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Desmin: its value as a marker of muscle derived tumours using a commercial antibody

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Summary. This study examined the staining reactions of a commercially available anti desmin antibody in 192 soft tissue sarcomas, 30 carcinomas and 22 malignant melanomas. Only 63% of rhabdomyosarcomas and 50% of leiomyosarcomas showed a positive reaction. Three non muscle sarcomas also reacted with the antibody. No positivity was seen amongst the carcinomas or the melanomas. These results do not compare favourably with the documented results of non commercial desmin antibodies in the literature. The most likely cause of the discrepancy in the varying results is the differing source of the antibody.

Key words: Desmin – Leiomyosarcomas – Rhabdomyosarcomas – Sarcomas – Carcinomas

Introduction

Reports in the literature suggest that desmin, an intermediate filament, can be a useful marker of tumours showing smooth and skeletal muscle differentiation. However many of these are based on studies using non commercially available antibodies and using tissues and tumours fixed in a variety of different ways. This investigation was carried out to evaluate the usefulness of a commercially available desmin antibody as a muscle tumour marker in formalin fixed material and to compare it to the non commercial desmin antibodies in terms of its sensitivity and specificity by a review of the literature. One hundred and ninety two sarcomas, 30 carcinomas and 22 malignant melanomas were reacted with an anti desmin antibody obtained from Labsystems. The results are presented.

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Material and methods

All tumours listed in Table 1 were examined in the study. All had been formalin fixed and paraffin embedded. A precise time for duration of fixation is not known as this was a retrospective study and the blocks ranged in age from 1 year to 24 years. The desmin antibody was obtained from Labsystems and had been raised against purified desmin isolated from cow Purkinje fibres. The peroxidase anti peroxidase (PAP) method was used. After treatment in 3% H_2O_2 in methanol alcohol for 25 min and after trypsinization in 1% trypsin +1% $CaCl_2$ for 30 min the sections were incubated at room temperature in monoclonal mouse IgG antibodies to desmin (Labsystems) diluted 1:175 with phosphate buffered saline (PBS). This was followed by washing and by incubation for 45 min at room temperature with Peroxidase conjugated rabbit anti mouse immunoglobulin (Dakopatts P260) diluted 1:50 with PBS +1% Bovine serum Albumin (BSA). After washing, the sections were stained with diaminobenzidine and counterstained with Haematoxylin. All the incubation steps were performed in a humidity chamber and with gentle agitation.

All sections were coded and were examined in the absence of the H&E diagnosis. Discrete cytoplasmic staining indicated a positive result.

Table 1. Anti Desmin staining of sarcomas, carcinomas and melanomas

Tumours	No	+ve	−ve
Leiomyosarcomas	22	14	8
Rhabdomyosarcomas	21	11	10
Liposarcomas	22	1	21
Malignant fibrous histiocytomas	24	1	23
Neurofibrosarcomas	16	0	16
Synovial sarcomas	20	0	20
Angiosarcomas	7	0	7
Haemangiopericytomas	18	0	18
Kaposi's sarcomas	7	0	7
Clear cell sarcomas	8	0	8
Epithelioid sarcomas	7	0	7
Fibrosarcomas	16	0	16
Sarcomas undifferentiated	4	1	3
Carcinomas	30	0	30
Melanomas	22	0	22

