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Cytokeratin-positive cells in bone marrow in comparison with other prognostic factors in colon carcinoma

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Abstract *Background and aims:* Despite the use of radical locoregional therapeutic methods and although conventional methods of diagnosis give no indication of metastases at the time of operation, distant metastases develop in approximately 50% of carcinoma patients within 5 years. While local relapses after the R0 resection of solid tumors are mainly a matter of concern for the surgeon, distant metastases can be traced back to the systemic dissemination of tumor cells at the time of operation. *Patients/methods:* A prospective study is presented in which 145 patients suffering from colon carcinoma were analyzed for the prognostic relevance of isolated disseminated tumor cells detected in the bone marrow (IDT BM). The patients were operated on between 1993 and 1997 and subsequently observed until 1999. *Results:* The monoclonal antibody A45-B/B3 was used with the immunocytochemical standard method for detecting IDT BM. For the purpose of cell cultivation, the cells were marked with the HEA-125 antibody and separated by means of magnetic cell sorting (MACS). *Conclusion:* In this investigation the presence of isolated disseminated tumor cells, as indicated by the A45-B/B3 antibody, proved to be an independent prognostic factor for survival time. The risk of an earlier death increased in node-negative and metastases-free patients with the detection of IDT BM by a factor of 12.60. The detection of IDT BM also represented an independent prognostic factor for the time until advancement of the tumor. The risk of an earlier relapse increased with the detection of disseminated tumor cells in the bone marrow containing the A45-B/B3 antibody by a factor of 18.02. A generally acknowledged standardization of the method is desirable. Due to the importance of the independent prognostic IDT BM factor, this method of ascertaining the pathological stage should be established at institutions of higher learning.

Key words Colon carcinoma · Isolated disseminated tumor cells · Bone marrow

Introduction

Despite the use of radical locoregional therapeutic methods and although conventional methods give no indication of metastases at the time of operation, distant metastases develop in approximately 50% of carcinoma patients within 5 years [12]. While local relapses after the R0 resection of solid tumors are mostly a matter of concern for the operating surgeon, distant metastases can be traced back to the systemic dissemination of tumor cells at the time of operation [14].

Following intensive testing of various detection methods for "minimal residual disease," the generally accepted term *isolated disseminated tumor cells in bone mar-*

row (IDT BM) was coined [12]. The International Union Against Cancer (UICC) acknowledged this by introducing an M1(i) stage [27].

Immunocytochemical analysis, which various research groups have been developing for over 10 years, has also been validated in terms of its clinical relevance [1, 2, 4, 12, 14,21]. At present, this analysis is regarded as the standard method for detecting occult, early dissemination of solid tumor cells [12,14]. Large-scale microscopic screening of large amounts of cytological preparations can be simplified by an automatic analysis of the marked cells with the help of an image analysis system which has been developed by several firms and is presently being tested clinically [14].

An increasing number of publications report on the application of polymerase chain reaction (PCR) for detecting isolated disseminated tumor cells of epithelial tumors [28,29]. However, the data thus far available do not suggest that the sensitivity of PCR is any higher than that of the immunocytological method. Moreover, using PCR analysis means that the possibility of characterizing morphology and phenotype is lost. In addition, the prognostic significance of disseminated tumor cells identified by PCR has not yet been sufficiently backed up by relevant data [12,24]. Using this complicated and expensive tool as a screening method is almost out of the question today because of financial pressures.

An alternative to the analysis of large volumes of samples is tumor cell concentration using "magnetic cell sorting" (MACS) methods [12]. Several studies have shown that IDT BM represent a prognostic deterioration of afflicted patients. However, the results are still contradictory as regards lymph glands and there are too few data as regards peritoneal lavage and venous blood [1, 2, 6, 7, 12,25]. The goal of our prospective, 6-year on-going study is to compare the rating of IDT BM with established prognosis factors so as to develop recommendations for treatment.

