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Human chorionic gonadotrophin and alpha-fetoprotein in testicular germ cell tumours: a retrospective immunohistochemical study

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A series of testicular germ cell tumours (46 seminomas and 27 non-seminomas) was studied immunohistochemically with regard to the presence of αFP and HCG. In three seminomas, HCG reactive syncitiotrophoblast-like giant cells (STLG) were found. Immunoreactive αFP did not occur in seminomas. In differentiated mature teratomas HCG or αFP could not be demonstrated. In embryonal carcinomas with or without teratoma (MTI/MTU/MTT) HCG immunoreactivity was found in 83%, usually localized in STLG. In 75% of these tumours αFP could be demonstrated. This protein was localized in foci of endodermal sinus or yolk sac differentiation, but also in single cells and cell clusters in areas of embryonal carcinoma. In some cases syncitial cells were present which contained both HCG and αFP. Immunostaining of tumour markers appeared not to provide important additional criteria for classification of these tumours in the currently available classifications. The significance of HCG containing STLG in seminomas deserves further investigation. Prospective studies of embryonal carcinoma with or without teratoma (MTI/MTU/MTT) will be necessary to evaluate the possible prognostic importance of the presence of αFP or HCG or both.

Keywords: germ cell tumours, testis, immunoperoxidase, alpha-fetoprotein, chorionic gonadotrophin

Introduction

Germ cell tumours of the testis are often associated with high blood levels of α_1-fetoprotein (αFP) and chorionic gonadotrophin (HCG) (Braunstein, McIntire &

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Waldmann 1973, Abelev 1974, Kohn, Orr & McElwain 1976). Elevated levels of these substances are found in the blood in up to 75% of non-seminoma tumour patients (Waldmann & McIntire 1974). In seminomas measurable blood levels of HCG have been reported in up to 20% of the cases (Newlands *et al.* 1976). In these tumours αFP does not or only rarely occurs. Blood levels of these markers are used to monitor the reaction of the tumour to treatment (Schultz *et al.* 1978) and for the early detection of recurrence (Lange *et al.* 1976, Perlin *et al.* 1976).

Based on clinicopathological association, αFP was thought to be produced in endodermal sinus tumour (Itoh *et al.* 1974, Nørgaard-Pedersen, Albrechtsen & Teilum 1975) or areas of this histological type of growth in teratomas and HCG in choriocarcinoma or syncitiotrophoblast-like giant cells (STLG) in other germ cell tumour types. Recent immunohistochemical investigations (Kurman *et al.* 1977, Javadpour, McIntire & Waldmann 1978, Hedinger, von Hochstetter & Egloff 1979) confirmed these observations. In addition a new classification of germ cell tumours was proposed on immunohistochemical staining patterns (Kurman *et al.* 1977, Taylor, Kurman & Warner 1978).

It seems unlikely, however, that immunohistochemical staining for these marker substances will provide more discriminating criteria than the presently available classifications because either one or both occur in up to 75% of non-seminoma germ cell tumours. Accordingly, areas of endodermal sinus tumour or choriocarcinoma and syncitiotrophoblast-like giant cells are frequently found in embryonal carcinoma (Talerman 1975) and less frequently also in teratoma (Kurman *et al.* 1977). Therefore the present investigation was undertaken to study:

1 The value of immunohistological criteria for testicular germ cell tumour classification according to Pugh & Cameron (1976) and to Mostofi & Price (1973).
2 The histological structures which contain immunoreactive HCG and αFP.
3 Whether or not these substances occur in completely different histological structures and cell types.

Materials and methods

CASES

From the files of the Department of Pathology 73 cases of germ cell tumour of the testis were selected. These cases were orchidectomy specimens, obtained at surgery between 1956 and 1979, of which embedded tissue blocks were available. All tissues had been fixed in buffered neutral 4% formaldehyde and embedded in paraffin (until 1970) or paraplast. Serial 4 μm sections were subjected to H & E staining and the horseradish peroxidase (HRP) labelled antibody method for the localization of HCG and αFP. Tumours which were found to contain HCG as well as αFP were subjected to a double staining method for the simultaneous localization of both these substances in the same section.

