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Diagnostic tools for differentiating between pleural mesothelioma and lung adenocarcinoma in paraffin embedded tissue. Part I: immunohistochemical findings

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Abstract. Specimens of 27 histologically definite mesotheliomas and 34 proven adenocarcinomas were examined with a panel of 14 antibodies: pan-epithelial antibody Lu-5, anti-keratin-18, anti-keratin-7, Ber-EP4, anti-Leu-M1, HEA-125, anti-carcino-embryonic antigen (CEA), anti-blood group-related antigens (anti-BGR A, B, H), B 72.3, anti-placental alkaline phosphatase (PLAP), anti-vimentin and BMA-120 used to determine their value in the differentiation between pleural mesothelioma and lung adenocarcinoma. Lu-5, anti-cytokeratin-7 and -18, B 72.3 and PLAP reacted in a high percentage of cases with both mesothelioma and adenocarcinoma. Anti-CEA and anti-Leu-M1 did not react with any of the 27 mesotheliomas tested but showed a reaction in 75% (anti-CEA) and 66% (anti-Leu-M1) of the lung adenocarcinomas. Seventeen percent of the adenocarcinomas and 96% of the mesotheliomas showed a positive reaction with anti-vimentin. Ber-EP4 was demonstrated in all lung adenocarcinomas, but only in 2 mesotheliomas in a focal manner (7%). HEA-125 and anti-BGR A, B, H reacted with 83% (HEA-125) and 75% (anti-BGR A, B, H) of the lung adenocarcinomas. The statistical parameters, sensitivity and efficiency were estimated and a normogram for judging the diagnostic power of a single antibody for the differential diagnosis of mesothelioma versus adenocarcinoma was developed. According to this, Ber-EP4, HEA-125, anti-BGR A, B, H and anti-CEA were, in descending order, the most powerful discriminatory antibodies.

Key words: Malignant mesothelioma – Lung adenocarcinoma – Ber-EP4 – BMA-120 – Blood-group-isoantigen

Introduction

Light microscopy, histochemistry, electron microscopy and more recently immunohistochemical examination with panels of mono- and polyclonal antibodies are all used to differentiate between mesothelioma and adenocarcinoma of the lung (Dewar et al. 1987; McCaughey et al. 1991; Sheibani et al. 1991a).

Antibodies of diagnostic relevance include carcinoembryonic antigen (CEA), Leu-M1, B 72.3, HEA-125, anti-vimentin, and a variety of anti-keratins (Battifora and Kopinski 1985; Blobel et al. 1985; Bollinger et al. 1989; Brockmann et al. 1990; Chenard-Neu et al. 1990; Churg 1985; Ghosh et al. 1987; Holden and Churg 1984; Kahn et al. 1986; Loosli and Hurlimann 1984; Momburg et al. 1987; Mullink et al. 1986; Ordonez 1989; Otis et al. 1987; Pfaltz et al. 1987; Sheibani et al. 1985; Soosay et al. 1991; Warnock et al. 1988; Wick et al. 1989, 1990; Wirth et al. 1990). More recently, blood group-related antigens A, B, H (anti-BGR A, B, H) (Alvarez-Fernandez and Carretero-Albinana 1991; Jordon et al. 1989; Kawai et al. 1988, 1991; Noguchi et al. 1989; Sheibani et al. 1991a; Wick et al. 1990), the endothelial marker BMA-120 (Alles and Bosslet 1986; Berghäuser et al. 1989) and Ber-EP4 (Latza et al. 1990; Sheibani et al. 1991b) have been evaluated.

The immunohistochemical diagnosis of mesothelioma in paraffin-embedded material is still made by exclusion of markers for tumours of other origins. The lack of mesothelioma-specific antibodies is further aggravated by contradictory reports about frequency patterns of the antigen expression (Azumi et al. 1992; Chang et al. 1992; McCaughey et al. 1991; Sheibani et al. 1991a). These immunohistochemical discrepancies may arise from impaired antigen preservation due to tissue fixation and embedding, the type and source of the antibody and immunohistochemical procedure used (Otis et al. 1987; Pfaltz et al. 1987; Wirth et al. 1990).

To overcome these problems, we developed a normogram on the basis of immunohistochemical results with 14 antibodies applied to two defined groups. The most important antibodies were Ber-EP4, vimentin and HEA125.

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Materials and methods

The tumour samples were fixed in buffered 4%-formalin and embedded in low-melting paraffin (Paraplast). They were classified according to the recommendations of the US/Canadian Mesothelioma Panel (MCaughey et al. 1991) as definite mesotheliomas, if they had had definite microscopic features of epithelial mesotheliomas: cuboidal to polygonal cells forming sheets, papillary structures, acinar spaces, or lining cleft; cells with single prominent nucleoli and a constant nuclear/cytoplasmic ratio. The conventional histological examinations were supplemented by electron microscopy in 21 cases as well as by clinical and radiological findings. PAS with and without diastase-pretreatment and Alcian blue staining with and without hyaluronidase digestion were used to test for mucins and glycogen.