Patients and methods

Patients and sampling method

We present details of a prospective study involving 145 patients with colon carcinoma in any stage who were operated on between 1994 and 1997 and who were subsequently observed until 1999 (mean observation time was 39 months, range 12–62 months). A total of 22.1% (n=32) of the patients subsequently received a bone marrow biopsy. We used the UICC's 1993 TNM classification of malignant tumors to classify the different stages [26].

The average age of the patients was 64.5 years. , Curative surgery was performed in 116 (80%) and palliative surgery in 29 (20%) patients with malignant tumors. In cases of distant metastases surgery was carried out in 18 (12.4%) patients and a local tumor R2 resection was carried out in 11 (7.6%) patients. Punch biopsy of the iliac crest was taken on both sides using the customary method under general anesthesia immediately after the operation as well as under local anaesthetic for the subsequent biopsy, when 5 ml of aspirate was drawn.

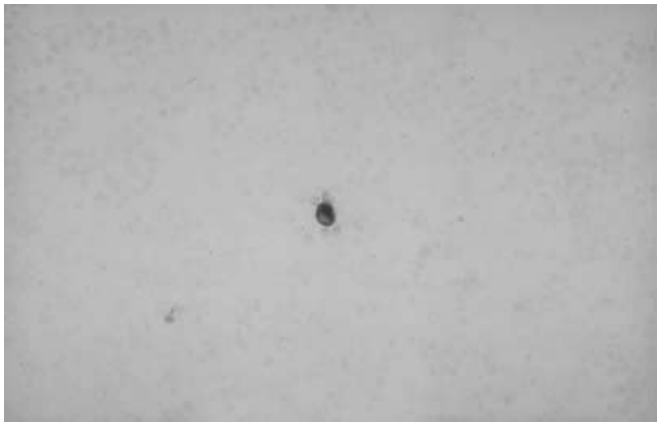

Fig. 1 Cytokeratin positive cells with the A45-B/B3 antibody

Immunocytochemistry

We used a diffused immunocytochemical double marking [1, 11, 15, 17,22]. In the course of four cytocentrifugation steps, we separated the cells from the bone marrow aspirate using Ficoll-Paque density centrifugation and made a cytospin preparation. We adjusted the cell concentration in order to have 100,000 cells per microscope slide. Five slides were prepared for each punch biopsy of the iliac crest; thus, 1 million cells per patient could be analyzed. At the beginning of the coloration process, we incubated the cytospin with a 10% AB serum in order to block any unspecific bonding sites. Immunocytological identification was achieved using an epithelial cell detection kit (EPIMET, Mikromet GmbH/Baxter Deutschland GmbH, Germany), which contains a monoclonal pan-cytokeratin antibody against A45-B/B3. Following cytocentrifugation, the cells are incubated with fixation solutions A and B; the prepared antibody solution is then added and the microscope slides are colored. Two independent researchers examined the cell preparations using transmitted light microscopy. At 200× and 400× magnification, strong red colored cytokeratin-positive cells could easily be identified and counted (Fig. 1).

For quality control, we conducted a negative control without antibodies, a positive control of the coloration, and a positive control using a series of tumor cell cultures. Fifty-one patients without detectable carcinoma were examined for IDT BM using the same method. None of these patients showed IDT BM with the A45-B/B3 antibody.

For in vitro cell separation of viable tumor cells, we drew 10 ml of venous blood and separated the cells using cytocentrifugation and Ficoll-Paque density centrifugation. We employed human epithelial antigen (HEA-125) MicroBeads (Milteny Biotec) to mark the cells. These are colloidal supramagnetic MACS MicroBeads conjugated with monoclonal mouse anti-human epithelial HEA-125 antibodies: isotype Mouse IgG1. MACS separation columns (capacity 2–10 µl) were used; three runs were carried out in each case. Specificity was increased using the FcR blocking reagent from Milteny Biotec. The reagent blocks the bonding of cells expressing human FcR receptor, e.g., monocytes and macrophages.

Statistical analysis

The following statistical procedures were used for the evaluations: the Wilcoxon test, chi-square test, Fischer's exact test, the Kaplan-Meier-Schätzer test, log rank test and Cox regression [3].