SERA

Antibodies to whole human chorionic gonadotrophin (Pregnyl, Organon, Oss, The Netherlands) were raised in rabbits by repeated intramuscular injection as described previously (Nieuwenhuijzen Kruseman *et al.* 1977). In order to remove anti-α chain activity the antiserum was absorbed repeatedly with bovine FSH (Sigma Chemical Co., St Louis, Missouri, USA). Specificity of the antibody was finally tested with a modified enzyme-linked immunosorbent assay (Nieuwenhuijzen Kruseman 1976). Anti-HCG-IgG was prepared by ammonium sulphate precipitation and was conjugated with HRP (Sigma type VI, RZ $\geq$ 3.0) using the periodate method as described by Wilson & Nakane (1978).

Antiserum against αFP was purchased from Dako (Copenhagen, Denmark, lot no. 048A). This antiserum was absorbed with cross-linked pooled whole human serum (Avrameas & Ternynck 1969) and subsequently with a crude liver extract (Merck, Darmstadt, FRG). The resulting antiserum gave only one precipitation line against fetal cord blood on immunoelectrophoresis.

HRP labelled goat-antirabbit IgG was prepared by conjugation of a commercially available IgG preparation (Miles Yeda, Israel, Lot no. G-156) with HRP (Sigma) using the periodate method as described above.

Tissue controls were: 1 Sections incubated with the conjugated second antibody only, 2 Sections incubated with specific antibody which had been preabsorbed with the antigen of interest and 3 Sections incubated with a non-immune serum. In addition, with each staining series, known positive and negative control tissues were included.

IMMUNOHISTOCHEMICAL PROCEDURES

Tissue sections were deparaffinized in xylene and rehydrated in a graded ethanol series. Prior to immunostaining the sections were washed in three changes of phosphate buffered saline (PBS) with 1% bovine serum albumin (BSA, Sigma, type V) and incubated for 15 min in 5% human serum. The sections were incubated in the first antibody at room temp for 30 min (anti HCG lot no. 1736 at a dilution of 1:1000, anti αFP at a dilution of 1:50; both in PBS-BSA). After three washes (10 min each) in PBS the sections were incubated with the goat-antirabbit peroxidase conjugate in a dilution of 1:200 for 30 min. After washing in PBS and finally in 0.05 M Tris-HCl buffer (10 min) the sections were reacted with 3'-3-diaminobenzidine (DAB, Sigma) as described by Graham & Karnowsky (1966). After slight counterstaining in haematoxylin the sections were dehydrated and mounted.

For double staining of both αFP and HCG in the same section the selected sections were first reacted with anti HCG-HRP conjugate and stained with DAB. After washing, the sections were incubated with anti-αFP antibodies followed by goat-antirabbit-HRP conjugate and stained with 4 chloro-1 naphthol (CN) according to Nakane (1968). These sections were mounted in a glycerol : gelatin mixture (9 : 1 v/v). As additional controls for this procedure, sections incubated for αFP were stained with CN followed by DAB and sections incubated for HCG were stained with DAB followed by CN.

HISTOLOGICAL EVALUATION

All tumours were classified on H & E sections using both the classifications of Mostofi & Price (1973) and of Pugh & Cameron (1976). Independently the results of the immunohistochemical staining were evaluated. Finally, in respect of the αFP and/or HCG reactive cases, the sections were reexamined for the histological identification of immunoreactive tissue structures.

Results

HISTOLOGY

Histological classification of the tumours is presented in Table 1. In this series 46 tumours were seminomas, one of these of the spermatocytic type. In several cases syncitial multinucleated giant cells were encountered. In many cases foci of epithelioid cells with multinucleated giant cells occurred. In three cases seminoma was found together with teratoma and/or embryonal carcinoma (MTI/MTU).

