 0.49; Figure 3).

ROC curves of the tumor markers used to distinguish thymic carcinoma and thymoma are shown in Figure 4. The ROC curve demonstrated that the AUC for CYFRA 21-1 was 0.86 (95% CI: 0.74–0.93; Figure 4a). When the cutoff value of CYFRA 21-1 was set as 2.7 ng/ml, the sensitivity and specificity were 68.8% and 95.2%, and the positive and

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**Figure 3** Comparison of serum CYFRA 21-1 levels between patients with thymic squamous cell carcinoma and those with non-squamous thymic carcinomas. CYFRA 21-1 level did not differ between patients with thymic squamous cell carcinoma and those with other thymic carcinomas (squamous cell carcinoma: 4.4 [1.95–11.5] ng/ml vs. nonsquamous cell carcinoma: 4.2 [2.4–5.7] ng/ml; p = 0.49 as per the Mann–Whitney U test).

**Figure 4** ROC curves of tumor markers for distinguishing thymic carcinoma from thymoma. (a) The ROC curve of CYFRA 21-1 demonstrated that CYFRA 21-1 was useful for distinguishing thymic carcinoma and thymoma. When the cutoff value was set as 2.7 ng/ml, the sensitivity and specificity were 68.8% and 95.2%, respectively. (b) The ROC curve for SCC and (c) CEA showed that they were not useful predictors of thymic carcinoma. AUC, area under the curve; CEA, carcinoembryonic antigen; CI, confidence interval; ROC, receiver operating characteristic; SCC, squamous cell carcinoma-related antigen.
negative predictive values were 88.0% and 85.5%, respectively. In contrast, SCC (AUC = 0.60; 95% CI: 0.47–0.71; Figure 4b) and CEA (AUC = 0.55; 95% CI: 0.43–0.67; Figure 4c) were not useful in predicting the diagnosis of thymic carcinoma.

The univariable analysis using the logistic regression model showed that sex (male/female, \( p = 0.01 \)), CYFRA 21-1 level (\( \geq 2.7 < 2.7 \text{ ng/mL} \), \( p < 0.01 \)), and TNM stage (IV/I–III, \( p < 0.01 \)) were associated with the diagnosis of thymic carcinoma (Figure 5). Further, multivariable analysis, including sex (male/female), history of smoking (yes/no), history of other malignancies (yes/no), CYFRA 21-1 level (\( \geq 2.7 < 2.7 \text{ ng/mL} \)), maximum diameter of the tumor in the axial section of the CT (\( \geq 49 < 49 \text{ mm} \)), and TNM stage (IV/I–III), demonstrated that CYFRA 21-1 level and TNM stage were independent predictors of thymic carcinoma (odds ratio = 25.6; 95% CI: 4.6–141.6; \( p < 0.01 \)), although TNM stage IV was also independently associated with thymic carcinoma (odds ratio = 11.7; 95% CI: 1.9–71.8; \( p < 0.01 \); Figure 5).

CYFRA 21-1 levels according to the TNM stage and WHO type are summarized in Figure 6. CYFRA 21-1 levels were significantly higher in patients with thymic carcinoma than in those with thymoma when limited to patients with stage IV (\( p = 0.01 \)) disease; whereas, no significant difference was observed between the two groups when limited to patients with stage I disease (\( p = 0.09 \); Figure 6a). Further, in patients with thymoma, there was no significant difference in CYFRA 21-1 levels according to the WHO types (\( p = 0.29 \); Figure 6b).

Thirteen patients with thymic carcinoma underwent curative surgery. Among them, both pre- and postoperative CYFRA 21-1 levels were available for seven patients. In all seven cases, CYFRA 21-1 levels decreased postoperatively (\( p = 0.02 \); Figure 7).

**Immunohistochemical evaluation**

Immunohistochemical staining for CK19 revealed intense expression both in a thymic squamous cell carcinoma (stage III, CYFRA 21-1 level of 5.9 ng/mL) and a type A thymoma (stage I, CYFRA 21-1 level of 1.0 ng/mL). Obviously, a positive immunoreaction was frequently observed only in the thymic carcinoma (see Figure S3).

