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Bone metastases of differentiated and medullary thyroid gland carcinomas

Usefulness and limitations of immunohistology performed on undecalcified plastic-embedded tissue specimens*

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Summary. Undecalcified methylmethacrylate(MMA)-embedded biopsies and surgical specimens from 20 bone metastases of differentiated or medullary thyroid carcinomas or prostate carcinomas were investigated immunohistologically for the presence of thyroglobulin, cytokeratin, vimentin, and CEA. The immunoreactions on MMA-sections revealed the same staining patterns as those demonstrated using paraffin sections of the primary lesions. Conversely, immunohistological examination of decalcified paraffin-embedded specimens of the same metastases yielded either false-negative results or results that did not allow an exact evaluation. The findings demonstrate the usefulness and limitations of immunohistology when performed on undecalcified plastic-embedded material.

Key words: Immunohistology – Methylmethacrylate-technique – Bone metastases – Thyroid carcinomas

Introduction

The achievement of artefact-free bone histology is a fundamental prerequisite for the evaluation of metabolic bone diseases and localised neoplastic processes in this tissue. Since the conventional acid decalcification methods for paraffin-histology of hard-tissues are coupled with a substantial loss of microscopic detail, the introduction of the undecalcified methylmethacrylate(MMA)-embedding and cutting technique (Delling 1972) represented a considerable diagnostic advance. This study describes a successful attempt to combine the advantages of undecalcified bone histology with the possibilities of immunohistology and demonstrates the relevance of these methods on examples of thyroid carcinoma bone metastases.

* Dedicated to Prof. Dr. G. Seifert on the occasion of his 65th birthday

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Material and methods

Secondary bone tumours from the files of Hamburg University's Department of Bone Pathology from the past 5 years were analysed retrospectively. In 12 cases the histology and/or clinical case history were indicative of metastases of differentiated thyroid carcinomas (papillary carcinomas (PC) $n=4$, follicular carcinomas (FC) $n=8$). Three cases are included in which the immunostaining had already been used for primary tumour diagnosis.

Further sections were prepared from the routinely formalin fixated MMA-embedded tissue samples. These were tested immunohistologically for the presence of thryroglobulin (TG), cytokeratin (KT), vimentin (VIM), and carcinoembryonic antigen (CEA). Instead of the usual cutting procedure, the sections were mounted on poly-L-lysine lined slides, deplasted for 3×10 min in (2-methoxyethyl)-acetate, rehydrated in 100% and 96% alcohol (each 2×10 min), and treated with 1% solution of periodic acid so as to block endogenous peroxidase. Specifications regarding source and dilution of the used antibodies are listed in Table 1. All immunohistological incubations were performed using the avidin-biotin-immunoperoxidase procedure (ABC-method: Hsu et al. 1981).

For checking purposes, the same incubation series were carried out on MMA-embedded bone metastases of 5 medullary thyroid carcinomas (MC) and 3 prostate carcinomas (PrCa). Furthermore, attempts were made – in the latter tumours as well as in 5 of the aforementioned differentiated thyroid carcinomas – at an immunohistological demonstration of calcitonin (CT) and of the prostate-specific antigen (PSA).

From 6 metastases (3 FC, 1 MC, 1 PC, 1 PrCa), paraffin-blocks of conventionally decalcified tumour tissue (Ossa-Fixona®: Fa. Roehm-Pharma, D-6108 Weiterstadt, 4 days at room temperature) were also available for study. Paraffin sections of these cases were analysed immunohistologically with the same antibodies as listed above.

Table 1. Source and dilution of the employed antibodies

	Antibodies		
Antigen	Clone	Source	Dilution
TG	J7-B49	Fa. Dianova, Hamburg, FRG	1:50
KT	KL 1	Fa. Dianova, Hamburg, FRG	1:1,000
VIM	V 9	Fa. Boehringer, Mannheim, FRG	1:20
CEA	431-31	Fa. Behringwerke, Marburg, FRG	1:3

Results

The immunohistological findings on MMA-sections are listed in Table 2. The monoclonal KT-antibody used in this study produced positive results with all carcinomas investigated. All PC and FC showed TG-positivity, the volume portions of labelled cells ranging between 5% and almost 100%. MC and PrCa were absolutely devoid of TG-immunoreactivity. CEA only was detectable in nearly all tumour cells of the 5 MC. In the 4 PC and in each 3 of the 8 FC and 5 MC cases, almost all tumour cells revealed cytoplasmic VIM-positivity. VIM could not be detected in the 3 PrCa cases.

