Immunohistochemical differentiation between primary adenocarcinomas of the ovary and ovarian metastases of colonic and breast origin. Comparison between a statistical and an intuitive approach

J H Lagendijk, H Mullink, P J van Diest, G A Meijer, C J L M Meijer

Abstract

***Aim*—To discriminate between adenocarcinomas that are primary to the ovary and metastatic to the ovary, especially of colonic and breast origin, by immunohistochemistry, using stepwise discriminant analysis or a decision tree.**

***Methods*—312 routinely processed, formalin fixed tissue specimens were used. The tumours were divided into a learning set (n = 159), composed of primary tumours of ovary, breast, and colon, and a test set, comprising 134 metastases from these sites and an additional 19 primary ovarian carcinomas. The immunohistochemical panel was composed of antibodies against cytokeratin 7 (CK7) and 20 (CK20), CA125, vimentin, carcinoembryonic antigen (CEA), gross cystic disease fluid protein-15 (GCDFP-15), and the oestrogen receptor (ER). The staining results of the tumours were expressed as the product of the staining intensity and the percentage of positive tumour cells. Analyses were first performed on the learning set and then evaluated on the test set.**

***Results*—Although the immunostaining patterns showed a considerable overlap between the three types of adenocarcinoma, the breast carcinomas were typically positive for GCDFP-15 and often for ER, and negative for vimentin. Ovarian carcinomas were always positive for CK7 and to a lesser extent for CA125. Colonic carcinomas showed prominent positivity for CEA and CK20, while no staining was seen for ER and vimentin. In discriminant analysis, six antibodies (αCK7, αCK20, αCA125, αCEA, αER, and αGCDFP-15) appeared to be necessary for optimal classification: 89% of the learning set and 82% of the test set were classified correctly. In the decision tree, only four antibodies (αCK7, αCEA, αER, and αGCDFP-15) were used to obtain a correct classification score of 89% for the learning set and 84% for the test set.**

***Conclusions*—Using a semiquantitative assessment of the immunostaining results by a restricted panel of six antibodies with stepwise discriminant analysis, 80–90% of the adenocarcinomas of colon, breast, and ovary can be correctly classified. Discriminant analysis is computer aided and therefore an easy method and for each case a probability value of the classification result is obtained. The intuitive decision tree method provides a slightly better result, requires only four antibodies, and offers a more practical method for the surgical pathologist.**

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Ovarian cancers constitute about 2.3% of all cancers. In the USA it is the fourth most common source of cancer death in women, exceeded only by breast, colorectal, and lung malignancies.[1] Cancer of the ovary constitutes about 25% of the gynaecological cancers. Up to 7% of the ovarian cancers, however, comprise metastases from various other locations.[2–4] About 40% of these originate from the colon.[5–7] These metastases often form large cystic masses or show abundant necrosis, mimicking primary endometrioid or mucinous ovarian carcinoma. They sometimes precede the diagnosis of the primary tumour. Other primary tumours that metastasise rather frequently to the ovary are breast carcinomas. Although the histological pattern is often suggestive of a metastasis from the breast, the growth pattern of the tumour can mimic that of a primary carcinoma of the ovary.[8 9] Moreover, the existence of a prior tumour in another organ may not be known.[10]

Because of the different therapeutic approaches to metastatic carcinomas of ovary, colorectum, and breast, it is important to discriminate between metastases of these tumours. Immunohistochemistry can be of great help in this, but individual antibodies are often not specific and sensitive enough. Therefore panels of several antibodies with a higher combined sensitivity and specificity should be used.

As a model to study the discriminative capacity of such a panel for metastatic tumours, primary tumours were used as a learning set and metastatic tumours as a test set. A prerequisite for this is that the immunohistochemical profiles of the primary adenocarcinomas and their metastases are comparable. Although there were strong indications for this in published reports,[11] it was checked once

more for the staining results of our samples before they were further evaluated.

We used antibodies against cytokeratin 7 (CK7)[12-15] and cytokeratin 20 (CK20),[15-16] vimentin,[17-19] CEA,[20-24] CA-125,[25-27] gross cystic disease fluid protein 15 (GCDFP-15),[11 28] and the oestrogen receptor (ER),[29-32] all of which have a certain selective reactivity for these adenocarcinomas.