The present study is descriptive in character, i.e. the results should be regarded as a description because of the multitude of

tests carried out on one and the same data set. Thus, the problem of multiple testing arises, which can be circumvented by adjusting the levels of significance. Each test delivers a significant finding only when the *P* value is less than 0.05 per number of tests. Therefore, with 10 tests, each individual test is only significant if $P<0.005$. The total probability of error then remains at 5%. Only major significant effects can be proven in this manner; significant tests should be seen as expected tendencies, not as statistical proof. Interesting phenomena can thus be filtered out.

Results

Description of the data

A total of 24.8% (36/145) of the patients were cytokeratin positive with the A45-B/B3 antibody. There were no noticeable differences between sides of the iliac crest as regards cytokeratin positivity. In patients who were cytokeratin positive, 1–20 – in one case even 500 – cytokeratin-positive cells were detected out of 10^6 mononuclear cells.

Simple statistics

Median comparisons and correlation analysis

Administration of the A45-B/B3 antibody did not prove any evident correlation between tumor size (pT), lymph node involvement (pN), distant metastasis (M), and cytokeratin positivity.

Univariate analysis of survival time

For univariate analysis, parameters for the multivariate Cox analysis are pre-selected. With the A45-B/B3 antibody, there was a correlation between cytokeratin positivity and survival time and also with the time until tumor progression. The correlation between the conventional prognostic factors and the (relapse-free) survival time is represented in Table 1. Selected survival curves for time to progression of tumor and survival time for cytokeratin-positive vs cytokeratin-negative patients can be seen in Fig. 2.

Table 1 Univariate analysis of survival time

Influence of factor	Survival	Time to progression
A45-B/B3 positivity	0.0058	<0.001
pT 1–2/2–3	<0.001	<0.001
pN 0/1–2	<0.001	<0.001
M 0/1	<0.001	<0.001
R 0/2	<0.001	<0.001
Age (division on median)	0.0367	0.0116

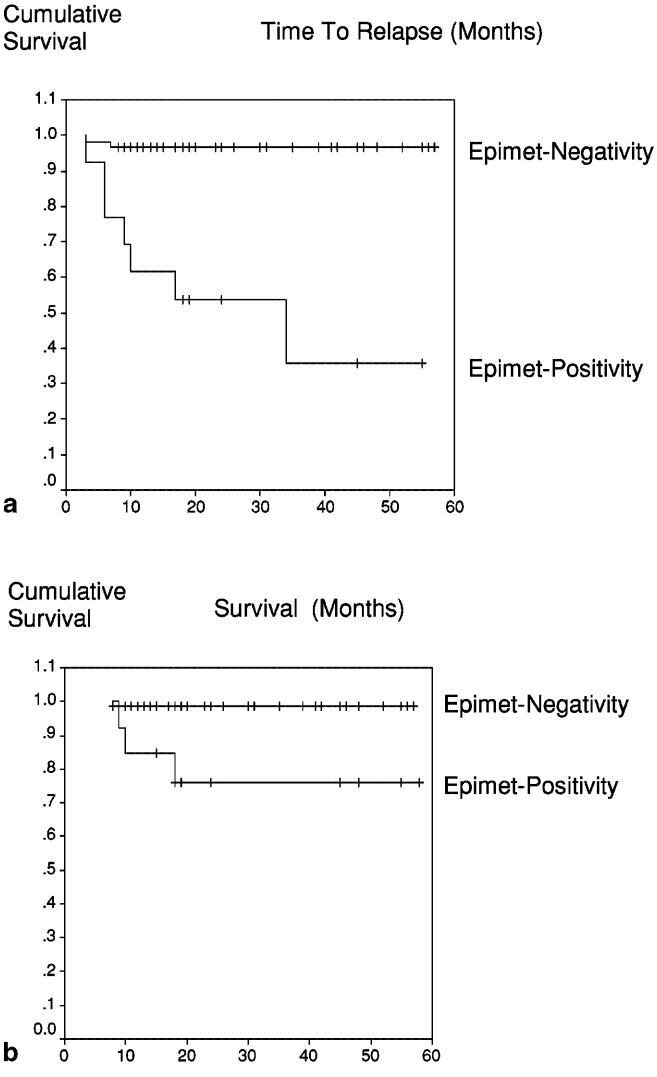

Fig. 2 Survival curves for (**a**) cytokeratin-positive vs (**b**) cytokeratin-negative patients

Multivariate analysis of survival time (Cox regression)

Distant metastases (M), lymph node (pN) status, and tumor size (pT) were taken for multivariate analysis. The analysis was carried out for the whole group first. In the second step, risk assessments were made according to the IDT BM for the R0, pN0, and M0 patient groups.

