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Epithelial membrane antigen expression in cholangiocarcinoma

An useful immunohistochemical tool for differential diagnosis with hepatocarcinoma

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Summary. Twenty-two cases of primary hepatic tumours (16 hepatocarcinoma and 6 cholangiocarcinoma) have been studied by immunoperoxidase technique, for the presence of Epithelial Membrane Antigen (EMA). All 6 cases of cholangiocarcinoma showed positive reaction for the presence of EMA while 14 out of 16 hepatocarcinomas were completely negative. In two cases of hepatocarcinoma focal positive cells were present. The results obtained suggest that EMA could be of valuable use, in surgical pathology, for discriminating hepatocarcinoma from cholangiocarcinoma.

Key words: Epithelial membrane antigen – Immunohistochemistry – Hepatic tumours

Anti Epithelial Membrane Antigen (E.M.A.) serum was first obtained immunizing rabbits with milk fat globule membrane prepared by defatting human cream (Ceriani et al. 1977; Heyderman et al. 1979). This antiserum (which succesfully detects EMA on paraffin embedded specimen and is now commercially available) reacts with various normal and neoplastic epithelia and is heterogeneously distributed in different tissues (Heyderman et al. 1979; Sloane et al. 1981 and 1982).

In normal liver EMA is found only in the cells lining bile ducts whereas hepatocytes are consistently negative (Sloane et al. 1981).

This observation prompted us to evaluate the potential use of anti EMA serum in the differential diagnosis of cholangiocarcinoma versus hepatocarcinoma. In fact this differential diagnosis when based only on morphological grounds can often be difficult.

In this paper we report the results obtained in 6 cases of cholangiocarcinoma and 16 cases of hepatocarcinoma immunohistochemically studied for the presence of EMA.

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Table 1

Case	Sex	Age	Source	EMA staining	
				Luminal	Cytoplasmic
Cholangiocarcinoma					
1	M	69	Liver biopsy	+ +/+ + +	+/+ +
2	F	51	Liver biopsy	+ +/+ + +	+/−
3	M	33	Liver biopsy	+/+ +	+/−
4	F	32	Liver biopsy	+ +/+ + +	+/−
4b			Lymph node met.	+/+ +	+/+ +
5	M	58	Hemihepatectomy	+/+ +	+/−
6	M	56	Liver biopsy	+/+ +	+/+ +
Hepato cellular carcinoma					
1	M	58	Liver biopsy	−	−
2	M	72	Liver biopsy	−	−
3	M	56	Hemihepatectomy	−	−
4	M	76	Liver biopsy	−	−
5	M	67	Liver biopsy	−	−
6	M	64	Hemihepatectomy	+/− (focal)	−
7	F	52	Hemihepatectomy	−	−
8	F	67	Hemihepatectomy	−	−
9	M	61	Hemihepatectomy	−	−
10	F	63	Hemihepatectomy	+/− (focal)	−
11	M	78	Liver biopsy	−	−
12	M	66	Hemihepatectomy	−	−
13	F	67	Hemihepatectomy	−	−
14	M	64	Liver biopsy	−	−
15	M	57	Hemihepatectomy	−	−
16	M	53	Hemihepatectomy	−	−

Materials and methods

Twenty-two surgical specimens of primary hepatic carcinoma (6 cholangiocarcinomas and 16 hepatocarcinomas) were selected from the files of our department; in addition one lymph node with metastasis of cholangiocarcinoma was added (case 4, Table 1).

Only histologically typical cases were included in this study.

4–6 μm sections were cut from routinely formalin fixed, paraffin embedded blocks, deparaffinized with Xylene and rehydrated in buffered saline. Immunoperoxidase staining was performed using adsorbed goat antiserum specific for EMA (Sera-lab) (dilution 1:800, incubation 1 h) using the PAP method of Sternberger (1974). Rabbit anti-goat Ig (dilution 1:50, incubation 30 min) and PAP complex (dilution 1:100, incubation 30 min) were purchased from DAKO.

The final staining step for peroxidase was performed using 3-3′ diaminobenzidine in ethylenglycolemonomethylether and hydrogen peroxide (Chilosi et al. 1981).

Positive controls were obtained including in every run a section of normal breast tissue, and, for negative controls, a section of lymph node.

Additional negative controls were obtained substituting goat antiEMA serum with PBS.

Positive findings were graded from + to + + + according to the intensity of reaction, and described as luminal or cytoplasmic, according to the location of reaction.

