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Diagnostic tools for differentiating pleural mesothelioma from lung adenocarcinoma in paraffin embedded tissue

II. Design of an expert system and its application to the diagnosis of mesothelioma

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Abstract. A panel of 14 antibodies (panepithelial antibody Lu-5, anti-keratin-18, anti-keratin-7, Ber-EP4, anti-Leu-M1, HEA-125, anti-carcinoembryonic antigen, anti-blood group-related antigens A, B, H, B72.3, anti-placental alkaline phosphatase, anti-vimentin and BMA-120), which have been evaluated for use in differentiating mesothelioma from lung adenocarcinoma, was applied to a group of 24 suspected mesotheliomas. Using the established qualitative, descriptive criteria derived from monovariate statistical analysis of the tumour control groups (definite mesotheliomas, adenocarcinomas), a definitive allocation was possible in only 25% of suspected cases. We therefore constructed two "expert systems", based on multivariate discriminant analysis with either the ALLOC 80 program for ordinal data or a newly developed analysis program for binomial data. With these two systems diagnostic allocation of suspected mesotheliomas was improved to 75% and 79%. The use of binomial data ("positive" versus "negative") in conjunction with the probability-based test system is of particular interest because the primary data are easy to record and the test results have a higher statistical probability.

Key words: Malignant mesothelioma – Lung adenocarcinoma – Immunohistochemistry – Expert system – Discriminant analysis

Introduction

Despite a broad panel of diagnostic tools, differential diagnosis between mesothelioma and adenocarcinoma of the lung remains difficult; no single organ- or tumour-specific marker system is available (McCaughey et al. 1991; Warnock et al. 1988).

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In a preceding paper (Moch et al. 1993) we analysed the discriminatory power of 14 antibodies on two groups of unequivocal pulmonary adenocarcinoma and "definite" mesothelioma (control groups). Each antibody was graded according to its discriminatory efficiency for this specific differential diagnosis. However, the criteria derived from monovariate statistics used in our previous study are not satisfactory for questionable mesotheliomas. A precise diagnosis may not be possible because the expression of diagnostic antigens is absent or atypical.

To evaluate this common diagnostic problem better, we studied an additional group of 24 tumours which could not be allocated to either group definitively. Only a quarter of such cases could be classified by the qualitative, descriptive criteria derived from monovariate statistics. However, by incorporating the previous control groups (proven mesotheliomas and adenocarcinomas) as training groups and applying multivariate test procedures to give a statistical probability for each suspected mesothelioma, we designed a computer-assisted decision system which defines the diagnostic probability of a given case in a manner similar to other "expert systems" (Hufnagl et al. 1988; Kolles and Remberger 1991; Zadeh 1989).

Materials and methods

Seventy-five tumours of the lung, with clinically proven or suspected features of mesothelioma or pulmonary adenocarcinoma, were classified by light microscopy according to the recommendations of the US/Canadian Mesothelioma Panel (McCaughey et al. 1991) into three diagnostic groups: "definite" mesothelioma ($n=27$; group 1), adenocarcinoma ($n=24$; group 2), and "probable" mesothelioma ($n=24$; group 3). The patients of groups 1 and 2 were described in our earlier paper (Moch et al. 1993) and are now designated as "training" groups for the respective tumour types. Group 3 is a new group of cases, which could not be allocated to either group on the basis of microscopic features, histochem-

istry, or clinical or radiological information. Data from this group were tested against the two training groups.

The avidin-biotin complex technique and the characteristics of the antibodies used for immunohistochemical staining have been described previously (Moch et al. 1993). Briefly, the following antibodies were employed: panepithelial antibody Lu-5, anti-keratin-18, anti-keratin-7, Ber-EP4, anti-Leu-M1, HEA-125, anti-carcinoembryonic antigen (CEA), anti-blood-group-related antigens (BGR; A, B, H), B72.3, anti-placental alkaline phosphatase (PLAP), anti-vimentin and BMA-120. The portion of labelled tumour cells in relation to all tumour cells was semiquantitatively estimated (ordinal data) into five classes of staining intensity: class 1, labelling portion: 0% ("negative"); class 2, labelling portion: 1–5% ("single cells"); class 3, labelling portion: 6–50% ("focal"); class 4, labelling portion: 51–75% ("extensive") and class 5, labelling portion: 76–100% ("diffuse").

