

Antibodies to cytokeratin and vimentin in testicular tumour diagnosis *

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Summary. Thirteen primary and metastatic testicular germ cell tumours, including classical and anaplastic seminomas, and non-seminomatous testicular tumours were examined for their intermediate filament protein (IFP) types. The seminomas were shown to react with a monoclonal and a polyclonal antibody to bovine lens vimentin, while non-seminomatous germ cell tumours were strongly positive for a polyclonal and a monoclonal antibody to cytokeratin.

In one case of seminoma with elevated serum levels of β HCG and α FP, cytokeratin positive tumour cells were found. In the case of teratocarcinoma, several components of the tumour could be distinguished using a combination of antisera in double-label immunofluorescence microscopy. The glandular component of this tumour was positive with the polyclonal antikeratin, but also with the monoclonal cytokeratin antibody specific for glandular epithelia (RGE 53). However, the squamous component was negative with this latter antibody. Strikingly, the spindle cell component showed focal positivity for vimentin, with coexpression of cytokeratin and vimentin in some cells.

Our data show that antibodies to cytokeratin and vimentin can be helpful in the diagnosis of testicular germ cell tumours, especially in the differentiation between seminomas and non-seminomatous tumours.

Key words: Cytokeratin – Vimentin – Testis – Seminoma – Embryonal cell carcinoma – Endodermal sinus tumour – Teratocarcinoma

Introduction

Identification of the intermediate filament protein types in cells of an unknown tumour offers the opportunity to obtain more information about

* This study was supported by a grant from the Dutch Cancer Foundation, The Queen Wilhelmina Fund (KWF), project NUKC 1981-12

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its origin and nature (for reviews see Osborn and Weber 1983; Ramaekers et al. 1983a). Antisera specific for intermediate filament proteins have proven to be useful immunohistochemical tools in surgical pathology especially in those cases where routine histological techniques do not permit a clear cut (differential) diagnosis. For example, antibodies to cytokeratins and vimentin can be used to differentiate between anaplastic carcinomas and lymphomas or melanomas (Gabbiani et al. 1981; Altmannsberger et al. 1982a; Schlegel et al. 1980; Ramaekers et al. 1983b, c, 1985a), while an antibody to desmin allows a distinction between rhabdomyosarcomas and leiomyosarcomas from other soft tissue tumours (Altmannsberger et al. 1982b; Denk et al. 1983). Cytokeratins, the epithelial type of intermediate filament proteins, comprise a family of 19 closely related, though biochemically distinct, polypeptides which to a certain extent are distributed in a tissue specific manner throughout human epithelia (Moll et al. 1982). As a result monoclonal antibodies to distinct cytokeratin polypeptides have been shown to distinguish between different types of epithelia using immunohistochemical techniques (Lane 1982; Tseng et al. 1982). For example, antisera to cytokeratin 18 recognize glandular epithelia and not squamous epithelia (Debus et al. 1982; Ramaekers et al. 1985b). Such monoclonal antibodies also distinguish adenocarcinomas from squamous cell carcinomas (Debus et al. 1984; Ramaekers et al. 1983d). The present report describes the use of monoclonal and polyclonal antibodies to the intermediate filament proteins vimentin and the cytokeratins in the diagnosis of primary and metastatic testicular tumours. Our findings show that such antibodies can distinguish seminomas from non-seminomatous tumours which may be helpful in surgical pathology, especially in those cases where the tumour cells exhibit an anaplastic appearance.

Materials and methods

In addition to a panel of human malignant tumours used to test further the monoclonal vimentin antiserum we investigated seven samples of seminomas and seminoma metastases, and six cases of non-seminomatous testicular tumours, both primary and metastatic. Table 2 summarizes the relevant clinicopathological data of the tumours investigated. Furthermore, we have investigated the intermediate filament distribution in five normal human testes, which were removed on clinical indications.

A portion of each tumour was fixed in formalin, embedded in paraffin and used for routine histology using H&E staining. A second portion was frozen in liquid nitrogen immediately after surgery and either stored in liquid nitrogen or cut immediately into frozen sections.

Immunohistochemistry. Frozen tissue material was cut on a cryostat and 5–7 µm thick frozen sections were fixed with methanol (–20° C, 5 min) and acetone (room temp., 10 to 30 sec) or in acetone alone (5 min).

Air dried sections were either rehydrated with phosphate buffered saline (PBS) or used directly for incubation with the first antiserum. Incubation procedures for single and double labeling indirect immunofluorescence have been described before (Ramaekers et al. 1981, 1982, 1983d, e) as has the immunoperoxidase (PAP) staining method used for the four cases of paraffin embedded normal testis (Ramaekers et al. 1983a).

The following antibody preparations were used in this study:

1. An affinity purified antiserum to human callus keratins (pKer) raised in rabbits and affinity-purified on human keratins. The serum was diluted 1:10 to 1:20 for immunofluorescence and 1:50 for immunoperoxidase assays. For preparation and specificity testing see Ramaekers et al. (1982, 1983a, b).

2. The mouse monoclonal antibody RGE 53 (available from Euro-Diagnostics BV Apeldoorn, The Netherlands) specific for cytokeratin 18. Preparation and specificity testing of this antibody have been described (Ramaekers et al. 1983d).

3. An antiserum to calf lens vimentin (pVim), raised in a rabbit. Preparation and specificity of this antiserum have been described (Ramaekers 1981, 1983a, c).

4. A monoclonal antibody to calf lens vimentin (MVI) purchased from Euro-Diagnostics B.V., Apeldoorn, The Netherlands. The antibody preparation was diluted 1:5 to 1:10.

5. A rabbit antiserum to chicken gizzard desmin (pDes), prepared as described before (Ramaekers 1983a).

6. A monoclonal antibody to chicken gizzard desmin (RD 301), specific for desmin as demonstrated by immunoblotting and immunofluorescence assays (to be published).

7. A monoclonal neurofilament antiserum (MNF), purchased from Euro-Diagnostics B.V. (Apeldoorn, The Netherlands) reactive with the 70 kDa and 200 kDa neurofilament proteins.

Corresponding fluorescein isothiocyanate- and Texas-red conjugated second antibodies were obtained from Nordic, Tilburg, The Netherlands, and from New England Nuclear, Boston, MA.

In control experiments either second antibodies alone or primary antibodies to GFAP and a monoclonal antibody to epidermal keratin (RKSE 60 which reacts only with keratinizing epithelial cells) were used (Ramaekers et al. 1983a).

