

# Immunohistochemical study of a case of malignant Müllerian mixed tumor in comparison with the activity of normal uterine tissue

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**Summary.** A case of malignant Müllerian mixed tumor of the uterus, exhibiting a histology of heterologous osteosarcomatous differentiation, is presented. Special emphasis is placed on the characteristic immunohistochemical reactivity of the tumor tissue in comparison with that of normal uterine tissue in the proliferative phase. Keratin, cytokeratin, epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), and human chorionic gonadotropin (HCG) were found only in the carcinomatous element. However, neuron specific enolase (NSE), S-100 protein (S 100) and vimentin were identified in almost all tumor tissue elements. Desmin and actin were not stained in any elements. Myoglobin was only detected weakly in the squamous carcinomatous element. Undifferentiated cell element, composed of small, round, spindle, or polygonal cells showed positive reactions to NSE and S 100, but not to any other antibodies. As compared to the reactivities of the normal proliferative endometrium, the glandular epithelial cells were positive with NSE, S 100, vimentin and CEA, but the stromal cells were only positive with vimentin. Such a multitudinous and concomitant expression of antigenicity to the different tumor elements indicates a close relationship to its mesodermal Müllerian origin, and NSE, S 100 and vimentin might be most adequate indicators of these types of tumors.

**Key words:** Uterine sarcoma, Müllerian tumor, Immunohistochemistry

## Introduction

Malignant Müllerian mixed tumor of the female genitalia is histologically characterized by the presence of both carcinomatous and sarcomatous elements. This

unusual tumor among gynecologic malignancies can be histologically classified mainly into three types; a homologous type, with a nonspecific mesenchymal element which is also called a carcinosarcoma, a heterologous type, with well differentiated mesenchymal elements, such as striated muscle, cartilage, fat, osteoid or bone (Genton, 1983), and a distinctive type of Müllerian adenosarcoma (Clement and Scully, 1974).

In the present report, different types of tumor cells, probably of mesodermal origin in the Müllerian mixed tumor, are immunohistochemically examined for various epithelial and non-epithelial antigens, including some intermediate filaments in comparison with the reactivities of the normal proliferative endometrium and other uterine tissues, using the avidin-biotin immunoperoxidase complex (ABC) method.

## Materials and methods

### Case report

A 57-year-old woman was admitted complaining of consistent lower abdominal pain and vaginal bleeding. The uterus was enlarged over fist-size ( $6.5 \times 6.0 \times 6.0$  cm). Subtotal hysterectomy with right salpingo-oophorectomy was performed, uterine adenomyosis having been diagnosed.

The cut section of the uterus and ovary with the fallopian tube revealed that a large part of the uterine cavity and tubal lumen was filled with blood. But in the uterine cavity, a polypoid tumor protruding from the endometrium was found, which showed irregular areas of hemorrhage, necrosis, myxoid degeneration, or sand-like ossification which had invaded the middle layer of the myometrium (Fig. 1).

Formalin-fixed and paraffin-embedded blocks of the tumor and the normal uterus in the proliferative phase were cut on serial sections, 5  $\mu$ m in thickness. Routinely deparaffinized sections of both tissues were provided for the histological and histochemical examinations using

hematoxylin and eosin stain (HE), periodic acid-Schiff stain (PAS), Azan-Mallory stain, alcian blue stain, reticulin stain and phosphotungstic acid-Schiff stain (PTAH), and for immunohistochemical examination.

Immunohistochemical stains were carried out with a staining kit using the ABC method produced from various sources, and following 11 different types of antibodies (immune animal, source) were used in the present study: keratin (rabbit; 44-57 KD, Biomeda), cytokeratin (mouse, Lipshaw/Immunon), EMA (mouse, Lipshaw/Immunon), HCG (rabbit, Biomeda), CEA (rabbit, Biomeda), NSE (rabbit, BioGenex), S 100 (rabbit, Biomeda), vimentin (mouse, BioGenex), actin (mouse, BioGenex), myoglobin (rabbit, Biomeda), and desmin (mouse, Biogenex). Each of the stains was provided together with approved sections for positive controls, and omissions of the primary antibodies were done for the negative controls.

