Flow-Cytometric Diagnosis of Thymoma Using Needle Biopsy Specimens

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Abstract

Introduction. Thymoma is a neoplasm of thymic epithelial cells which is characteristically associated with a large number of non-neoplastic T lymphocytes. These T cells are induced by epithelial cells and show a unique phenotype of CD4⁺CD8⁺ double positive (DP), when studied by flow cytometry. This DP phenotype can be used as one of the diagnostic criteria to indicate a thymoma. The preoperative diagnosis of thymoma and other thymic tumors is an important problem because the treatment differs according to the diagnosis.

Methods. A flow-cytometric analysis of needle biopsy specimens was performed on ten thymic tumors. The results were then compared with the findings of a histological diagnosis using conventional hematoxylin–eosin (H&E) staining.

Results. Six cases with high frequencies of DP cells were diagnosed as thymoma by a flow-cytometric analysis, and were confirmed by a histological analysis of the resected specimen. Flow cytometry did not suggest a thymoma in the other four cases with few DP cells. The final diagnoses of the resected specimen of these four cases were: one thymic carcinoma, one malignant lymphoma, and two germ-cell tumors. The accuracy and specificity of diagnosis of thymoma using a fluorescence-activated cell sorting analysis from needle biopsy specimens were 6/6 (100%). On the other hand, the specificity of H&E staining from needle biopsy specimens for the diagnosis of thymoma was 6/6 (100%), but the accuracy was only 6/9 (66.7%).

Discussion. Flow cytometry can be applied to needle biopsy specimens and thus is considered to offer useful information for the preoperative diagnosis of thymic tumors.

Key words. Thymoma · Needle biopsy · Flow cytometry · Mediastinal tumor

Introduction

Thymoma is a neoplasm of thymic epithelial cells. Thymoma seems to maintain the ability of normal thymic epithelial cells to induce T-cell differentiation. This is indicated by the large number of normal immature thymocytes associated with thymoma.¹–³ A preoperative diagnosis of thymoma and other thymic tumors is often difficult, partly because of the small size of the biopsy materials.⁴,⁵ This is especially a problem because the treatment differs according to the diagnosis. For example, a malignant thymic germ-cell tumor should be treated with chemotherapy.⁶,⁷ On the other hand, the majority of thymomas are resectable and surgery is the first treatment of choice.⁸–¹⁰

In a previous study, we analyzed the phenotype of lymphocytes in thymic tumors and showed the CD4⁺CD8⁺ double-positive (DP) phenotype to be unique to thymoma (except for malignant lymphoma).¹¹ When more than 3% of the lymphocytes in a thymic tumor were DP, the tumor was most likely a thymoma. For a tumor with fewer than 3% DP cells, a tumor other than thymoma was suggested; however, a thymoma could still not be ruled out. In the present study, we used flow cytometry to study the lymphocytes recovered from needle biopsy specimens of ten thymic tumors. We were able to correctly diagnose six thymomas. The other four cases were not thymomas and the flow-cytometric findings suggested that they were not likely to be thymomas.
Patients and Methods

All patients with an anterior mediastinal tumor who underwent a needle biopsy at our hospital between June 1997 and March 1999 were included in the study. Prior to the needle biopsy, the patients were informed of the nature of the study and gave their informed consent to undergo a needle biopsy and allowed us to use the resected material. The patient age ranged from 18 to 64 years. There were eight men and two women. We performed an echo-guided biopsy with an 18-gauge needle, the size of a typical sample being about 0.5 × 7 mm. The sample was minced with small scissors and suspended in phosphate-buffered saline. The sample was passed through a nylon mesh. The yield of mononuclear cells was about 1.6–24 × 10^6 (mean 7.2 × 10^6). Separated mononuclear cells were incubated with fluorescein isothiocyanate-conjugated antihuman CD8 antibody and phycoerythrin-conjugated antihuman CD4 antibody (Pharmingen, San Diego, CA, USA) for 20 min. They were washed twice, and 1 × 10^6 cells were analyzed using flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA, USA). A pathological diagnosis was made by one pathologist using hematoxylin-eosin (H&E)-stained sections without any knowledge of the flow-cytometric data.

Results

In all ten cases a sufficient number of cells was recovered from needle biopsy specimens, thereby allowing us to make a preliminary diagnosis before we obtained an official histology report. The proportion of DP cells in the thymic tumor-associated lymphocytes in the ten cases was 34.7% ± 34.1% (mean ± standard deviation).