The group of 27 mesothelioma included 12 epithelial, 13 biphasic and 2 sarcomatous malignant mesotheliomas.

The adenocarcinoma group comprised 24 proven primary tumours of the lung.

For immunohistochemistry 5 µm thick sections were deparaffinized in xylene and rehydrated in descending grades (100%–70%) of ethanol. To enhance the immunostaining of selected antibodies, the sections were incubated with 0.1% protease (type XIV; Sigma, St. Louis, Mo., USA; 0.5 M TRIS-buffer; pH, 7.5) for 30 to 60 min. The enzymatic reaction was stopped in cold TRIS-buffer (0.5 M; pH, 7.55). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in absolute methanol (30 min). Nonspecific binding was blocked by pre-incubation with normal serum of the species of the bridging antibody (dilution 1:10, 30 min).

The avidin-biotin-complex technique (Hsu et al. 1981) was employed using the ABC-elite kit (Vector, Burlingame, Calif., USA) according to instructions of the supplier. The sections were incubated with the primary antibodies listed in Table 1 overnight at 4° C in a moist chamber. The immunoperoxidase was visualized with 3.3′ diaminobenzidine (Serva, Chemie Brunnschwig, Basel). The intensity of the staining product was increased by treatment of the slides with 1% osmium tetroxide for 2 min. The slides were counterstained with haematoxylin (Shandon, IG Instrumenten-Gesellschaft, Zürich), rehydrated and mounted in Eukitt (Kindler, Freiburg, Germany).

When incubating for blood group antigens, washing between the incubation steps was done with a TRIS-buffered saline (TBS)/Tween-buffer (1.25 g Tween-20/1 L TBS-solution) to reduce background staining.

Staining controls were carried out by replacing the primary antibody by PBS, verifying internal tissue controls and simultaneous staining of known positive tissue controls.

The portion of labelled tumour cells in relation to all tumour cells was semi-quantitatively classified as "negative" (0%), "single cells" (1–5%), "focal" (6–50%), "extensive" (51–75%) and "diffuse" (76–100%).

In the present analysis no cut-off value was used, but, rather, single cell staining was taken as a positive result.

Table 1. Antibodies used in the study

Antibody	Source	Type of reactivity	Dilution
Lu-5[a] (MAb)[c]	Hoffmann-LaRoche, Basle, Switzerland	c[h]	1:1000
Anti-Keratin-18[a,b] (MAb)	Progen, Heidelberg Germany	c	1:5
Anti-Keratin-7[a] (MAb)	Sigma, St. Louis USA	c	1:5000
Ber-EP4[a] (MAb)	Dr. Stein, Berlin, Germany	m[g]	1:64
Anti-Leu-M1 (MAb)	Dako Corporation, Denmark	m	1:400
HEA-125[a] (MAb)	Read Systeme AG, Bad Zurzach, Switzerland	m	1:40
Anti-CEA (MAb)	Amersham International PLC, Zurich, Switzerland	c	1:1
Anti-BGR A[d] (MAb)	Dako Corporation Denmark	m	1:400
Anti-BGR B (MAb)	Dako Corporation, Denmark	m	1:400
Anti-BGR H (MAb)	Dako Corporation, Denmark	m	1:20
B72.3 (MAb)	Dr. Schlom, NIH[e], Bethesda, USA	m, c	1:200
PLAP (PAb)[f]	Dako Corporation, Denmark	m	1:80
Anti-vimentin (MAb)	Boehringer, Mannheim, Germany	c	1:160
BMA-120 (MAb)	Behring, Marburg, Germany	m	1:80

[a] After pronase pretreatment of the slides, [b] Anti-Keratin-18 Ks 18.04, [c] MAb: Monoclonal antibody, [d] Anti-BGR: Anti blood group related antigen, [e] NIH: National Institute of Health, [f] PAb: Polyclonal antibody, [g] m: membraneous, [h] c: cytoplasmic

Table 2. Results of the 2×2 table analysis, the Kolmogoroff-Smirnoff test and the odds ratio of the various antibodies for the two groups mesothelioma and adenocarcinoma, respectively