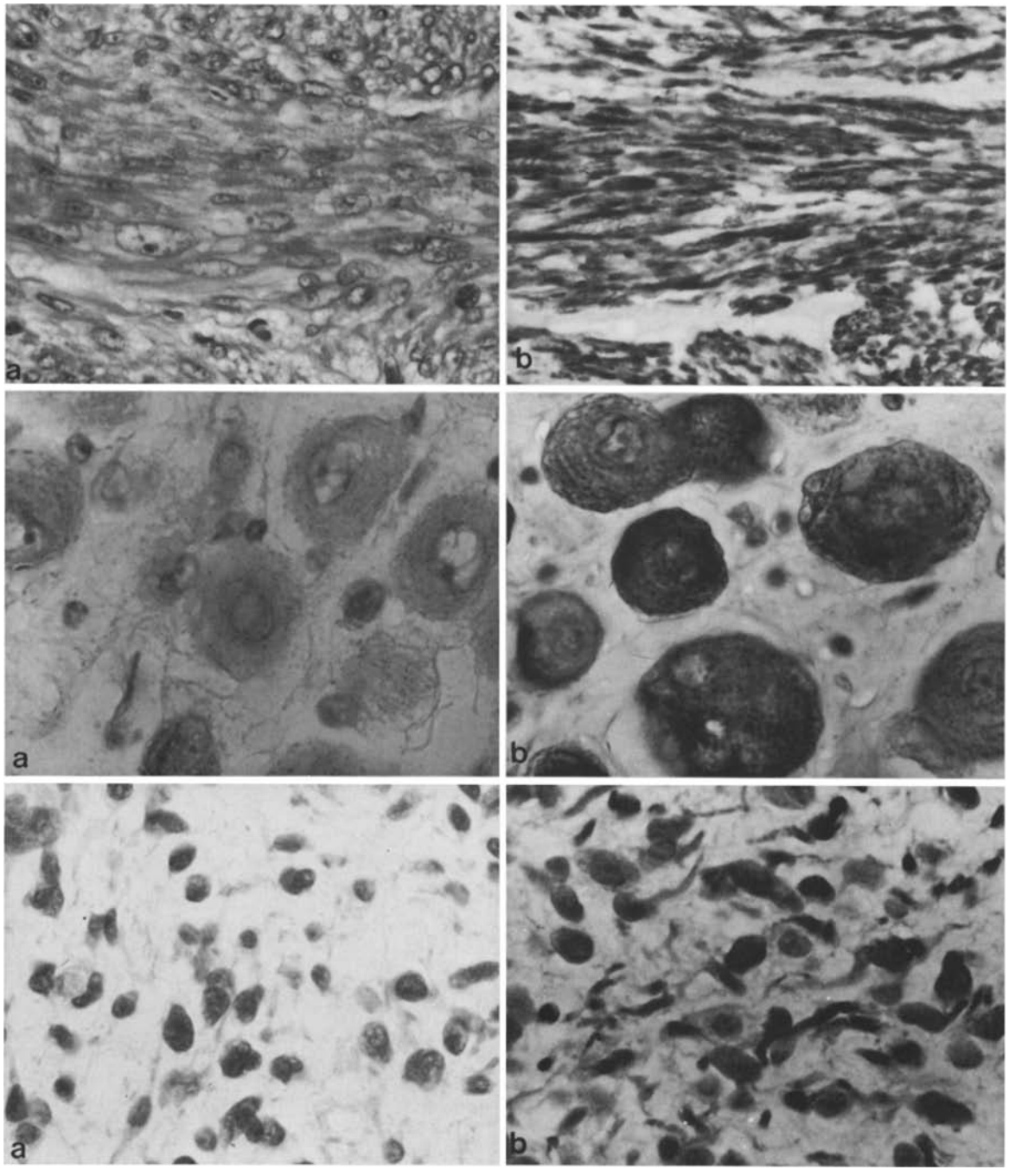
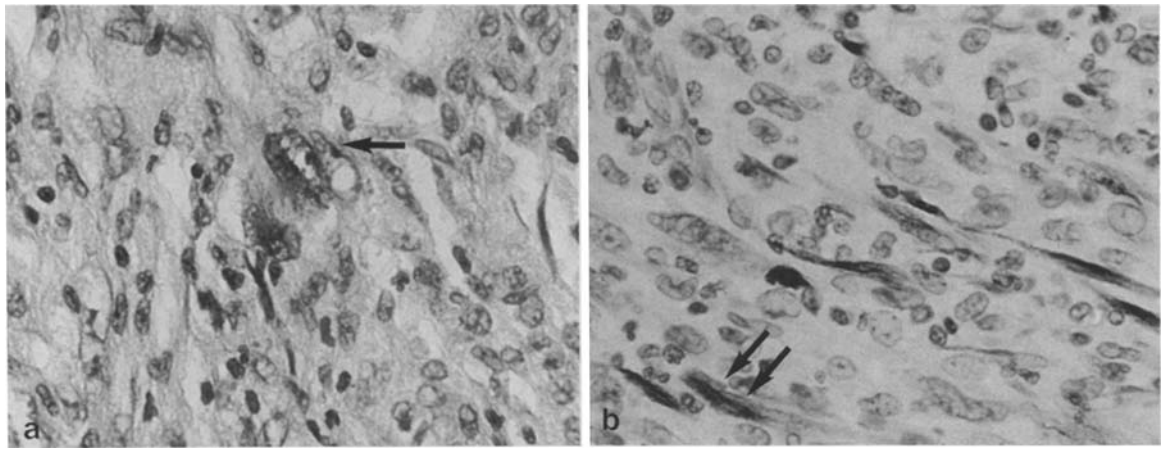