Staining results were scored semiquantitatively as described before,[33] and were evaluated using two different approaches: (1) stepwise discriminant analysis, and (2) a decision tree. For both, the staining results were analysed using the learning set, and the classification rules obtained were evaluated on the test set. The stepwise discriminant analysis was carried out with a suitable computer program, and the decision tree was constructed intuitively. Based on the results obtained with each of the two approaches, antibody panels could be composed whereby the great majority of the samples could be correctly classified.

Methods

TISSUE

Formalin fixed, paraffin embedded tissue blocks of human adenocarcinomas of the breast, colorectum, and ovary, as well as metastases from these primary tumours (especially those to the ovary), were randomly selected from the archives of the departments of pathology of the General Hospital Gooi-Noord, Blaricum, Stichting Deventer Ziekenhuizen Deventer, and the Free University Hospital, Amsterdam, The Netherlands. In all cases the most representative block was selected, as judged on the haematoxylin and eosin stained sections.

Fifty four primary colorectal, 55 primary breast, and 50 primary ovarian carcinomas were used as a learning set. The colorectal tumours were of various differentiation grades (two well differentiated, 32 moderately differentiated, 14 poorly differentiated, and six undetermined) and included different histological types, among which were a few colloid carcinomas and signet ring cell carcinomas. The set of breast carcinomas comprised 49 ductal carcinomas, five lobular carcinomas, and one medullary carcinoma. The group of ovarian carcinomas comprised serous, mucinous, and endometrioid types of all differentiation grades (seven well differentiated, 16 moderately differentiated, and 27 poorly differentiated).

The test set comprised 134 adenocarcinoma metastases (46 colonic, 64 breast, and 24 ovarian) and 19 primary ovarian adenocarcinomas (two well differentiated, six moderately differentiated, and 11 poorly differentiated).

Twenty of the metastases were localised in the ovary, 12 originating from the breast and eight from the colorectum. The other metastases, from colorectal and breast primaries, were mainly derived from locoregional lymph nodes, liver, bones, and skin (n = 116), and those from ovarian primaries were predominantly found in the peritoneum and omentum (n = 24).

IMMUNOHISTOCHEMISTRY

The antibodies were selected because of their known reactivity with adenocarcinomas of the ovary, colorectum, or breast (table 1) and were suitable for formalin fixed, paraffin embedded tissue.

Four micron thick sections were cut and deparaffinised in graded alcohols and xylene. The sections were then subjected to immunostaining, applying a standard avidin-biotin-peroxidase complex (ABC) method by means of an automatic staining device (Ventana 320, Ventana Medical Systems). For CK7, CK20, and GCDFP-15, the sections were digested with alkaline protease (proteinase 2, provided by Ventana), which was done automatically in the apparatus. Before immunostaining with CA-125 the sections were digested with 1% pronase. Before vimentin and ER immunostaining, antigen retrieval was carried out by boiling the sections in citrate buffer (pH 6.0) for 2 × 10 minutes using a microwave oven. Only the immunostaining for the ER was done by hand, using an overnight incubation at 5°C. In all staining sessions, appropriate positive and negative control sections were included.

SCORING OF THE IMMUNOSTAINING

The immunostaining was scored semiquantitatively by means of a modified histoscore method,[34] taking into account the staining intensity and the percentage of positive tumour cells as described before.[33] Briefly, for each stained section the estimated percentage of tumour cells was multiplied by the intensity

Table 1 Antibodies used for immunostaining of colonic, ovarian, and breast carcinomas and their metastases

*Antibody**	*Dilution*	*Antigen*	*Main specificity*	*Source*	*Reference No*
Parlam 1	1:2000	CEA	Gastrointestinal tract epithelium	NKI, Amsterdam, The Netherlands	20, 23
Vim 9	1:2000	Vimentin	Mesenchymal cells	NKI, Amsterdam, he Netherlands	18
OVTL 12/30	1:80	Cytokeratin 7	Glandular epithelium except colon	Sanbio, Uden, The Netherlands	12
Ks 12.8	1:100	Cytokeratin 20	Colonic epithelium	Dako, Glostrup, Denmark	16
OC-125	1:100	CA-125	Ovarian epithelium	ITK, Uithoorn, The Netherlands	25
BRST-2	1:100	GCDFP-15	Breast epithelium	Signet, Dedham, Mississipi, USA	11, 29
Anti-ER	1:50	ER	Nuclei of breast epithelium	Dako, Glostrup, Denmark	30, 32

* All antibodies were mouse monoclonal.

value (0, 1, 2, or 3). This was taken as the immunohistochemical score (IS), which could vary between 0 and 300. An IS value > 10 was considered positive.