The most frequent non-seminoma diagnosis was embryonal carcinoma with or without teratoma (MTI/MTU/MTT). Pure choriocarcinomas did not occur. Only 6 cases of pure embryonal carcinoma (MTU) were found. Usually these tumours showed areas with irregular clusters of large pleomorphic cells, mostly solid, but occasionally with tubular or papillary differentiation. In several cases embryoid bodies were found. In six cases, areas of endodermal sinus tumour (yolk sac tumour)

Table 1. Marker presence according to diagnosis

Diagnosis	No.	αFP	HCG
Seminoma	43	0	0
Seminoma + STLG	3	0	3
teratoma differentiated	3	0	0
Seminoma with EC and T*	3	1	3
Seminoma with MTI/MTU**	3	1	3
Embryonal carcinoma*	6	4	6
MTU**	4	3	4
Embryonal carcinoma with T*	15	13	11
MTI**	14	12	10
MTT**	3	2	3
Total	24	18	20
Total	73	18	23

* Mostofi & Price (1973). ** Pugh & Cameron (1976). Abbreviations: STLG, syncitiotrophoblast-like giant cells; EC, embryonal carcinoma; T, teratoma; MTI, malignant teratoma intermediate; MTU, malignant teratoma undifferentiated; MTT, malignant teratoma trophoblastic.

consisting of microcystic, reticular or papillary structures, occasionally accompanied by Schiller–Duval bodies, occurred. Syncitial multinucleated giant cells, resembling syncitiotrophoblastic cells, were encountered in most of the non-seminomatous tumours (17 cases). These cells were usually found in clusters, in close proximity to blood vessels and either surrounded by connective tissue stroma or intermingled with other tumour elements. In three cases typical trophoblastic areas consisting of

Table 2. Testicular germ cell tumours: tissue types and marker presence

	Serum			EC					
Case	αFP	HCG	EST† with αFP	with αFP	without αFP	STLG‡ with HCG	SEM	TDM	TDI
1	nd	nd				+	+		
2	nd	nd				+	+		
3	−	+				+	+		
4	nd	nd						+	
5	nd	nd						+	+
6	nd	nd						+	
7	nd	nd		+		+			+
8	nd	nd	+	+		+		+	+
9	nd	nd	+	+		+		+	
10	nd	nd		+		+			+
11	nd	nd		+		+			+
12	nd	nd			+			+	+
13	+	+	+	+		+		+	
14	nd	nd		+		+		+	
15	+	+		+		+			+
16	−	−			+			+	
17	+	+	+	+		+		+	
18	−	−		+				+	+
19	+	−	+	+				+	+
20	+	+		+		+	+	+	
21	−	+			+	+	+		+
22	nd	nd		+		+		+	
23	nd	nd			+	+	+		
24	nd	−		+		+			
25	+	+		+		+			
26	−	−			+	+			
27	+	+		+		+			
28	nd	nd			+	+*			
29	+	+	+	+		+*		+	
30	+	+		+		+*			

EST, endodermal sinus tumor; EC, embryonal carcinoma; SEM, seminoma; TDM, teratoma differentiated mature; TDI, teratoma differentiated immature. nd, not done; † all cases with EST showed αFP immunoreactivity; ‡ all cases with STLG showed HCG immunoreactivity; * malignant teratoma trophoblastic (MTT).