In this investigation, the detection of IDT BM cells using the A45-B/B3 antibody, the detection of lymph node metastases, and the detection of distant metastases represented independent prognostic factors for the survival time and for the time until tumor progression (Table 2). The other factors examined did not have this prognostic relevance. The risk of an earlier death increased with the detection of cytokeratin-positive cells

Table 2 Multivariate analysis of survival time

Influence of factor	Survival		Time to progression	
	P-value	95% confidence interval	P-value	95% confidence interval
A45-B/B3 positivity	0.0090	1.2–4.3	<0.001	2.8–8.1
Lymph node –metastases (pN0/pN positive)	0.0088	1.3–7.6	<0.001	1.6–5.9
Distant metastases (M0/1)	<0.001	2.8–11.9	<0.001	2.4–7.8

in bone marrow by a factor of about 1.2–4.3 [confidence interval (CI)] compared with cytokeratin-negative patients; with the detection of lymph node metastases at the time of operation, it increased about 1.3 to 7.6 compared with node-negative patients; and with the detection of distant metastases at the time of operation, it increased about 2.8 to 11.9 compared with metastasis-free patients.

The risk of experiencing a tumor relapse earlier increased with the detection of IDT BM with the A45-B/B3 antibody by about 2.8–8.1 compared with cytokeratin negative patients; with the detection of lymph node metastases at the time of operation, it increased about 1.6 to 5.9 compared with node-negative patients; and with a distant metastases at the time of operation, it increased about 2.4 to 7.8 compared with metastasis-free patients.

Analysis of the R0, pN0, and M0 patient groups gave an increase in risk of 18.0 (3.7–86.9 for CI 95%) for time to progression and of 12.6 (1.3–121.7 for CI 95%) for survival (stratification: age, tumor size).

Additional investigations

Follow-up biopsy of patients examined at the time of operation for IDT BM. A control puncture biopsy was carried out during the follow-up examination period using the same method on 32 (19.2%) of the 167 patients examined at the time of operation. With the A45-B/B3 antibody, disseminated tumor cells were not detectable in 65.6% (n=21) of the patients but were detectable in 34.4% (n=11) . Convincing statistical calculations cannot be made because of the limited number of cases.

A quantitative analysis of the follow-up puncture biopsies showed six cases of tumor progression in A45-B/B3-positive patients; however, five patients remained tumor-free.

Comparison of the initial and multiple follow-up biopsies with respect to quantitative cytokeratin positivity shows a correlation with the clinical development in the majority of cases.

In vitro cell separation of IDT BM and viable tumor cell cultures. Disseminated tumor cells from the venous blood of seven patients with metastatic colorectal carcinomas were marked by the monoclonal HEA-125 antibody and were isolated and accumulated usingMACS. After 7 days, growth of tumor cells was ascertainable, which later stagnated (Fig. 3).

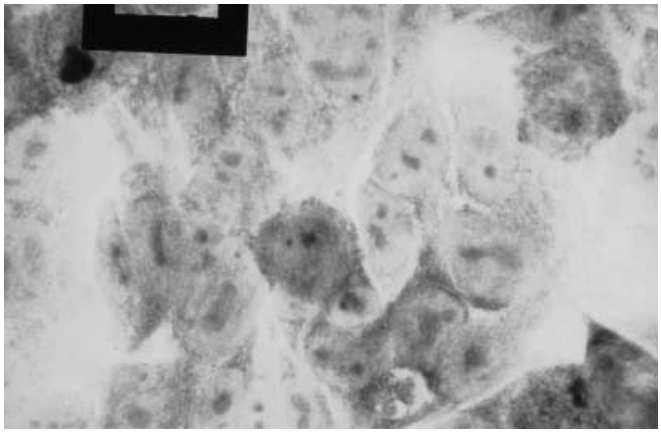

Fig. 3 Carcinoma cell cultures from the venous blood of a patient with metastatic colorectal carcinoma after 7 days

