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Histopathologic changes of thymoma preoperatively treated with corticosteroids

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Abstract Preoperative treatment of thymoma in advanced stages with corticosteroids may reduce the size of the tumor, but no precise histologic evaluation has been performed. We examined the histopathologic features of pretreatment biopsy and posttreatment surgical specimens of eleven cases of thymoma with such treatment to see the changes of the histologic subtypes based on Muller–Hermelink classification. All specimens were also assessed immunohistochemically for MIB-1 labeling and apoptotic cells to verify the effectiveness of this pretreatment. Seven tumors clinically diminished in size after the treatment with corticosteroids. Fungal infection occurred in three cases postoperatively. The histology of mixed thymomas (two cases) was converted to that of medullary thymoma. Predominantly cortical thymomas (four cases) and cortical thymomas (three cases) changed to show similar histologic features; both became epithelial-rich thymoma with large polygonal tumor cells having indistinct cell borders. In contrast, two well-differentiated thymic carcinomas showed at surgery more prominent squamoid appearance with distinct cell borders. The apoptotic indices of epithelial cells were increased (P=0.001), and the MIB-1 indices tended to be decreased with corticosteroid treatment. These results suggest that there may be a histogenetic relationship between medullary and mixed thymomas and also between predominantly cortical and cortical thymomas. Corticosteroids may cause degenerative changes in the epithelial cells and lymphocytes and, in thymomas in advanced stages, corticosteroid pretreatment may be warranted, although attention should be paid to infection after surgery.

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Introduction

Thymoma is a neoplasm arising from thymic epithelial cells associated with variable numbers of non-neoplastic T lymphocytes [17, 27]. The classification of thymomas and designation of the subsets have been a subject of debate because of their heterogeneity of histologic features and clinical behaviors [14]. The classification system proposed by Muller–Hermelink and colleagues (M-H classification) [12, 13, 18] is based on the cytologic resemblance of the tumor epithelial cells to the respective counterparts in the normal thymus. According to their classification, thymic epithelial tumors are divided into medullary thymoma, mixed medullary and cortical thymoma, predominantly cortical thymoma, cortical thymoma, well-differentiated thymic carcinoma (WDTC), and high-grade thymic carcinoma. This classification system is reproducible and related to the tumor grades [16, 21, 24] and has been adopted in the recently published classification system by the World Health Organization (WHO) [25]. The latter uses types A, AB, B1, B2, B3, and C, corresponding to the subtypes in M-H classification, implying that the topographical designations still have to be refined. Furthermore, the relationship among the subtypes in these classification systems needs to be clarified.

The primary treatment of thymoma is surgical excision. In the cases at advanced stages, postoperative radiotherapy or chemotherapy can be considered [3, 27]. Preoperative treatment for thymoma has not been established, although corticosteroids may be preoperatively used for treatment of myasthenia gravis, one of the most common complication of thymoma, to suppress production of the antibody [6]. At the Nagoya City University Medical School affiliated hospitals, thymoma patients in advanced stages have, since 1995, been treated with corticosteroids before surgical resection; in consequence,

several tumors were diminished in size, and their surgical resection became easier in comparison with those without pretreatment.

We report herein the histopathologic changes of thymomas upon corticosteroid treatment, comparing the features with those of the pretreatment specimens. The tumors were subtyped according to the M-H classification system, and the changes by treatment provided some insight into the relationship of these subtypes and the effectiveness of the corticosteroids. Although the histologic features of thymoma after corticosteroid treatment have been described previously [13, 32], a comparative study using both pretreatment biopsy and posttreatment surgical materials has not been performed.

Apoptotic cells in both pre- and posttreatment sections were examined using the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labeling (TUNEL) technique [8] and the proliferative activity of the tumor cells was estimated by immunostaining of the Ki-67 antigen.

Materials and methods

The files of the Departments of Pathology and Surgery of Nagoya City University Medical School and the Division of Thoracic Surgery of Seirei Mikatabara General Hospital revealed that 16 cases of thymoma had been treated with corticosteroids before surgery. All patients gave informed consent to the treatment method. Selected in the present study were eleven cases, in which both pretreatment biopsy and posttreatment surgical materials were available for the histopathologic examination. Needle biopsy had been performed in ten cases and open biopsy was performed in one case. The biopsied tumors were subtyped according to M-H and WHO classifications. Only the hematoxylin and eosin-stained sections of biopsy specimens were available in case 5 and case 10 because the biopsies had been performed at different hospitals. Case 9 was inoperable since the thymoma involved the superior vena cava and only the incisional biopsy was performed at thoracotomy; this case was excluded from immunohistochemical study because both pretreatment and posttreatment biopsy specimens were not enough to evaluate the apoptotic and MIB-1 indices. More than four paraffin-embedded tissue blocks were available in ten resected tumors; in two cases (case 2 and case7), about 30 blocks were obtained from each resected tumor to examine the histologic features of a whole gross section.