The incubation series with 3 different commercially available CT antibodies resulted in a diffuse cytoplasmic and nuclear immunostaining, not only in all 5 MC but also in all of the other tumours. No specific immunoreaction, on the other hand, was achieved either in the 3 PrCa or in the remaining tumours when using the polyclonal PSA antibody. The immuno-

Table 2. Immunohistological findings on bone metastases

Tumour	*n*	TG	KT	VIM	CEA
FC	8	8/8	8/8	3/8	0/8
PC	4	4/4	4/4	4/4	0/4
MC	5	0/5	5/5	3/5	5/5
PrCa	3	0/3	3/3	0/3	0/3

FC, PC, MC = follicular, papillary, medullary thyroid carcinoma; PrCa = prostate carcinoma

histological incubations with TG, KT, VIM, and CEA antibodies performed on paraffin section of decalcified tumour specimens each time yielded negative results except for one of the FC with a doubtful uncertain positivity for TG.

The immunohistological demonstration of CEA in one MC case is shown in Fig. 1. Our findings among the differentiated thyroid carcinoma cases will be described briefly by means of two case reports.

Case 1. A 64-year-old man was admitted to the surgical clinic for osteosynthetic treatment of a spontaneous fracture of the neck of the right femur. Roentgenograms demonstrated multiple pulmonary nodules as well as numerous osteolyses of the thoracic spine.

During surgery, bone fragments were removed from the fracture gap. The MMA-histology revealed the metastasis of a tubular adenocarcinoma. Upon TG-immunohistology, about 60% of the tumour cells gave an intense cytoplasmic positivity (Fig. 2a). The lesion was thus diagnosed as metastatic follicular thyroid carcinoma, and a total thyroidectomy with bilateral modified radical neck dissection was performed.

The thyroidectomy specimen contained a grossly invasive tumour 4.5 cm in diameter that invaded the cervical soft tissue. Histology revealed the tumour to be entirely follicular in structure, the neoplastic thyrocytes containing nuclei of the prominent ground-glass appearance characteristic of the follicular variant of papillary thyroid carcinoma. Additional immunohistology carried out both on the paraffin-embedded primary lesion and on the MMA-embedded metastasis disclosed that all tumour cells contained KT and VIM (Fig. 2b and c).

Radioiodine ablation of residual thyroid parenchyma was performed postoperatively. The patient is presently undergoing radioiodine treatment, showing intense radioiodine uptake of the roentgenologically demonstrated pulmonary and osseous metastases.

Case 2. A 39-year-old female patient had noticed a slowly growing slightly elevated tumour growing in the region of the left skull cap during the last 2 years. Historically, it was known that there had been a subtotal resection of the left thyroid lobe 7 years before. Histology at that time had established the diagnosis of a microfollicular thyroid adenoma.

