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Tumour cells in the tumour draining vein of patients with non-small cell lung cancer: detection rate and clinical significance☆

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Abstract

Objectives: This prospective study was performed to examine whether tumour cells are detectable in the tumour draining vein of patients with non-small cell lung cancer. Furthermore, the impact of these cells on the clinical course was analysed. **Patients and Methods**: Sixty-two consecutive patients with completely resected primary non-small cell lung cancer (pT1-4 pN0-2 M0) were admitted to the study. Pulmonary venous blood was drawn at the time of surgery for primary non-small cell lung cancer. The tumour draining vein was punctured subsequent to thoracotomy prior to manipulation of the tumour. The blood samples were examined for occult tumour cells by immunocytochemical staining of cytospins using the pancytokeratin antibody A45-B/B3 (murine immunoglobulin G1; Micromet, Munich, Germany). **Results**: Disseminated cancer cells in pulmonary venous blood were observed in 11 of 62 patients (18%) and did not correlate with standard clinicopathological parameters. In patients without involvement of mediastinal lymph nodes (pN0–pN1), detection of occult tumour cells was an independent prognostic parameter for unfavourable outcome: log rank analysis showed a significant association of occult tumour cells in pulmonary venous blood with shortened cancer-related survival ($P = 0.019$) and multivariate regression analysis demonstrated an independently significant ($P = 0.004$) prognostic impact. **Conclusion**: The present study shows that disseminated cancer cells in the pulmonary venous blood are detectable in about 20% of the patients with operable non-small cell lung cancer and that they are associated with a poor clinical outcome. Therefore, the detection of such cells might be useful for the identification of patients who benefit from adjuvant therapy. Furthermore, in order to avoid an additional systemic spread of tumour cells intraoperatively, the pulmonary veins should be ligated first during lung cancer surgery.
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Keywords: Pulmonary vein; Circulating cells; Non-small cell lung cancer; Survival rate; Survival analysis; Neoplasm staging

1. Introduction

The failure to reduce mortality of operable non-small cell lung cancer (NSCLC) most likely results from early dissemination of cancer cells, which is usually missed by conventional staging procedures at the time of surgery. Because adjuvant therapy aims to eradicate occult disseminated tumour cells before metastatic disease becomes clinically evident, the early detection of occult tumour cells could identify patients who might benefit from such treatment. Detection of cancer cells in secondary sites like pulmonary venous blood or peripheral blood indicates overall hematogenous dissemination. Analysis of pulmonary venous blood draining the lung cancer investigates the primary source of hematogenous cancer cell dissemination. The latter kind of analysis potentially detects patients' present status of hematogenous dissemination, but so far, the clinical impact of disseminated tumour cells in pulmonary venous blood has not been investigated. Therefore, this prospective study was designed to evaluate the prognostic impact of occult tumour cells in pulmonary venous blood drawn at the time of primary surgery for non-small cell lung cancer. Occult cancer cells in pulmonary venous blood were detected using immunocytochemistry to analyse their impact on cancer-related survival. This is the first report demonstrating that occult tumour cells in

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pulmonary venous blood are of independent prognostic value in operable NSCLC.

2. Patients and methods

2.1. Patients

The study was activated in January 1996 and the latest follow-up was updated in October 2001. After approval by the ethical committee of the University of Munich and after written informed consent, 62 consecutive patients with completely resected primary non-small cell lung cancer and without overt distant metastases (pT1–4 pN0–2 M0) were admitted to the study. The tumours were classified according to the international union against cancer's TNM-classification [1]. The preoperative staging of all patients had resulted in resectable tumours (T1–T4) without evident distant metastasis (M1) or contralateral or supraclavicular lymph node involvement (N3). All samples of pulmonary venous blood were drawn after written informed consent. The tumour-draining pulmonary vein was punctured subsequent to thoracotomy and prior to intrathoracal preparation for lung resection. In general, a lobectomy or pneumonectomy with systematic mediastinal lymphadenectomy was performed. Patient characteristics are shown in Table 1. The median age at time of surgery was 62 years with a range of 44–85. Patients whose primary tumours were classified as pT3 or pT4 received adjuvant percutaneous radiotherapy of the tumour bed and patients with mediastinal lymph node involvement (pN2) received percutaneous radiotherapy of the entire mediastinum.