Immunohistochemical staining with the streptavidin-biotin-immunoperoxidase method [7] was performed using HISTOFINE immunostaining kits (Nichirei, Tokyo, Japan). The primary antibodies used in this study were anti-cytokeratin antibodies AE1/3 (Dako, Carpinteria, Calif.; 1:50) and CAM5.2 (Becton Dickinson Immunocytometry Systems, San Jose, Calif.; prediluted), anti-bcl-2 oncoprotein (124, Dako; 1:25), anti-CD1a (O10, Immunotech, Marseille Cedex, France; prediluted), anti-CD99 (12E7, Dako; 1:50), and anti-Ki-67 antigen (MIB-1, Immunotech; 1:50). Sections except for anti-CD99 antibody were subjected to appropriate antigen retrieval methods: CAM5.2, was digested with 0.1% trypsin solution for 10 min, AE1/3 and anti-bcl-2 oncoprotein antibody microwaved in a 0.01 M citrate buffer, pH 6.0, for 20 min [9], and MIB-1 was immersed in a citrate buffer (pH 6.0) and then exposed for 40 min in a stainless steel pressure cooker [20]. The catalyzed signal amplification method [1] using Dako CSA System peroxidase kits was applied for anti-CD1a antibody [10]. Apoptotic cells were visualized with an enzymatic reaction using the TUNEL method. This was performed using the ApopTag kit (Oncor, Gaithersburg, Md.) as described previously [31]. The positively stained cells were considered as epithelial cells when these cells were large enough when compared with lymphocytes. The apoptotic index and MIB-1 index were defined as described in the previous study [30]. Student's *t*-test was used for statistical analysis of apoptosis and MIB-1 indices between pre- and posttreatment sections. The difference was considered significant when the *P* value was less than 0.05.

Results

Clinical findings

The clinicopathologic data of the patients are summarized in Table 1 and Table 2. According to the criteria of Masaoka and colleagues [19], all but two cases were clinically in stage IV. From the findings of a computed tomographic scan, seven cases significantly diminished in size after treatment with corticosteroids; the reduction rate was more than 60% in case 3 and case 4 and between 30% and 60% in cases 1, 5, 6, 7, and 10, but less than 30% in the other cases (cases 2, 8, 9, and 11). After surgery, fungal infection occurred in three cases; cryptococcal meningitis in case 3, pulmonary candidiasis in case 4, and cutaneous candidiasis in case 10. Follow-up data were available in all cases. Eight patients were alive without evidence of disease (five cases) or with disease (three cases) between 1 year and 3 years after surgery. One patient (case 9) died of the disease which was inoperable. The other two patients died of unrelated diseases.

Histologic findings

Histologic examination of the pretreatment biopsy specimens disclosed that two tumors (cases 1 and 2) were composed of mixed polygonal and spindle epithelial cells associated with a moderate number of lymphocytes and were classified as mixed thymoma (type AB according to the WHO classification) (Fig. 1A, B). The other nine tumors were composed mainly of polygonal epithelial cells; four with predominant lymphocytic component (Fig. 2A, B), three with a moderate number of lymphocytes (Fig. 3A, B), and two with scant lymphocytes and with the epithelial cells showing squamoid features and mild cellular atypia focally (Fig. 4A, B). They were classified as predominantly cortical thymoma (type B1), cortical thymoma (type B2), and WDTC (type B3), respectively. No mitotic figures were found in the biopsy sections in each case.

Resected specimens after treatment with corticosteroids revealed histologic changes in various degrees compared with the pretreatment biopsy specimens. The resected tumors contained no areas showing the same histologic features as those in biopsy sections. Case 1 and case 2 with the initial diagnosis of mixed thymoma showed diffuse growth of mostly spindle epithelial cells with elongated nuclei, dispersed chromatin, and inconspicuous nucleoli, reminiscent of medullary thymoma (Fig. 1C, D). Glandular and hemangiopericytoma-like structures were seen in part. Small epithelial cells with round nuclei were

Table 1 Clinical findings. *M* methylpredonisolone for intravenous injection; *P* predonisolone for oral administration; *AND* alive with no evidence of disease; *AWD* alive with disease; *DOC* died of other cause or complication; *DOD* died of disease

Case no.	Age/Gender	Stage[a]	Corticosteroids		Reduction rate of tumor size (%)	Follow-up		Complication
			Total dose (duration)	Interval between drug cessation and surgery		Outcome	Duration	
1	56/Female	II	M 3.0 g (4 days)	6 days	32	AND	3 years, 1 month	
2	52/Female	II	M 3.0 g (3 days)	3 days	8	AND	9 months	
3	73/Female	IV	P 1180 mg (50 days), M1.5 g (3 days)	12 days	70	DOC	4 months	Fungal infection
4	68/Female	IV	P 380 mg (12 days), M 2.0 g (3d)	1 days	62	DOC	1 year	Fungal infection
5	26/Female	IV	P 480 mg (12 days), M 3.0 g (3 days)	15 days	37	AWD	3 years, 3 months	
6	55/Male	IV	M 3.0 g (3 days)	10 days	45	AND	1 year	
7	51/Male	IV	M 3.0 g (3 days)	18 days	38	AND	1 year	
8	50/Male	IV	M 3.0 g (3 days)	14 days	5	AWD	2 years, 6 months	
9	50/Female	IV	M 4.0 g (4 days)	12 days	11	DOD	3 months	
10	78/Female	IV	P 510 mg (26 days)	1 days	39	AWD	3 years, 5 months	Fungal infection
11	45/Male	IV	M 3.0 g (3 days)	4 days	16	AND	3 years, 2 months	

[a] According to Masaoka's classification

Table 2 Histopathologic findings. *Med* medullary thymoma; *Mixed* mixed thymoma; *Pcor* predominantly cortical thymoma; *Cor* cortical thymoma; *WDTC* well-differentiated thymic carcinoma; *Pre* before corticosteroid treatment; *Post* after corticosteroid treatment