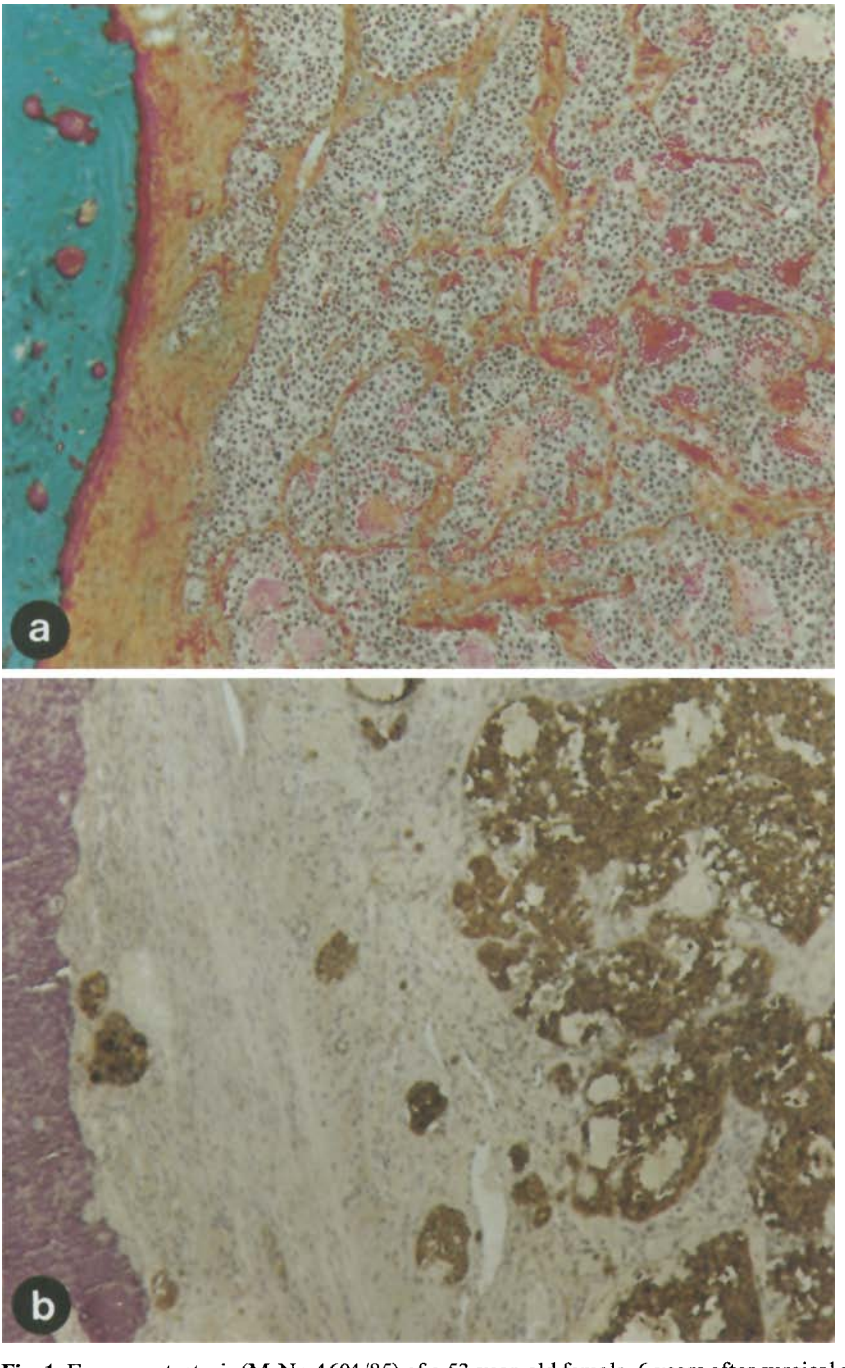

Fig. 1. Femur metastasis (M-Nr. 1601/85) of a 53-year-old female, 6 years after surgical removal of a medullary thyroid carcinoma. **(a)** Masson-Goldner ×72.5; **(b)** CEA ×72.5