**DISCUSSION**

In the present study, it was found that the CYFRA 21-1 level in patients with thymic carcinoma was significantly higher than that in patients with thymoma; whereas, SCC and CEA levels showed no significant differences between the two groups. The ROC curve demonstrated that when the cutoff

| Predictive factors                | Univariable Analysis | Multivariable Analysis |
|-----------------------------------|----------------------|------------------------|
| Sex (Male/Female)                 | 0.01                 | 0.35                   |
| History of smoking (Yes/No)       | 0.11                 | 0.43                   |
| Previous malignancy (Yes/No)      | 0.49                 | 0.81                   |
| CYFRA 21-1 level (\( \geq 2.7 < 2.7 \text{ ng/mL} \)) | \(<0.01\)             | \(<0.01\)               |
| Maximum diameter (\( \geq 49 < 49 \text{ mm} \))  | 0.71                 | 0.06                   |
| TNM Stage (IV/I–III)              | \(<0.01\)             | \(<0.01\)               |

FIGURE 5 Univariable and multivariable analyses and forest plots of thymic carcinoma predictors. In the univariable analysis, sex, CYFRA 21-1 level (\( \geq 2.7 \text{ ng/mL} \)), and tumor-node-metastasis (TNM) stage were associated with the diagnosis of thymic carcinoma. Multivariable analysis demonstrated that CYFRA 21-1 level and TNM stage were independent predictors of thymic carcinoma. The TNM stage is based on the eighth edition of the classification by the Union for International Cancer Control/American Joint Committee on Cancer.
value was set as 2.7 ng/mL, the sensitivity and specificity to distinguish thymic carcinoma from thymoma were 68.8% and 95.2%, respectively, with an AUC of 0.86. Although in our study, patients with thymic carcinoma had more advanced disease than those with thymoma, multivariable analysis showed that CYFRA 21-1 level of ≥2.7 ng/mL was an independent predictor of thymic carcinoma. Furthermore, in the analysis of seven patients with thymic carcinoma, the CYFRA 21-1 levels decreased after curative surgery. These findings suggest that serum CYFRA 21-1 level might act as a distinguishing factor between thymic carcinoma and thymoma and also indicate disease-free status following tumor resection. To the best of our knowledge, this is the first study to distinguish thymic carcinoma from thymoma using CYFRA 21-1.

The major differential diagnoses of anterior mediastinal tumors include lymphomas, germ cell tumors, and thyroid tumors. Measuring serum tumor markers, including alpha-fetoprotein and beta subunit of human chronic gonadotropin, is recommended to rule out malignant germ cell tumors.1 Similarly, high serum soluble interleukin-2 receptor level has been reported to predict the presence of lymphoma.18-20 Benign mediastinal teratomas can often be distinguished based on CT findings, such as fat density area.21 Thyroid tumors involving the anterior mediastinum can be identified on CT scans as contiguous with the thyroid gland.1 However, no previous reports have identified useful tumor markers for the diagnosis of TETs. In this study, CYFRA 21-1 level was significantly higher in patients with thymic carcinoma than in those with thymoma.

CK19 is one of the molecular species of cytokeratins, which are a multigene family of polypeptides expressed in various epithelial cells.22 Weissferdt and Moran14 studied 31 cases of thymic carcinoma, including both squamous cell carcinoma and nonsquamous cell carcinomas, and immunohistochemical analysis revealed that all the tumors were positive for cytokeratins; however, CK19 expression itself has not been evaluated specifically. In the present study, CK19 expression in the thymic squamous cell carcinoma directly indicated that an increase in the serum CYFRA 21-1 level was attributable to the tumor cells. The mechanism by which CK19 is released into the serum from the cytoskeleton remains unclear; however, it has been speculated that CK19 fragments are released into the blood circulation.
because of destruction of cells induced by apoptosis or necrosis. In this study, although viable tumor cells showed an intense expression of CK19 in both the types, CK19-immunoreactive coagulation necrosis was found only in thymic carcinoma but not in thymoma. These findings, supported by the hypothesis of the studies mentioned above, can explain the significant increase in serum CYFRA 21-1 levels in the thymic carcinoma group. Further, in this study, the serum CYFRA 21-1 level showed no significant difference between squamous cell carcinoma and nonsquamous cell carcinomas. These findings suggest that CK19 might be expressed in not only thymic squamous cell carcinoma but also in nonsquamous thymic carcinomas, and that CYFRA 21-1 can aid in distinguishing thymic carcinoma regardless of histological subtypes. However, further studies using immunohistochemical evaluation for nonsquamous subtypes are needed to confirm these hypotheses.