STATISTICAL ANALYSIS

For statistic analysis, the SPSS statistical program was used. To evaluate the power of the respective antibodies to discriminate between the three primary sites, stepwise discriminant analysis was performed as described previously.[33] In this way, the best combination of discriminatory antibodies was selected and classification functions were composed to classify each case as either colonic, mammary, or ovarian carcinoma.

The posterior probability values of the classification of the individual cases were also obtained. Such values can be useful in daily practice as an indication of the certainty of the classification result. We introduced the condition that the posterior probability value had to be more than 0.8; this means that the certainty of a classification result is more than 80%. We feel that in daily practice this is about the minimum acceptable certainty.

DECISION TREE APPROACH

In order to get a more practical approach for the surgical pathologists to discriminate between the different adenocarcinomas, a flow chart was developed, also based on the IS values of the learning group specimens. This approach was carried out intuitively, using as few steps and as few antibodies as possible.

Results

STAINING RESULTS OF THE LEARNING SET

Table 2 shows the mean IS values for the different antigens obtained in the learning and test sets of the colorectal, ovarian, and breast carcinomas. In table 3 the percentage of samples positive (IS > 10) for the different markers is shown.

In ovarian carcinomas, cytoplasmic staining was generally seen with antibodies against CK7 and, when present, also with α-vimentin. Most tumours showed expression of CA125 at the cell membrane, usually at the luminal site but occasionally also in the cytoplasm. Only in mucinous ovarian carcinomas was a cytoplasmic staining for CK20 and a membranous staining for CEA sometimes seen. One third of the tumours of the ovary showed some (nuclear) positivity for ER. From the ovarian carcinomas, the mucinous subtype had a slightly different staining pattern: the mean IS_{CEA} and IS_{CK20} values in this subtype were 66 and 75, while the mean IS values for these antibodies in the total set of ovarian carcinomas were only 30 and 27, respectively. The mucinous tumours were also less intensely stained for CA125 (mean IS, 36) than all ovarian carcinomas as a group (mean IS, 64).

Most colorectal carcinomas showed an abundant strong cytoplasmic staining for CEA and to a lesser extent for CK20, while CEA often showed strong membrane staining as well. Occasionally a certain degree of cytoplasmic staining for CK7 was seen, but the intensity was rather weak and the number of positive tumour cells was limited. CA125 showed only weak membranous staining in a few samples. Most colorectal carcinomas were positive for CEA and CK20. In signet ring cell carcinomas the IS for CK20 was very low: the mean IS value was 3, compared with 138 for the total group of colorectal carcinomas. In the colloid subtype the IS_{CK20} varied considerably as compared with the mean IS of all colorectal carcinomas. Other significant differences in the IS in subtypes of the colorectal carcinomas were not found.

Table 2 Immunoscore (IS) for each antibody in the learning and test sets

	n	*GCDFP-15*	*CEA*	*ER*	*CK20*	*CK7*	*CA125*	*Vimentin*
Learning set								
Colorectal carcinoma	48	0 (1)	**185 (73)**	0 (0)	138 (87)	22 (53)	6 (16)	1 (2)
Breast carcinoma	43	69 (61)	26 (45)	**56 (81)**	16 (35)	211 (93)	11 (27)	5 (15)
Ovarian carcinoma	43	1 (4)	30 (59)	7 (19)	27 (59)	**161 (97)**	64 (66)	40 (66)
Test set								
Metastatic colorectal carcinoma	46	0 (2)	**190 (79)**	0 (0)	153 (94)	37 (88)	6 (15)	3 (18)
Metastatic breast carcinoma	64	**70 (73)**	40 (77)	43 (65)	11 (32)	**197 (111)**	11 (35)	7 (25)
Metastatic ovarian carcinoma	24	0 (1)	29 (47)	15 (30)	42 (63)	**225 (73)**	87 (91)	32 (63)
Primary ovarian carcinoma	19	1 (3)	20 (44)	4 (14)	29 (57)	**174 (96)**	63 (66)	35 (63)

Values are mean (SD).

