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Clinical and Immunohistochemical Analysis of Patients with Unknown Primary Tumour. A Search for Prognostic Factors in UPT

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Abstract. *Background: The unknown primary tumour (UPT) is an intriguing clinical finding in approximately 5% of all newly diagnosed patients with cancer. To evaluate a correlation between the specific immunohistochemical alterations in UPT cells and the unique clinical features of UPT patients, to define the natural history of UPT and to verify prognostic factors, we undertook a detailed clinical and immunohistochemical analysis of patients with the diagnosis of adenocarcinoma of UPT. Results: Patients with UPT present with a short history and have a poor prognosis. Univariate analysis was performed with clinical, biological and immunohistochemical variables. Patients with a higher age (>60 years), a poor performance score (2-3), liver metastases or more than two organ sites involved, or patients with elevated LDH-levels, were found to have worse prognosis. We confirm that the prognostic model published by Culine is a valuable model for the prediction of prognosis in patients with UPT. Immunohistochemical detection of proliferation (MIB-1), p53, vascular endothelial growth factor-A, CD34, CD44v6 and Her2neu indicated that these factors were of no prognostic value. Conclusion: In conclusion, patients with UPT have a very poor median prognosis of 12 weeks. Prognostically favourable factors are young age, good performance status, no liver metastases and normal LDH level. We found no relationship with immunohistochemical factors.*

Unknown primary tumour (UPT) is defined as biopsy-proven metastasis of a malignancy in the absence of an identifiable primary site after a complete history and physical examination have been carried out, along with basic laboratory studies, chest X-ray and, if indicated, additional symptom-directed studies (1-3). The diagnosis of an UPT is made in approximately 5% of all newly diagnosed patients with cancer(2-4). UPT is predominantly classified as adenocarcinoma (50-60%) or poorly-differentiated adenocarcinoma or carcinoma (30-40%). Only 5-8% of the UPT are a squamous cell carcinoma and 2-5% an undifferentiated malignancy (2-4). In some cases the primary tumour becomes apparent after several months. In 60-80% the primary is found by autopsy(5,6). In most clinical studies the prognosis for patients with UPT is poor except for treatable subgroups. Median survival from the time of diagnosis ranges from 3 to 11 months, depending on the selection of patients (3,4,7,8).

At present, different theories exist to describe the heterogeneous UPT syndrome. UPT may be considered as metastases in patients in whom the primary tumour has not been found and did not result in clinical signs. Otherwise UPT may represent a separate group of cancers with genetic and phenotypic characteristics or even unusual primary tumours mimicking metastatic disease (in case of one identified tumour site). Advances in understanding the basic biology of UPT can have a direct impact on clinical care. As we described earlier, only scant information exists on the biological characteristics of UPT (9).

Since UPT is an aggressive type of tumour with a high metastatic potential, a high expression of p53, MIB-1, VEGF and CD 44v6 and a high microvessel density might be expected. To evaluate a correlation of specific immunohistochemical alterations in UPT cells with the unique clinical features of UPT patients, to define the natural history of UPT and to verify prognostic factors, we undertook a detailed clinical and immunohistochemical analysis of patients with the diagnosis of adenocarcinoma of UPT.

Patients and Methods

Patients. From 1990 to 1996, all consecutive patients with an adenocarcinoma of unknown primary were analysed in the St. Elisabeth Hospital in Tilburg, the Netherlands. The data of patients were obtained from the national pathological computerised archive (Pathologisch Anatomisch Landelijk Geautomatiseerd Archief), from

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our hospital and from the Eindhoven Cancer Registry (codes 196 to 199 ICD-O). In this way 160 patients were selected. However, after reviewing the clinical charts of these patients, only 72 patients were biopsy-proven adenocarcinoma of UPT (of the other 88 patients, in 55 the primary tumour became known, for 20 patients no histology was available and for 13 patients only cytology was available). After reviewing the histological data, 2 biopsies were not adenocarcinoma. So 70 patients were selected according to the following criteria: clinical and histological material available, an adenocarcinoma and no primary tumour existing at the time of diagnosis. The clinical and histological information on all patients was entered into a computerized database and analysed by SPSS 10.0.