Fig. 1a, b. This is a leiomyosarcoma showing fascicles of tumour cells in longitudinal and cross-section. Intense staining with the desmin antibody is shown. **a** H.E. ×400; **b** Anti Desmin ×400

Fig. 2a, b. This is an embryonal rhabdomyosarcoma. Comparison between the H.E. stained section (**a**) and the anti desmin stained section (**b**) demonstrates the intense reactivity with the desmin antibody. **a** H.E. ×400; **b** Anit Desmin ×400

Fig. 3a, b. This tumour is a myxoid liposarcoma composed of ovoid and round cells set in a myxoid stroma. Anti desmin staining is evident in **a** H.E. ×400; **b** Anti Desmin ×400



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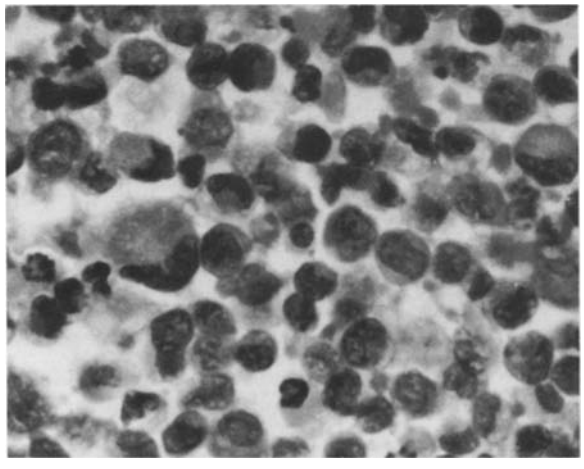

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Fig. 4a, b. This is a malignant fibrous histiocytoma. The tumour is composed of spindle and ovoid cells arranged in a storiform pattern (not illustrated). Large histiocytic cells were present (*arrow*). The spindle shaped cells (*double arrows*) stained with the desmin antibody. **a** H.E. ×400; **b** Anti Desmin ×400

Fig. 5. This is an embryonal rhabdomyosarcoma from the leg of a 4 year old boy. Despite staining with anti desmin on two occasions and despite positive staining of controls in the same batch of sections, the tumour did not react with the desmin antibody. Anti Desmin ×400

The following control sections were used: a positive control (small bowel wall), a negative control (omission of the primary antibody), and an endogenous peroxidase control where H_2O_2/Methanol was omitted.

Results

The results are summarised in Table 1. Of the 192 soft tissue sarcomas included in the study positive staining was found in 14/22 leiomyosarcomas (Fig. 1), 11/21 rhabdomyosarcomas (Fig. 2), 1/22 liposarcomas (Fig. 3), 1/24 malignant fibrous histiocytomas (Fig. 4) and in 1/4 sarcomas unclassified. The number of positively staining tumour cells per section varied considerably amongst the leiomyosarcomas and rhabdomyosarcomas, in some more than 90% of tumour cells stained but in others less than 10 cells per section stained. Therefore it was essential to examine each section carefully using a high magnification.