## Results

### *Microscopic and histochemical findings*

The tumor was admixed with carcinomatous, undifferentiated carcino-sarcomatous, and spindle cell sarcomatous elements (Figs. 2-4). Carcinomatous element was composed of tubular adenocarcinoma for the most part, with some keratinizing squamous cell carcinoma (SCC). Sarcomatous element consisted of spindle cells resembling endometrial stromal cells, fibroblasts, or smooth muscle cells, in which osteoid, or bone, with or without calcification, was massively formed. In the area of undifferentiated cell element, small anaplastic cells in round, polygonal, or stellate shapes proliferated in sheet-like or somewhat epithelial arrangement, and showed a transition to spindle sarcoma element, and to SCC element.

Histochemically, dense collagen fibers and fine reticulin fibers were found not only in osteoid, or bone, but also in other matrices. Myxoid change was seen, too. A little PAS-positive mucus was found in the adenocarcinoma area, but much alcian blue-positive mucus was found in both carcinomatous and sarcomatous areas. In the undifferentiated area, a few large spindle cells and bizarre multinucleated cells were seen but did not demonstrate cytoplasmic striation with PTAH stain. Other heterologous elements such as striated muscle, fat or cartilage could not be detected in the tumor tissue.

### *Immunohistochemical findings*

The reactivity of the four types of tumor cells (adenocarcinoma cells, SCC cells, sarcoma cells and undifferentiated cells) and four types of uterine tissue cells (endometrial glandular epithelial cells, endometrial stromal cells, myometrial smooth muscle cells and endothelial cells of the blood vessels) were investigated and compared with each other. The results are as shown in Table 1.

Keratin was intensely positive only on the SCC cells

(Fig. 5). Cytokeratin and EMA were weakly to moderately positive on only carcinoma cells of both SCC- and adenocarcinoma-elements. The endometrial gland cells were also weakly positive on cytokeratin. HCG and CEA were weakly positive on only the SCC cells, but not on the sarcoma cells and undifferentiated cells. Interestingly, CEA was moderately positive on the endometrial gland cells, secreting positive fluid into the lumen (Fig. 6). Particularly, NSE and S 100 were weakly to intensely positive on all elements in the tumor tissue. That is, NSE was moderately positive on the sarcoma cells and intensely positive on the undifferentiated cells (Fig. 7) and also moderately positive on the endometrial gland cells (Fig. 8). S 100 was moderately positive on all the elements (Fig. 9) and also to both endometrial epithelium (Fig. 10) and myometrial muscle cells. Vimentin was intensely positive on the spindle sarcoma cells (Fig. 11), weakly positive on the adenocarcinoma cells, moderately positive on the endometrial epithelium, and weakly positive on the endometrial stromal cells (Fig. 12), moderately positive on the myometrial muscle cells, and intensely positive on the endothelial cells of the blood vessels, but negative on the undifferentiated tumor cells. Myoglobin was weakly positive only on SCC cells in the tumor elements, but moderately positive on the myometrial muscle cells. Actin and desmin were negative to most of the elements, but intensely positive on only the myometrial muscle cells.

## Discussion

The present study demonstrated the immunohistochemical diversity of histological elements, epithelial or mesenchymal in nature, in malignant Müllerian mixed tumor originating in the endometrium. The results may well reflect the origin of this type of uncommon endometrial malignancy which was considered to be the mesodermal Müllerian tube.

Under electron microscope, endometrial sarcomas of the uterus revealed a basically immature cell type in all endometrial sarcomas, irrespective of whether they were of the pure homologous or of the mixed type, which suggested that cells of the early proliferating endometrium should all originate as endometrial sarcomas from immature stromal cells because of the striking ultrastructural similarity between these cells (Böcker and Stegner, 1975).

Lifschitz-Mercer et al. (1987) have reported that the tumor cells of the endometrial stromal sarcomas, as well as the mesenchymal elements of the mixed Müllerian tumor, were decorated exclusively with antibodies to vimentin, but desmin was not demonstrated in these tumor cells. According to the most recent report by Chung et al. (1988), both epithelial and mesenchymal elements of mixed Müllerian tumor are demonstrated well with vimentin rather than S 100, but desmin was only found in the components of leiomyosarcomas or rhabdomyosarcomas. This data may be understandable because of the data from Gown and Gabbiani's study (1984) that vimentin can be demonstrated from the early



1. Extirpated uterus showing polypoid tumor in the dilated endometrial cavity with blood. The tumor reveals areas of hemorrhage, necrosis and calcification, with invasion into the myometrium.