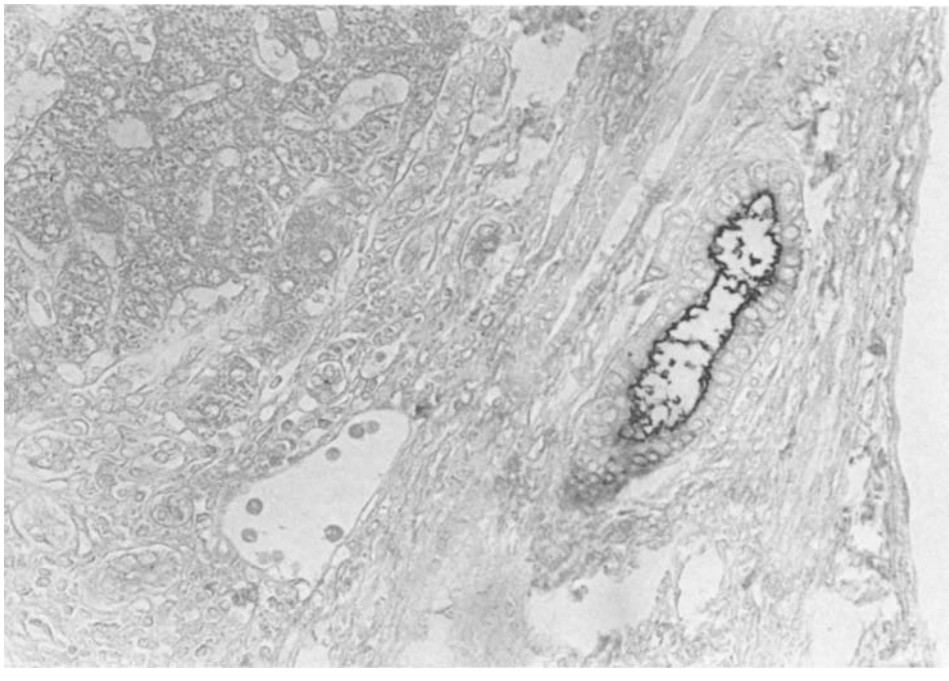

Fig. 1. Large portal tract in normal liver; positive reaction (*black*) in luminal membranes of bile duct-lining cells (*on the right*). Hepatocytes (*on the left*) are negative. (Immunoperoxidase for Epithelial Membrane Antigen ×250; no counterstain)

Results

Normal liver

Bile ducts present in normal liver sorrounding tumours constantly exhibited specific staining for EMA while hepatocytes were completely negative. The positivity was mainly located on luminal borders and graded +/++ (Fig. 1).

Cholangiocarcinoma

A specific immunostaining for EMA was evident in all the 6 specimens of cholangiocarcinoma. The neoplastic cells which formed glandular structures constantly exhibit a strong luminal and, with less intensity, cytoplasmic staining (Fig. 2a, b) (Table 1).

Hepatocarcinoma

The 16 cases of hepatocarcinoma showed different histological pattern and were classified according to Peters (1976) as trabecular, acinar, pseudoglandular and adenoid.