Data were designed as binary if they contained only two values describing two different states ("negative" or "positive" staining).

The semi-quantitative data of the staining reactions (negative, 0; single cells, +; focal, ++; extensive, +++; diffuse labelling, ++++) were subjected to a stepwise non-parametric statistical procedure (ALLOC 80 program; Hermans et al. 1982). ALLOC 80 is based on a non-parametric density estimation. The aim of applying such a procedure was to allocate a case of uncertain origin to one of k populations optimally ($k=2$ in the present study).

The binary data were processed by a new test procedure developed by one of us (M.B.). The program was written in Microsoft Excel for Apple Macintosh computers.

The method was based on the primary likelihood (PL_g):

$$PL_g = \prod_{a=1}^{A} [(1-t_a)-(-1)\, t_a \cdot r_{ga}],$$

where a, attributes (=antibody); $\prod$, sign for product; $a=1$, first attribute (antibody of the test set) t_a, taxon: binary data of the test case in question; r_{ga}, ratio between the sum of the value of the attribute and the total number of cases in the group g; A, total number of attributes (antibodies); g, group (definite mesothelioma or adenocarcinoma).

In a first step, the allocation probabilities of all probable mesothelioma ($n=24$) was determined using the staining values for all 12 antibodies.

In a second step, the two original learning groups were supplemented by the correctly classified (allocation probability 100%) cases of the training group (possible mesothelioma). The cases remaining in the possible mesothelioma group with allocation probabilities <100% were compared again, this time with the expanded learning groups.

Results

The results of the immunohistological staining reactions of group 1 (definite mesothelioma) and group 2 (adenocarcinoma) have been published previously (Moch et al. 1993). Those of group 3 (possible mesothelioma) are shown in Table 1.

Using the criteria established in the preceding paper, 6 cases (25%) could be satisfactorily classified into one of the diagnostic groups. Ber-EP4, HEA-125 and CEA, the most important markers of lung adenocarcinoma, were expressed in three cases of possible mesothelioma (cases 1, 3 and 7; Table 1) which accordingly could be reclassified as adenocarcinoma. Absence of this phenotype, together with the complete lack of BGR, Leu-M1, and B72.3 in 3 of the remaining 21 cases (cases 4, 19 and 24; Table 1), was interpreted as favouring definite mesothelioma.

In the remaining 18 cases (75% of the original possible mesothelioma) an unequivocal diagnosis of mesothe-

Table 1. Probable malignant mesotheliomas: immunoreactivity for selected antibodies

Case number	CEA	HEA-125	Leu-M1	B72.3	Ber-EP4	BGR A,B,H	Vimentin
1	+++	++	++	++++	+++	++++	0
2	0	++	0	++	+	0	++
3	++	+	+	+++	++	++	+++
4	0	0	0	0	0	0	++
5	0	0	0	++	0	0	+++
6	0	0	0	++	0	0	+++
7	++	++	0	++	++	++	++
8	0	0	0	+	0	0	+++
9	0	0	0	+	0	0	++
10	0	0	0	++	+	0	+++
11	0	0	0	+	0	0	+++
12	0	++	0	++++	0	0	0
13	0	+	0	0	0	0	+++
14	0	0	0	+	0	0	++
15	0	0	0	++	+	0	++++
16	0	0	0	+	0	0	+++
17	0	0	0	++	0	0	+++
18	0	0	0	+++	0	0	+++
19	0	0	0	0	0	0	+++
20	0	0	0	+++	++	0	++
21	0	0	0	+++	0	0	+
22	0	0	0	+	0	0	0
23	0	0	0	++	0	0	+++
24	0	0	0	0	0	0	++