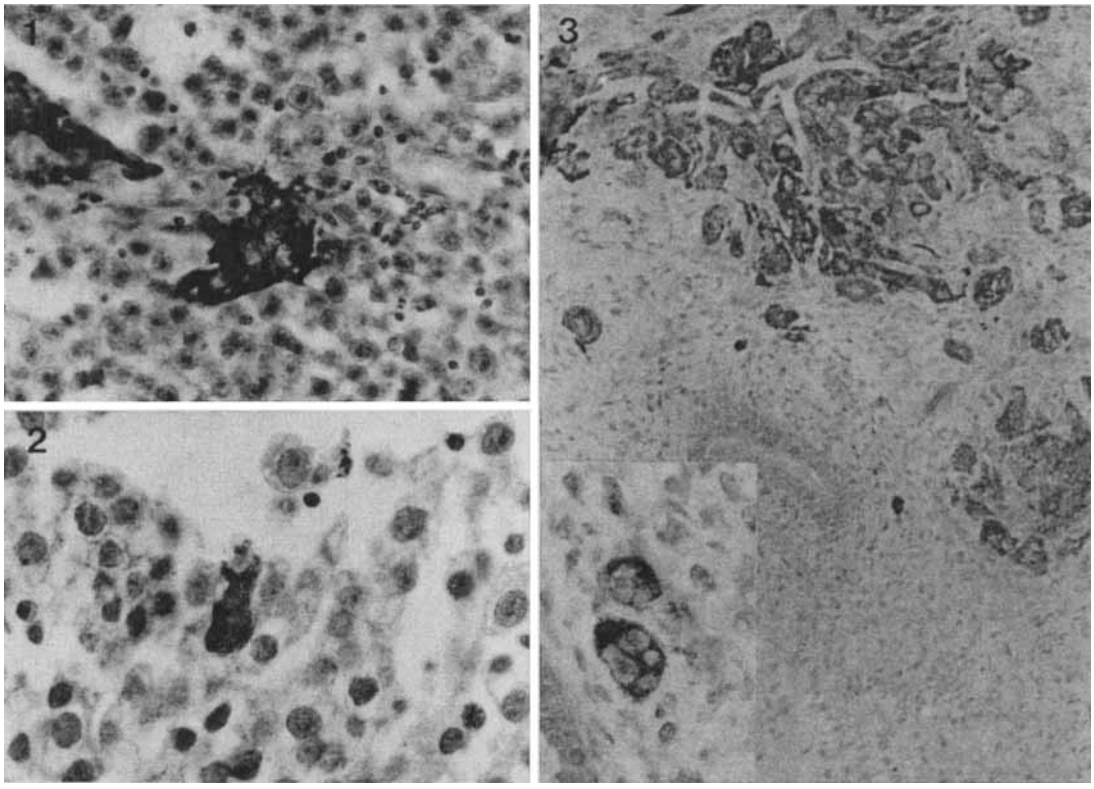

Figure 1. HCG reactive syncitiotrophoblast-like giant cells in seminoma. × 225

Figure 2. Same tumour, mononuclear HCG reactive cell. × 360.

Figure 3. Cluster of HCG reactive syncitiotrophoblast-like giant cells in an embryonal carcinoma with teratoma (MTI). × 100. *Inset*: × 225.

papillary clusters of cytotrophoblastic cells lined with syncitiotrophoblastic giant cells and surrounded by blood-filled spaces, were found (MTT).

Pure teratoma, containing cell types derived from three germ cell layers, was found in three cases. In these tumours mature cells in organoid arrangements predominated. In one of these, areas of immature embryonal mesenchyme were found without unequivocal histological signs of malignancy. In none of these tumours were syncitial giant cells found.

IMMUNOHISTOCHEMISTRY

Results of immunoperoxidase staining for HCG and αFP are summarized in Table 2. No differences were found between paraffin and paraplast embedded material. Immunostained cells or structures were morphologically identified if possible.