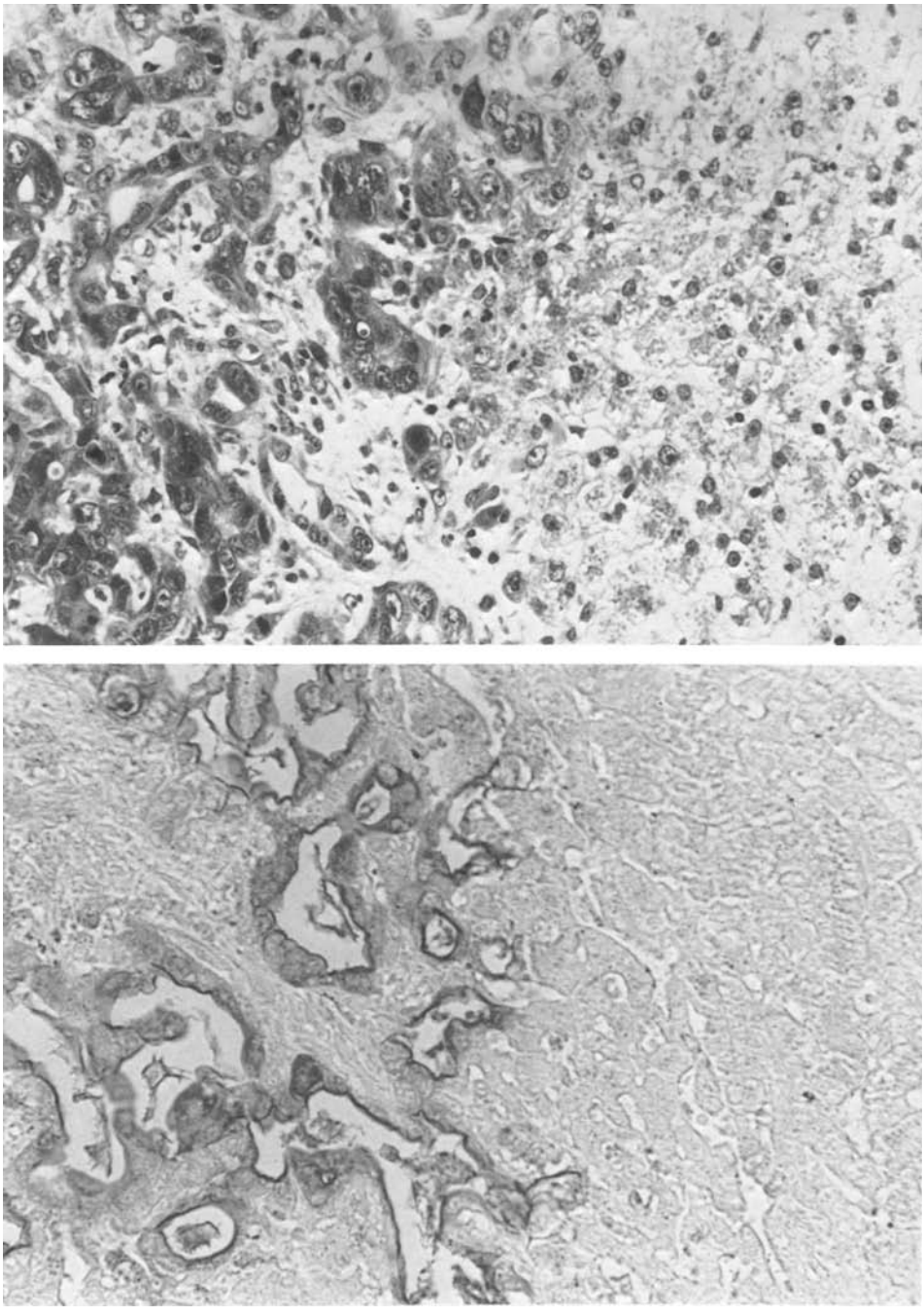

Fig. 2. **A** Cholangiocarcinoma (*left*) infiltrating sorrounding liver (*right*). (HE ×250) **B** Same area of **A** in sequent section. Cholangiocarcinoma (left) stains positively on luminal borders (*black*) of glandular spaces while non-neoplastic liver (*right*) is negative. (Immunoperoxidase for Epithelial Membrane Antigen ×250; no counterstain)