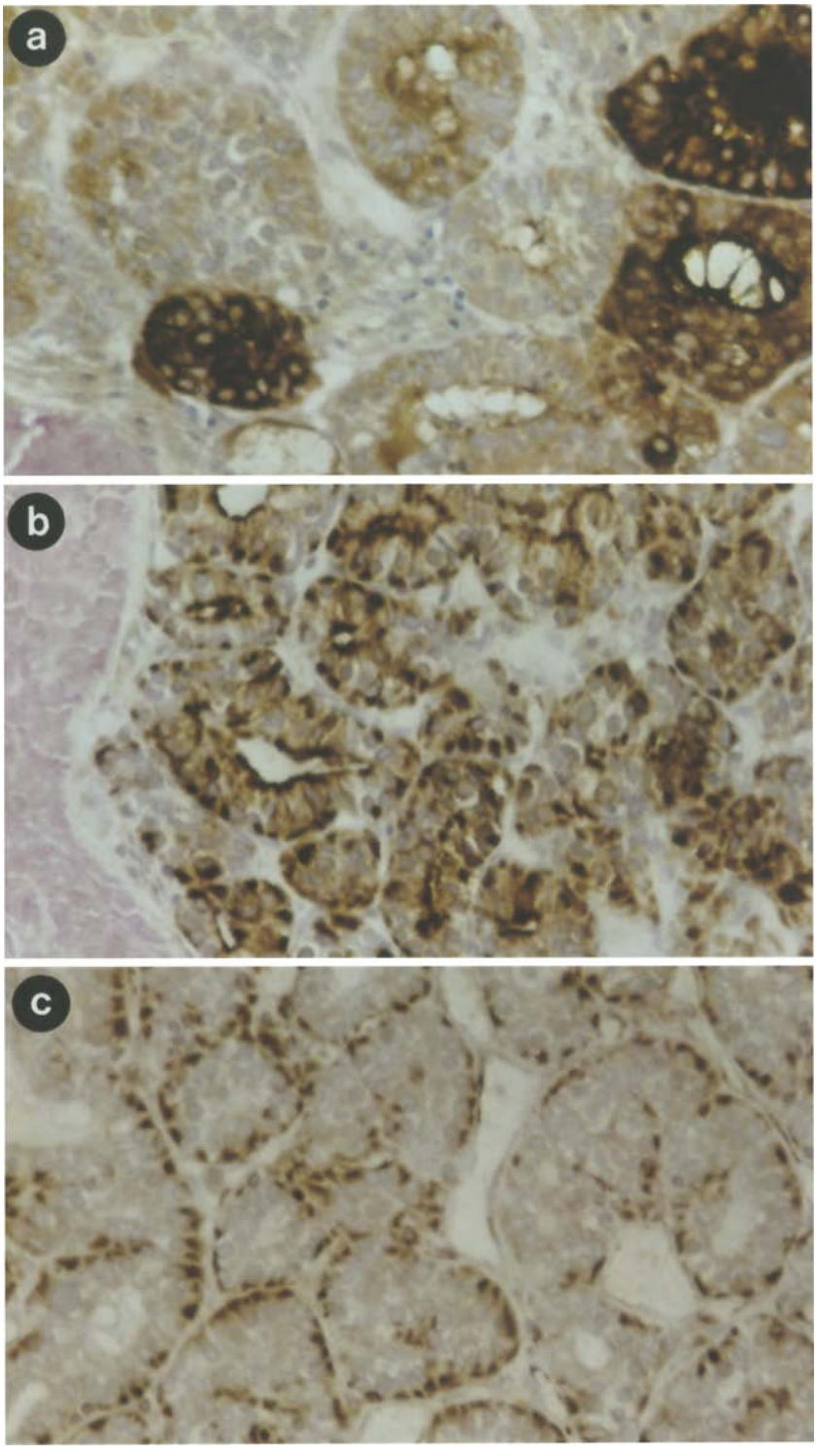

Fig. 2. Femur neck metastasis (M-Nr. 379/84) of a papillary thyroid carcinoma. (**a**) TG ×288; (**b**) KT ×288; (**c**) VIM ×288