Immunohistochemical staining. Next to immunohistochemical prognostic factors, we also performed analysis of Her2neu receptor, for this can have therapeutic consequences(10).

From the routine formalin-fixed paraffin-embedded archival tumour blocks, slides of 3 μm thickness were prepared. Immunohistochemical staining was performed using mouse monoclonal antibody against MIB-1 (Dianova, Hamburg, Germany; 1:100, one-hour incubation at room temperature), mouse monoclonal antibody against p53 (D07, DAKO A/S, Denmark, 1:200, two-hour incubation at room temperature), mouse monoclonal antibody against CD34 (NCL-END, Novocastra Laboratories, Newcastle, UK; 1:50), mouse monoclonal antibody against VEGF (MAB293, R&D Systems, UK) and mouse monoclonal antibody against CD44v6 (R&D Systems, 1:1000, one-hour incubation at room temperature) as described by the manufacturer. Staining for Her2-neu was performed using an automated stainer (DAKO, Carpentaria, CA, USA) and the polyclonal Her-2 antibody NonHercepTest (DAKO) in conjunction with 20 minutes of antigen retrieval (60˚C) in a steam bath.

Immunohistochemical score. Immunohistochemical countings were analysed as continuous data for MIB-1, p53 and CD44v6. The cut-off values used in this study were defined as follows. MIB-1 staining greater than 20 was regarded as high expression (11). Tumours were considered p53-positive when more than 10% of cells showed positive staining (12). The tumour was considered CD44v6-positive when more than 5% of tumour cells showed CD44v6 expression (13,14). Also other cut-off points were analysed for MIB-1, p-53 and CD44v6. The intensity of staining for VEGF was graded on a scale of 0 to 3+, with 0 representing no detectable stain and 3+ representing the strongest stain under a x200 field (15,16). Microvessel density was measured by selecting the highest vascularised areas at low power and counting the microvessels using a 200x magnification. The MVD was expressed as the number of vessels/mm^2. The criteria for microvessel recognition were the same as described by Vermeulen *et al.* (17). At least 2 mm^2 tumour were evaluated and the median value (56 vessels/mm^2 in this study) was used as cut-off value. Her2-neu staining was quantified using the following scale: 0, no membrane staining; 1+, barely perceptible light membranous rimming that may not totally encircle the cell membrane; 2+, light to moderate membranous rimming that totally encircles the membrane; 3+, moderate to strong rimming that totally encircles the membrane. Tumours were considered to overexpress Her2-neu if membrane staining of 2+ or 3+ intensity was present(18).

Two investigators scored all slides independently.

Table I. *Patient characteristics.*

Characteristic	No. of UPT (%)
Age	
40-49	7 (10)
50-59	10 (14)
60-69	23 (33)
≥ 70	30 (43)
Median	66
Range	42-88
Sex	
Male	39 (56)
Female	31 (44)
PS*	
0	26 (37)
1	19 (27)
2	14 (20)
3	11 (16)
No. organ sites	
1	35 (50)
2	
1 of 2	16 (23)
≥ 3	19 (27)
Primary site	
Liver	30 (43)
Lung	10 (14)
Lymph node	9 (13)
Bone	7 (10)
Peritoneum	6 (9)
Other	8 (11)
Primary tumour	
Found	15 (21)
Not found	55 (79)
Treatment	
Yes	33 (47)
No	37 (53)

* Performance status

Statistical analysis. Survival was calculated from the date of diagnosis, which was the date on which the biopsy was performed. Estimates of the survival distribution of patients were made using the method of Kaplan and Meier. Differences between survival curves for various patient subgroups were assessed by the log-rank test. Multivariate analysis was done by Cox-regression analysis according to a backward stepwise selection.