The median follow-up duration was 25 months (range 5–66 months). Follow-up studies included physical examination, chest X-ray and blood tests in 3 months interval and an additional thoracic computer tomographic (CT) scan, abdominal ultrasound and bronchoscopy in 6 months interval. Close follow-up was documented by contacting family practitioners with questionnaires concerning local relapse, distant metastasis and death. If possible a relapse was confirmed at our institution and the patient was admitted for subsequent therapy.

2.2. Occult dissemination of tumour cells into pulmonary venous blood

The tumour-draining pulmonary vein was punctured subsequent to thoracotomy and prior to intrathoracal preparation for lung resection. Ten millilitres of pulmonary venous blood were drawn into a sterile syringe. The obtained pulmonary venous blood, yielding between 5×10^6 and 6×10^7 (mean 2.5×10^7) mononuclear cells,

Table 1
Patient characteristics and distribution of disseminated tumour cells in pulmonary venous blood

Variable	No. of patients	No. of patients with CK positive[a] blood samples	*P* value[b]
Total	62	11 (18%)	
Tumour extension			
pT1–pT2	50	9 (18%)	
pT3–pT4	12	2 (17%)	1.00
Lymph node involvement			
pN0–pN1	49	10 (20%)	
pN2	13	1 (8%)	0.43
Tumour histology[c]			
Adenocarcinoma	19	1 (5%)	
Squamous cell carcinoma	28	6 (22%)	
Miscellaneous	15	4 (27%)	0.21
Age			
<62 years	27	4 (15%)	
≥62 years	35	7 (20%)	0.74
Sex			
Female	17	3 (18%)	
Male	45	8 (18%)	1.00

[a] Detection of one or more immunocytologically stained cells per 2.5×10^6 mononuclear cells was considered positive concerning dissemination of occult tumour cells.
[b] Two-sided *P* values determined by Fisher's exact test show possible significance of correlation between disseminated tumour cells and clinicopathological parameters.
[c] 'Miscellaneous' represents eight mixed histologies and seven large cell carcinomas.

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was centrifuged through 20 ml Ficoll–Hypaque for 30 min at 1200 × *g*. The interface layer, which contains mononuclear cells was collected and brought to a final concentration of 10^6 cells/ml. These interphase cells were cytocentrifuged (Hettich, Tuttlingen, Germany) on six glass slides at 200 × *g* for 3 min and dried overnight for immediate staining or storage at −80°C.

Occult tumour cells in cytospins of pulmonary venous blood were detected by immunohistochemical staining using the monoclonal antibody A45-B/B3 (Micromet, Munich, Germany) that binds to an antigen on cytokeratins 8, 18, and 19 [2]. Cytokeratins are expressed by simple epithelia and tumours derived thereof [3]. High sensitivity and specificity of the immunocytochemical detection of disseminated tumor cells using the antibody A45-B/B3 has been demonstrated previously [4].

For visualization of antibody binding, the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique combined with the Neufuchsin method [5] was employed as reported previously [4]. Briefly, after incubation with A45-B/B3 at a concentration of 2.0 μg per ml, the polyvalent rabbit anti-mouse immunoglobulin antiserum (Z259, Dako) and preformed complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase antibodies (D651, Dako) were applied at the appropriate dilutions for 30 min. In all experiments an isotype-matched, irrelevant murine monoclonal antibody (MOPC 21, IgG_1, Sigma) served as negative control on one glass slide per patient.

2.3. Evaluation

Occurrence of disseminated cancer cells in pulmonary venous blood was assessed by examination of five glass slides per patient yielding approximately 5×10^5 mononuclear cells per slide. Samples of pulmonary venous blood with at least one immunocytochemically stained cell were considered positive concerning occult tumour cells. All cytospins were examined independently by two observers who were unaware of the clinical data. Slides with discrepant evaluations were re-evaluated and a consensus was reached ($n = 3$). The slides were examined under light-microscopes using objectives with ×10 and ×40 magnification.

2.4. Statistical analysis

Statistical analysis was performed using the SPSS software package, version 11.0 (SPSS, Inc, Chicago, IL). The threshold for statistical significance was chosen 0.05. Two-tailed Fisher's exact test was used to analyse the association between occult cancer cells in pulmonary venous blood and categorical clinicopathological variables. For this purpose, all variables were dichotomised. For analysis of follow-up data, survival curves were calculated with the Kaplan–Meier method and survival distributions were compared by log-rank test. The primary end point was cancer-related survival, as measured from the date of surgery to the time of the last follow-up or cancer-related death. Data on patients who were still alive at the end of the study or who died of cancer-unrelated causes were censored. The joint effects of other prognostically relevant variables were further examined via Cox proportional hazards analysis. The respective covariables were entered stepwise forward into the model to assess possible independence of the prognostic value of disseminated tumour cells. The 0.05 level of significance was used for entering or removing a covariable from this model.