Antibody	Chi-square test	Fisher's exact test (2P)	CC	Komogoroff-Smirnofftest (2P)	Odds ratio	
					Mesothelioma	Adenocarcinoma
Lu-5	–	–		ns	∞*	∞*
Anti-Keratin-18	2.34*	0.2165	0.210	ns	∞*	0.0
Anti-Keratin-7	0.04	1.0000	0.029	0.17	1.2	0.86
Ber-EP4	43.59	0.0001	0.679	0.01	0.0	∞*
Anti-Leu-M1	26.23	0.0001	0.583	0.01	0.0	∞*
HEA-125	33.26	0.0001	0.628	0.01	0.01	130.0
Anti-CEA	31.30	0.0001	0.617	0.01	0.0	∞*
Anti-BGR A, B, H	30.35	0.0001	0.611	0.01	0.01	98.8
B 72.3	18.14	0.0001	0.512	0.01	0.05	22.0
PLAP	12.36	0.0006	0.442	0.01	0.10	9.6
Anti-vimentin	33.26	0.0001	0.628	0.01	130.1	0.08
BMA-120	1.63	0.3539	0.176	ns	4.0	0.25

CC, Contingency coefficient;
* Division by zero. Figure correspond to a theoretical value of one.
** When the Chi-square value >3.84, then $2P<0.05$.

Table 3. Example of the terms sensitivity, efficiency and odds ratio of the various antibodies for recognizing the tumour type analysed (e.g. mesotheliomas). n=a+b+c+d

	Negative staining	Positive staining
Tumour type analysed (e.g. mesothelioma)	a	b
Reference tumour type (e.g. adenocarcinoma)	c	d

Differences of staining patterns between the two groups were tested by the chi-square test and the exact Fisher test using the BMDP programme (Dixon et al. 1990) as well as the Kolmogoroff-Smirnoff test (Sachs 1978). Test results were generally considered to be statistically significant if the two-sided error probability was equal to or lower than 5% ($2P \leq 0.05$). Deviations are indicated in the text (Table 2).

Sensitivity and efficiency of the different antibodies as well as odds ratios, i.e. the probability that a positive reaction indicates the presence of the tumour of interest, were calculated for mesothelioma according to the following formulas (see also Table 3):

$$\text{Sensitivity (\%)} = b/(a+b)\ 100 \quad (1)$$
$$\text{Efficiency (\%)}_{\text{original}} = (b+c)/n\ 100 \quad (2)$$
$$\text{Efficiency}_{\text{derived}} = 2\ \text{efficiency}_{(\text{original})} - 100 \quad (3)$$
$$\text{Odds ratio} = b\ c/a\ d \quad (4)$$

A measure for the discriminating power of a given antibody is the absolute value of the difference between the sensitivities for mesothelioma and adenocarcinoma, respectively (|Δ sensitivity|, Table 4).

The efficiency calculated according to formula (3) permits a biologically oriented interpretation of the values since a high positive or negative value of $\text{efficiency}_{\text{derived}}$ indicates a high biological discriminating power, values near zero can be observed for antibodies without discriminating importance for the tumour in question (Table 4).

When calculating the odds ratio, unallowed divisions by zero can occur. These situations are indicated in the result Table 2.

The relationship among the staining intensity of the antibodies was tested by carrying out a principal component analysis (Feldman et al. 1987). For determining the number of factors to extract, two methods were applied: the criterion of 75% variance and the root curve criterion. As soon as the sum of the proportionate contributions of the Eigenvalues exceeded 0.75, it was assumed that all relevant matrix variance had been accounted for. Inasmuch as the Eigenvalues were always determined in order from largest to smallest, the rank order of the Eigenvalue associated with the point of inflection was considered to be an estimation of the number of factors. For calculating the principal components, the larger of the numbers of factors determined by either the 75% variance rule or by the root curve analysis was taken.

Results

The results of the immunostainings of the two groups are summarized in Table 5. Typical staining patterns are shown in Figs. 1–8. The observed frequencies clearly show that cytokeratins were prevalent in both tumour groups, whereas antibodies recognizing differentiation or membrane-bound antigens common to secretory cells were rare in mesotheliomas and, if present, in a focal manner only.

The statistical analysis singled out the antibody Ber-EP4 as the most discriminatory antibody as evidenced by the highest Chi-square value and contingency-coefficient. This was followed by the group of HEA-125, CEA, BG related antigens A, B, H and Leu-M1, all of which were significantly discriminatory but less powerful as can be seen from the odds ratio. Vimentin turned out to be a significantly discriminatory antibody with a high odds ratio in favour of mesothelioma in the same way that HEA-125 was for adenocarcinoma.

The sensitivities of the various antibodies for each tumour group and the differences of the sensitivities are shown in Table 4. According to this Ber-EP4 (92.6%), followed by anti-vimentin and HEA-125 (79.6%, each)