Results

The 70 patients with biopsy-proven adenocarcinoma of unknown primary tumour (AUPT) represent 1.9% of the total patient population with malignancies presented in the Sint Elisabeth Hospital in Tilburg, The Netherlands. The demographic characteristics and clinical findings are listed in Table I. The UPT patients with adenocarcinoma were elderly patients. At presentation one third were already in a poor condition with performance status 2 or worse and

Table II. *Univariate analysis with clinical variables.*

Variable	No. of patients	Median survival in weeks	*p*-value
Age			
<60	17	30	
≥60	53	12	0.016
Sex			
Male	39	12	
female	31	13	0.226
Performance status			
0-1	45	21	
2-3	25	6	0.000
Liver metastases*			
Yes	30	10	
No	40	13	0.039
Lymph nodes*			
Yes	9	65	
No	61	12	0.101
No. of metastatic sites			
1-2	51	17	
≥3	19	7	0.004
Primary tumour			
Found	15	12	
Not found	55	13	0.506
Treatment			
Yes	33	22	
No	37	9	0.001

* primary site at presentation

Table III. *Univariate analysis of immunohistochemical parameters.*

	Number		No.	Survival in weeks	*p*-value
MIB1	40	< 20	17	11	0.07
		≥ 20	23	13	
P53	48	≤ 10	25	11	0.74
		> 10	23	13	
VEGF	46	0	28	12	0.38
		1	6	13	
		2/3	12	12	
CD34	36	≤ 56/mm^2	19	12	0.37
		> 56/mm^2	17	13	
CD44v6	40	≤ 5	19	12	0.95
		> 5	21	11	
Her2neu		0/1	29	12	0.47
		2/3	16	13	

over 20% of patients had more than 2 organs involved, predominantly the liver. Less than 50% of the patients received cancer-related therapy. (Table I)

The patients presented with general symptoms (not feeling well, weight loss *etc.*) and/or symptoms related to the metastatic site (dyspnoea, jaundice, palpable lymph node *etc.*). The duration of the symptoms was not clear.

Radiological and/or endoscopical examinations were performed. In 66 (94%) patients an X-ray of the thorax and in 62 (88%) patients an ultrasound of the upper abdomen were performed. In 20 (64%) of the women a mammography was done. Neither 48 CT-scannings of the thorax and/or abdomen nor 46 analyses of the digestive tract revealed the primary tumour (18 barium enema, 7 coloscopy, 20 gastroscopy, 1 gastric X-ray).

In 15 out of 70 patients the primary was identified during follow-up (4) or at autopsy (11). When autopsy was performed the primary was found in all cases. In these patients the appropriate investigation for the particular tumour had been performed at diagnosis (X-mammography, CT of the abdomen, CT of the thorax) but did not reveal the primary at that time. The primary tumour was predominantly located in lung (4/15) and pancreas (4/15). Other primary sites were breast (2), bile duct (1), colon (1), stomach (2) and ovary (1). The metastatic pattern of these tumours seemed not to differ much from metastatic patterns of patients in whom the specific primary tumour was identified at diagnosis: for example, pleuritis carinomatosa, adrenal, brain and pulmonal metastases in lung cancer, peritonitis carcinomatosa and liver and bone metastases with a pancreatic primary.

The overall survival of 12 weeks was poor. To evaluate clinical prognostic factors univariate analysis was performed with clinical variables. The worst prognosis was observed in patients older than 60 years, patients with a poor performance score (2-3), patients with liver metastases, patients with more than two organ sites involved and untreated patients (Table II). In univariate analysis, only lactate dehydrogenase had prognostic relevance among the studied biological parameters. Haemoglobin, alkaline phosphatase, bilirubin and gamma-glutamyl transferase showed no significant influence.

In 48 of the 70 patients there was sufficient material to perform additional immunohistochemical staining, to evaluate different immunohistochemical prognostic factors (Table III). When 48 patients with immunohistochemical staining were compared to 22 patients without staining, there was no difference in survival, age, sex, PS, liver metastases or LDH. The only difference was that, in the group of 48 patients with immunohistochemical-staining, the primary was found in 14 patients (data not shown). This was because in 11 of these patients autopsy was performed, which provided sufficient material for immunohistochemical staining.

In an univariate analysis, only MIB-1 dichotomised at 20% showed a trend to have prognostic value. Strikingly, high proliferation was related to better survival. Looking into this further, 5 out of 7 patients belonging to a treatable subgroup had high MIB-1 staining. All other factors were of no prognostic value. A remarkable observation was made that different metastases within a patient showed a similar expression of the markers.