Complete surgical resection is the most important prognostic factor in patients with thymic carcinoma and thymoma. Here, the CYFRA 21-1 level in patients with thymic carcinoma was significantly higher than that in patients with thymoma when limited to stage IV disease; whereas, there was no significant difference when limited to stage I disease. For patients with stage IV thymic carcinoma or thymoma, it is generally difficult to achieve complete resection; however, several studies have suggested the benefits of maximal debulking surgery or resection for pleural dissemination on survival in patients with thymoma. Nevertheless, the benefits of these procedures have not been established in patients with thymic carcinoma. Even when patients have stage IV disease, distinguishing thymic carcinoma from thymoma is important for selecting an optimal treatment strategy.

Here, the cutoff value (2.7 ng/ml) was lower than the upper limit of the normal range of CYFRA 21-1 in our institute (<3.5 ng/ml), and has also been reported as a cutoff value for primary lung cancer. High specificity with a low cutoff level indicates that patients with thymoma rarely show a high CYFRA 21-1 level even when the thymoma is advanced. In addition, a CYFRA 21-1 level of ≥2.7 ng/ml was an independent predictor of thymic carcinoma after adjusting for possible confounding factors, such as the TNM stage. Although the distribution of TNM stage in this study was not equivalent between patients with thymic carcinoma and those with thymoma, these findings suggest that CYFRA 21-1 may be a potential predictor of thymic carcinoma regardless of the TNM stage.

Several reports have described that elevated CYFRA 21-1 level is associated with thymic carcinoma. Yoshiike et al. reported a case of thymic squamous cell carcinoma with an extremely high CYFRA 21-1 level of 310 ng/ml (normal level, <3.5 ng/ml). However, the status of CYFRA 21-1 after treatment has not yet been described. Two other researchers have described cases of thymic carcinoma with high CYFRA 21-1 levels. In both cases, the tumors shrank, and CYFRA 21-1 level decreased to its normal range after chemotherapy. Suzuki et al. reported that high serum CYFRA 21-1 levels were detected in five out of 11 patients with stages III–IV thymic carcinoma. In the present study, all seven patients who underwent curative surgery for thymic carcinoma showed a decrease in CYFRA 21-1 levels after surgery. Furthermore, CYFRA 21-1 level in patients with stage IV thymic carcinoma tended to be higher than that in patients with stage I thymic carcinoma. These findings suggest that CYFRA 21-1 levels may represent the tumor stage and also disease-free status. However, further studies should address whether CYFRA 21-1 levels increase when the tumor shows recurrence.

Our study has several limitations. First, this was a retrospective, single-center study with a small sample size. Because thymic carcinoma is a rare mediastinal tumor, larger multicenter studies are needed to confirm our results. Second, we did not include every potential confounding factor in our multivariable model. In our study, the proportion of stage IV disease in patients with thymic carcinoma was high, whereas that in patients with thymoma was low. Although the CYFRA 21-1 level was an independent predictor of thymic carcinoma after adjusting for possible confounding factors, including the TNM stage, residual confounding cannot be completely ruled out. As patients with thymoma usually present with an early stage tumor and those with thymic carcinoma at an advanced stage, further studies with larger sample size are warranted. Whether CYFRA 21-1 can distinguish thymic carcinoma from thymoma in the early stages should be determined in further studies. Third, immunohistochemical staining for CK19 was performed in only two representative cases. Further studies
using a larger sample size are needed to confirm these results. Fourth, previous malignancies may have affected the levels of tumor markers, although only a small proportion of patients had a history of malignancies, and all previous malignancies were treated and controlled without recurrence. In the multivariable analysis, the CYFRA 21-1 level was an independent distinguishing factor after adjusting for histories of malignancy; however, further studies are needed to address the influence of previous malignancies on the diagnostic accuracy of tumor markers. Finally, CYFRA 21-1 measurement methods have changed twice during the 20-year study period. Although correlations between the measurement methods were strong, the differences between the methods might have affected the outcomes. In addition, the optimal cutoff value may be different for other measurement methods. Nevertheless, the present study indicated the potential value of CYFRA 21-1 for diagnosing thymic carcinoma.

In conclusion, our study suggests that the serum levels of tumor marker CYFRA 21-1 can aid in distinguishing between thymic carcinoma and thymoma, and also indicate disease-free status following tumor resection. However, further studies are necessary to confirm our results and determine whether CYFRA 21-1 can predict thymic carcinoma at an early stage.

ACKNOWLEDGMENTS
We would like to thank Editage (www.editage.com) for English language editing. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

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How to cite this article: Shiiya H, Ujiie H, Hida Y, Kato T, Kaga K, Wakasa S, et al. Elevated serum CYFRA 21-1 level as a diagnostic marker for thymic carcinoma. Thorac Cancer. 2021;12:2933–42. https://doi.org/10.1111/1759-7714.14158