Seminoma

In three seminoma cases (7%) HCG immunoreactivity was encountered. In addition

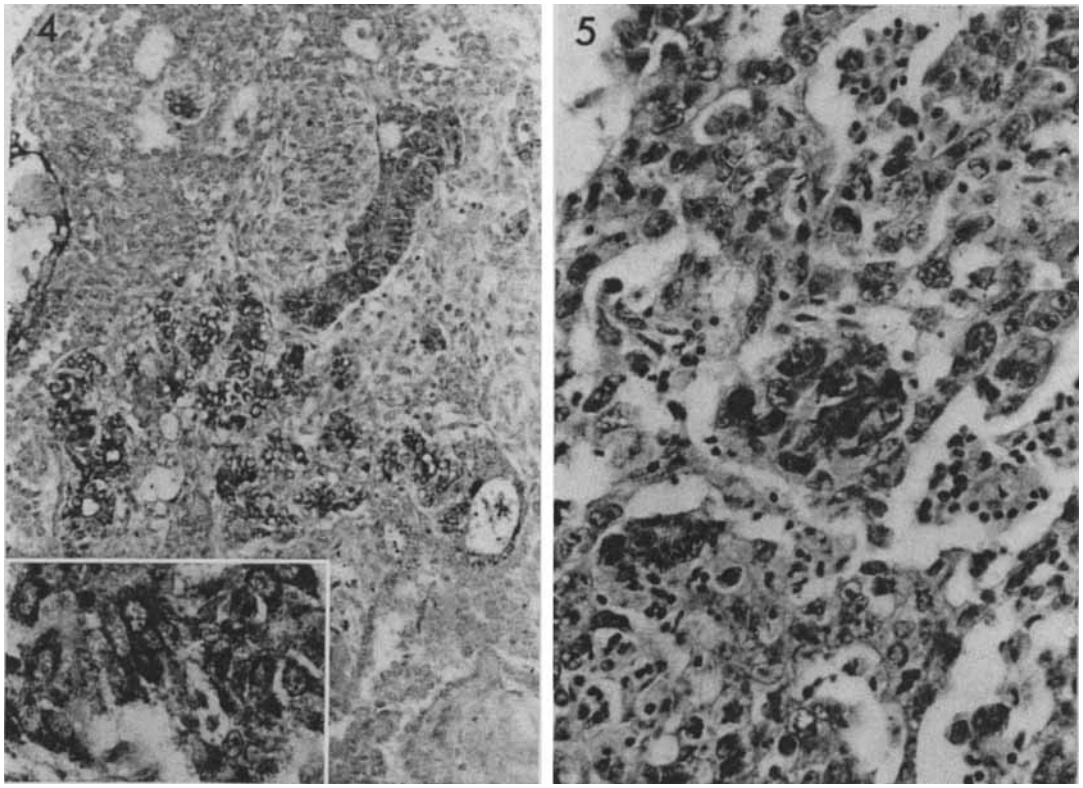

Figure 4. Clusters of αFP reactive cells in embryonal carcinoma (MTU). × 100. *Inset*: × 360.
Figure 5. Tubular structure containing αFP in embryonal carcinoma (MTU). × 225.

to STLG (Figure 1) staining was also found in some seminoma-like cells (Figure 2). The STLG often occurred adjacent to thin walled blood vessels. None of the seminomas contained αFP immunoreactivity.

Embryonal carcinoma (MTI/MTU/MTT)

In 20 (83%) of these neoplasms HCG reactive cells were found, usually recognizable as STLG (Figure 3). In all cases with STLG, HCG immunoreactivity was found. The staining intensity of the individual cells, however, varied appreciably. αFP immunoreactivity occurred in 18 cases (75%) and was mostly found in undifferentiated embryonal carcinoma cells or in syncitial cell masses (Figure 4). In addition staining was occasionally noted in areas of papillary or tubular differentiation (Figure 5). In the six cases with foci of endodermal sinus tumour, αFP immunoreactivity was found in reticular, microcystic, tubular and papillary types of growth (Figures 6 & 7). The five cases without αFP immunoreactivity, besides the absence of endodermal sinus tumour foci, did not show particular histological features.