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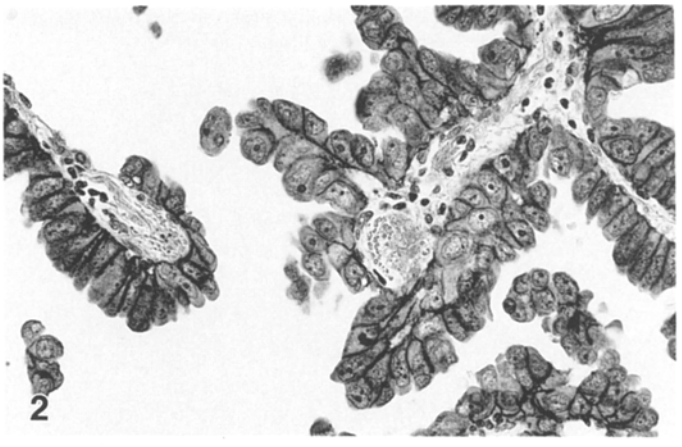
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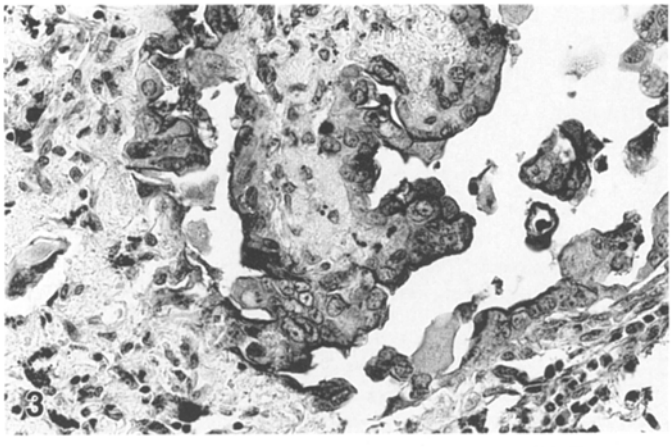
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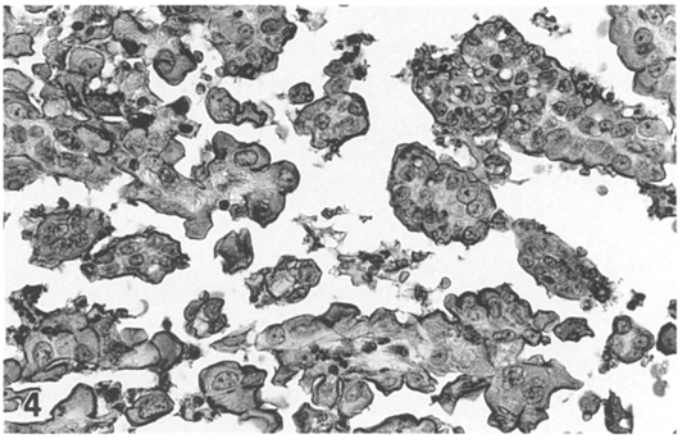
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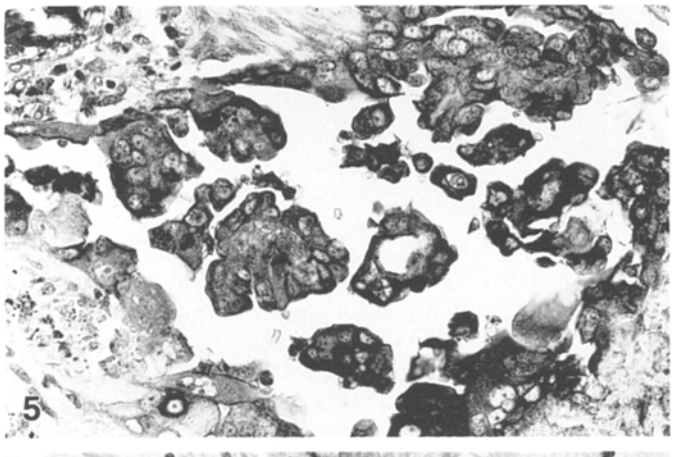
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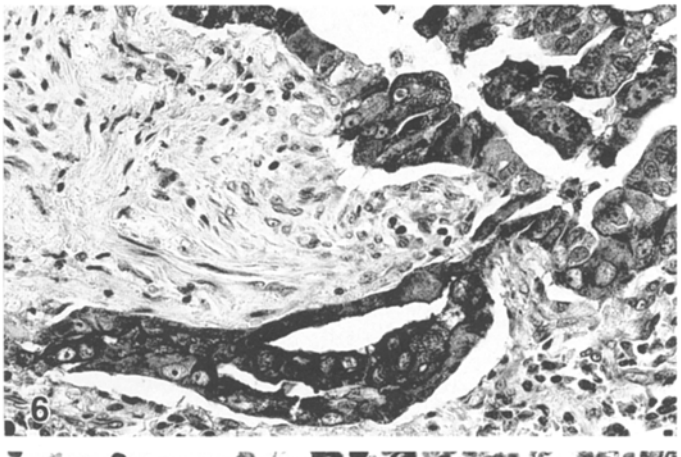
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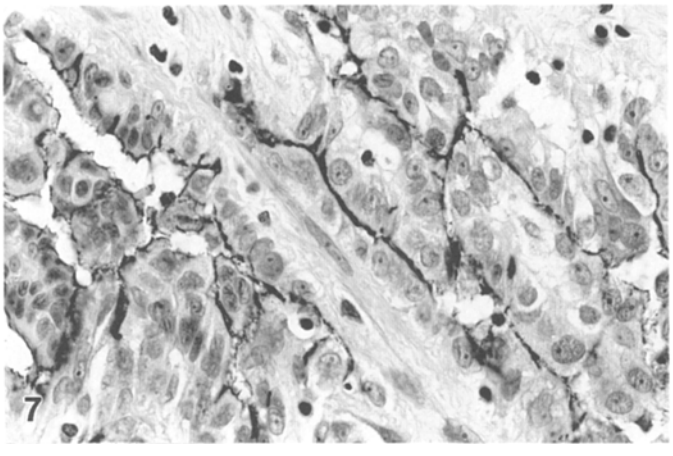
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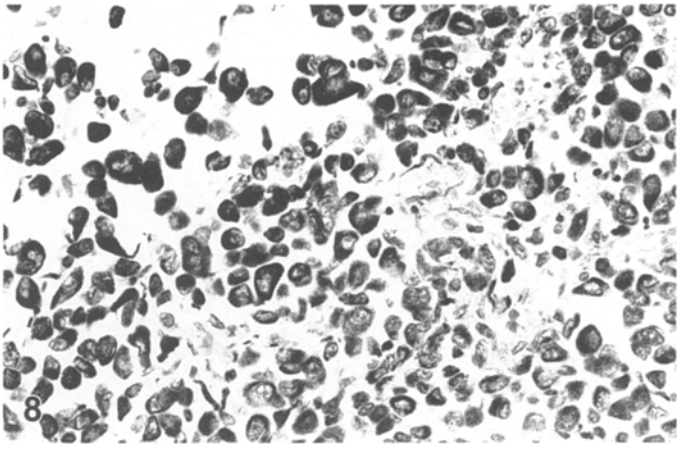
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Table 4. Sensitivity (in %) of the various antibodies for the groups mesothelioma and adenocarcinoma, absolute values of differences of sensitivities and efficiency $_{derived}$ for the mesothelioma group, considering influence of single cell staining