Gel electrophoresis and immunoblotting. Cytoskeletal preparations from calf lens, bovine liver hepatocyte cultures, and LLCMK-2 cultures were made by extracting cell pellets with 1% Triton X-100 in PBS, containing 0.01 mM phenylmethylsulfonyl fluoride (PMSF). The insoluble fraction was washed with PBS and applied to gel electrophoresis in sodium dodecylsulfate containing 10% polyacrylamide slab gels as described by Laemmli (1970). Vimentin was purified from bovine lens as described before (Ramaekers et al. 1981). For immunoblotting experiments the electrophoretically separated polypeptides were transferred to nitrocellulose sheets using an electrophoresis buffer containing 25 mM Tris-HCl, 192 mM glycine and 20% methanol (pH 8.3), essentially as described by Towbin et al. (1979). The sheets were then exposed at room temperature and under constant shaking to the following incubation steps in sequence: preincubation for 3 h in buffer I, a 3% solution of bovine serum albumin (BSA from Sigma) in 350 mM NaCl, 10 mM Tris-HCl, pH 7.6, and 0.5 mM PMSF (Sigma); 3 washing steps for 10 min each in buffer II (0.5% Triton X-100 from BDH in PBS); incubation for 18 h with the first antibody in buffer III (0.3% BSA, 150 mM NaCl, 10 mM Tris-HCl, pH 7.6, 0.1 mM PMSF, 1% Triton X-100, 0.5% sodium deoxycholate from Merck, and 0.1% sodium dodecylsulfate (SDS from BDH). The vimentin monoclonal antibody was diluted 1:20; 3 washing steps, 10 min each in buffer III; 3 washing steps, 10 min each in buffer II; incubation for 60 min in peroxidase-conjugated rabbit anti-mouse IgG (Nordic, Tilburg, The Netherlands) diluted 1:200 in buffer IV (0.5% Triton X-100, and 0.5% BSA in PBS); 3 washing steps, 10 min each in buffer II. Peroxidase activity on the immunoblot was detected using 4-chloro-1-naphthol from Merck (Darmstadt, FRG). 50 mg of 4-chloro-1-naphthol was dissolved in 5 ml ethanol, added to 95 ml PBS and stirred for 5 min. After filtration, 30% H₂O₂ was added to the solution to a final concentration of 0.06% (40 µl 30% H₂O₂ per 20 ml) and the peroxidase reaction developed by gentle shaking of the blot in this solution during 5 min. The sheets were then rinsed with tap water for 10 min and dried at room temperature between filter paper.

Results

Antibodies

The specificity and reactivity patterns of most of the antibodies used in this study have been described extensively before (Ramaekers et al. 1981, 1982, 1983a-e, 1984a, b).

In brief, the rabbit antiserum to callus keratins (pKer) reacted exclusively with most epithelial tissues, while the monoclonal antibody RGE 53 reacted

with a subgroup of epithelial tissues, i.e. glandular and columnar epithelial cells and mesothelial cells. This antibody reacts specifically with cytokeratin 18 as detected by one- and two-dimensional immunoblotting assays and does not seem to interact with other cytokeratin polypeptides or any of the other types of intermediate filament proteins.

The rabbit antiserum to vimentin (pVim) was shown to react exclusively with the 57 kD vimentin protein in immunoblotting experiments (Herman et al. 1983). In general it is specific for cells of mesenchymal origin and does not react with epithelial cells *in vivo* (for exceptions see for example Herman et al. 1983).

The commercially available monoclonal antibody to calf lens vimentin (MVI) was further characterized, using the indirect immunofluorescence technique on frozen sections and cell cultures as well as immunoblotting assays. It was obvious that the antibody reacted only with tissues of mesenchymal origin, such as fibroblasts, endothelial cells, some blood vessel smooth muscle cells (Fig. 1a), glomeruli in the kidney (Fig. 1b) etc., but did not stain epithelial tissues. In cultured cells (bovine lens cells, BHK-cells, PtK-cells, HeLa cells, Kb cells and cultures from bovine hepatocytes) the antibody gave a filamentous staining pattern (Fig. 1c), identical to that obtained with the polyclonal antiserum to calf lens vimentin. Moreover, this antibody was tested on a series of frozen sections from different types of human malignant tumours including astrocytomas, transitional cell carcinomas of the bladder, endometrial tumours, renal cell tumours, mesotheliomas, several other types of carcinomas and some sarcomas. From these experiments it was obvious that the monoclonal vimentin antibody gave a staining pattern virtually identical to that of the polyclonal vimentin antiserum (results not shown).

In the immunoblotting technique (Fig. 1e) the antibody gave a specific positive reaction coinciding with the vimentin band seen at 57 kD in the Coomassie-stained gel strip or its replica. Cytoskeletal extracts from bovine lens and LLCMK-2 cell cultures next to purified bovine lens vimentin were used as antigen preparations.

Table 1. Distribution of intermediate filament proteins (IFP) in different cell types of normal testis as detected with several IFP-antibodies

Cell type	pKer	RGE53	pVim	MVI	pDes	RD301	MNF
Spermatogonia	—	—	—	—	—	—	—
Sertoli cells	—	—	+	+	—	—	—
Leydig cells	—	—	+/-	+/-	—	—	—
Myoid cells surrounding seminiferous tubules	—	—	+/-	+/-	+	+	—
Rete testis lining cells	+	+	+	+	—	—	—
Ductus efferens lining cells	+	N.T. ^a	—	N.T.	—	N.T.	—
Nerve cells	—	—	—	—	—	—	+