Table IV. *Cox multivariate regression analysis including clinical variables and biological parameters.*

Variable	Relative death rate	*p*-value
Age		
<60	1	0.04
≥60	2.6	
Performance status		
0-1	1	0.005
2-3	6.6	
Organ sites involved		
1-2	1	0.25
≥ 3	1.2	
Liver metastases		
Yes	1	0.006
No	2.1	
Lactate dehydrogenase		
Normal	1	0.003
> normal	2.5	

In a multivariate analysis of clinical and biologic parameters, age, performance status, liver metastases, as well as LDH were of prognostic relevance (Table IV) .

Discussion

The 70 consecutive patients with biopsy-proven adenocarcinoma of UPT formed 1.9% of all the malignancies in that period. This is less than the figure of approximately 5% mentioned in the literature(2-4). However, when patients with poorly-differentiated carcinoma, squamous carcinoma and undifferentiated carcinoma were included, as well as the patients with UPT proven by cytology or only a clinical diagnosis, they formed 4.5% of all the malignancies in that period. It is also important to keep in mind that only patients with a biopsy are included. As found earlier in a population based study, these patients have a slightly better prognosis than patients who are clinically diagnosed (11 *vs.* 7 weeks) (4).

The 70 patients with UPT fit most of the clinical features of UPT as mentioned earlier (1-4,7). First they present with a short history of non-specific symptoms. Secondly, in most cases the primary tumour remains unidentified but, if found during life or by autopsy, it is a small asymptomatic tumour, often localised in the lung or pancreas. Thirdly, over 25% of the patients presented with 3 or more metastases. Fourthly, the prognosis for these unselected patients was very poor with a median survival of 12 weeks, but when patients belonged to a treatable subgroup and were treated the prognosis was much better, as also is mentioned by others. However, new, more effective chemotherapeutic agents are available and in more recent studies, with selected patients from poor prognostic groups, median survival from 9-13 months is reached, with >40% of patients alive 1 year after diagnosis (19-22).

Independent good prognostic factors were young age, good performance status, no liver metastases and normal LDH-level, as in other studies (2,7,8,23,24). In contrast to other studies, alkaline phosphatase and lymph node metastases were of no prognostic value (2,7,8,23). When we used the prognostic model presented by Culine *et al*., the good prognostic group (PS=0 or1 and no liver metastases) contained 22 patients with a median survival of 26 weeks, the intermediate group (PS>1 or liver metastases) contained 32 patients with a median survival of only 12 weeks and the poor prognostic group (PS>1 and liver metastases) contained 12 patients with a median survival of 4 weeks (24). So we can confirm this prognostic model, which seems to be a powerful tool in predicting prognosis in patients with UPT.

In the 15 patients in whom the primary tumour became obvious, we found no unusual metastatic pattern. This in contrast to earlier studies (6,25,26). It should be mentioned, however, that we only could investigate a small group of patients.

Immunohistochemical data were only gathered in 55-60% of the patients. But these patients formed a representative group of all 70 UPT's, considering age, performance score and survival. We expected overexpression of factors that are correlated to poor prognosis in known primaries, but we did not find any correlation between UPT and any of these factors. The results of other investigators who performed some of these prognostic factors on UPT are contradictive. As Bar-Eli, we found no high expression of p53, which in contrast to Briasoulis *et al*. who found over-expression of p53 in 70% of all patients with UPT (27,28). The low expression of p53 suggests p53 mutation may not play an important role in the development of this aggressive tumour. However, one should keep in mind that all three studies were performed in small groups (23-48 patients) and used different tests. Also a high MVD as a prognostic indicator described earlier by our group could not be confirmed in this study (29).

In conclusion, patients with UPT have a very poor prognosis with a median survival of 12 weeks. Young age, good performance status, the absence of liver metastases and a normal LDH level are favourable prognostic factors. Further research is necessary to answer the question as to whether UPT is a specific entity. Therefore we are performing a study to investigate specific genetic and immunohistochemical alterations in UPT.

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