Table 3 Positive samples (immunostaining score > 10) for each antibody in learning and test sets

	n	*GCDFP-15*	*CEA*	*ER*	*CK20*	*CK7*	*CA125*	*Vimentin*
Learning set								
Colorectal carcinoma	48	0 (0)	**47 (98)**	0 (0)	42 (87.5)	11 (23)	6 (12.5)	0 (0)
Breast carcinoma	43	**33 (77)**	17 (40)	**25 (58)**	8 (19)	40 (93)	10 (23)	4 (9)
Ovarian carcinoma	43	0 (0)	12 (28)	5 (12)	16 (37)	**39 (91)**	27 (63)	16 (37)
Test set								
Metastatic colorectal carcinoma	46	0 (0)	**46 (100)**	0 (0)	40 (87)	8 (17)	7 (15)	2 (4)
Metastatic breast carcinoma	64	**47 (73)**	26 (41)	**27 (42)**	11 (17)	54 (84)	11 (17)	5 (8)
Metastatic ovarian carcinoma	24	0 (0)	9 (38)	5 (21)	12 (50)	**23 (96)**	18 (75)	7 (29)
Primary ovarian carcinoma	19	0 (0)	4 (21)	1 (5)	10 (53)	**19 (100)**	15 (79)	6 (32)

Values are n (%).

Table 4 Classification rules obtained for the primary sites of colorectal, breast, and ovarian carcinomas

Colorectal carcinomas	−0.010 * (IS)GCDFP-15 + 0.046 * (IS)CEA− 0.008 * (IS)ER + 0.021 * (IS)K20 + 0.019 * (IS)K7 + 0.124 * (IS)CA-125 − 6.907
Breast carcinomas	0.350 * (IS)GCDFP-15 + 0.016 * (IS)CEA + 0.024 * (IS)ER + 0.001 * (IS)K20 + 0.031 * (IS)K7 − 0.013 * (IS)CA-125 − 6.175
Ovarian carcinomas	−0.127 * (IS)GCDFP-15 + 0.008 * (IS)CEA + 0.003 * (IS)ER + 0.006 (IS)K20 + 0.021 * (IS)K7 + 0.031 (IS)CA-125 − 3.998

Breast carcinomas usually showed cytoplasmic staining for CK7 and GCDFP-15 and nuclear ER staining. αGCDFP-15 showed an exclusive positivity in breast carcinomas, and also $IS_{ER} > 95$ could only be found in these tumours. In a few cases some cytoplasmic staining for vimentin and CK20 was seen, as well as combined membranous and cytoplasmic positivity for CEA. Some cases showed weak CA125 membranous staining. No differences were found in immunostaining between subtypes of breast carcinomas.

TEST SET

Comparison of the immunohistochemical profiles of primary tumours (IS_{prim}) and metastases (IS_{meta}) showed only a few minor differences for colorectal and breast carcinomas (table 2). In some ovarian carcinomas slight variations between primary tumours and metastases were observed, but these were not statistically significant. The mean (SD) IS_{ER} was more than doubled in the metastases (IS_{prim}, 7 (19) and 4 (14) in the learning and test sets, respectively; IS_{meta}, 15 (30)), with a greater variation. The metastases of ovarian carcinomas also showed a higher mean IS_{CK7} (IS_{meta}, 225 (73); IS_{prim}, 161 (97)). Other antibodies did not show clear differences between the primaries and the metastases.

STEPWISE DISCRIMINANT ANALYSIS

In the learning set, the antibodies against CEA, GCDFP-15, CA125, CK7, ER, and CK20 were selected in this order by stepwise discriminant analysis. The obtained classification rules for each primary localisation are given in table 4. In this way the individual samples were classified (according to the highest score from either formula) and for each classification the posterior probability was obtained. For the learning set a correct classification of 98% (53/54) of the colorectal carcinomas, 88% (44/50) of the tumours of the ovary, and 80% (44/55) of the breast carcinomas was obtained. The overall percentage correctly classified tumours was 89%. The classification results were about equally good for the different differentiation grades (data not shown), so poorly differentiated cancers were also usually correctly classified. Classification results were also nearly equally good for the different subtypes of breast cancer (data not shown).