Antibody	Sensitivity		|Δ-sensitivity|		Efficiency $_{derived}$[a]	
	Meso-thelioma	Adeno-carcinoma	with single cells	without single cells	with single cells	without single cells
Lu-5	100.0	100.0	0	0	5.9	5.9
Anti-Keratin-18	100.0	91.7	8.3	4.6	13.7	9.8
Anti-Keratin-7	81.5	79.2	2.3	1.4	5.9	2.0
Ber-EP4	7.4	100.0	92.6	96.3	−92.2	−96.1
Anti-Leu-M1	0	66.7	66.7	66.7	−68.6	−68.6
HEA-125	3.7	83.3	79.6	83.3	−80.4	−84.3
Anti-CEA	0	75.0	75.0	75.0	−76.5	−76.5
Anti-BGR A, B, H	3.7	75.0	71.3	71.3	−72.5	−72.5
B 72.3	33.3	91.7	58.3	65.7	−56.9	−64.7
PLAP	14.8	66.7	51.9	55.6	−52.9	−56.9
Anti-vimentin	96.3	16.7	79.6	79.6	80.4	80.4
BMA-120	14.8	4.2	10.6	0.5	5.9	− 5.9

[a] Compare formula (3)

had the highest values for sensitivity, indicating their high diagnostic power for discriminating mesothelioma from adenocarcinoma.

The efficiency calculated for mesothelioma is shown in Table 4. The diagnostic impact of these values is best appreciated when efficiency is plotted against Δ-sensitivity as depicted in Fig. 9. This analysis clearly show that anti-cytokeratin antibodies and BMA-120 were not contributory to differential diagnosis. Exclusion of mesothelioma is best accomplished by Ber-EP4, Leu-M1 or blood group iso-antigens and Leu-M1, in this order. The only positive mesothelioma marker is vimentin, which is constantly coexpressed with cytokeratin 18, since all mesotheliomas are also positive for this intermediate filament.

The principal component analysis of the semi-quantitative data of the two tumour groups (Table 6) revealed a strong relationship among the antibodies usually considered to be specific markers of adenocarcinomas (Ber-EP4, anti-Leu-M1, HEA-125, anti-CEA, anti-BG related antigens A, B, H, B 72.3 and PLAP). This relationship was indicated by the high loading values on the first extracted factor, which explained just 57.6% of the total variance. Additionally, this group of antibodies is also teamed up with anti-vimentin. However, anti-vimentin showed a negative loading value. BMA-120, a marker of endothelial cells, lies alone on the third factor with a very high loading value of 0.952.