Labelling portion: 0, 0% (negative); +, 1–5% (single cells); ++, 6–50% (focal); +++, 51–75% (extensive); ++++, 76–100% (diffuse)
CEA, Carcinoembryonic antigen; BGR, blood-group-related antigens

Table 2. Comparison of immunohistochemical diagnosis with that of the "expert system" using binomial or ordinal data

Case number	Immuno-histochemistry (descriptive, qualitative)	"Expert system" diagnosis (% statistical probability) to belong either in the group definite mesothelioma or adenocarcinoma	
		Binomial data	Ordinal data
1	AdCa	AdCa (100%)	AdCa (100%)
2	?	AdCa (92%)	Meso (74%)
3	AdCa	AdCa (100%)	AdCa (61%)
4	Meso	Meso (100%)	Meso (100%)
5	?	Meso (100%)	Meso (100%)
6	?	Meso (100%)	Meso (100%)
7	AdCa	AdCa (100%)	AdCa (61%)
8	?	Meso (100%)	Meso (100%)
9	?	Meso (100%)	Meso (100%)
10	?	Meso (92%)	Meso (74%)
11	?	Meso (100%)	Meso (100%)
12	?	Meso (90%)	Meso (100%)
13	?	Meso (100%)	Meso (100%)
14	?	Meso (100%)	Meso (100%)
15	?	Meso (92%)	Meso (74%)
16	?	Meso (100%)	Meso (100%)
17	?	Meso (100%)	Meso (100%)
18	?	Meso (100%)	Meso (100%)
19	Meso	(Meso (100%)	Meso (100%)
20	?	Meso (92%)	AdCa (62%)
21	?	Meso (100%)	Meso (100%)
22	?	Meso (100%)	Meso (100%)
23	?	Meso (100%)	Meso (100%)
24	Meso	Meso (100%)	Meso (100%)

?: Clear diagnosis not possible; AdCa, adenocarcinoma of the lung; Meso, malignant mesothelioma

lioma was not possible because of extensive labelling with the anti-vimentin antibody or focal staining for Ber-EP4, HEA-125, B72.3 or PLAP (Table 1).

The discriminant analysis carried out with all 24 cases of possible mesothelioma (test group) and the two learning groups (definite mesothelioma and adenocarcinoma) showed that 92.6% of the histologically definite mesotheliomas ($n=27$) and all lung adenocarcinomas ($n=24$) could be properly classified. For this discrimination among the two learning groups, a single immunohistochemical marker, Ber-EP4, was decisive (Moch et al. 1993).

From the training group (probable mesothelioma) 17 cases could be allocated with an allocation probability of 100% to the definite mesothelioma group and 1 case to the definite adenocarcinoma group (Table 2). Three cases of the 24 probable mesotheliomas (cases 2, 10, and 15) were all recognized as suspected mesothelioma with a probability of 74%. Three other tumours (cases 3, 7 and 20) were labelled as adenocarcinoma rather than mesothelioma with a probability of 61%, based on the ordinal data.

For a second analysis, two learning groups were supplemented by these cases which were allocated to either group with a probability of 100% ($n=18$), thus increasing the sample size in the total learning set from 51 to 69. In a following discrimination analysis with these augmented learning groups, 97.1% of these 69 cases were classified in either of the learning groups (definite mesothelioma or adenocarcinoma). The allocation probability of the 3 above-mentioned cases (2, 10 and 15) for belonging to the mesotheliomas was reduced to 69%. The allocation probability of cases 3, 7 and 20, however, was increased from 61% to 65% in favour of an adenocarcinoma (Table 2).

Nineteen of the 24 cases of the probable mesothelioma group (79.2%) were classified as mesothelioma or as adenocarcinoma (100% probability). On the basis of the binomial data, cases 3 and 7 were classified as adenocarcinoma (allocation probability 100% each). However, case 2 was recognized as adenocarcinoma with 92% probability. Five cases were not as firmly allocated and therefore remained in the doubtful mesothelioma group. However, the probability was in the vicinity of 90% for mesothelioma (cases 10, 12, 15 and 20).