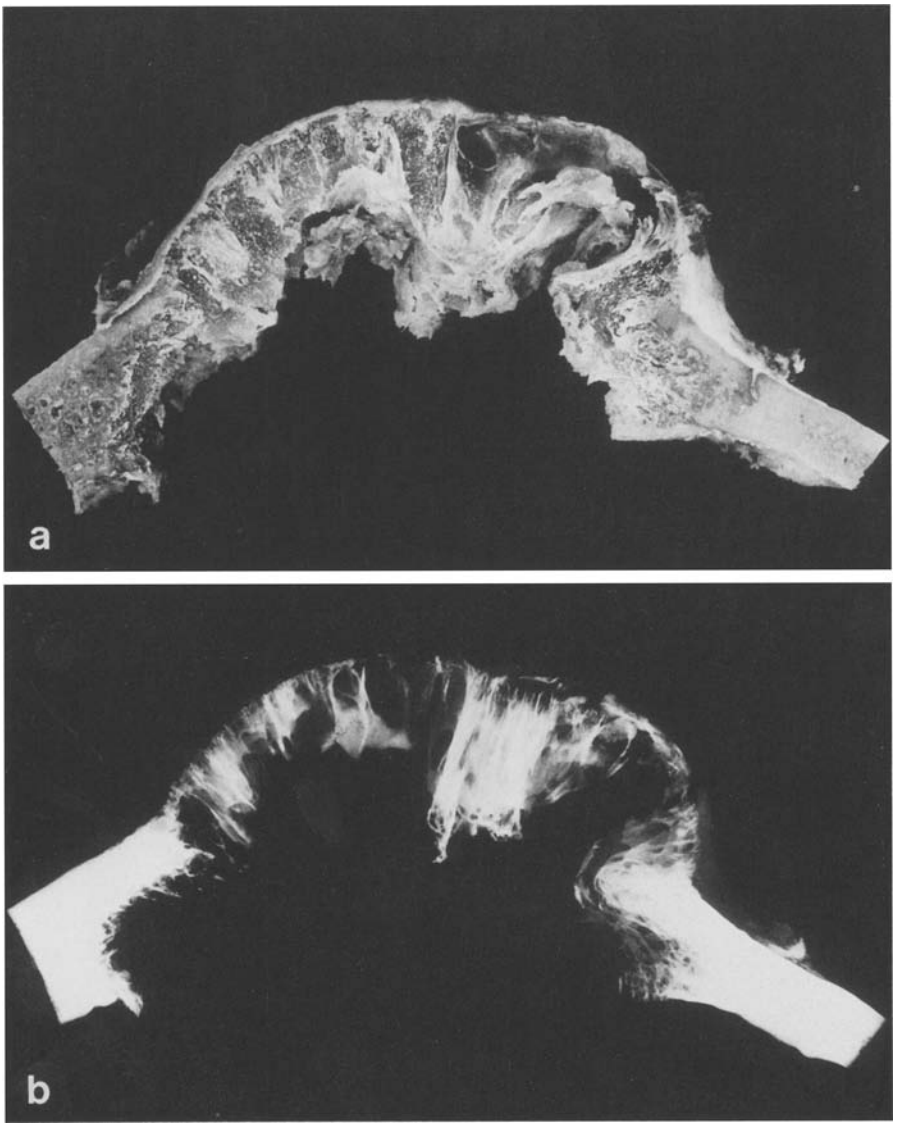

Fig. 3. Skull metastasis (M-Nr. 130/85) of a highly differentiated follicular thyroid carcinoma. (**a**) Macroscopic aspect and (**b**) roentgenogram of the surgical specimen

On admission to the neuro-surgical clinic, the tumour was roentgenologically shown to have a diamater of 8 cm. Due to the tentative diagnoses "eosinophilic granuloma" or "giant cell tumour", the tumour-bearing part of the skull was resected. Macroscopy and roentgenogram of the surgical specimen are illustrated in Fig. 3. The MMA-histology showed the bone metastasis of a follicular structured tumour (Fig. 4a), which upon immunohistology gave positive reactions for TG (Fig. 4b and c) and KT.