Double staining of αFP and HCG reactive tumours showed that usually these substances occurred in different cell populations. In some areas of several tumours

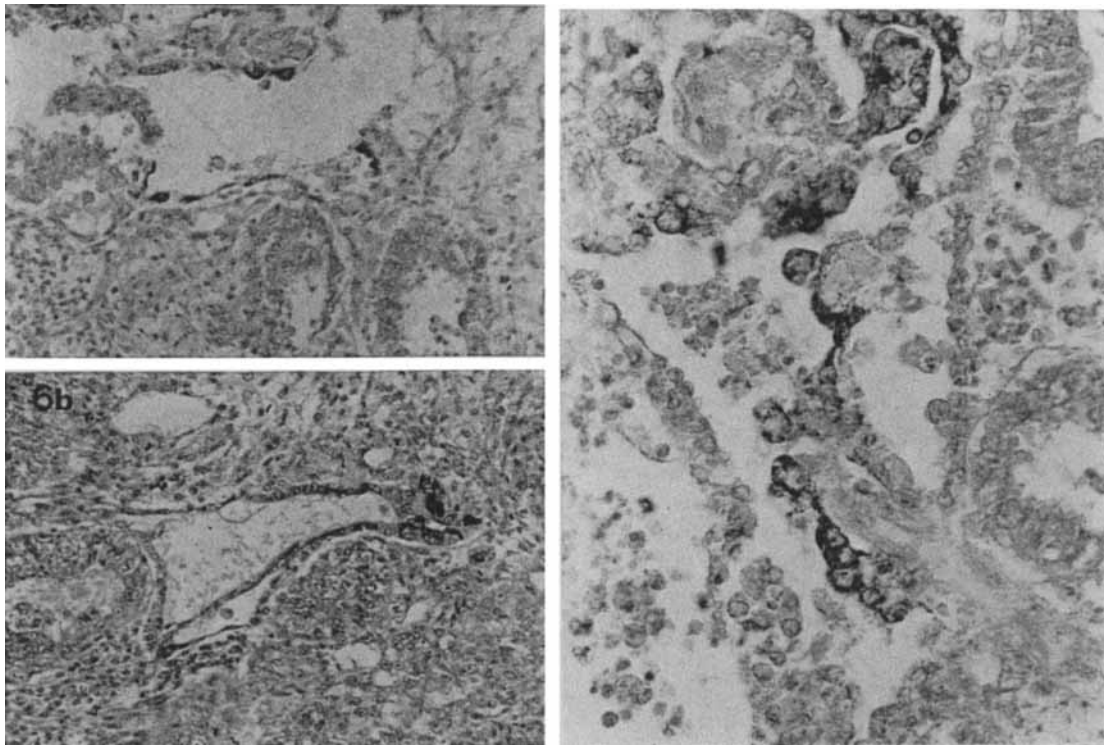

Figure 6. Microcystic pattern in focus of endodermal sinus tumour containing αFP reactive cells. Both **a** & **b** ×90.

Figure 7. Papillary structure in focus of endodermal sinus tumour containing αFP reactive cells. ×225.