^a N.T.: not tested

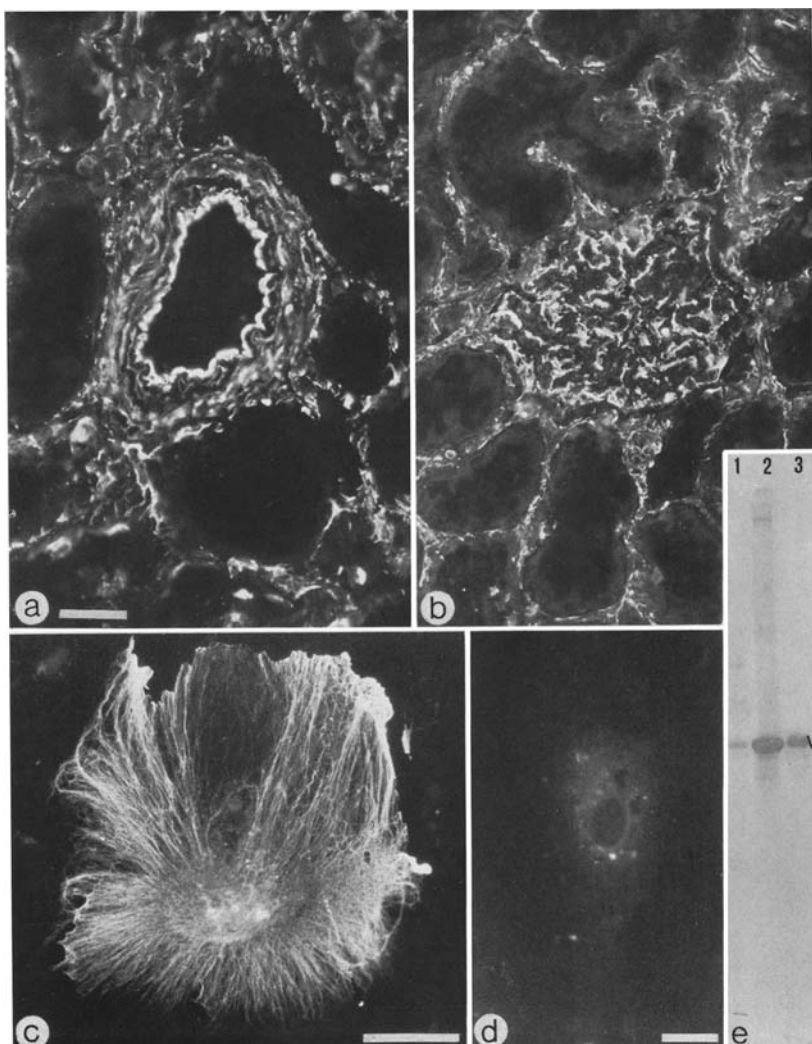


Fig. 1 a–e. Specificity test of the monoclonal antibody to vimentin (MVI). **a, b** Staining pattern on human kidney frozen sections showing a positive reaction in endothelial cells, blood vessel smooth muscle cells, stromal cells and glomeruli, but no reaction in epithelial cells. **c, d** Bovine cultured lens cells showing a fibrillar staining pattern after incubation with the MVI antibody (**c**) but not with the FITC-conjugated goat-anti-mouse antibody alone (**d**). **e** Immunoblotting of MVI on a cytoskeletal preparation from LLCMK-2 cells (lane 1), bovine lens (lane 2) and on purified bovine lens vimentin (lane 3). Note only a strong reaction with vimentin (V). Bars indicate 50 μ m

Intermediate filament pattern in normal human testis

When normal human testes, either as frozen sections or as paraffin sections were incubated with the antisera described above and in the Materials and Methods section, the following distribution of IFP was observed (see also Table 1).

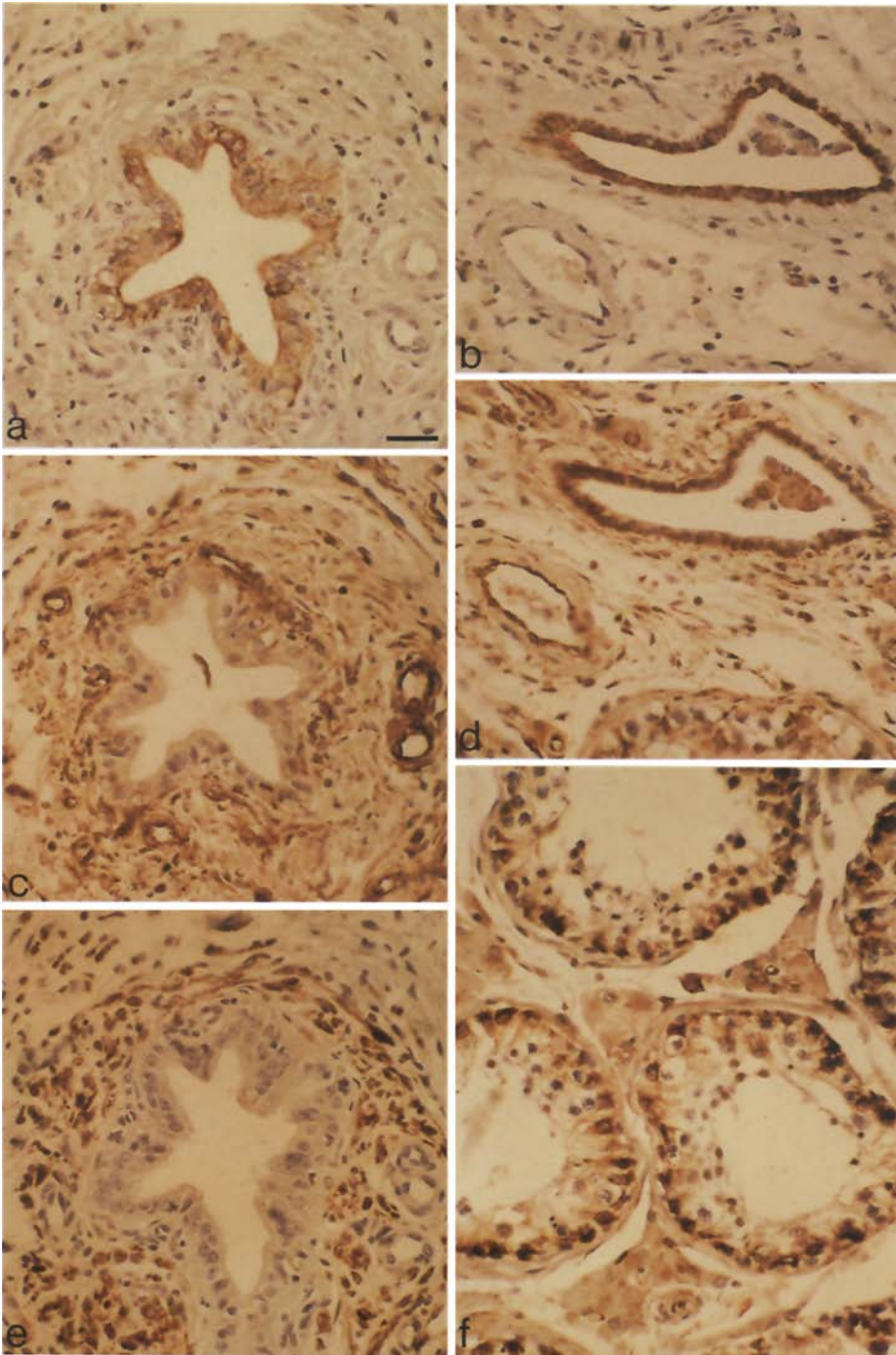


Fig. 2a-f. Immunoperoxidase (a-f) and double-label indirect immunofluorescence (g-j) staining of normal human testis tissues with the antibodies to cytokeratin (a, b, g), vimentin (c, d, f, h, j) and desmin (e, i) showing the ductus efferens (a, c, e) the rete testis (b, d, g, h) and seminiferous tubules (f, i, j). Bars indicate 50 μ m