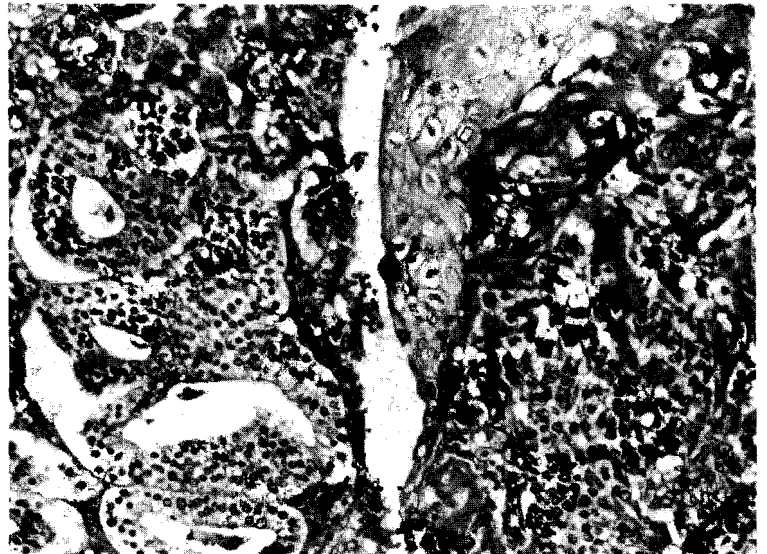
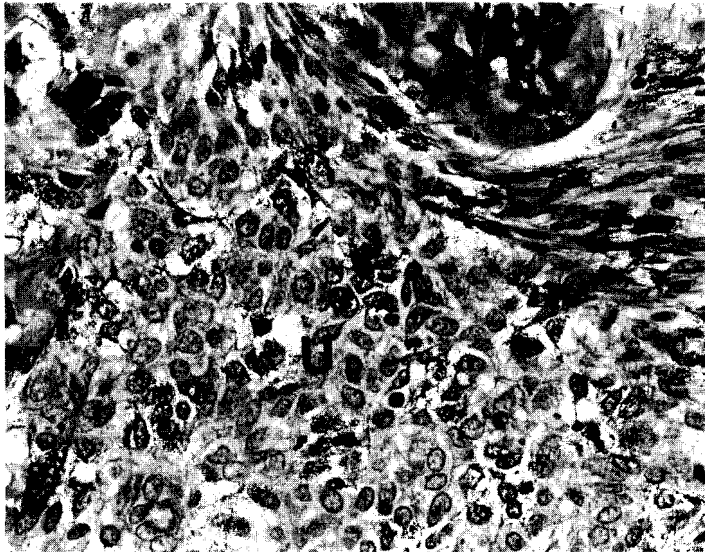


Fig. 2. The tumor showing histologically different elements of well-differentiated adenocarcinoma (left one-third), keratinizing SCC (middle) and osteoblastic sarcoma (right one-third). HE,  $\times 100$  (original).



3. The tumor showing calcified bone and osteoid formation in osteoblastic sarcoma, undifferentiated cell areas (U) and spindle cell sarcoma. HE,  $\times 200$  (original).

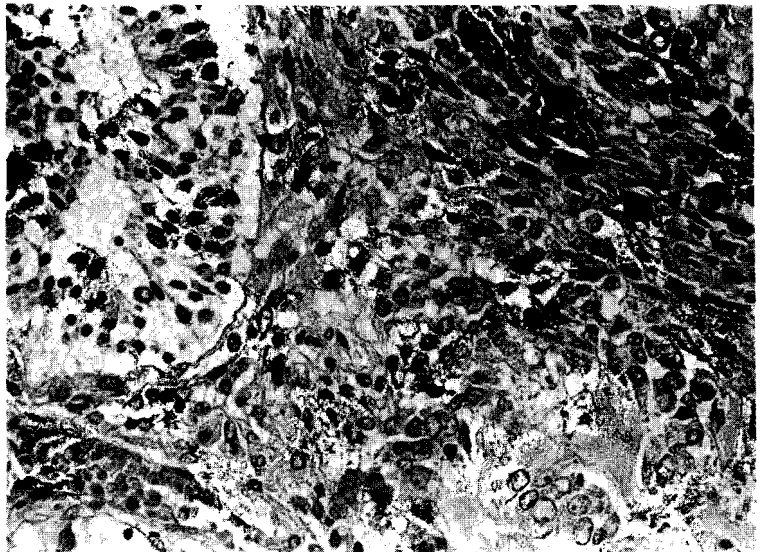
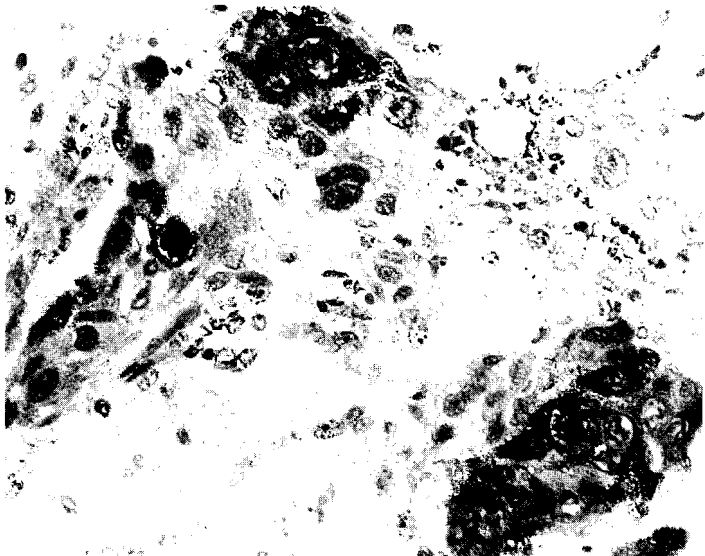