Discussion

For the diagnosis of malignant mesothelioma morphological, clinical and radiological criteria are required. The histological features have been well-defined (McCaughey et al. 1991), but additional helpful features include the absence of epithelial mucin, carcino-embryonic antigen and of the Leu-M1 epitope as well as the presence of keratin in neoplastic cells (McCaughey et al. 1991; Sheibani et al. 1991a, b; Wirth et al. 1990). In view of the lack of a single marker sensitive and specific enough for the identification of mesothelioma in paraffin-embedded tissue, several groups have used antibody panels and introduced new diagnostic reagents as outlined in the introduction. A comparison of these publications (Table 7) shows that there are several potent antibodies, the diagnostic value of which is confirmed by the present study. However, there are also conflicting immunohistochemical data regarding frequencies and ranking of individual antibodies, for example for the antibodies B 72.3, Leu-M1 and vimentin. Different immunohistochemical staining techniques, different procedures of tissue preparation and variations of the monoclonal and polyclonal antibodies used, may all be re-

Fig. 1. Pulmonary adenocarcinoma stained with Ber-EP4 antibody. Strong predominantly membrane staining of neoplastic cells. (ABC stain; ×100)

Fig. 2. Pulmonary adenocarcinoma with luminal reaction for HEA-125. (ABC stain; ×100)

Fig. 3. Focal membranous staining with anti-Leu-M1 in a pulmonary adenocarcinoma. (ABC stain; ×100)

Fig. 4. Adenocarcinoma cells showing peripheral staining for blood-group A. (ABC stain; ×100)

Fig. 5. Adenocarcinoma with strong cytoplasmic staining for CEA. (ABC stain; ×100)

Fig. 6. Adenocarcinoma cells with cytoplasmic and peripheral staining for B 72.3 (ABC stain; ×100)

Fig. 7. Peripheral immunoreactivity for BMA-120 in a case of malignant mesothelioma. (ABC stain; ×100)

Fig. 8. Epithelioid mesothelioma showing strong staining for vimentin. (ABC stain; ×100)

Table 5. Immunoreactivity of mesotheliomas ($n=27$) and adenocarcinomas of the lung ($n=24$)

Antibody	Positive reactions		Grading of reactivity				
	Absolute	in %	negative	single	focal	extensive	diffuse
Mesothelioma:							
Lu-5	27/27	100	0	0	4	4	19
Anti-Keratin-18	27/27	100	0	1	6	6	14
Anti-Keratin-7	22/27	82	5	1	14	5	2
Ber-EP4	2/27	7	25	1	1	0	0
Anti-Leu-M1	0/27	0	27	0	0	0	0
HEA-125	1/27	4	26	1	0	0	0
Anti-CEA	0/27	0	27	0	0	0	0
Anti-BGR A, B, H	1/27	4	26	0	1	0	0
B 72.3	9/27	33	18	2	6	1	0
PLAP	4/27	15	23	1	3	0	0
Anti-vimentin	26/27	96	1	0	12	9	5
BMA-120	4/27	15	23	3	0	1	0
Adenocarcinoma:							
Lu-5	24/24	100	0	0	4	5	15
Anti-Keratin-18	22/24	92	2	4	3	6	9
Anti-Keratin-7	19/24	79	5	8	6	4	1
Ber-EP4	24/24	100	0	1	2	9	12
Anti-Leu-M1	16/24	67	8	7	5	4	0
HEA-125	20/24	83	4	5	8	7	0
Anti-CEA	18/24	75	6	0	6	4	8
Anti-BGR A, B, H	18/24	75	6	3	5	9	1
B 72.3	22/24	92	2	4	8	7	3
PLAP	16/24	67	8	12	2	2	0
Anti-vimentin	4/24	17	20	2	1	1	0
BMA-120	1/24	4	23	0	0	1	0

negative, 0% of tumor cells stained; single, 1–5% of tumor cells stained; focal, 6–50% of tumor cells stained; extensive, 51–75% of tumor cells stained; diffuse, 76–100% of tumor cells stained

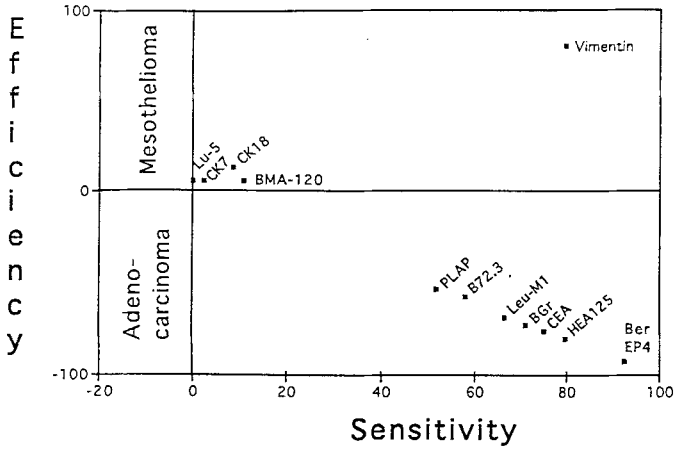

Fig. 9. Discriminating power of the panel antibodies using efficiency $_{derived}$ and Δ sensitivity