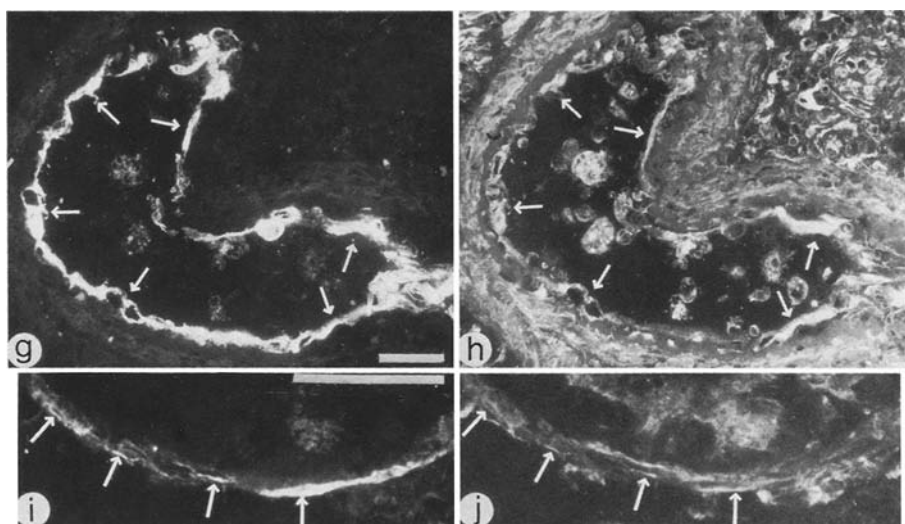


Fig. 2g-j.

With the cytokeratin antibodies pKer and RGE53 a strongly positive reaction was seen only in the epithelial cells lining the ductus efferens and the rete testis (Fig. 2a, b). No other cell types were positive. The antibodies to vimentin did not stain the epithelial cells of the ductus efferens (Fig. 2c). Strikingly, however, a considerable part of the epithelial cells of the rete testis were also positive for vimentin (Fig. 2d). Double-label experiments, using pKer and MVI or pVim and RGE 53 as primary antibody mixtures, clearly demonstrated this co-expression of cytokeratin and vimentin in rete testis lining epithelial cells (Fig. 2g, h). Furthermore, a positive reaction with the vimentin antibodies was found in Sertoli cells and in Leydig cells (Fig. 2f). The latter cells, however, showed a variable reaction, with some Leydig cells negative and others containing perinuclear clusters. Fibroblasts and blood vessels were also stained (Fig. 2c, d, f). Myoid cells surrounding the seminiferous tubules were strongly positive with the antibodies to desmin (Fig. 2i). Some of these myoid cells could be shown to co-express desmin and vimentin as demonstrated in the double-label indirect immunofluorescence technique using pDes and MVI or pVim and RD 301 as primary antibody mixtures (Fig. 2i, j). The desmin antibodies did not stain any other cell types than muscle cells (see also Fig. 2e). The neurofilament antibody only stained nerve cells.

Testicular tumours

The staining patterns obtained with the cytokeratin and vimentin antibodies in the different testicular germ cell tumours are summarized in Table 2 and depicted in Figs. 3–5.

Table 2. Clinicopathological data and immunohistochemical detection of intermediate filament proteins (IFP) in human testicular germ cell tumors^a

Patient age	Tumor location and diagnosis	Stage	Follow up (Yrs)	Clinical status	Levels of α FP and/or β HCG	Reactivity of IFP antisera in the tumour cells			
						pKer	RGE53	pVim	MVI
1-39	Anaplastic seminoma in the testis	pT ₂ (T ₃)N ₀ M ₀ (A)	3.7	A	normal	- ^b	- ^b	+ ^c	
2-26	Anaplastic seminoma in the mediastinum	TxNxMx (C)	1.3	D	normal	-	-	+ ^c	
3-48	Seminoma in the testis	pT ₁ N ₄ M ₁ (C)	0.3	D	elevated	+/- ^b	+/- ^b	+ ^c	+ ^c
4-27	Seminoma in the testis	pT ₁ N ₀ M ₀ (A)	0.3	A	normal	-	-	+ ^c	+ ^c
5-39	Seminoma in the testis	pT ₁ N ₂ M ₀ (B)	0.3	A	normal	- ^b	- ^b	+ ^c	+ ^c
6-50	Seminoma in the testis	pT ₁ N ₀ M ₀ (A)	0.5	A	normal	- ^b	- ^b	+ ^c	+
7-32	Lymph node metastases of an anaplastic seminoma	pT ₁ N ₄ M ₁ (C)	1.5	A	normal	- ^b	- ^b	+ ^c	
8-40	Embryonal cell carcinoma in the testis	pT ₁ N ₀ M ₀ (A)	2.0	A	elevated	+	+	-	
9-34	Embryonal cell carcinoma mixed with seminoma in the testis	pT ₁ N ₁ M ₀ (B)	0.3	A	normal	+	+	- ^d	- ^d
10-19	Endodermal sinus tumor/local recurrence in scrotum	pT ₄ N ₂ M ₀ (B)	2.4	D	elevated	+	+	-	
11-20	Metastasis of an endodermal sinus tumor on the omentum	pT ₄ N ₂ M ₀ (B)	2.4	D	elevated	+	+	-	
12-29	Lymph node metastasis of an embryonal cell carcinoma with a choriocarcinomatous component	pT ₁ N ₂ M ₀ (B)	1.3	A	elevated	+	+	-	
13-26	Teratocarcinoma in the testis: -glandular component -squamous component -spindle cell component -loose dispersed cells	pT ₁ N ₂ M ₀ (B)	1.5	A	elevated	+	+	-	-
						+	-	-	-
						+ ^e	+ ^e	+ ^e	+ ^e
						+			

^a Abbreviations: A: alive; D: dead; α FP: Alpha fetoprotein; β HCG: Beta human chorionic gonadotropin

^b Occasionally a few cytokeratin positive cells were seen. It has not been possible so far to determine whether these cells were malignant or whether they represented preexisting epithelial cells. In case nr. 3, however, it was quite obvious that also tumour cells contained cytokeratins. In case nr. 7 the primary tumour in the testis contained a Yolk-sac component, as detected in H&E stained paraffin sections

^c Also (considerable amounts of) vimentin negative tumour cells were seen

^d Only a few vimentin positive cells were found

^e Some of the cells in the spindle cell component seem to co-express cytokeratin and vimentin as detected by double label immunofluorescence (see text).