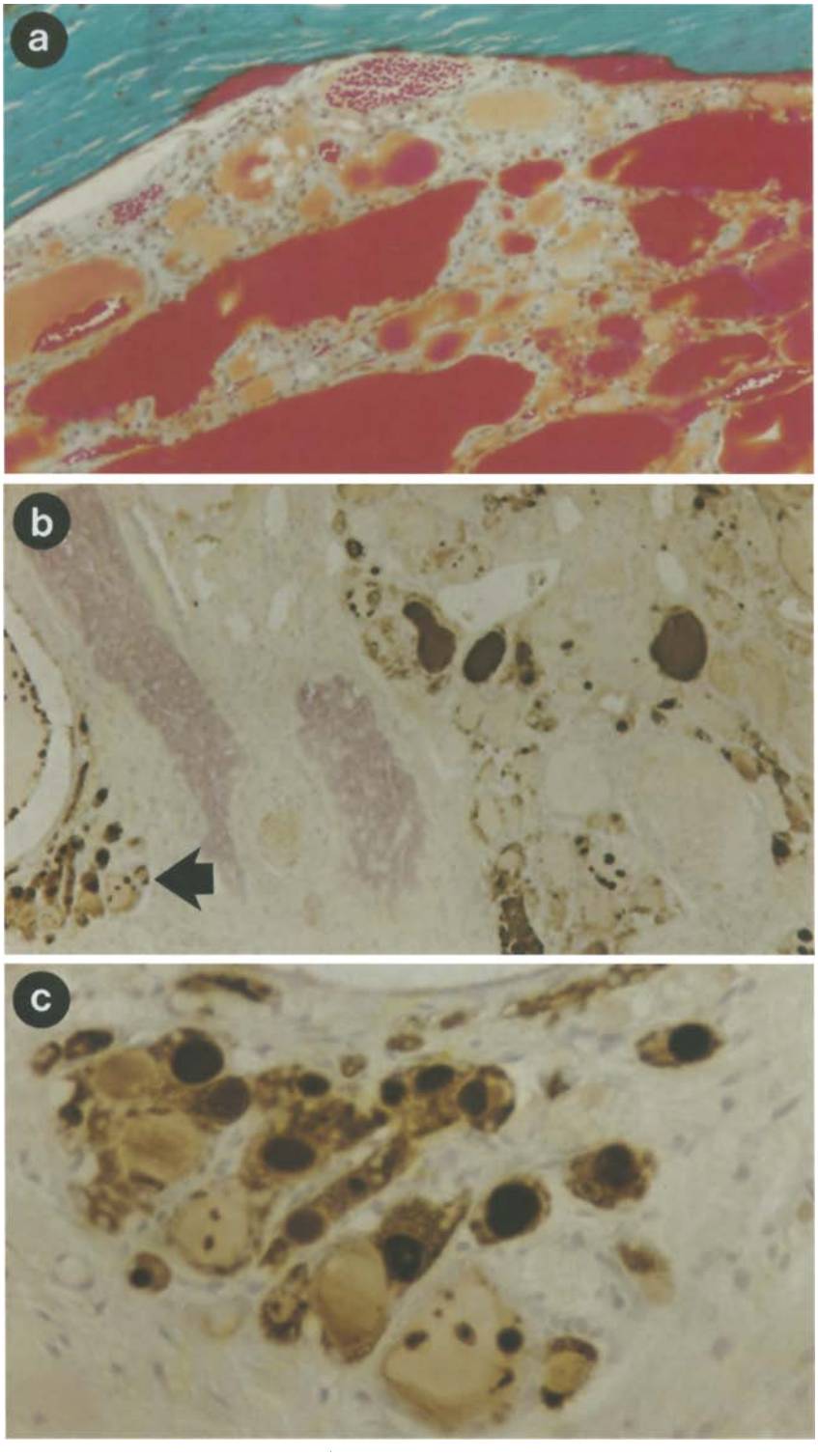

Fig. 4. Same case as Fig. 3: (**a**) Masson-Goldner ×115; (**b**) TG ×72.5, *arrow*= (**c**) TG ×288

Subsequently, the residual thyroid was removed surgically. Histological evaluation showed no trace of a tumour. Paraffin blocks of the thyroid adenoma removed 7 years before in another hospital and originally described as being 3.5 cm in diameter, were obtained to prepare further sections. These revealed a microfollicular and trabecular structured tumour without nuclear pleomorphism or evidence of mitoses. The tumour capsule was found to be intact in each of the 4 paraffin blocks.

Despite absent histological hallmarks of malignancy, this lesion was reclassified as an encapsulated follicular carcinoma having regard to the clinical course of disease. Roentgenologically as well as scintigraphically there was no evidence of further metastases. The patient receives thyroid hormone substitution and is now symptom-free 13 months later.

Discussion

During the last decade, immunohistology has gained entry into the routine of diagnostic histopathology. For the thyroid gland, this holds true especially for the detection of TG (Böcker et al. 1981) which is specific to this organ and to follicle cell tumours and next to this for CT (DeLellis 1981) and CEA (DeLellis et al. 1978) as marker substances of thyroid C-cells and medullary thyroid carcinomas. The commercially available monoclonal and polyclonal antibodies for the named antigens supply reliable results when used on paraffin and on cryostat material.