Table 6. Results of the principal component analysis (Confidence limits: – 0.280 to 0.280)

Antibody	Factor 1	Factor 2	Factor 3	Factor 4
Lu-5	0.107	0.757	0.159	0.125
Anti-Keratin-18	−0.053	0.921	−0.100	−0.140
Anti-Keratin-7	−0.049	0.002	0.001	0.885
Ber-EP4	0.902	−0.212	0.140	0.014
Anti-Leu-M1	0.735	0.121	−0.130	−0.002
HEA-125	0.851	−0.030	−0.049	−0.009
Anti-CEA	0.846	0.036	−0.077	0.065
Anti-BGR A, B, H	0.856	0.165	−0.100	0.004
B 72.3	0.775	0.193	0.022	−0.179
PLAP	0.698	−0.082	−0.017	0.339
Anti-vimentin	−0.799	0.147	−0.117	0.167
BMA-120	−0.043	0.032	0.952	−0.008
Total explained variance	57.6	19.0	11.1	12.3

sponsable for the uncertainty still present (Azumi et al. 1992; Chang et al. 1992; McCaughey et al. 1991; Sheibani et al. 1991a; Wirth et al. 1990).

Staining performance may vary from one laboratory to the other. We therefore suggest that each laboratory should analyse the behaviour of its working antibody panel, particularly when an immunohistological tumour diagnosis has to be done by exclusion.

The stepwise characterization by monovariate statistics is exemplified in this paper. Classical comparative

Table 7. Results of previous immunohistochemical studies of adenocarcinomas (CA) of the lung and malignant mesotheliomas (MM) using different antibodies

Authors	Percentage of positive stained cases (in %)													
	Anti-vimentin		B 72.3		HEA-125		Anti-Leu-M1		Anti-BGR A, B, H		Ber-EP4		PLAP	
	MM	CA	MM	CA	MM	CA	MM	CA	MM	CA	MM	CA	MM	CA
Berghäuser et al. 1989	70	10	0	90	10	50	0	75	–	–	–	–	–	–
Chenard-Neu et al. 1990	57	0	–	–	14	100	0	60	–	–	–	–	–	–
Gaffey et al. 1992	71	–	2	–	–	–	0	–	–	–	20	100	–	–
Kawai et al. 1991	–	–	–	–	–	–	–	–	0	83	–	–	–	
Latza et al.1990	–	–	–	–	–	–	–	–	–	–	0	50	–	–
Moch et al. 1992	96	17	33	92	4	75	0	67	4	75	7	100	15	67
Mullink et al. 1986	92	50	–	–	–	–	–	–	–	–	–	–	–	–
Ordonez 1989	26	13	5	83	–	–	0	61	–	–	–	–	–	–
Otis 1987	–	–	0	50	–	–	0	50	–	–	–	–	–	–
Pfaltz et al. 1987	43	14	–	–	–	–	–	–	–	–	–	–	–	–
Sheibani et al. 1991a	–	–	–	–	–	–	–	–	–	–	1	100	–	–
Szpak et al. 1986	–	–	45[a]	86	–	–	–	–	–	–	–	–	–	–
Warnock et al. 1988	–	–	0	86	–	–	0	57	–	–	–	–	–	–
Wick et al. 1990	41	17	0	83	–	–	0	100	0	67	–	–	0	19
Wirth et al. 1990	86	0	48	75	–	–	8	80	–	–	–	–	–	–

[a] Including cases with immunoreactivity in single cells in contrast to the author

analysis identifies Ber-EP4, HEA-125 and CEA as the most significant discriminatory antibodies. However, it does not specify their diagnostic power in the given setting. This can be accomplished by calculation of the odds ratio, sensitivity, efficiency and principal component for the various antibodies. The probability of detecting a mesothelioma or a reference tumour is well reflected by the odds ratio. By this parameter, a positive reaction with anti-vimentin can be identified as a high probability index for having mesothelioma. A low probability exists when the antibodies Ber-EP4, anti-Leu-M1 and anti-CEA do not stain the tumour. Additionally, there is a high probability for an adenocarcinoma when a positive staining with the antibody HEA-125 is observed. The odds ratio for an adenocarcinoma and a positive staining with the antibody Ber-EP4 cannot be given for methodological reasons (division by zero) but supposedly this value would be high. The results of the odds ratios confirm, that Ber-EP4, anti-Leu-M1, anti-CEA, HEA-125 and anti-vimentin are the antibodies which discriminate best mesothelioma from adenocarcinoma. However, it is not possible to decide which of these five antibodies has the greatest differential-diagnostic power from the odds ratios.