3. Results

3.1. Detection of disseminated tumour cells in pulmonary venous blood

Pulmonary venous blood was obtained from 62 consecutive patients with newly diagnosed primary non-small cell lung cancer and without overt distant metastases (pT1–4 pN0–2 M0). At the time of initial surgery of the primary tumour, prior to intrathoracal preparation for lung resection, 11 patients (18%) had cytokeratin-positive tumour cells in their pulmonary venous blood draining the lung cancer. The median frequency of occult cancer cells per blood sample was one cell per 2.5×10^6 mononuclear pulmonary venous blood cells analysed (range 1–4).

3.2. Patient characteristics

Primary tumours were classified as pT1 in 11 patients (18%), as pT2 in 39 patients (63%), as pT3 in eight patients (13%) and as pT4 in four patients (6%). Although tumour extension is a standard risk factor used for prognostic evaluation in NSCLC, we found that the incidence of disseminated tumour cells in pulmonary venous blood was similar in patients with smaller (pT1–pT2) and larger (pT3–pT4) tumour extensions ($P = 1.00$, Fisher's exact test; Table 1).

Routine histopathologic lymph node examination exhibited pN0-status in 28 patients (45%), pN1-status in 21 patients (34%) and pN2-lymph node involvement in 13 patients (21%). Only one patient with pN2-lymph node involvement was positive concerning disseminated cancer cells in pulmonary venous blood. Fisher's exact test revealed that the lymph node status was not associated with occurrence of disseminated tumour cells in pulmonary venous blood ($P = 0.43$, Fisher's exact test; Table 1). Further characteristics like tumour histology, patients' age and sex did not correlate with occurrence of disseminated tumour cells either (Table 1).

3.3. Survival analysis

The median follow-up duration was 25 months (range 5–

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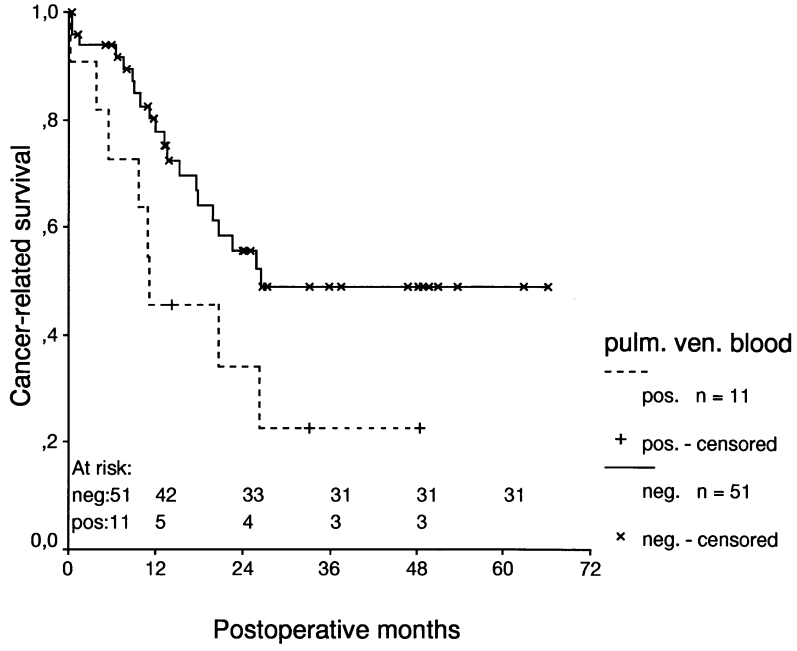

Fig. 1. Relationship between detection of disseminated tumour cells in pulmonary venous blood and cumulative cancer-related survival in NSCLC. Detection of one or more immunocytologically stained cells was considered positive concerning dissemination of occult tumour cells. Survival distributions were calculated with the Kaplan–Meier method and compared by log-rank analysis: $P = 0.054$. All 62 patients were eligible for survival analysis. One patient died of cancer-unrelated cause and was censored at the time of death.