Fig. 4. The tumor showing areas of osteoblastic sarcoma, spindle cell sarcoma and adenocarcinoma. HE,  $\times 200$  (original)



5. Tumor element of SCC showing intensely positive reaction with keratin, immunohistochemistry.  $\times 200$  (original).

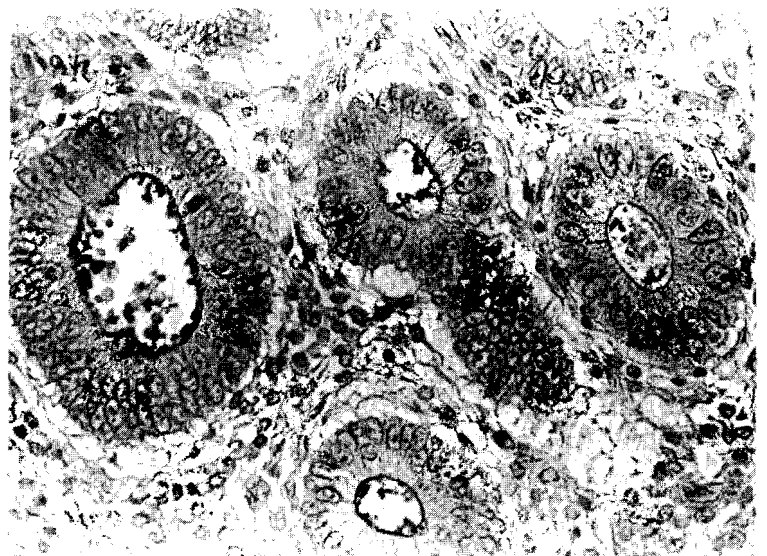
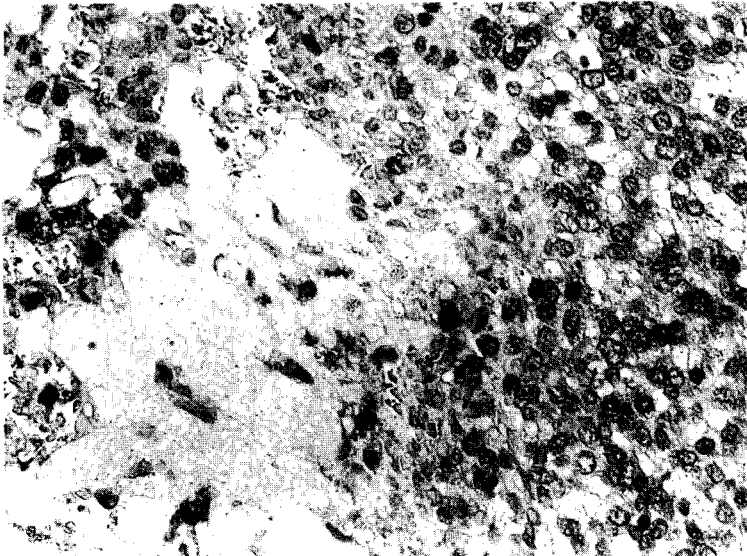