Discussion

In the present study, data obtained for a test group of suspected mesotheliomas were analysed against two tumour reference groups by different approaches.

One approach was based on descriptive criteria (staining results with individual antibodies) which had been ranked in our earlier paper by monovariate statistics. This approach comes close to the empirical judgement of staining reactions in surgical pathology. This is sufficient if the criterion used obeys the all-or-nothing rule. Mesothelioma does not behave this way, because it shares properties not only with adenocarcinoma of the lung, but also with other tumours not considered here. Accordingly, it is not surprising that even usage of ranked markers was helpful only in a quarter of the test cases.

In contrast to this monovariate approach, multivariate methods are increasingly gaining importance in surgical pathology because they better reflect the needs of modern diagnostic histology, which uses objective and quantifiable diagnostic criteria. Two aspects must be considered in working with numerical data. Quantitative information extracted from descriptive information (e.g. a semi-quantitative score of staining reactions) does not guarantee, per se, that the conclusion deduced from this information is true and statistical results always express probabilities only (Wonnacott and Wonnacott 1977).

Systems which imitate the decision process of a surgical pathologist in equivocal situations and allow such multivariate decisions on a statistical basis can be designated as "expert systems".

Modern computer systems intended to assist the pathologist in this process can be divided into three different types: rule-based systems (Bartels and Hiessl 1989; Bartels and Thompson 1989; Bartels and Weber 1989; Smeulders and van Ginneken 1989), systems based on discriminant analysis (Hufnagl et al. 1989; Oberholzer et al. 1989) and systems based on a simple stochastic approach, resembling Bayesian statistics (Kolles and

Remberger 1991). The system presented here can be considered as a mixture of the last two types of such an "expert" system.

Whereas monovariate analysis only allowed the definitive allocation of 6 cases to either the group of mesothelioma or adenocarcinoma, multivariate procedures allocated 12 (binomial data) and 18 (ordinal data), respectively, to one of the tumour groups with 100% probability. In 4 cases (2, 10, 15 and 20) the probability in both multivariate procedures was less than 100%. In 2 of 4 cases the allocation was different with the two test systems, indicating that a probability of less than 100% should be considered doubtful. In general, by using binomial data, the allocation probability of each case was achieved with a higher statistical probability. When compared with immunohistochemical results, binomial data were more informative. Cases 3 and 7, with immunoreactivity for CEA, were allocated with an probability of 100% to the adenocarcinoma group compared with a probability of 61% using ordinal data. Also the obvious advantage of the binary identification analysis on binomial data presented here is that it produces an allocation probability without having semi-quantitative data.

A disadvantage of the binary identification analysis is the absence of information concerning the safety of the learning groups used. The ALLOC 80 program, however, allows a discriminant analysis of the training groups themselves by self-testing the individual members. A correct allocation of all cases belonging to the definite mesothelioma and definite adenocarcinoma groups was made in 51 cases, resulting in correct allocation of 96.1%. When these two histologically defined groups were supplemented by the correctly classified possible mesothelioma and the stepwise discrimination analysis was repeated, the percentage of correctly classified cases in the two learning groups was 97.1%.

The results of the multivariate analyses carried out on immunohistochemical staining patterns of mesotheliomas and adenocarcinomas agree well with the monovariate findings published in the previous paper (Moch et al. 1993). The described test procedure provides further information on the probability of the correct diagnosis in uncertain cases. The presented "expert system", however, is not designed to make histological diagnosis but to add objective criteria in favour of the most probable diagnosis. In the form presented it concerns only immunohistological findings. However, it could easily be amplified by incorporating additional morphological criteria or relevant clinical symptoms. The stability of the two learning groups also suggests that the system is self-learning and by accumulating control cases one may gain statistical security. We believe that it will become essential to use such tools in order to improve the quality of diagnosis and improve assessment of ambiguous tumour histology.

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