Case no.	Histologic subtype[a]		Other findings after corticosteroid treatment	Epithelial cells				Number of immature T-lymphocytes	
	Pre	Post		Apoptotic index (%)		MIB-1 index (%)		Pre	Post
				Pre	Post	Pre	Post		
1	Mixed	Med		1.5	1.8	4.4	3.7	Moderate	Few
2	Mixed	Med		1.2	2.2	3.2	2.1	Moderate	Few
3	Pcor	Cor[b]	Fibrous replacement	2.0	5.2	3.4	3.2	Abundant	Few
4	Pcor	Cor[b]	Large cystic spaces	2.0	3.7	4.0	2.7	Abundant	Few
5	Pcor	Cor[b]	Large cystic spaces	NA	4.3	NA	3.0	Abundant	Few
6	Pcor	Cor[b]		1.3	4.0	4.1	3.7	Abundant	Scant
7	Cor	Cor[b]	Focal coagulative necrosis	2.1	3.4	4.2	3.3	Moderate	Scant
8	Cor	Cor[b]		2.2	4.5	4.0	6.4	Moderate	Few
9	Cor	Cor[b]		NP	NP	NP	NP	Moderate	Few
10	WDTC	WDTC	Focal coagulative necrosis	NA	6.2	NA	4.8	Scant	Few
11	WDTC	WDTC	Bizarre and multinucleated tumor cells	2.5	5.0	6.5	4.0	Scant	Few

[a] According to the Muller–Hermelink classification system
[b] Epithelial-rich thymoma with cortical type tumor cells

also intermingled. Cellular atypia was not apparent, although some epithelial cells showed slightly irregular and indented nuclear contours with more dispersed chromatin. Furthermore, epithelial cells with condensed nuclei and eosinophilic cytoplasm were focally observed. Lymphocytes were markedly decreased in number and some lymphocytes, with occasional nuclear fragmentation, were observed in clusters. Foci of fibrosis with mild sclerosis were scattered in the tumor nests.

The tumor in case 3 was considerably replaced by sclerotic fibrous tissue accompanied by many foamy macrophages, cholesterol clefts, hemorrhages, and areas of coagulative necrosis. Several small nests of large polygonal epithelial cells with vesicular nuclei and distinct nucleoli were seen. Some tumor cells showed condensed nuclei with eosinophilic cytoplasm. There were many macrophages and cholesterol clefts in the tumor nests as well. Although intracytoplasmic vacuolization was seen