Case 10 and 11 represent the same patient

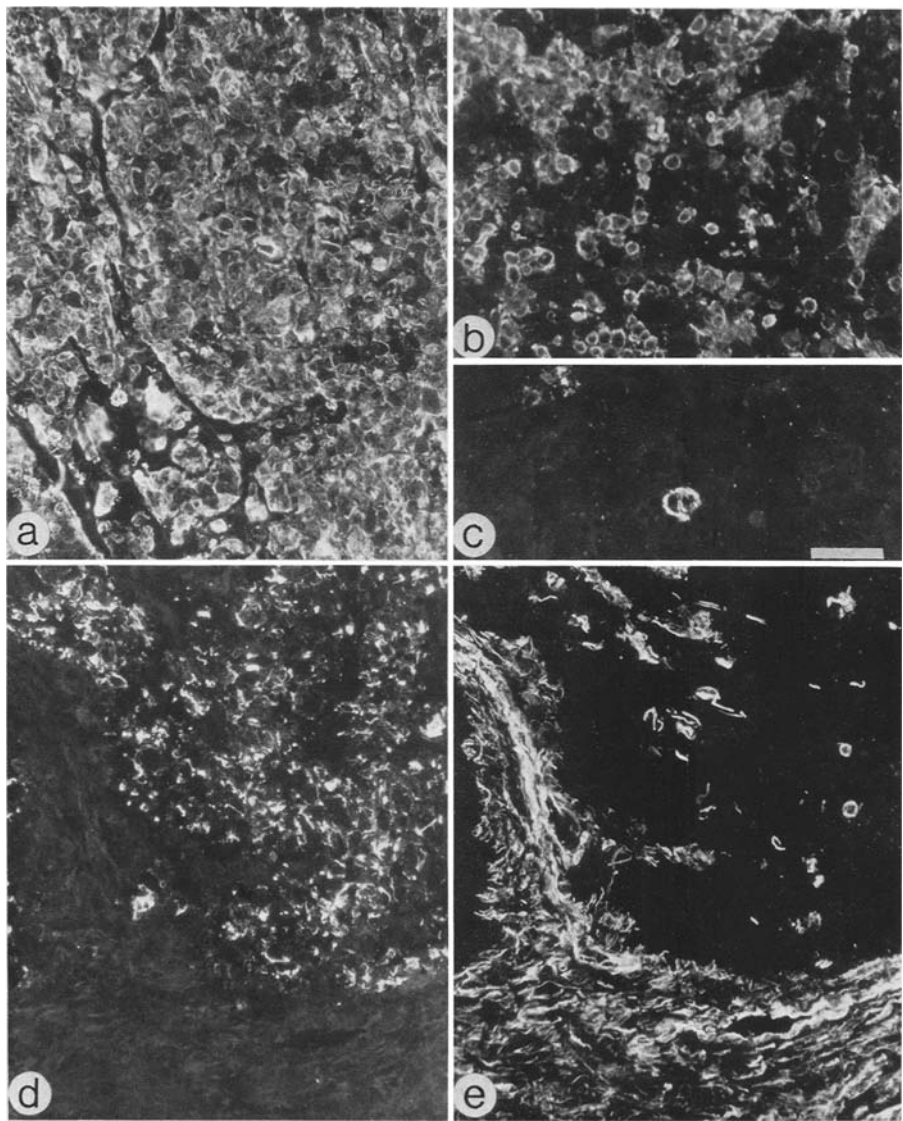


Fig. 3a–d. Seminoma frozen sections, incubated with the polyclonal antibody to cytokeratins (c) and RGE 53 (d). d and e represent double-label immunofluorographs of case nr. 3 in an area with a high concentration of cytokeratin-positive cells. Bar indicates 50 μ m

Seminomas. Four classic seminomas, an anaplastic seminoma in the testis and two metastases of anaplastic seminomas were examined for their IFP-content using the antibodies to vimentin and cytokeratin. It was obvious that in all cases tumour cells were positive for vimentin as detected by the polyclonal antiserum (Fig. 3a) but also a considerable number of vimentin negative tumour cells were seen. Four cases were examined with the monoclonal anti-vimentin antibody, which gave results similar to those ob-

tained with the polyclonal antiserum. Again vimentin-negative tumour cells were seen. The vimentin antibody also stained stromal components (fibroblasts, blood vessels) and Sertoli cells. When incubated with the polyclonal or monoclonal antibodies to (cyto)keratins six out of seven cases were negative (Fig. 3b) although in four of these cases a few cytokeratin positive cells scattered through the tissue sections were seen. It was not possible to determine whether these latter cells were malignant tumour cells or if they represented preexisting epithelial cells from, for example, the rete testis.

In case no. 7, a lymph node metastasis which was morphologically pure anaplastic seminoma, a few cytokeratin positive cells were detected. This correlates well with the finding that the primary tumour in the testis was a mixed Yolk-sac tumour/seminoma. From earlier studies (Ramaekers et al. 1983d) we know that this latter type of germ cell tumour is positive for cytokeratin but negative for vimentin. Double labeling studies performed on frozen sections from case no. 7 supported this suggestion. A complete overlap of the reaction pattern of the polyclonal and the monoclonal anti-cytokeratin antibodies was seen with the reaction of the monoclonal antibody stronger than that of the polyclonal antiserum. No overlap was observed between the reactivity patterns of the polyclonal vimentin antiserum and RGE 53. We have, however, also observed tumour cells that were negative for both cytokeratin and vimentin antibodies. Interestingly, in one of the cases (no. 3 which had elevated levels of serum markers) it was quite obvious that tumour cells were also positive for cytokeratin (Fig. 3c). Double-label studies in this case again showed a total overlap between reactivity patterns of the two cytokeratin antibodies, the monoclonal antibody reacting more strongly than the rabbit antiserum. No overlap was seen in the reactivity patterns of the polyclonal vimentin antiserum and RGE 53 on one hand or between the monoclonal vimentin antibody and the polyclonal keratin antiserum on the other (Fig. 3c, d). Furthermore, it was clear that tumour cells staining neither for cytokeratin nor vimentin were also present in this case. In no case of seminoma was a positive reaction found in the tumour cells with antibodies to desmin, GFAP or the monoclonal antibodies RKSE 60 or MNF. The desmin antibodies did, however, stain preexisting and vascular smooth muscle tissues, while MNF stained nerve cells.

Non-seminomatous testicular tumours. Five non-seminomatous testicular tumour cases, including two primary and one metastatic embryonal cell carcinoma, one primary and one metastatic endodermal sinus tumour and a teratocarcinoma, were tested for their IFP-type. The two samples of the endodermal sinus tumour (cases 10 and 11) were from the same patient, obtained about one year apart.