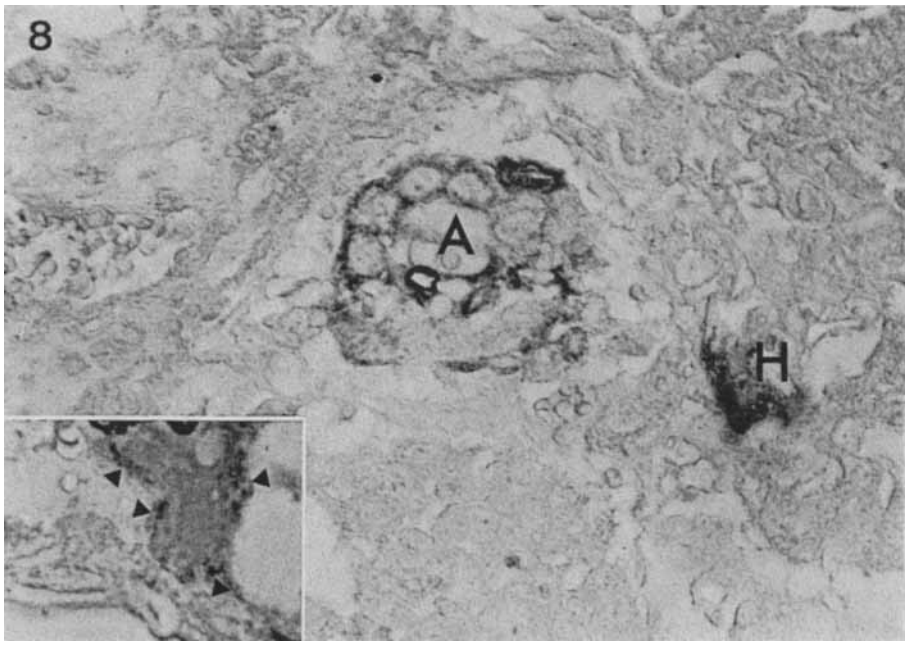

Figure 8. Double staining of HCG (H) and αFP (A) reactive structures in embryonal carcinoma with teratoma (MTI). ×100. *Inset*: double staining of syncitial cell in the same tumour; the light central part of the cytoplasm contains αFP whereas the slightly darker granular staining in the periphery (▲) represents HCG. ×600.

both cell types intermingled and, in a few tumours, cells were found which contained both immunoreactive HCG and αFP (Figure 8).

In a number of cases prior to orchidectomy serum HCG and αFP levels had been determined by radio-immunoassay (Table 2). In 13 of these cases tissue immunoreactivity corresponded with the presence of the substances in the blood. In case 26 very few scattered HCG immunoreactive STLG were found whereas no radioimmunoassayable HCG was found in the serum. In case 24 serum HCG was found to be negative (with a pregnancy test) whereas several clusters of HCG-immunoreactive STLG were found.

Discussion

Germ cell tumours of the testis display a wide variety of histological structures and growth patterns. This complexity is reflected in the different classifications (Mostofi & Price 1973; Pugh & Cameron 1976) and also in the numerous more or less synonymous terms which have been used to designate some of these tumours. The frequent presence of foci of choriocarcinoma or STLG in non-seminoma germ cell tumours, tentatively explained the occurrence of high serum levels of HCG, as this substance was proven to be produced in syncitiotrophoblast (Midgley & Pierce 1969).

The association of endodermal sinus tumour with high serum levels of αFP (Ballas 1973, Wilkinson, Friedrich & Hosty 1973, Tsuchida *et al.* 1973, Itoh *et al.* 1974, Talerman & Haye 1974), which in the embryo is produced in the yolk sac (Gitlin & Perricelli 1970), suggested that foci of endodermal sinus tumour could be responsible for αFP production.

Immunohistochemical localization of HCG (Heyderman & Neville 1976) and of both HCG and αFP (Kurman *et al.* 1977) in non-seminoma germ cell tumours indeed showed that HCG is produced in foci of choriocarcinoma or in STLG and αFP in foci of endodermal sinus tumour but also in embryonal carcinoma (undifferentiated teratoma) cells. These findings led Kurman *et al.* (1977) and Taylor *et al.* (1978) to propose a new classification for germ cell tumours of the testis that incorporated immunohistochemical results. Against this background the present retrospective study was focused primarily on the potential value of immunohistochemistry for tumour classification.

The occurrence of HCG reactive STLG in a small proportion of seminoma cases (7% in the present series) is an interesting phenomenon that was recently reported also by Javadpour *et al.* (1978) and Hedinger *et al.* (1979). Thackray & Crane (1976) found a slightly (but statistically not significant) lower 10-year survival rate for these patients than in cases without tumour giant cells. We agree with Hedinger *et al.* (1979) that the presently available data do not allow definitive conclusions regarding this problem. Therefore, a larger number of similar cases should be studied to determine the prognostic significance of HCG reactive STLG in seminoma.