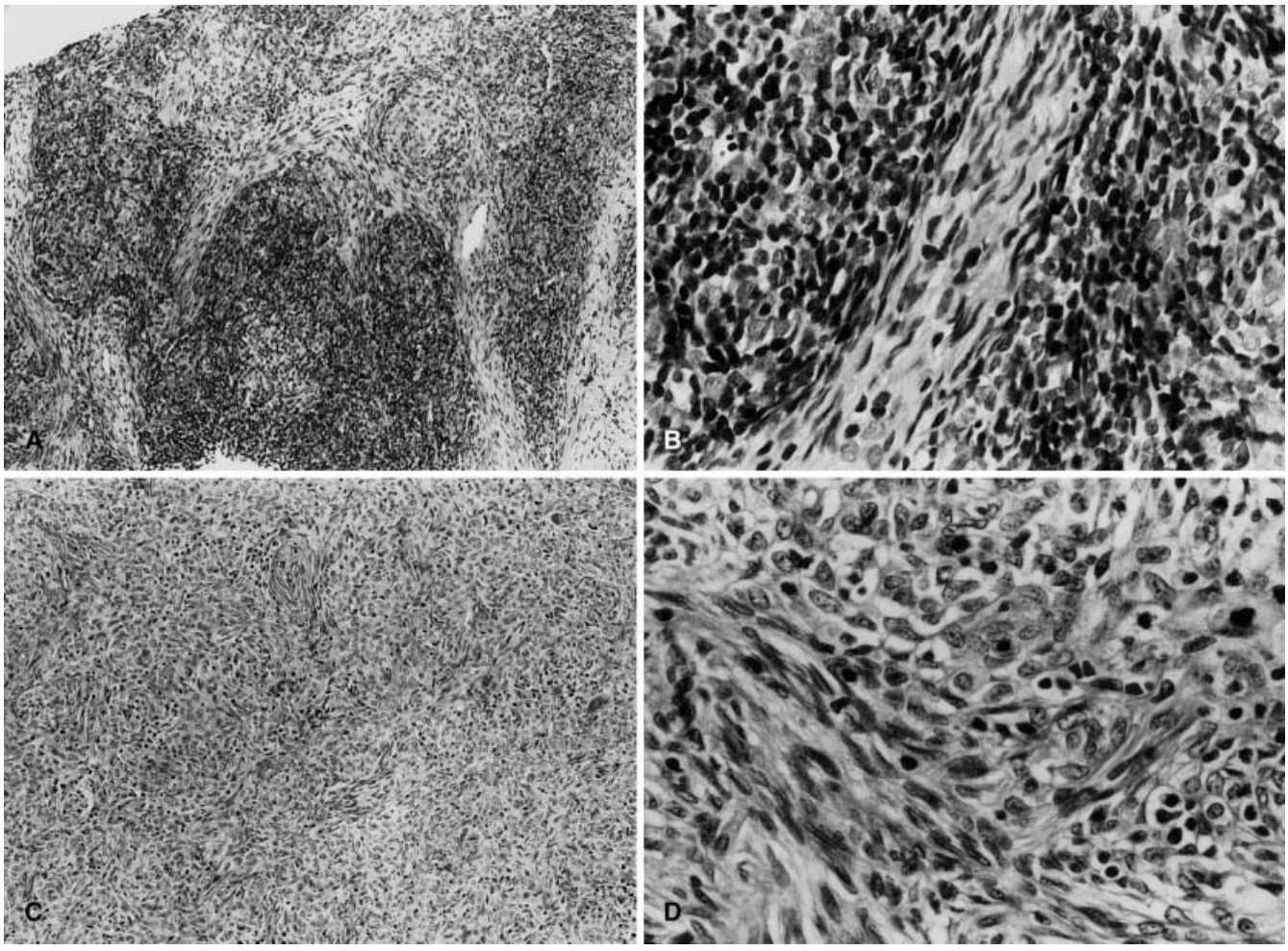

Fig. 1 Mixed thymoma (case 1). Lower (**A**) and higher (**B**) magnification of biopsy specimen showing mixed polygonal and spindle epithelial cells with moderate number of lymphocytes (hematoxylin and eosin, ×100 and ×400). Lower (**C**) and higher (**D**) magnification of resected specimen showing mostly spindle epithelial cells with dispersed nuclear chromatin and some epithelial cells with condensed nuclear chromatin (hematoxylin and eosin, ×100 and ×400)

in some tumor cells, this was also observed in those of the pretreatment biopsy sections. Lymphocytes were moderately decreased, and many of them had fragmented or irregular-shaped nuclei.

On lower magnification, the other eight cases disclosed essentially similar histologic features (Fig. 2C, Fig. 3C, and Fig. 4C). The tumors were divided into lobules of variable sizes by strands of hyalinized stroma, and the lobules were composed of polygonal epithelial cells with scant lymphocytes. On higher magnification, however, the features of polygonal epithelial cells were slightly different among these tumors. The epithelial cells of cases 4–9, similarly to those of case 3, were large with round to oval nuclei, vesicular chromatin, and relatively distinct nucleoli (Fig. 2D and Fig. 3D). Some epithelial cells possessed condensed or pyknotic nuclei and eosinophilic cytoplasm. The epithelial cells of these cases were closely packed, and cell borders were generally indistinct. In some areas, squamoid foci with nests or a whorl-like arrangement of polygonal epithelial cells having abundant eosinophilic or clear cytoplasm and distinct cell borders were observed in all cases except for case 9, in which only incisional biopsy had been performed. Perivascular spaces surrounded by epithelial palisade were recognized in cases 4–8. Several areas of case 7 showed coagulative necrosis associated with hemorrhages, foamy macrophages, cholesterol clefts, and sclerosing fibrosis. In addition, focally hemorrhagic, large cystic spaces of varying sizes containing lymphocytes, foamy macrophages, and fibrin were seen in case 4 and case 5 (Fig. 2E). Although some cystic spaces contained small vessels in the lumen, the walls of the cysts were devoid of epithelial lining. The lymphocytes of these cases varied in number among lobules, and many of them possessed fragmented or irregular nuclei.

The epithelial cells of case 10 and case 11 showed slightly smaller nuclei with vesicular chromatin and less distinct nucleoli than those of cases 4–9. The cell border was well-defined and squamoid appearance was more prominent (Fig. 4D). The irregularity of nuclear contour was recognized as well. Both cases showed several clus-