However, as our own experience shows, they can not be utilized for diagnostic purposes on decalcified material where they produced either false-negative results or results that did not allow an exact evaluation. Restrictively one has to note that we did not carry out incubation series on hard tissue specimens decalcified by different methods but merely by one acid-decalcification procedure, routinely used in our institute. Furthermore it is necessary to mention that the loss of antigenicity we demonstrated for TG, KT, VIM, and CEA can not automatically be assumed for other marker substances. Recently Schulz et al. (1985) reported on the successful immunohistological detection of osteonectin in acid-decalcified bone tumour specimens.

Since, however, during the last few years the method of artefact-free MMA-histology has gained increasing acceptance with numerous institutes and departments of pathology adopting this method as a matter of routine, it appeared also reasonable to examine the possibility of performing immunohistology on plastic-embedded tissues.

For our experiments, we chose 4 marker substances identified by corresponding antibodies which we were familiar with from diagnosis on paraffin and cryostat material. The specific reactivity of the monoclonal anti-cytokeratin antibody KL1 with all MMA-embedded carcinomas corresponded to its reliable immunoreaction with all epithelial tissues on frozen and paraffin sections (Viac et al. 1983).

The staining properties of the monoclonal VIM-antibody used in this study are well documented in the literature (Osborn et al. 1984). We tested

the expression of VIM as a second type of intermediate filament protein since it has recently been demonstrated not only as a cytoskeletal component of mesenchymal tissues but also of many epithelial tumours such as thyroid carcinomas (Miettinen et al. 1984; Droese et al. 1985) and renal carcinomas (Waldherr and Schwechheimer 1985). It thus gains relevance for the immunohistological differential diagnosis of occult carcinomas.

Our results regarding the reactivity and distribution of VIM in MMA-embedded metastases of differentiated thyroid carcinomas were identical to our findings with the respective paraffin-embedded primary tumours, 7 of which were available for study. In accordance with Altmannsberger et al. (1986) we found a coexpression of VIM and KT for each of the PC tested as opposed to such a co-expression detectable only for a minority of the FC.

The follicle cell origin of the 12 metastases of differentiated thyroid carcinomas could be confirmed in all cases by a specific intense diffuse cytoplasmic TG-positivity. As expected, the MC and PrCa cases were devoid of TG immunoreaction.

In order to verify the diagnosis of a metastatic MC immunohistologically in the 5 cases with a histologically verified primary C-cell carcinoma, we had, at first, carried out incubations with 3 different polyclonal CT-antibodies. These, however, produced non-specific positivity when incubating follicle-cell as well as prostate carcinomas. Hence, in the next stage, we examined the staining pattern of a commercially available monoclonal CEA-antibody (Bosslet et al. 1985) which according to our own experience on a large scale of PC, FC, and MC primaries does not react with non-C-cell thyroid carcinomas (unpublished results). This antibody thus substantially differed from the polyclonal CEA antibodies, recently described by Vogel et al. (1985) – despite absorbtion – to show immunoreaction with up to 40% of follicle cell carcinomas.

Using the monoclonal CEA-antibody on our MMA-embedded material led to the same results as noted above for our paraffin-embedded thyroid carcinoma primaries. It was thus possible to ascertain the possibility of using CEA as a tumour-typical although not tumour-specific antigen of MC in immunohistological diagnosis on undecalcified hard tissue.

Our results show that immunohistology yields diagnostically relevant reliable results when performed on MMA-embedded material. Its utilization is recommended to those institutes of pathology where the method of hartcut histology has been introduced. This is especially relevant for evidence of the thyroid associated antigen TG, and for the intermediate filament proteins KT and VIM as well as for CEA using the monoclonal antibodies we examined. Our hitherto unsatisfactory attempts to illustrate CT and PSA show that – as in the case of acid decalcification – a loss of antigenicity can occur also in the MMA-embedding process and that systematic test series should be employed before diagnostic utilization of these and other markers.

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