When applying the classification formulas obtained to the test set, 91% (42/46) of the colorectal metastases, 79% (19/24) of the ovarian metastases, and 74% (47/64) of the breast metastases were correctly classified. Of the 20 metastases to the ovary, 65% were correctly classified. Ninety per cent of primary tumours of the ovary were correctly classified in the test set (17/19). The overall correct classification of the test set samples (metastases and primaries) was 82%.

When considering only the "certain" classifications with a posterior probability value of 0.8 or more, 122 tumours of the learning set (77% of the total) could be classified. Of these, 98% of the carcinomas of the colorectum, 95% of the breast, and 94% of the ovary were correct (table 5). Of the 122 tumours with a posterior probability value of 0.8 or more, the overall correct classification was 96%; one colorectal, two breast, and two ovarian carcinomas were classified falsely as originating from the ovary, the ovary, and the colorectum, respectively. The results correspond to correct identification in 91% of the colorectal carcinomas, 69% of the breast carcinomas, and 60% of the ovarian carcinomas from the original learning group (n = 159). In this setting, the differentiation grade had no impact on the classification results.

Using the posterior probability of > 0.8 as the threshold value for the test set, 77% (117/153) were classified as "certain"—100% (38/38) of the colorectal carcinomas, 83% (39/47) of the breast carcinomas, and 100% (32/32) of the carcinomas of the ovary. Two of the falsely classified metastases of the breast were misclassified as originating from the colon, while the other six were classified falsely as carcinomas of the ovary. The overall correct classification of the 117 tumours of the test set was 93% (table 5). These results correspond to a correct identification of 81% of the adenocarcinomas of the colon, 60% of the breast, and 67% of the ovary from the original test set.

Table 5 Classification results considering only classification with a posterior probability (PP) > 0.8 ("certain")

	n	Number with PP >0.8 (%)	Classified as PP >0.8 (%)		
			Colonic	Breast	Ovarian
Learning set					
Colorectal carcinoma	54	50 (93)	**49 (98)**	0	1 (2)
Breast carcinoma	55	40 (73)	0	**38 (95)**	2 (5)
Ovarian carcinoma	50	32 (64)	2 (6)	0	**30 (94)**
Test set					
Metastatic colorectal carcinoma	47	38 (81)	**38 (100)**	0	0
Metastatic breast carcinoma	65	47 (72)	2 (4)	**39 (83)**	6 (13)
Metastatic ovarian carcinoma	24	17 (71)	0	0	**17 (100)**
Primary ovarian carcinoma	19	15 (79)	0	0	**15 (100)**

DECISION TREE ANALYSIS

A decision tree was developed intuitively, based on the IS values of the learning set samples and the number of correctly classified samples, in such a way that the number of correctly classified samples was as high as possible in each