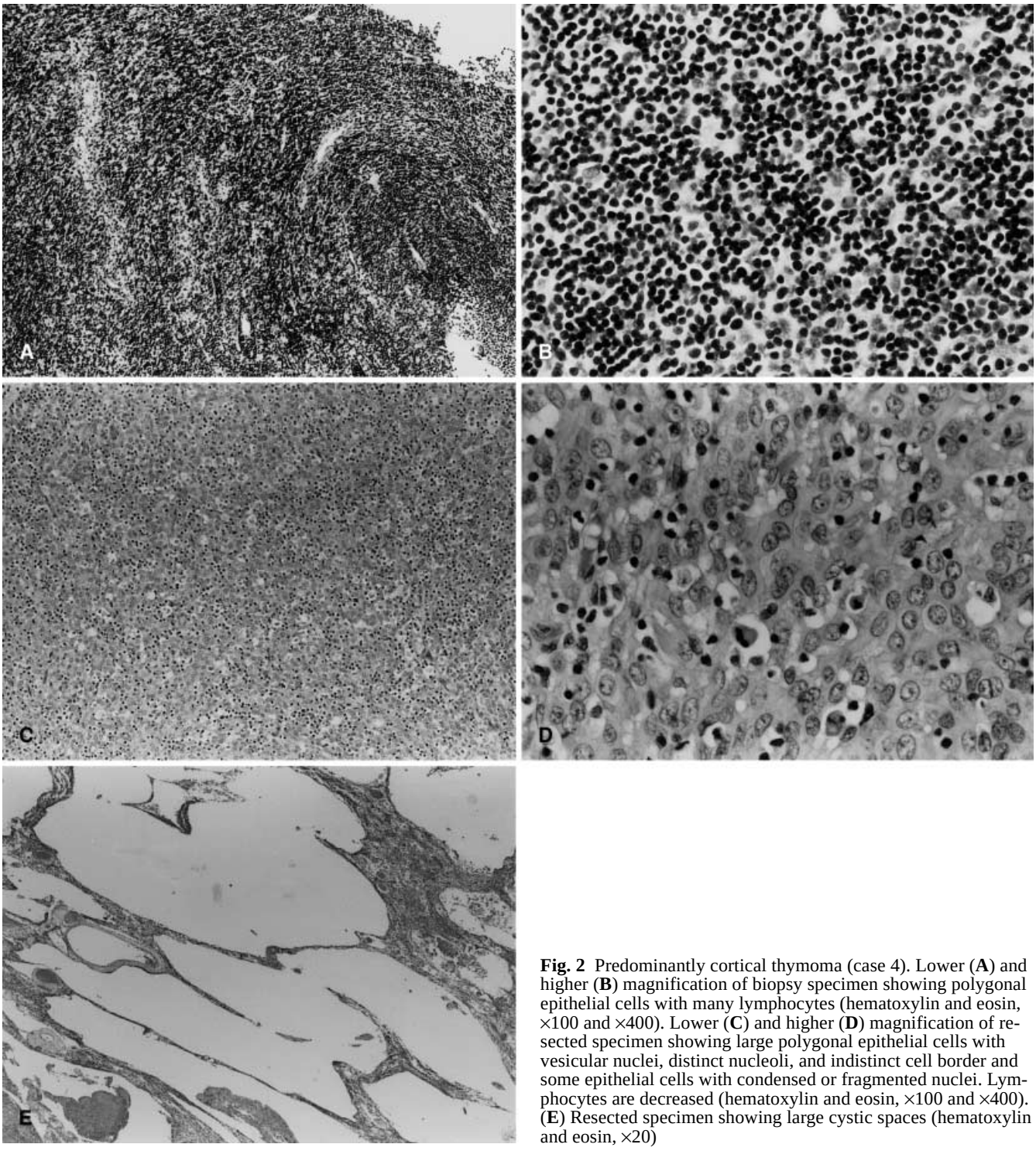

Fig. 2 Predominantly cortical thymoma (case 4). Lower (**A**) and higher (**B**) magnification of biopsy specimen showing polygonal epithelial cells with many lymphocytes (hematoxylin and eosin, ×100 and ×400). Lower (**C**) and higher (**D**) magnification of resected specimen showing large polygonal epithelial cells with vesicular nuclei, distinct nucleoli, and indistinct cell border and some epithelial cells with condensed or fragmented nuclei. Lymphocytes are decreased (hematoxylin and eosin, ×100 and ×400). (**E**) Resected specimen showing large cystic spaces (hematoxylin and eosin, ×20)

ters of epithelial cells with clear cytoplasm. Many perivascular spaces with epithelial palisade were seen throughout the tumors. Cystic structures similar to those in case 4 and case 5 were also focally observed in these cases. Furthermore, tumor cells with large, bizarre, and occasionally multilobulated nuclei were found in some lobules of case 11 (Fig. 4C). In these lobules and the surrounding hyalinized fibrous strands, small foci of calcification were present. Some areas of coagulative necrosis were found in this case. The number of lymphocytes of case 10 and case 11 was scant and showed no remarkable changes between sections of before and after the corticosteroid treatment. However, lymphocytes with fragmented nuclei were easily found in the latter. Mitotic figures were not found in the resected specimens except for that of case 8, which showed one mitosis per 10 HPF.