When incubated with the different types of antibodies the tumour cells in all five embryonal cell tumours reacted only with the polyclonal and monoclonal (cyto)keratin antibodies (Fig. 4a, c, d) but not with the polyclonal or monoclonal vimentin antiserum (Fig. 4b). The reaction of RGE 53 was normally slightly more intense than the reaction with the rabbit anti-keratin antiserum pKer (compare Fig. 4a and 4d). Stromal components

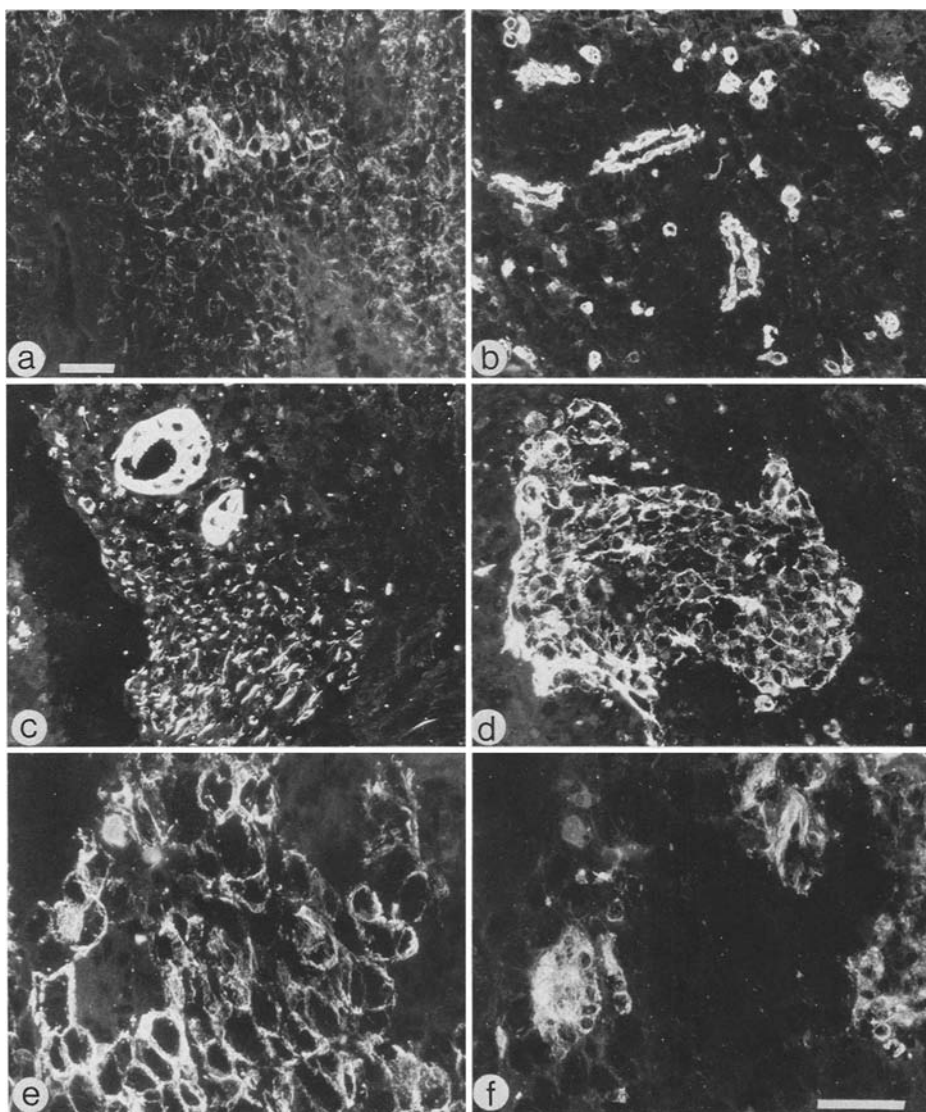


Fig. 4a–f. Embryonal cell carcinoma frozen sections, incubated with the polyclonal antibody to cytokeratins (a), the polyclonal antibody to vimentin (b, f) and RGE 53 (c, d, e). e and f represent double-label immunofluorographs. Bars indicate 50 μ m

of the tumour were positive for vimentin only. When double-label immunofluorescence microscopy was applied to cases nr. 8 and 9 using the rabbit antiserum to vimentin in combination with RGE 53 or the monoclonal antibody to vimentin in combination with the polyclonal anti-cytokeratin antiserum, it was obvious that both reactions are mutually exclusive (Fig. 4e, f). No reaction was found in the tumour cells with the antibodies to desmin, GFAP, MNF, or with RKSE 60.

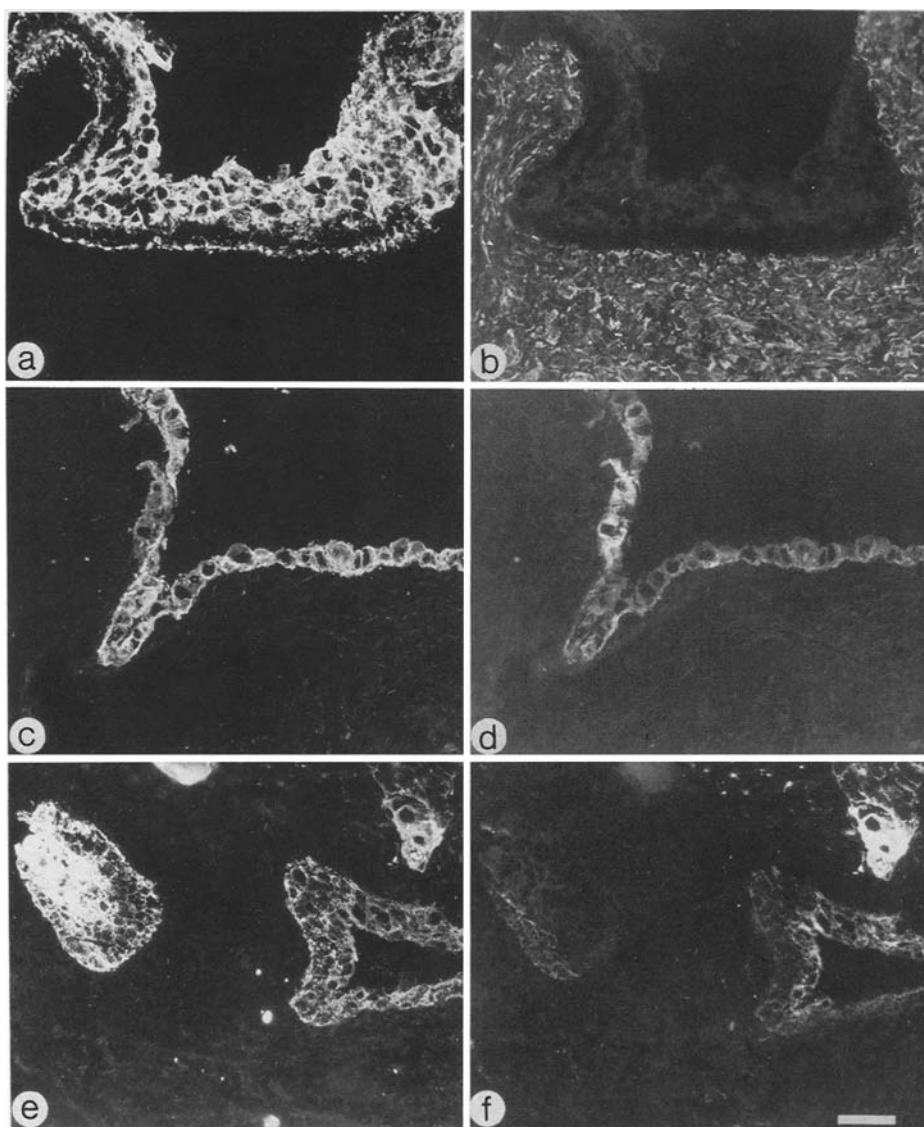


Fig. 5a-f. Double-label indirect immunofluorescence staining patterns of the teratocarcinoma, incubated with the cytokeratin antibodies (**a, c, e**, pKer; **d, f, g, i**, RGE 53) and the vimentin antibody (**b, h, j**). Bar indicates 50 μ m