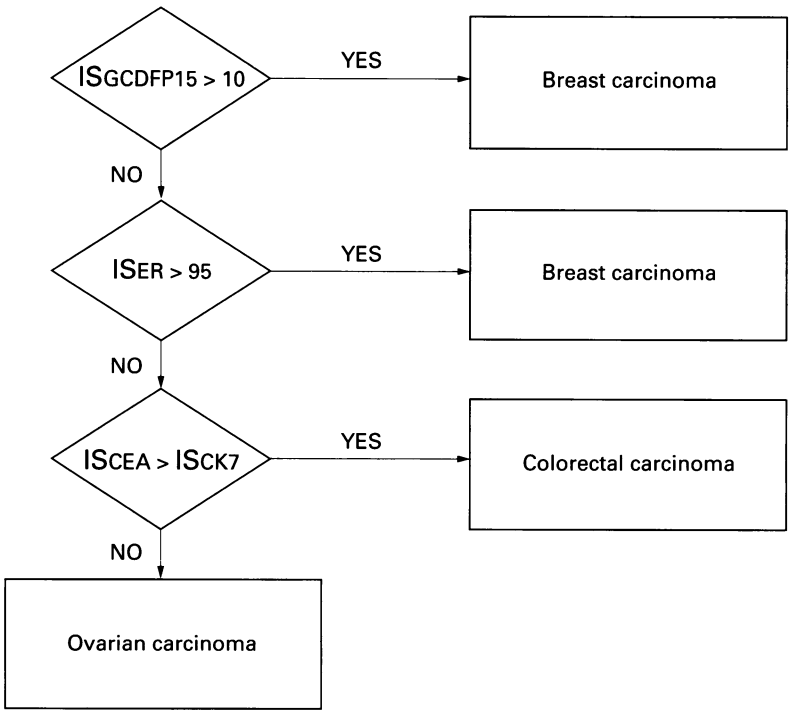

Figure 1 Decision tree for the differential diagnosis of adenocarcinomas of colorectum, breast, and ovary. ISCEA, immunoscore for carcinoembryonic antigen; ISCK7, immunoscore for cytokeratin 7 antigen; ISER, immunoscore for oestrogen receptor; SGCDFP, immunoscore for gross cystic disease fluid protein-15.

step. The flow chart constructed was eventually composed of only three discriminating steps (fig 1).

In the first two steps, tumours were classified as of breast origin when the IS score for GCDFP-15 was > 10 (step 1) or the IS score for ER was > 95 (step 2). In these two steps 44 of 55 breast carcinomas of the learning set could be classified correctly. In the third step, tumours with an IS_{CEA}/IS_{CK7} ratio more than 1 were classified as colorectal carcinomas. Here all colorectal carcinomas of the learning group were correctly classified (54/54), while seven adenocarcinomas of the ovary (43/50) and one carcinoma of the breast (1/10) were misclassified as of colorectal origin.

The remaining group of tumours, which were classified as ovarian carcinoma, consisted of 43 carcinomas of the ovary and 10 misclassified breast carcinomas. In this way, an overall correct classification of the learning set of 89% was obtained. No relation was seen between the differentiation grade or breast cancer subtype and the classification results.

Table 6 Results of decision tree analysis of learning and test sets

		Step 1	*Step 2*	*Step 3*	*Rest*	
		Classified as:				*Correctly classified (%)*
	n	*Breast*	*Breast*	*Colonic*	*Ovarian*	
Learning set						
Colorectal carcinoma	54	0	0	**54**	0	100
Breast carcinoma	55	**42**	**2**	1	10	80
Ovarian carcinoma	50	0	0	7	**43**	86
Test set						
Metastatic colorectal carcinoma	46	0	0	**41**	5	89
Metastatic breast carcinoma	64	**46**	**3**	3	12	72
Metastatic ovarian carcinoma	24	0	0	1	**23**	96
Primary ovarian carcinoma	16	0	0	1	18	95

When testing this flow chart on the test set samples, in the first two steps no misclassifications of ovarian or colonic carcinomas occurred and also 49 of 64 metastases of the breast carcinomas were classified correctly, corresponding to 77% correct classification. In the third step, 41 of 46 metastases of adenocarcinomas of colonic origin could be correctly classified, while there were four falsely classified metastases (one of ovarian and three of breast origin). The remaining group, classified as ovarian, included 23 metastases of ovarian, 12 of breast, and five of colorectal origin. Of the 20 metastases to the ovary, 90% were correctly classified as of either colonic or breast origin. The overall correct classification of the metastases was 82% (table 6). Of the 19 primary adenocarcinomas of the ovary in the test set, 18 were correctly classified. In all, 84% of the test group samples were correctly classified.

Discussion

Metastatic adenocarcinomas to the ovary are a diagnostically important group of ovarian neoplasms[2] because they often are misinterpreted as primary tumours. One of the reasons is that the metastases originate from an unknown primary site. The largest group of metastases to the ovary are derived from primaries within the gastrointestinal tract (47%), of which the great majority was reported as colorectal,[4] while metastases from the breast are the second largest group. Gagnon and Têtu[9] described 165 ovarian metastases of which 59 (38%) originated from the breast.

Recognition of metastases to the ovary also depends on the knowledge of the frequency with which certain primary tumours metastasise to this site, the clinical history, a thorough examination of the patient, and the evaluation of macroscopic and microscopic characteristics,[10] extended by specialised techniques such as immunohistochemistry.