Staining was repeated in the group of negatively staining leiomyosarcomas and rhabdomyosarcomas so as to minimise the possibility of technical error. No additional staining was identified. Repeat staining of the "false positive" liposarcoma, the malignant fibrous histiocytoma and the unclassified sarcoma was also performed and a positive reaction was again obtained with approximately 10% of the tumour cells staining in the liposarcoma, 20% in the malignant fibrous histiocytoma and over 90% in the unclassified sarcoma. The liposarcoma, which was a classical example of the myxoid variant, was composed of predominantly round cells set in a loose myxoid stroma (Fig. 3). The malignant fibrous histiocytoma was composed mainly of spindle and ovoid cells arranged in a storiform pattern with a considerable number of tumour giant cells (Fig. 4). The positive tumour in the sarcoma unclassified category was an undifferentiated tumour composed of sheets of ovoid and spindle cells with vesicular nuclei and prominent nucleoli arranged in a haphazard manner. Many tumour giant cells were present. No cross striations or myofilaments were evident. The appearances suggested a malignant fibrous histiocytoma but this could not be conclusively confirmed and so it was included in the sarcoma unclassified group.

Discussion

Desmin is regarded as a useful immunocytochemical marker of tumours showing muscle differentiation. However until now the sensitivity and specificity of a commercially available anti desmin antibody have not been adequately tested. Review of the literature with regard to the staining reactions of desmin in leiomyosarcomas and rhabdomyosarcomas (some unfixed, some alcohol fixed and some formalin fixed) suggests that these tumours show almost a 100% rate of positive staining (Miettinen et al. 1982; Bonazzi del Poggetto-C 1983; Denk et al. 1983; Schauer et al. 1984; Altmannsberger et al. 1985; Miettinen et al. 1985). This is in marked contrast to the present study where only 63% of leiomyosarcomas and 50% of rhabdomyosarcomas stained. The discrepancy in results does not seem to be related to the duration of or method of fixation of the tumours as the same group of tumours was examined with a panel of antibodies and a high rate of appropriate reactivity was found (Leader et al. 1986a; Leader et al. 1987). Nor can the use of trypsinization be incriminated as positive control sections stained intensely. It has also been shown that trypsinization improves the reliability of the PAP technique in formalin fixed paraffin embedded material (Mepham et al. 1979; Isaacson et al. 1980; Hautzer et al. 1980). Radiation can alter tumour epitopes and produces misleading immunocytochemical results (Leader et al. 1986b). However review of the unstained leiomyosarcomas in this study did not reveal a higher incidence of radiation exposure. Finally the question of tumour heterogeneity must be raised. If this was the explanation for the negative staining of smooth muscle tumours in this study it would also have been noted in other studies using desmin antibodies. For these reasons the discrepancy between the staining reactivity of the desmin antibody in this study and others is almost certainly the differing source of antibody used. All of the studies quoted above refer to non commercially available desmin antibodies whereas the desmin antibodies used in this study were obtained from a commercial source (Labsystems).

The reason for the false positive staining in three non muscle sarcomas may at first seem surprising. However two of the tumours were probable malignant fibrous histiocytomas. Cells resembling myofibroblasts have been seen in malignant fibrous histiocytomas at ultrastructural level and anti desmin reactivity has recently been reported in 6/10 malignant fibrous histiocytomas (Lawson et al. 1987).

The cause of the positivity in the liposarcoma is more puzzling. It may be significant that this tumour was a recurrence of a liposarcoma that had been earlier treated by radiotherapy. It has been suggested that radiotherapy may alter a tumour's epitopes thus resulting in unexpected staining reactions with tumour markers (Leader et al. 1986b). Whilst desmin is the main subunit protein of intermediate filaments in mature muscle cells (Lazarides and Hubbard 1976; Altmannsberger et al. 1981); Bennet et al. (1979) have shown that vimentin is the predominant intermediate filament during the early stages of myogenesis and that desmin appears only with maturation. The expression of desmin in the smooth muscle of vessel walls is variable and surprisingly in the aorta vimentin is found in much greater quantities than desmin (Gabbiani et al. 1981; Frank and Warren 1981). It has therefore been suggested that it may be possible to distinguish vascular from non vascular leiomyosarcomas by their reactivity with desmin antibodies (Denk et al. 1983). Desmin has been detected in glomerular and extra glomerular mesangial cells supporting the theory that these arise from a specific subset of vascular smooth muscle cells (Bachmann et al. 1983).

In conclusion, this paper highlights the necessity for rigorous trial of commercial antibodies under controlled conditions before it can be accepted that these antibodies are as reliable as their non commercial equivalents.

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