The teratocarcinoma in the left testis (case 13) could be shown to contain different types of epithelial differentiation in H&E stained sections. In addition to keratinizing stratified squamous epithelium and ducts consisting of columnar and mucus producing epithelium, an additional component consisting of more atypical cells, focally forming ducts, and focally growing as solid nests, was observed. The stroma had a myxoid appearance. When

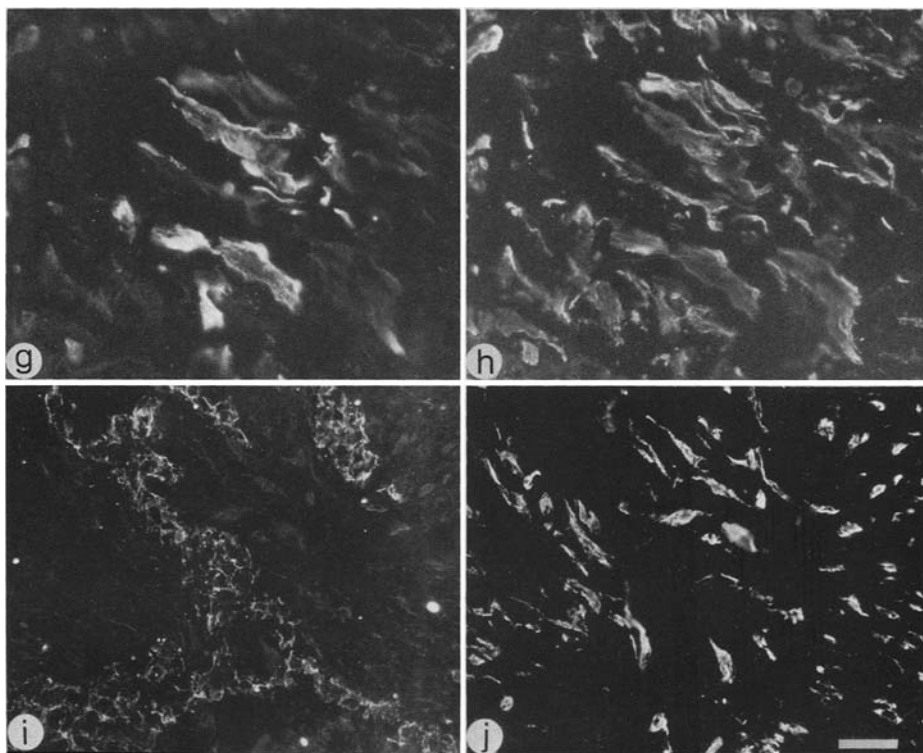


Fig. 5g-j.

frozen sections of this tumour were incubated with the rabbit antiserum to keratin, virtually all tumour cells, including the glandular-, squamous-, and spindle cell components as well as most dispersed tumour cells, were positive (Fig. 5a, c, e). When incubated with RGE 53 a distinction could be made between the glandular component (which was RGE 53 positive) and the squamous component (which was negative for RGE 53; compare Fig. 5c-f). Surprisingly, like all other components of the tumour, this latter component was negative with RKSE 60, the monoclonal antibody specific for keratinizing cells.

When incubated with the antibodies to vimentin, only the spindle cell component was stained in addition to (other) stromal elements (Fig. 5b). Double label immunofluorescence studies revealed a complete overlap between the staining patterns of the antikeratin rabbit antiserum and RGE 53 in the glandular component of the tumour and no overlap in the squamous component (Fig. 5c-f). An overlap between the staining patterns of the polyclonal antiserum to vimentin and RGE 53 on one hand or the polyclonal antiserum to keratin and the monoclonal antibody to vimentin on the other hand, was seen only in some spindle cells but not in other components of the tumour or stroma (Fig. 5g-j). Although not generally observed, co-expression of cytokeratins and vimentin has also been detected

in some other types of tumour cells (see for example Herman et al. 1983 and Ramaekers et al. 1983e). No reaction was seen in this tumour with the antisera to desmin, GFAP, or neurofilaments.

Discussion

In the practical management of malignant germ cell tumours of the testis, the most important diagnostic distinction is between seminomas on the one hand and non-seminomatous tumours including embryonal carcinoma, endodermal sinus tumour, teratocarcinoma and choriocarcinoma on the other (Paulson et al. 1982; Mostofi 1973). In practice the only situation in which real diagnostic difficulties may be encountered is the distinction between anaplastic seminomas and embryonal cell carcinomas. This distinction is an important one, however, since these tumours show significant differences in sensitivity to irradiation and in prognosis. Therefore, proper diagnosis of these germ cell tumours is essential for the selection of treatment modalities.

In some of the tumours an overlapping of diagnostic criteria such as cell and nuclear size, pleomorphism, and arrangement, as well as presence of a lymphocytic infiltrate may cause confusion. The results described here with intermediate filament protein antibodies show that seminomas contain mainly vimentin as their intermediate filament protein. In contrast, embryonal cell carcinomas usually contain cytokeratin but not vimentin.

Some tumours which are histologically classified as seminomas may show elevated serum levels of β HCG and/or α FP. It has been proposed that these seminomas have a significantly worse prognosis than those with normal serum levels of these markers (Morgan et al. 1982). However this interpretation remains controversial (Mauch et al. 1979). It is of interest that the one patient in our series who presented with a histologically pure seminoma but elevated serum levels of β HCG and α FP also showed an intermediate filament protein pattern intermediate between seminoma and embryonal cell carcinoma (see Table 2). However it is important to note that a mixed embryonal cell carcinoma/seminoma is characterized and recognized as such (because of extensive cytokeratin positive tumour areas) although the serum markers were not elevated.

Based on these studies and those previously reported (Battifora et al. 1984) we conclude that intermediate filament typing of testis tumours may provide a useful adjunct to routine histology and determination of serum β HCG and α FP in determining therapy and prognosis. The routine use of antibodies to vimentin in addition to cytokeratins provides a useful positive test to corroborate the cytokeratin negativity of seminoma.

Acknowledgements. We wish to thank Gert Westerhuis for excellent technical assistance with the immunofluorescence and immunoblotting assays. We thank Arie Kant for preparation and testing of the rabbit antisera. Mrs. Yvonne Stammes is acknowledged for excellent secretarial assistance in the preparation of the manuscript. Furthermore we thank our colleagues in the Pathology Departments for their help with tumour diagnosis and Ton van Eupen from the Medical Photography Department, Nijmegen for his help with the illustrations.