66 months). Within the observation period, a total of 28 patients among the 62 eligible patients (45%) died of cancer-related causes. One patient died of a cancer-unrelated cause. For analysis of cancer-related survival, his survival data was censored at the time of death. Kaplan–Meier survival analysis showed that there was a tendency towards an unfavourable outcome in patients with disseminated cancer cells which however, was not statistically significant in the total population ($P = 0.054$, log-rank test; Fig. 1). Among patients with pN0–pN1 lymph node status, the prognostic impact of disseminated tumour cells in pulmonary venous blood was of statistical significance

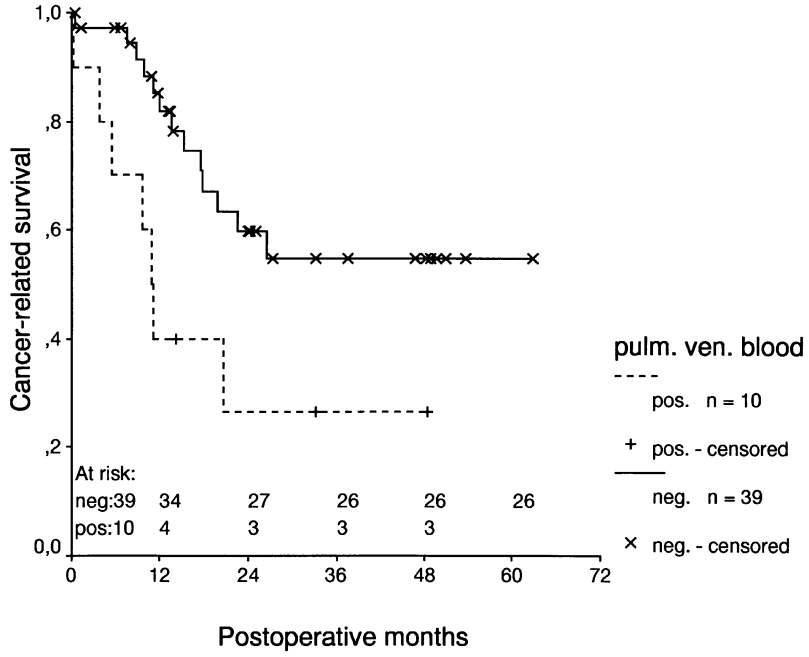

Fig. 2. Distribution of cancer-related survival of patients with pN0–pN1 lymph nodes. Detection of one or more immunocytologically stained cells was considered positive concerning dissemination of occult tumour cells. All 49 patients were eligible for survival analysis. Survival distributions were calculated with the Kaplan–Meier method and compared by log-rank analysis: $P = 0.019$.

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Table 2
Univariate and multivariate analysis of cancer-related survival in patients with pN0–pN1 lymph-nodes ($n = 49$)[a]

Risk factor	Univariate analysis	Multivariate analysis[a]		
	P value[b]	Relative risk	95% Confidence interval	P value
Disseminated tumour cells in pulmonary venous blood[c]	0.019	4.2	1.6–11.1	0.004
Tumour extension	0.0005	3.9	1.8–8.4	0.001
Tumour histology	0.018	[d]	[d]	[d]
Age	0.053	5.1	1.6–16.3	0.005

[a] Stepwise multivariate analysis was performed using the Cox proportional-hazard model.
[b] P-values of univariate analyses were determined by log-rank test.
[c] Detection of one or more immunocytologically stained cells per 2.5×10^6 mononuclear cells was considered positive concerning dissemination of occult tumour cells.
[d] The covariable 'tumour histology' was removed from the Cox regression model, since the variable was not significant on multivariate analysis.

($P = 0.019$, log-rank test; Fig. 2). Among the 49 patients with pN0–pN1 lymph nodes, seven of ten patients with cytokeratin-positive cells in pulmonary venous blood died of cancer-related causes (70%), whereas of 39 patients without occult tumour, only 13 died of cancer-related causes (33%).