The frequency of αFP and HCG production in the present series of non-seminoma germ cell tumours (67% and 75% respectively) is somewhat higher as was reported by Kurman *et al.* (1977). However, the reported frequency of occurrence of these

substances in the serum varies from 31% and 38% for αFP and HCG respectively (Schultz *et al.* 1978) to 84% for both markers (Newlands *et al.* 1976). Moreover, in the present series, whenever tested, immunohistochemical results and serum determinations were in close agreement. In two cases of embryonal carcinoma (MTU) a few HCG reactive STLG occurred without concurrent detectable blood levels of this substance. These results indicate that immunohistochemical detection of these substances closely correlates with the serum radioimmunoassay. It should be emphasized that adequate tissue sampling is of utmost importance as immunoreactive structures in the tumours can be very scanty and often appear in clusters rather than randomly scattered.

The results summarized in Table 1 further indicate that in general the presence or absence of one or both marker substances is not diagnostic for any single type of tumour. In this series, however, three tumours were diagnosed as differentiated teratoma. These tumours contained neither αFP or HCG as was also observed by Kurman *et al.* (1977). Absence of marker substances in a differentiated teratoma would warrant more extensive tissue sampling to ascertain or exclude the coexistence of other elements. A larger series of TD would have to be studied to evaluate the diagnostic value of the absence of markers for this tumour.

In general, immunostaining facilitates the identification of tissue elements which otherwise may escape attention. Foci of endodermal sinus tumour will be easily identified through αFP immunoreactivity and similarly trophoblastic foci (leading to the diagnosis of MTT) or STLG will be detectable through HCG immunoreactivity. The occurrence of these elements in a high proportion of non-seminoma germ cell tumours and the rarity of pure endodermal sinus tumour and choriocarcinoma (Mostofi & Price 1973, Pugh & Cameron 1976, Talerman 1975) imply that immunohistochemical classification as suggested by Kurman *et al.* (1977) and Taylor *et al.* (1978) offers no advantages over existing classifications. Detailed study of clinical, immunohistochemical and histopathological information may eventually result in redefinition of some of the existing categories and hopefully in one clinically valid and biologically relevant classification.

In pure embryonal carcinomas (MTU) and in cases of embryonal carcinoma with teratoma (MTI) some tumours did not contain αFP or HCG. Whether or not the presence or absence of marker proteins has a bearing on the prognosis remains an open question.

The occurrence of both αFP and HCG in the majority of non-seminoma germ cell tumours in parallel with the predominance of mixed histological types limits the validity of speculations about the histogenetic relationship between the different tumour types. It seems, however, justified to conclude that, in addition to the histologically identifiable differentiated (αFP containing yolk sac and HCG containing trophoblastic) structures, a population of undifferentiated cells exists which is also capable of production of these substances. Whether or not the histologically distinct structures are differentiation products of these cells remains to be elucidated. Our finding of cells containing both αFP and HCG is supportive evidence for the common origin of the different types of germ cell tumours, as postulated by Pierce (1966) and Pierce, Stevens & Nakane (1967).

In conclusion it can be stated that immunohistochemical detection of αFP and HCG in germ cell tumours closely correlates with elevated blood levels of these substances. For the currently available classifications of non-seminoma germ cell tumours immunohistochemical information appears to offer little additional discriminative possibilities. A larger series of differentiated teratoma should be studied to evaluate the diagnostic reliability of the absence of αFP and HCG in these tumours The prognostic value of HCG reactive STLG in seminomas and of αFP and HCG reactive structures in embryonal carcinoma with or without teratoma (MTI/MTU/MTT) also deserves further investigation. The occasional presence of both HCG and αFP in the same cell supports the theory of the common origin of germ cell tumours.

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