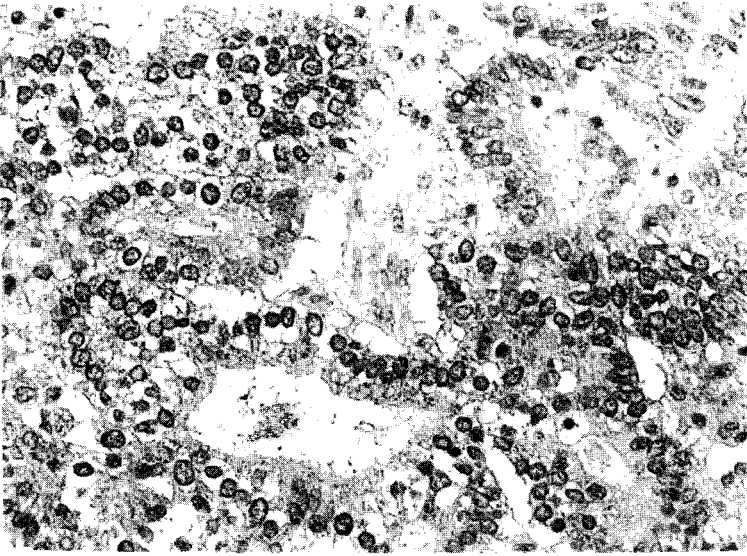
Fig. 6. Normal endometrium showing positive reaction of the glandular epithelium with CEA and also the secretory fluid into the lumen. Immunohistochemistry,  $\times 200$  (original).



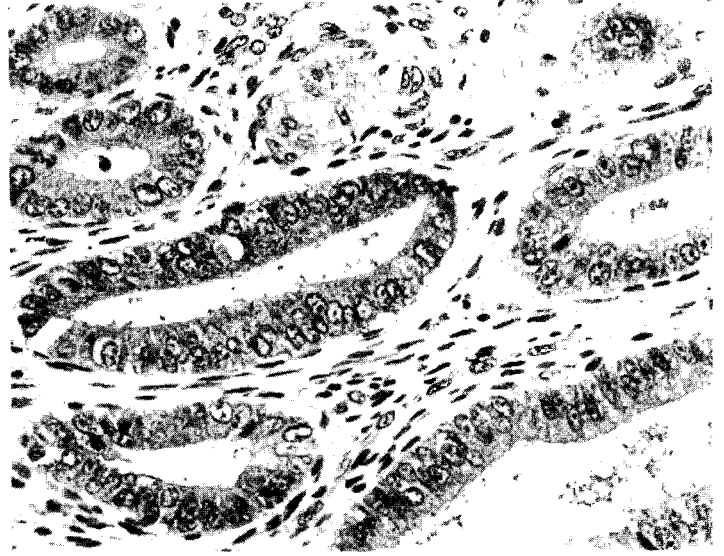
**Fig. 7.** Tumor element of undifferentiated cells showing intensely positive reaction with NSE. Immunohistochemistry.  $\cdot$  200 (original).



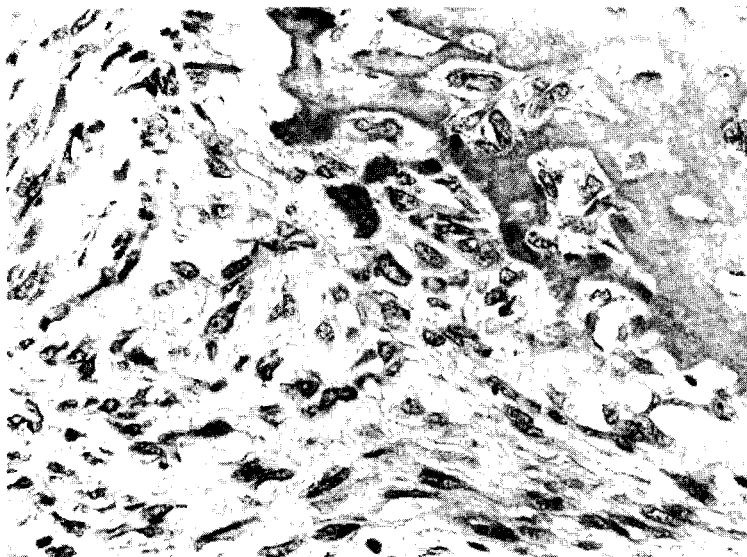
**Fig. 8.** Normal endometrium showing positive reaction of the glandular epithelium with NSE. Immunohistochemistry.  $\cdot$  200 (original).