The present study was performed to make an objective interpretation of immunohistochemical data in the differential diagnosis of metastases from colorectal and breast adenocarcinomas, and of metastatic or primary ovarian carcinomas. For this purpose we selected antibodies with certain well known specificities for various primary and metastatic tumours at the three sites. We started with the assumption that the immunohistochemical profiles of primary adenocarcinomas of colon, ovary, and breast are comparable with those of their metastases. Although there were some indications of this in published reports,[11] we checked the hypothesis further. The primary adenocarcinomas of colon and breast and their metastases showed no significant differences in expression. Only in ovarian carcinomas were some minor differences found between the IS values—the metastases had a higher IS for CK7 and ER. Because this difference did not seem important, the study was performed as planned. The results show that no real differences were found between the percentages of correctly classified ovarian metastases and primaries compared with the other adeno-

carcinomas (table 5). Therefore, our model was considered to be valid. The primary adenocarcinomas were used as a learning set to define the antigen profiles, and they were subsequently used to discriminate the test set of metastatic tumours supplemented by a limited number of primary ovarian adenocarcinomas. This supplement of 19 primary ovarian carcinomas was added to compare this group of tumours with the 20 ovarian metastases of colon and of breast origin. Immunostaining was scored semiquantitatively, taking into account staining intensity and the percentage of stained cells. This scoring system has proved to be reproducible[33] and has the advantage that tumours can be discriminated in spite of less obvious staining differences.

Two approaches were used to analyse the results of the immunostaining. The first method was a stepwise discriminant analysis, in which antibodies against CEA, GCDFP-15, CA125, CK7, CK20, and ER were needed for an optimum result. The best classification results were obtained for colorectal carcinomas and the worst for breast carcinomas, although for the latter the two most specific tumour markers are used, namely GCDFP-15 and ER (see tables 2 and 3). The reason for this seeming contradiction is because in stepwise discriminant analysis the combined sensitivity and specificity of all the antibodies determines the sequence of the antibodies to be entered in the analysis. In contrast, in the decision tree procedure, the sequence of the steps is determined by the specificity of the antibodies only.

Stepwise discriminant analysis and decision tree analyses showed nearly the same results. In both cases, the learning sets even showed exactly the same overall result. There were only some small differences when the results were compared for the different tumour groups. In the test set, the results of the decision tree analysis were slightly better: two more breast carcinomas and four more carcinomas of the ovary were classified correctly, with the use of fewer antibodies. The total proportion of correctly classified tumours in the test set was 82% for the stepwise discriminant analysis and 86% for the decision tree analysis. In addition, with the decision tree, more of the metastases to the ovary were correctly classified (90%) than with the stepwise discriminant analysis (65%). These results, together with the simplicity of the method, support the use of the decision tree approach.

Histological subtype or differentiation grade did not influence the classification results for the different cancers. This indicates that expression of the relevant antigens is retained in different histological subtypes and with increasingly poor differentiation grade, and also after dissemination. The proposed system is therefore considered to be robust, and useful in clinical practice where most problems are encountered with the most poorly differentiated cancers.

In the stepwise discriminant analysis, CEA turned out to be the tumour marker with the highest discriminative value. This was also found previously for the differential diagnosis of only ovarian *v* colorectal carcinoma.[33] Although its usefulness is doubtful for the discrimination of mucinous ovarian carcinomas, which, like colorectal carcinomas, often express CEA strongly,[6 7 22] we were able to classify 13 of 19 primary and eight of nine metastatic mucinous ovarian carcinomas correctly in the decision tree approach.

The effectiveness of αCEA in this differential diagnosis is due at least in part to the semiquantitative evaluation of the sections, which allows the evaluation of small differences in staining (table 2).