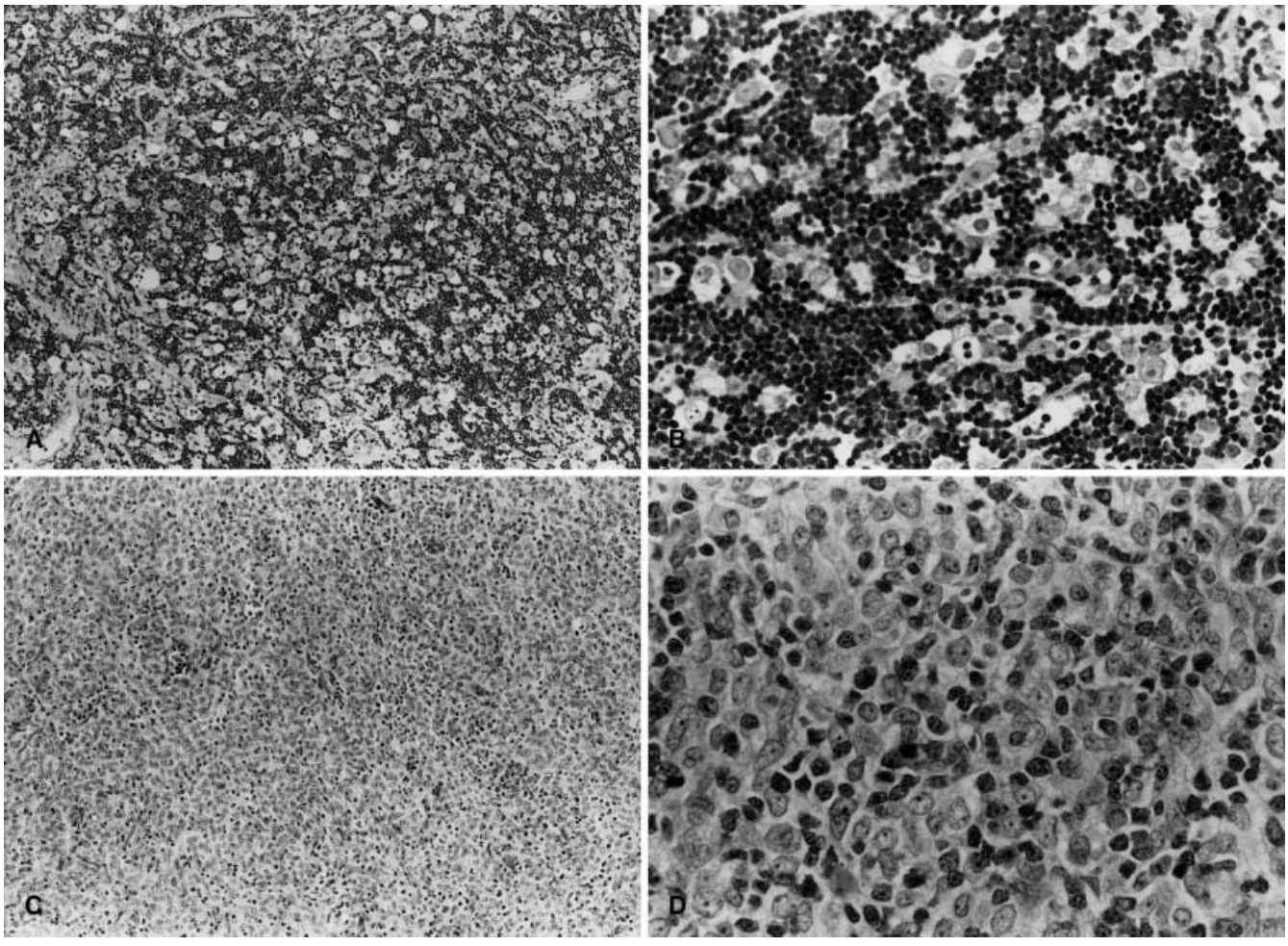

Fig. 3 Cortical thymoma (case 8). Lower (**A**) and higher (**B**) magnification of biopsy specimen showing large polygonal cell with a moderate number of lymphocytes (hematoxylin and eosin, ×100 and ×400). Lower (**C**) and higher (**D**) magnification of resected specimen showing large polygonal epithelial cells with vesicular nuclei, distinct nucleoli, and indistinct cell border. Lymphocytes are decreased (hematoxylin and eosin, ×100 and ×400)

Immunohistochemical findings

The epithelial cells of all cases showed positive staining for anti-cytokeratin antibodies AE1/3 and CAM5.2. The staining patterns of these antibodies were not different between pretreatment biopsy sections and posttreatment surgical sections. Bcl-2 protein expression was observed in case 1 and case 2, in both pretreatment and posttreatment sections, although the staining was weak. The apoptotic and MIB-1 indices of the epithelial tumor cells of biopsy sections showed no differences between lymphocyte-rich areas and lymphocyte-poor areas. The apoptotic indices were generally low in each tumor. However, the indices after treatment with corticosteroids were higher with statistical significance than those before the treatment (P=0.001; Fig. 5A, B). In cases 4, 5, 10, and 11, many epithelial cells around the cystic spaces showed positive staining with the TUNEL method in comparison with those of the other areas (Fig. 5C). The MIB-1 indices showed no significant difference between pretreatment and posttreatment sections (P=0.161), although the indices of the former tended to be higher than those of the latter (Fig. 5D). The epithelial cells with large and bizarre nuclei in some lobules of case 11 showed no increase in the MIB-1 labeling (Fig. 5E). As for immunoreactivity of lymphocytes, almost all lymphocytes of pretreatment sections reacted for CD1a and CD99, even in case 10 and case 11, which were associated with scant lymphocytes. After treatment with corticosteroids, many of the remaining lymphocytes were negative for these antigens.

Discussion

In the present study, in accordance with the previous reports [13, 32], the lymphocytes of all cases were diminished in number and, consequently, the tumors changed to predominantly epithelial-type thymoma after the treatment. Many of the remaining lymphocytes were negative for CD1a and CD99, the membrane antigens of immature T lymphocytes [4, 15], and several of them showed