References

- Altmannsberger M, Weber K, Hölscher A, Schauer A, Osborn M (1982a) Antibodies to intermediate filaments as diagnostic tools. Human gastrointestinal carcinomas express pre-keratin. *Lab Invest* 46:520–526
- Altmannsberger M, Osborn M, Trenner J, Hölscher A, Weber K, Schauer A (1982b) Diagnosis of human childhood rhabdomyosarcoma by antibodies to desmin, the structural protein of muscle specific intermediate filaments. *Virchows Arch [Pathol Anat]* 39:203–215
- Battifora H, Sheibani K, Tubbs RR, Kopinski MI, Sun T-T (1984) Antikeratin antibodies in tumour diagnosis. Distinction between seminoma and embryonal carcinoma. *Cancer* 54:843–848
- Debus E, Weber K, Osborn M (1982) Monoclonal cytokeratin antibodies that distinguish simple from stratified squamous epithelia: characterization on human tissues. *EMBO J* 1:1641–1647
- Debus E, Moll R, Franke WW, Weber K, Osborn M (1984) Immunohistochemical distinction of human carcinomas by cytokeratin typing with monoclonal antibodies. *Am J Pathol* 114:121–130
- Denk H, Krepler R, Artlieb U, Gabbiani G, Rungger-Brändle E, Leoncini P, Franke WW (1983) Proteins of intermediate filaments: an immunohistochemical and biochemical approach to the classification of soft tissue tumours. *Am J Pathol* 110:193–208
- Franke WW, Grund C, Schmid E (1979) Intermediate-sized filaments present in Sertoli cells are of the vimentin type. *Eur J Cell Biol* 19:269–275
- Gabbiani G, Kapanci Y, Barazzzone P, Franke WW (1981) Immunochemical identification of intermediate-sized filaments in human neoplastic cells. A diagnostic aid for the surgical pathologist. *Am J Pathol* 104:206–216
- Herman CJ, Moesker O, Kant A, Huysmans A, Vooijs GP, Ramaekers FCS (1983) Is renal cell (Grawitz) tumour a carcinosarcoma? Evidence from analysis of intermediate filament types. *Virchows Arch B* 44:73–83
- Lane EB (1982) Monoclonal antibodies provide specific intramolecular markers for the study of epithelial tonofilament organization. *J Cell Biol* 92:665–673
- Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680–684
- Mauch P, Weichselbaum R, Botnick L (1979) The significance of positive chorionic gonadotropins in apparently pure seminoma of the testis. *Int J Radiat Oncol Biol Phys* 5:887–889
- Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalog of human cytokeratins: patterns of expression in normal epithelia, tumours and cultured cells. *Cell* 31:11–24
- Morgan DA, Caillaud JM, Bellet D, Eschwege F (1982) Gonadotrophin-producing seminoma: A distinct category of germ cell neoplasm. *Clin Radiol* 33:149–153
- Mostofi SK (1973) Testicular tumours epidemiologic, etiologic and pathologic features. *Cancer* 32:1186–1201
- Osborn M, Weber K (1983) Biology of Disease. Tumor diagnosis by intermediate filament typing: a novel tool for surgical pathology. *Lab Invest* 48:372–394
- Paulson DF, Einhorn L, Peckham M, Williams SD (1982) Cancer of the testis. In: DeVita Jr VT, Hellman S, Rosenberg SA (eds) *Cancer, principles and practice of oncology* J B Lippincott Company, Philadelphia, USA, pp 786–822
- Ramaekers FCS, Puts JGG, Kant A, Moesker O, Jap PHK, Vooijs GP (1981) Use of antibodies to intermediate filaments in the characterization of human tumors. *Cold Spring Harbor Symp Quant Biol* 46:331–339
- Ramaekers FCS, Puts JGG, Kant A, Moesker O, Jap PHK, Vooijs GP (1982) Differential diagnosis of human carcinomas, sarcomas, and their metastases using antibodies to intermediate-sized filaments. *Eur J Cancer Clin Oncol* 18:1251–1257
- Ramaekers FCS, Puts JGG, Moesker O, Kant A, Huysmans A, Haag D, Jap PHK, Herman CJ, Vooijs GP (1983a) Antibodies to intermediate filament proteins in the immunohistochemical identification of human tumours: an overview. *Histochem J* 15:691–713
- Ramaekers FCS, Puts JGG, Moesker O, Kant A, Jap PHK, Vooijs P (1983b) Demonstration of keratin in human adenocarcinomas. *Am J Pathol* 111:213–223
- Ramaekers FCS, Puts JGG, Moesker O, Kant A, Vooijs GP, Jap PHK (1983c) Intermediate

- filaments in malignant melanomas. Identification and use as marker in surgical pathology. *J Clin Invest* 71:635–643
- Ramaekers F, Huysmans A, Moesker O, Kant A, Jap P, Herman C, Vooijs GP (1983d) Monoclonal antibody to keratin filaments specific for glandular epithelia and their tumors. Use in surgical pathology. *Lab Invest* 49:353–361
- Ramaekers FCS, Haag D, Kant A, Moesker O, Jap PHK, Vooijs GP (1983e) Coexpression of keratin- and vimentin-type intermediate filaments in human metastatic carcinoma cells. *Proc Natl Acad Sci USA* 80:2618–2622
- Ramaekers FCS, Vroom TM, Moesker O, Kant A, Scholte G, Vooijs GP (1985a) The use of antibodies to intermediate filament proteins in the differential diagnosis of lymphoma versus metastatic carcinoma. *Histochem J* 17:57–70
- Ramaekers FCS, Moesker O, Huysmans A, Schaart G, Westerhof G, Wagenaar SS, Herman CJ, Vooijs GP (1985b) Intermediate filament proteins in the study of tumor heterogeneity: an in-depth study of tumors of the urinary and respiratory tract. *Ann N Y Acad Sci* (in press)
- Schlegel R, Banks-Schlegel S, McLeod JA, Pinkus GS (1980) Immunoperoxidase localization of keratin in human neoplasms, a preliminary survey. *Am J Pathol* 101:635–641
- Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedure and applications. *Proc Natl Acad Sci USA* 76:4350–4354
- Tseng SCG, Jarvinen MJ, Nelson WG, Huang J-W, Woodcock-Mitchell J, Sun T-T (1982) Correlation of specific keratins with different types of epithelial differentiation: monoclonal antibody studies. *Cell* 30:361–372
- Vorstenbosch CJAHV, Colenbrander B, Wensing CJG, Ramaekers FCS, Vooijs GP (1984) Cytoplasmic filaments in fetal and neonatal pig testis. *Eur J Cell Biol* 34:292–299

Accepted August 9, 1985