The discriminative power of GCDFP-15 in this analysis was evident; between 70% and 80% of the primary or metastatic breast carcinomas could be classified, while there were no false positives. Although αGCDFP-15 is known to be positive not only in breast carcinomas but also in carcinomas of the salivary glands, sweat glands, and prostate and in some non-neoplastic tissue,[28] this does not play a role in the present differential diagnosis. The percentage of positive primary and metastatic breast carcinomas was in accordance with the results of Wick *et al.*[11] The CA125 antibody was the third one included in the stepwise discriminant analysis. It was reported to be relatively effective in differentiating Mullerian tissue derived adenocarcinomas from other types of adenocarcinoma.[27] In the stepwise discriminant analysis, we were able to confirm this. As described by Loy *et al*,[26] CA125 was expressed in about 60% of the ovarian adenocarcinomas, while in colonic carcinomas only 4% and in breast carcinomas 13% of the tumour cells were stained. In our study, seven of 63 primary colorectal carcinomas (11%) were positive and 42 of 63 (67%) showed no labelling at all, while in ovarian adenocarcinomas 47 of 69 (68%) were positive. Here, only 12 of 69 of the primary carcinomas (17%) were completely negative. The number of positive breast carcinomas fell in between (20%). The differences were sufficient for CA125 to play a role in the stepwise discriminant analysis. In contrast, CA125 was not used in the decision tree procedure. Further analysis of the decision tree rest group ("ovarian carcinomas") with CA125 (IS > 80) resulted in only one additional correctly classified carcinoma of the ovary.

CK7 was only included in the stepwise discriminant analysis at step 4. It separates the colorectal carcinomas from the other tumours. The primary mucinous ovarian tumours could also be differentiated very well from colonic carcinomas, owing to large differences in the mean IS (table 2).[33] Our results, however, were not as absolute as those described by Berezowski *et al*[15] and others,[12 14] who found that ovarian tumours, including the mucinous type, are consistently positive for CK7 whereas colonic carcinomas are consistently negative.

ER was only included in the stepwise discriminant analysis at step 5. The reason for this is probably the low mean IS value and its overlap with GCDFP-15 (table 2). In contrast to the reports by Takeda *et al*[31] and Ollayos,[32] all our colorectal carcinomas were negative. In the

decision tree procedure, the use of the ER antibody added two and three additional correctly classified cases to the learning set and test set, respectively. No ovarian carcinomas were falsely added to the group of breast carcinomas because of the IS threshold value of 95.

In recent reports,[2 16] CK20 was proposed as a important tool to differentiate between adenocarcinomas of colonic and ovarian origin because of its exclusive presence in colorectal carcinomas and only occasional positivity in mucinous ovarian carcinomas. As already described, CEA has much higher discriminatory power than CK20,[33] which is also illustrated by the mean IS values (table 2). CK20 was only included in the stepwise discriminant analysis in the last step and was not used in the decision tree procedure.

Vimentin did not contribute to the classification results in the stepwise discriminant analysis, although it was found in some ovarian carcinomas.[17-19] The mean IS value of ovarian carcinomas was probably too low to play a discriminating role in the stepwise discriminant analysis. It was also not used in the decision tree.

The results in this study are reasonably comparable for both analyses and lie in between 80% and 90% correctly classified cases. A difference between the stepwise discriminant analysis and the decision tree analysis is the number of antibodies to be used. Although this may vary a little because of variations in the particular antibodies and laboratory practice, in our study it turned out that six antibodies (αCEA, αGCDFP-15, αCA125, αCK7, αER, and αCK20) are needed in the stepwise discriminant analysis, and only four of them (αGCDFP-15, αER, αCEA, and αCK7) are needed in the decision tree. Therefore the decision tree method has the advantage that it is cheaper and, moreover, it can be performed immediately when evaluating the sections. It therefore seems to be a more practical method for the surgical pathologist. On the other hand, stepwise discriminant analysis provides a computer aided, statistics based method for the classification of the adenocarcinomas. With this method, probability values for a correct diagnosis are generated. Moreover, the results obtained can easy be stored in a computer and verified by clinical follow up, and the antibody performance can be evaluated periodically. Newly developed and clinically verified antibodies can be added to modify the classification rule. Also the clinically verified cases can be added to the learning set to enhance the reliability of the stepwise discriminant analysis.

Summarising, the method of scoring immunostaining semiquantitatively and analysing the results by either a stepwise discriminant analysis or a decision tree offers the possibility of differentiating primary ovarian carcinomas from metastatic colorectal and breast adenocarcinomas to the ovary. With both approaches it is possible to classify 85–90% of cases correctly.

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