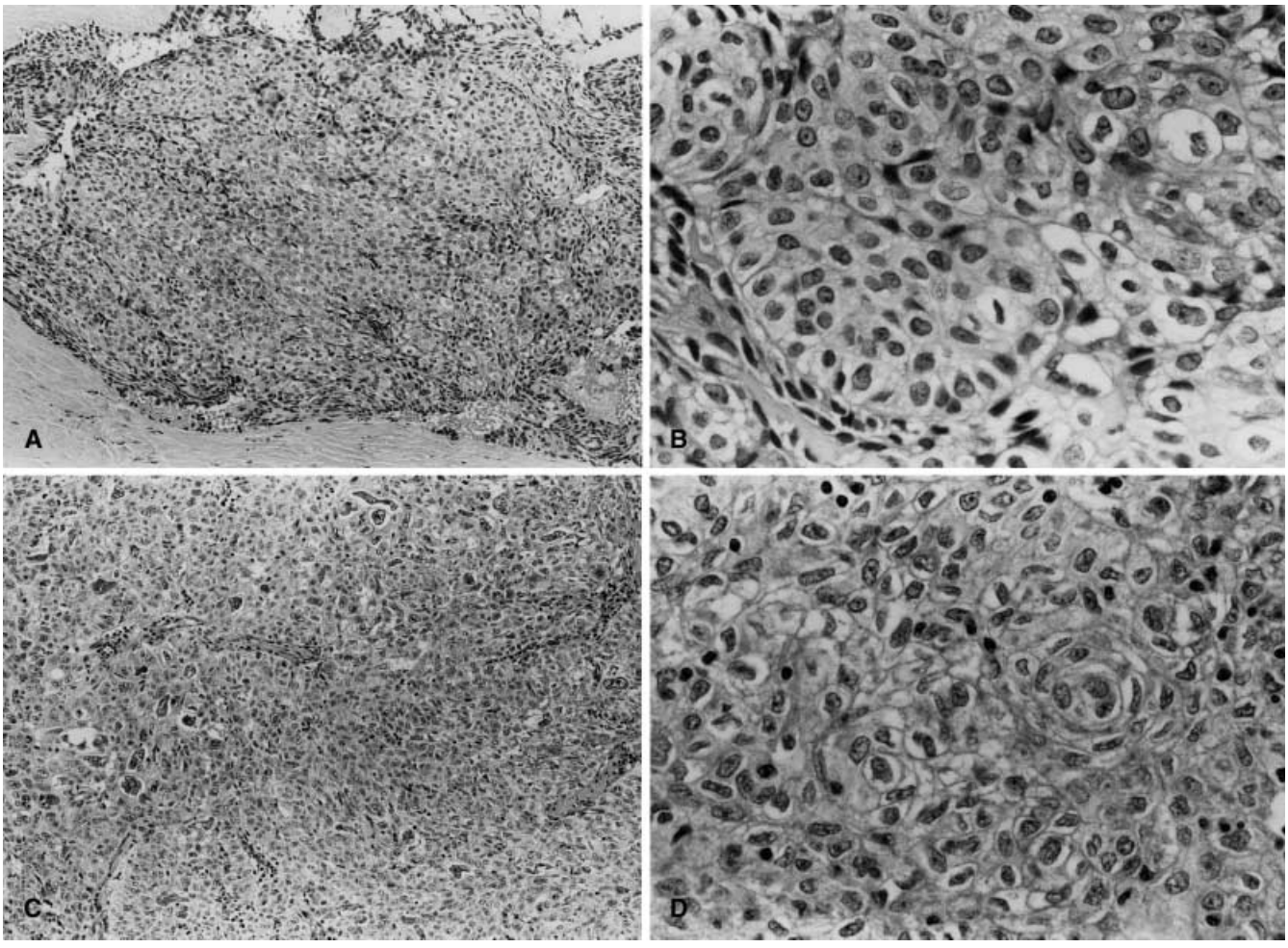

Fig. 4 Well-differentiated thymic carcinoma (case 11). Lower (**A**) and higher (**B**) magnification of biopsy specimen showing large polygonal epithelial cells with distinct cell border (hematoxylin and eosin, ×100 and ×400). Lower (**C**) and higher (**D**) magnification of resected specimen showing large polygonal epithelial cells with squamoid appearance. Some epithelial cells show large, bizarre, and multilobulated nuclei (**C**; hematoxylin and eosin, ×100 and ×400)

condensed or fragmented nuclei. These findings are in keeping with the observation that corticosteroids can cause destruction of immature T-lymphocytes by binding of the steroids to the lymphocytes [26]. Kirchner and colleagues [13] described that the epithelial cells of thymoma after the corticosteroid treatment revealed a sponge-like pattern of large cytoplasm due to the empty pores after the deletion of lymphocytes. In the present study, the prominent histologic features of the posttreatment tumors were the presence of epithelial cells with condensed or pyknotic nuclei and eosinophilic cytoplasm, similar changes as in the lymphocytes. These findings indicate that corticosteroids may cause degenerative changes in the epithelial cells and immature T lymphocytes. Intracytoplasmic vacuolization was also seen in some epithelial cells, but this may represent a nonspecific feature since it was also observed in the epithelial cells of pretreatment biopsy sections. Alternatively, the different findings may be due to the amount of corticosteroids given, which is not described in the previous reports.

Thymoma sometimes shows different histologic features from place to place and it may be impossible to diagnose the whole pretreatment tumor by the biopsy specimens which represent only a small part of the tumor. By investigating multiple blocks from the resected tumors, however, we confirmed that the latter contained no foci showing the features similar to those of the biopsy specimen. Therefore, we consider that the histopathologic differences between pre- and posttreatment tumors can be the changes induced by corticosteroids. A WDTC in biopsy diagnosis (case 11) showed epithelial cells with large, bizarre, and multilobulated nuclei in places after the treatment. Since these cells were accompanied by small foci of calcification and hyalinized fibrous tissue with many macrophages and cholesterol clefts and showed a low MIB-1 labeling index, such nuclear features probably represent degenerative changes. Cystic structures without epithelial lining and with focal hemorrhages, as seen in cystic thymoma [28], were also observed in the resected tumors in cases 4, 5, 10, and 11. The epithelial cells around the cystic spaces showed rel-