This information can be derived from sensitivity values measured for the two tumour groups and by the differences of sensitivities of the antibodies. A representative measure for the discriminating power of an antibody is the difference between its sensitivities for mesothelioma and adenocarcinoma, respectively (Table 4). By this measure, the most important antibody in the original panel must be Ber-EP4, because of a Δ sensitivity value of 92.6, followed by anti-vimentin and HEA-125 (79.6 for each), anti-CEA (75.0) and anti BGR A, B, H (71.3). From these data, the antibodies can be ranked according to their discriminating importance (power): 1. Ber-EP4, 2./3. anti-vimentin and HEA-125, 4. anti-CEA and 5. anti-BGR A, B, H. Note that anti-Leu-M1 is "replaced" by the anti-BGR A, B, H. This finding could be expected because of a very strong correlation between anti-CEA, anti-BGR A, B, H, documented by the same frequency of positive staining in lung adenocarcinoma (75% each, Table 5) and the low frequences of negative staining in mesothelioma (anti-BGR A, B, H: 4%, anti-CEA: 0%, Table 5). The existence of such a correlation among the two antibodies is also proven by the results of the principal component analysis (Table 6). A similar ranking is obtained by calculating the efficiency values.

Using Δ-sensitivity and efficiency, a simple normogram of the discriminating power of the panel antibodies can be constructed (Fig. 9). For our panel it clearly points out three clusters of antibodies. Anti-cytokeratin antibodies together with BMA-120 are found close to zero of the scale of efficiency. This group of markers is clearly separated from the antibodies marking cytoplasmic or surface components which are functionally associated with excretory cells of adenocarcinomas. The third "cluster" is represented by anti-vimentin as the only positive marker for mesothelioma. The high diagnostic impact of the co-expression of vimentin with cytokeratin, notably No. 18, is in contrast to some reports (Ordonez 1989, Pfaltz et al. 1987, Wick et al. 1990) but in line with others which found a significant, though weaker difference in the expression of vimentin (Berghäuser et al. 1989; Chenard-Neu 1990; Mullink et al. 1986; Wirth et al. 1990). Additionally, staining for vimentin, if present, is mostly focal in adenocarcinomas compared with mesotheliomas.

The interpretation of single cell staining is differen-

tially assessed. Some authors disregard staining of single cells. For methodological reasons we rated single tumour cell staining as an overall positive, because in view of tumour heterogeneity there is no definable cut-off value. Since the values will also be used for multivariate analysis on tumours with equivocal staining patterns, minimal but definite staining of single tumour cells was considered in all statistics. This strict procedure favours less stringent results when compared to studies using cut-offs.

This point is illustrated by the antibody B 72.3. Our staining results are similar to those of Wirth et al. (1990; Table 7). Two staining patterns were observed in our study: strong positive staining in the adenocarcinoma tested, with most specimens also exhibiting cell membrane staining. Moderate cytoplasmic staining was observed in 33% of the mesotheliomas. Other laboratories reported weak staining as a negative result for mesothelioma by using a cut-off value of 10% of positive tumour cells or by counting only membrane staining (Jordon et al. 1989; McCaughey et al. 1991; Ordonez 1989; Otis et al. 1987; Sheibani et al. 1991a; Szpak et al. 1986; Warnock et al. 1988; Wick et al. 1990).

As shown by Gaffey et al. (1992), focal immunoreactivity with Ber-EP4 does not exclude the diagnosis of malignant mesothelioma.

Taking single-cell staining as negative in the present series $|\Delta$ sensitivity$|$ and efficiency derived of Ber-EP4, HEA-125, B 72.3, PLAP and BMA-120 does not alter Table 4 significantly. The ranking order of these antibodies does not change, underlining the diagnostic stability of these antibodies.

The low ranking of BMA-120 should be interpreted with caution. It appears that the preservation of the antigen is very labile and depends on the fixation and/or storage (Berghäuser, personal communication; unreported observations).

In summary, by various descriptive statistical methods we have defined the discriminating power of antibodies commonly used in differentiating mesothelioma from adenocarcinoma of the lung. We suggest that for the practical work – as a minimum requirement for that purpose – the antibodies Ber-EP4, HEA-125, CEA and anti-vimentin should be included in a panel, supplemented by an anti-cytokeratin as a positive antibody control. HEA-125 could be replaced by blood group-related antisera or B 72.3. The performance of this panel was demonstrated by a normogram. It should be stressed that the values given in this paper apply only to the discrimination from adenocarcinoma of the lung and do not necessarily apply to metastic adenocarcinomas from other sites. This holds true particularly for vimentin. Furthermore, one should keep in mind that the present data are derived from typical tumour cases. It will be shown in an accompanying paper that in a group of uncertain mesotheliomas the rules derived from monovariate statistics will not satisfactorily solve all diagnostic problems.

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