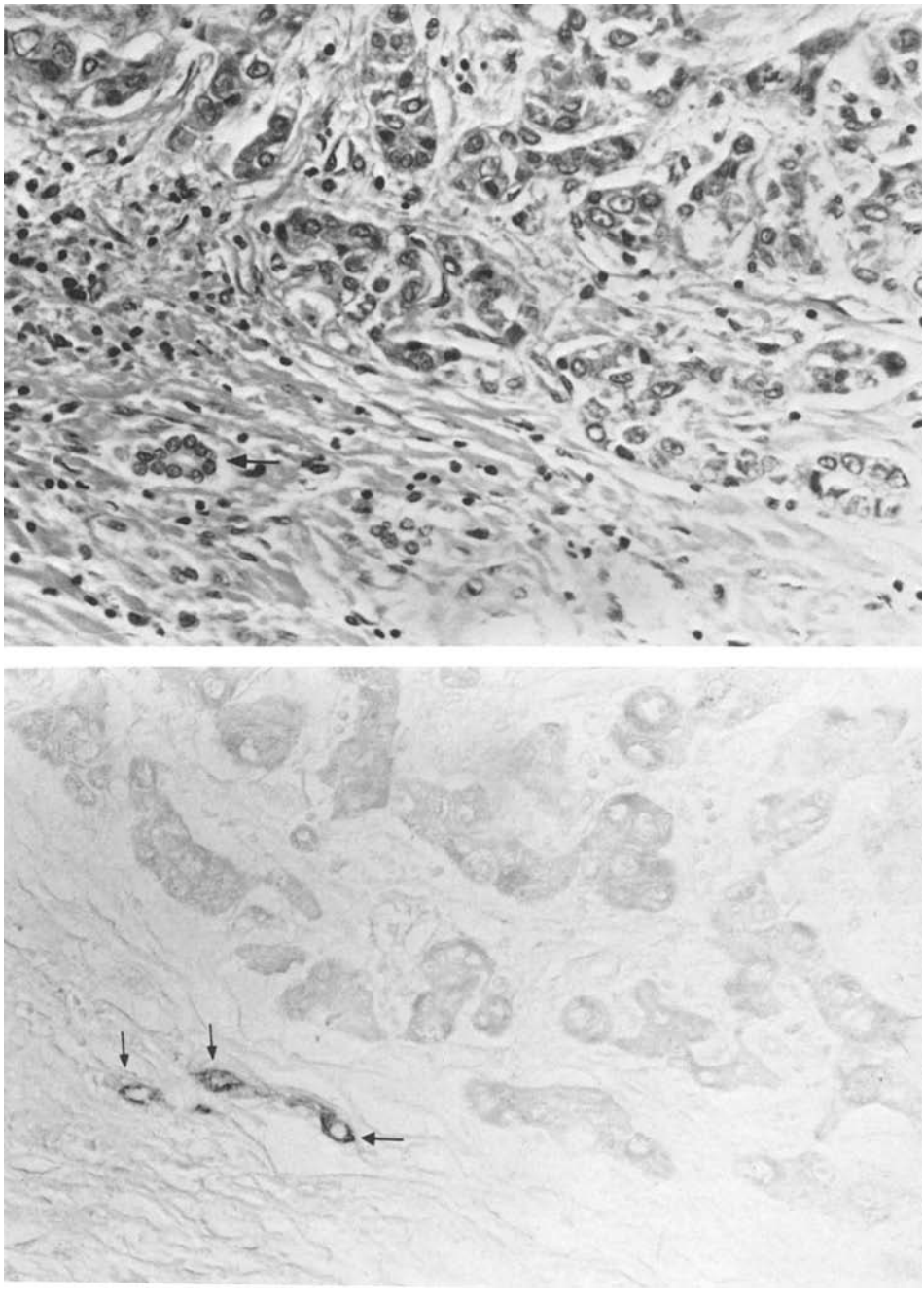

Fig. 3. **A** Hepatocarcinoma sorrounding portal tract, a normal bile duct is present on the left (*arrow*) (HE ×250). **B** Same area of **A** in sequent section; normal bile duct stains positively (*arrow*) while neoplastic hepatocytes are negative. (Immunoperoxidase for Epithelial Membrane Antigen ×250; no counterstain)

In most cases different histological patterns were evident but the trabecular type was predominant.

In one case (# 12) an adenoid pattern was prevailing. When studied by Immunoperoxidase, EMA was completely absent in both cytoplasm and membrane of neoplastic cells, in 14 out of 16 cases studied (Fig. 3).

In the other 2 cases (histologically described as trabecular and pseudoglandular) only focal areas with a weak membrane staining (graded +/−) were observed, while the rest of the tumor was negative (Table 1).

Discussion

Epithelial Membrane Antigen (E.M.A.) is widely distributed in, but confined to, epithelium and mesothelium and is expressed by many tumours derived from them (Sloane et al. 1981). Within the liver it is present in the epithelial cells lining bile ducts and negative in hepatocytes. Sloane et al. (1981) reported 3 cases of hepatocarcinoma and described all of them as EMA negative.

In this study we can confirm that observation in further 14 cases of hepatocarcinoma. In addition we could demonstrate a positive EMA immunostaining in all the cases of cholangiocarcinoma studied (6 cases).

In normal bile ducts EMA was confined to the membrane of cells lining bile ducts whereas in cholangiocarcinoma as observed in other neoplastic epithelia (Sloane et al. 1981) the antigen was found also within the cytoplasm of neoplastic cells.

The presence of EMA in all the cases of cholangiocarcinoma and the negative finding in 14 out of 16 cases of hepatocarcinoma show that primary hepatic tumors tend to retain the heterogeneous EMA expression of their normal counterparts.

The presence of EMA in 2 out of 16 hepatocarcinomas could indicate that hepatocytes through neoplastic transformation can rarely acquire this antigen which is not normally present.

As hepatocytes and cells lining bile ducts have a common origin, this finding is not surprising (Mac Sween and Scothorne 1979). The variable (+/−) distribution of EMA in these 2 cases could alternatively correspond to mixed form of hepato-cholangiocarcinoma lacking an evident histological appearance.

An extensive study of other types of hepatic tumours such as cholangiolocarcinoma and mixed form of hepato-cholangiocarcinoma could bring more information on this regard.

With the exception of these 2 cases where results for the presence of EMA were in contrast with histological type, it appears that EMA can be of valuable use in surgical pathology for discriminating hepatocarcinoma from cholangiocarcinoma especially when only small biopsies (i.e. needle biopsy) are available for study. Nevertheless it must be stressed that the presence of EMA can by no means be considered characteristic of cholangiocarcinoma as its presence has been reported in most carcinomas (Sloane and Ormerod 1981) and therefore it is of no use in discriminating between cholangiocarcinoma and hepatic metastases.

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