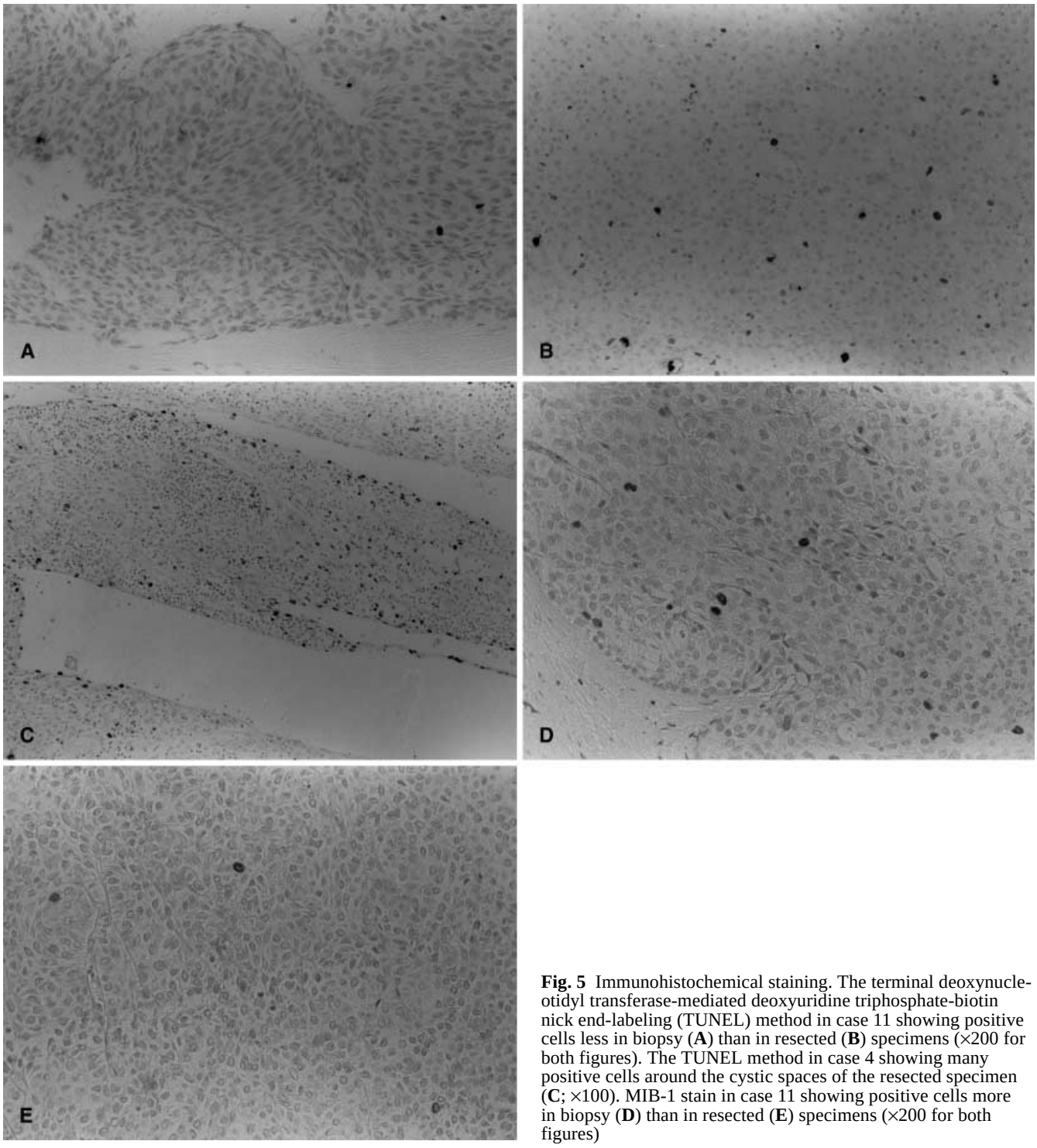

Fig. 5 Immunohistochemical staining. The terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-biotin nick end-labeling (TUNEL) method in case 11 showing positive cells less in biopsy (**A**) than in resected (**B**) specimens (×200 for both figures). The TUNEL method in case 4 showing many positive cells around the cystic spaces of the resected specimen (**C**; ×100). MIB-1 stain in case 11 showing positive cells more in biopsy (**D**) than in resected (**E**) specimens (×200 for both figures)

atively frequent apoptotic cells. Therefore, the cystic changes also seem to represent degeneration. Although these features could be observed in thymomas without corticosteroid treatment [27, 28], those in the present study may have been induced by corticosteroids since the tumors in cases 4, 5, and 10 were significantly diminished in size clinically after the treatment. Furthermore, in case 3, the resected tumor was markedly replaced by hyalinized fibrous tissue, with many remaining epithelial cells having irregular or condensed nuclei. Corticosteroids have also been known to have a direct effect on tumor regression or disappearance in a variety of tumors [22]. The degenerative changes in the epithelial cells may also be caused secondarily by depletion of lymphocytes and subsequent defects in lymphoepithelial interaction. Although such changes do not necessarily

mean a favorable clinical course of the tumors as previously pointed out [28], at least the surgical resection of the tumor becomes easier. Unfortunately, because of a short observation time, it is impossible to estimate the effect of corticosteroid pretreatment on the survival rate.

After treatment with corticosteroids, both predominantly cortical thymomas (cases 4, 5, and 6) and cortical thymomas (cases 7, 8, and 9) changed the histologic features to predominantly epithelial thymoma composed of large polygonal cells. The latter may be confused with WDTC as previously pointed out by Kirchner and colleagues [13]. Although such foci were observed in the present posttreatment specimens of both predominantly cortical and cortical thymomas, WDTC showed more prominent squamoid appearance with a well-defined cell border throughout the tumor. The epithelial cells of predominantly cortical and cortical thymomas showed larger nuclei with more distinct nucleoli and indistinct cell borders than those of WDTC, even after corticosteroid treatment. These histologic features make it possible to distinguish treated predominantly cortical and cortical thymomas from WDTC. In contrast, it seems to be impossible to distinguish predominantly cortical thymoma from cortical thymoma after they are treated with corticosteroids. These two types of thymoma may be closely related tumors with a different ability of attracting T lymphocytes.

The histology of mixed thymoma (case 1 and case 2) changed to that of medullary thymoma by treatment with corticosteroids. The latter included focal glandular and hemangiopericytoma-like features, which are often observed in medullary thymoma [23]. Although the present series contains only two such cases, medullary thymoma and mixed thymoma might also be a related tumor. The frequent coexistence of the components of medullary thymoma in mixed thymoma [23, 27] and bcl-2 oncoprotein expression in both types [5, 30] could support this hypothesis.

In the present study, fungal infection developed in three patients who were treated with oral corticosteroids for relatively long periods. While patients with unresectable or recurrent tumor showed clinical response to corticosteroids in several case reports [11, 22, 29], one such patient was complicated with nocardiosis after steroid therapy [2]. Apparently, investigation of an increased number of cases with steroid pretreatment is required. However, the increase of the apoptotic index and decrease of the MIB-1 proliferative index of the tumor cells after the present pretreatment may support the application of this type of therapy for thymomas in advanced stages, although attention should be paid to infection after surgery.

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