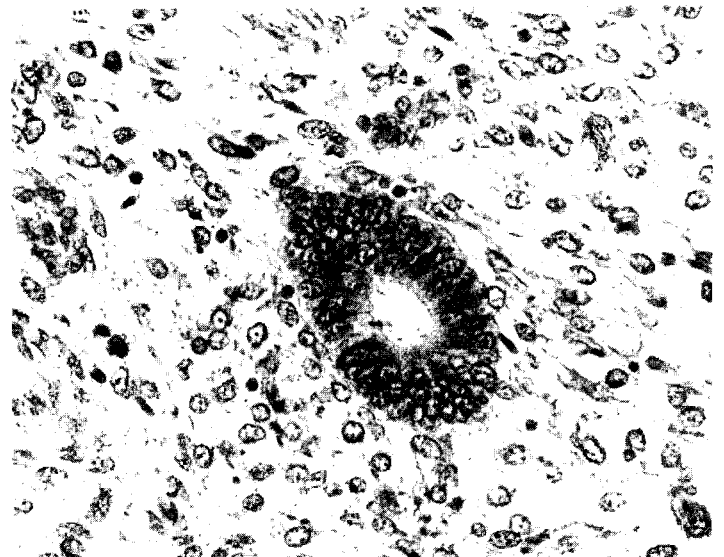
**Fig. 9.** Tumor element of adenocarcinoma showing positive reaction with S 100. Immunohistochemistry.  $\cdot$  200 (original).



**Fig. 10.** Normal endometrium showing positive reaction of the glandular epithelium with S 100.  $\cdot$  200 (original).



**Fig. 11.** Tumor elements of osteoblastic and spindle cell sarcoma showing intensely positive reaction with vimentin.  $\times$  200 (original).



**Fig. 12.** Normal endometrium showing positive reaction of the glandular epithelium with vimentin and also weakly positive reaction of the stroma. Immunohistochemistry,  $\times$  200 (original).

**Table 1.** Immunohistochemical reactivities of different types of tumor cells in malignant Müllerian mixed tumor in comparison with those of cells in normal uterine tissue in the proliferative phase.

Antibodies	Malignant Müllerian mixed tumor				Uterus on proliferative phase			
	Carcinoma cells		Sarcoma cells	undifferentiated cells	Endometrial cells		Myometrial cells	
	Adeno. car.	SCC			Glands	Stroma	Muscle	Endothelium
Keratin	-	3+	-	-	-	-	-	-
Cytokeratin	+	+	-	-	+	-	-	-
EMA	+	2+	-	-	-	-	-	-
HCG	-	+	-	-	-	-	-	-
CEA	-	+	-	-	2+*	-	-	-
NSE	+	+	2+	3+	2+	-	-	-
S 100	2+	2+	2+	2+	2+	-	2+	-
Vimentin	+	-	3+	-	2+	+	2+	3+
Actin	-	-	-	-	-	-	3+	-
Myoglobin	-	+	-	-	-	-	2+	-
Desmin	-	-	-	-	-	-	3+	-

-: negative, +: weakly positive, 2+: moderately positive, 3+: intensely positive SCC: squamous cell carcinoma, \*: expelling CEA-positive materials into the lumen

stage of embryogenesis, but that desmin is only present in mature muscle cells.

Chung et al. (1988) emphasized that keratin was detected in both epithelial and mesenchymal elements of malignant Müllerian tumor as well as the report by Geisinger et al. (1987), in which they described 3 possible explanations for the presence of epithelial antigen in the sarcomatous neoplastic cells. One of the explanations was that cells exhibiting keratin in the sarcomatous areas are mesenchymal cells with early epithelial differentiation. Contrary to these data, Bonazzi del Poggetto et al. (1983) and Ramaekers et al. (1985) reported no keratin in the mesenchymal elements, just as in the present data, but they furthermore described no vimentin in epithelial areas, in contrast to the present data. Manivel et al. (1986) also identified NSE, like the present study, and Leu 7 in two cases of mixed Müllerian tumor and claimed that this tumor was capable of differentiating towards becoming a neuroendocrine carcinoma.

In the immunohistochemical observations, the well differentiated elements exhibit marker antigens such as keratin for SCC and EMA for adenocarcinoma. However, the most interesting data in the present study are that the concomitant and multitudinous expression of various antigens on the epithelial, non-epithelial and undifferentiated tumor cells in the different elements of Müllerian mixed tumor, as exhibited in the epithelial and stromal cells of the endometrium, which must be a reflection of their immunohistological identity as a result of their concordant histogenesis deriving from the mesodermal Müllerian tube.

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