3.4. Disseminated tumour cells and other prognostic variables

In the 49 patients with pN0–pN1 lymph nodes, a multivariate analysis was conducted to evaluate whether the significant correlation between dissemination into pulmonary venous blood and shortened cancer-related survival results from an association with prognostic factors or whether occult cancer cells in pulmonary venous blood could maintain their own prognostic value (Table 2). Detection of occult cancer cells, tumour extension, tumour histology and patients' ages were tested for independence of their prognostic values. It was unnecessary to consider therapy as an extra variable since it was already taken into account by tumour size which considers pT3 and pT4 patients who received adjuvant radiotherapy. The multivariate regression analysis demonstrated that cancer cell dissemination into pulmonary venous blood was an independently significant ($P = 0.004$) prognosticator for reduced cancer-related survival in patients with pN0–pN1 lymph nodes. The relative risk for cancer-related death was 4.2 fold increased in case of detection of occult cancer cells in pulmonary venous blood (95% confidence interval 1.6–11.1). Compared to other factors, the risk for cancer-related death in patients with dissemination into pulmonary venous blood was approximately as much increased as in patients with pT3 and pT4 tumours.

4. Discussion

The presence of occult disseminated tumour cells which can not be detected by regular staging procedures at the time of primary surgery is one possible reason for unfavourable clinical courses of patients with operable NSCLC. Therefore, it has been suggested to include the detection of occult disseminated tumour cell into future staging nomenclatures [6,7]. Previously, prognostic relevance of disseminated cancer cells in NSCLC has been reported for the detection in bone marrow and regional lymph nodes [8,9], but so far the clinical impact of disseminated tumour cells in pulmonary venous blood has not been investigated. Recently, it has been shown that prior to resection of primary NSCLC, disseminated tumour cells can be detected in peripheral arterial blood that normally derives directly from pulmonary venous blood [10]. In the latter study, disseminated tumour cells were detected using reverse transcriptase-polymerase chain reaction (RT-PCR). This highly sensitive method [11] was positive in 16 (53%) of 30 patients undergoing surgery for NSCLC. However, RT-PCR is associated with the possibility of false positive results because circulating tumour transcripts may be detected besides intact tumour cells [12]. To investigate the frequency and the clinical impact of selectively intact, and therefore potentially metastasiogene tumour cells in blood that directly drains the primary tumour, the present study was performed using a different methodological setting.

Occult disseminated tumour cells in pulmonary venous blood were detected using immunocytochemistry with a monoclonal antibody (A45-B/B3) that binds to cytokeratins 8, 18, and 19. These cytokeratins are expressed by simple epithelia and tumours derived thereof [3]. In comparison to antibodies against single members of the cytokeratin family, A45-B/B3 is more sensitive [4]. Occurrence of multiple tumour-specific chromosomal aberrations in cytokeratin-positive cells detected by A45-B/B3 is of strong evidence that this antibody is capable to detect individual cancer cells [13]. Using this method, occult disseminated tumour cells were detected in 11 (18%) of 62 patients (Table 1).

In patients with pN0–pN1 lymph nodes, the prognostic impact of disseminated tumour cells in pulmonary venous blood was of independent statistical significance (Fig. 2, Table 2). Even in early stages of operable non-small cell lung cancer, 5 years survival rate remains at 50–60% in stages I and II [1,14]. Therefore, adjuvant therapy seems to

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be indicated also in patients with apparently resectable primary tumours in order to improve the clinical outcome. Early-stage patients with disseminated tumour cells in their pulmonary venous blood showed a reduced cancer related survival, suggesting that especially those patients at increased risk for unfavourable outcome might benefit from adjuvant treatment regimens. Standard therapeutic regimens for metastatic NSCLC consist of systemic administration of anti-proliferating agents. However, the clinical efficacy and response rate is low. One possible reason for this disappointing efficacy might be, that disseminated tumour cells are frequently not in a proliferating state. For example it has been shown that only about 4% of tumour cells, de-tected in the bone marrow of cancer patients, express proliferation markers like Ki-67, indicating that most of these cells remain in a dormant state [15]. Therefore, in addition to standard chemotherapeutic agents, adjuvant therapy with proliferation-independent agents, such as monoclonal antibodies [16] might be more appropriate, especially in early stage NSCLC.

The present study showed that occult tumour cells are present in pulmonary venous blood prior to any manipulation of the primary tumour or of the involved lung. Because the tumour-draining pulmonary vein was punctured immediately on opening the chest and because no dissection of the lung was performed prior to sampling of pulmonary venous blood, the detected single tumour cells are likely to be shed spontaneously. This spontaneous shedding might be increased by any manipulation of the primary tumour or of the involved lung. Therefore, in an effort to minimise tumour shedding, we recommend routine ligation of the pulmonary veins as the initial step prior to pulmonary arterial dissection in routine lobectomies or pneumonectomies for NSCLC.

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References

[1] Mountain CF. Revisions in the International System for Staging Lung Cancer. Chest 1997;111(6):1710–7.

[2] Stigbrand T, Andres C, Bellanger L, Bishr OM, Bodenmuller H, Bonfrer H, Brundell J, Einarsson R, Erlandsson A, Johansson A, Leca JF, Levi M, Meier T, Nap M, Nustad K, Seguin P, Sjodin A, Sundstrom B, van Dalen A, Wiebelhaus E, Wiklund B, Arlestig L, Hilgers J. Epitope specificity of 30 monoclonal antibodies against cytokeratin antigens: the ISOBM TD5-1 workshop. Tumour Biol 1998;19(2):132–52.

[3] Kasper M, Stosiek P, Typlt H, Karsten U. Histological evaluation of three new monoclonal anti-cytokeratin antibodies. 1. Normal tissues. Eur J Cancer Clin Oncol 1987;23(2):137–47.

[4] Pantel K, Schlimok G, Angstwurm M, Weckermann D, Schmaus W, Gath H, Passlick B, Izbicki JR, Riethmuller G. Methodological analysis of immunocytochemical screening for disseminated epithelial tumor cells in bone marrow. J Hematother 1994;3(3):165–73.

[5] Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, MacDonald S, Pulford KA, Stein H, Mason DY. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 1984;32(2):219–29.

[6] Cote RJ, Hawes D, Chaiwun B, Beattie EJ Jr. Detection of occult metastases in lung carcinomas: progress and implications for lung cancer staging. J Surg Oncol 1998;69(4):265–74.

[7] Hermanek P, Hutter RV, Sobin LH, Wittekind C. International union against cancer. Classification of isolated tumor cells and micrometastasis. Cancer 1999;86:2668–73.

[8] Passlick B, Kubuschok B, Izbicki J, Thetter O, Pantel K. Isolated tumor cells in bone marrow predict reduced survival in node-negative non-small cell lung cancer. Ann Thorac Surg 1999;68:2053–8.

[9] Kubuschok B, Passlick B, Izbicki JR, Thetter O, Pantel K. Disseminated tumor cells in lymph nodes as a determinant for survival in surgically resected non-small-cell lung cancer. J Clin Oncol 1999;17(1):19–24.

[10] Kurusu Y, Yamashita J, Hayashi N, Mita S, Fujino N, Ogawa M. The sequence of vessel ligation affects tumor release into the circulation. J Thorac Cardiovasc Surg 1998;116(1):107–13.

[11] Gerhard M, Juhl H, Kalthoff H, Schreiber HW, Wagener C, Neumaier M. Specific detection of carcinoembryonic antigen-expressing tumor cells in bone marrow aspirates by polymerase chain reaction. J Clin Oncol 1994;12(4):725–9.

[12] Zippelius A, Kufer P, Honold G, Kollermann MW, Oberneder R, Schlimok G, Riethmuller G, Pantel K. Limitations of reverse-transcriptase polymerase chain reaction analyses for detection of micrometastatic epithelial cancer cells in bone marrow [See comments]. J Clin Oncol 1997;15(7):2701–8.

[13] Klein CA, Schmidt KO, Schardt JA, Pantel K, Speicher MR, Riethmuller G. Comparative genomic hybridization, loss of heterozygosity, and DNA sequence analysis of single cells. Proc Natl Acad Sci USA 1999;96(8):4494–9.

[14] Martini N, Bains MS, Burt ME, Zakowski MF, McCormack P, Rusch VW, Ginsberg RJ. Incidence of local recurrence and second primary tumors in resected stage I lung cancer. J Thorac Cardiovasc Surg 1995;109(1):120–9.

[15] Pantel K, Schlimok G, Braun S, Kutter D, Lindemann F, Schaller G, Funke I, Izbicki JR, Riethmuller G. Differential expression of proliferation-associated molecules in individual micrometastatic carcinoma cells. J Natl Cancer Inst 1993;85(17):1419–24.

[16] Passlick B, Sienel W, Seen-Hibler R, Wockel W, Thetter O, Pantel K. The 17-1A antigen is expressed on primary, metastatic and disseminated non-small cell lung carcinoma cells. Int J Cancer 2000;87(4):548–52.

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