Discussion

In addition to the newer methods for detecting “minimal residual disease”, such as reverse transcription (RT) PCR [28,29], the immunocytochemical detection of IDT BM using monoclonal antibodies is currently considered the standard method [12, 13,14]. The antibodies we used against A45-B/B3 have been tested and acknowledged to be useful for detection purposes [1, 4, 14,22]. We prefer the A45-B/B3 antibody because of the higher specificity described in the literature. Conjugates in which the alkali phosphatase is directly bonded to the antigen-bonding fab fragment of the primary antibody, such as the A45-B/B3 antibody (epithelial cell detection kit), raise the specificity and simplify the implementation of the methods [1,13]. Moreover, a direct comparison between the studies is not possible because other monoclonal antibodies (17-1A) and antimuzin antibodies (RA96) [6,7] have been used in addition to the CK II antibody. A standardization of the method is needed to simplify this problem in the future.

Twenty-five per cent of patients were found to be cytokeratin positive with the A45-B/B3 antibody. Using immunocytochemical methods, Schlimok et al. [21] established the presence of IDT BM in about 30% of patients with metastasis-free colorectal carcinomas, whereas Pantel and Riethmüller [15] established the presence of IDT BM in 27% of M0 patients and in 39% of M1 patients, respectively, depending on the stage. In a meta-analysis of 20 studies of all types of carcinoma, these cells were found, on average, in 35% of patients [5].

Our analysis showed no relation between cytokeratin positivity and the pT, pN, and M stage. Most of the authors who have dealt with this detection method saw a clear correlation with tumor stage, lymph node involvement, and distant metastases, and also partially to grading and depth of the invasion [1, 2, 4, 6, 7, 14,23].

The conventional prognosis factors show a significant relationship between survival times and pT, pN, M and the time until progression of the tumor. These are known facts and are taken into consideration in the TNM classification [26,27].

There was a significant relationship between cytokeratin positivity and survival time. In accordance with this fact, Funke and Schraut [5] found a positive correlation between tumor cell detection and a reduction in the illness-free interval in 14 of 20 studies in their meta-analysis of 2494 patients from 1980 to 1997 by univariate analysis. Juhl et al. [6,7] also demonstrated a correlation with the survival rate of IDT BM-positive patients using an antibody panel as did Schlimok et al. [21], who observed a significantly shorter survival time for IDT BM-positive patients. Following a median observation time of 29.4 months, Broll et al. [1] reported that a relapse or development of distant metastasis occurred more often (21%) in patients with IDT BM than in patients without disseminated tumor cells (10%), even though differences in the survival time between the two groups could not be proven statistically.

The detection of IDT BM cells using the A45-B/B3 antibody, lymph node involvement, and distant metastases represent independent prognostic factors in the multivariate survival time analysis (Cox regression) for the survival times and the time until progression of the tumor[3]. The risk of an earlier death increased with the detection of IDT BM by 1.2 to 4.3; with the detection of lymph node metastases it increased in similar dimensions – by 1.3 to 7.6 – and with the detection of distant metastases by as much as a factor of 2.8 to 11.9. The risk of tumor progressing earlier was highest with the detection of IDT BM with the A45-B/B3 antibody. With the detection of IDT BM, the risk increased by 1.6 to 5.9, with the detection of lymph node metastases by 1.3 to 7.6, and with distant metastases by 2.4 to 7.8. In comparison with the literature, only five of the 11 studies of the meta-analysis conducted by Funke and Schraut [5] showed positive bone marrow evidence as an independent prognostic factor for a shorter disease-free interval. Moreover, five out of 12 studies provided univariate evidence, but only two studies provided multivariate evidence that bone marrow involvement is an independent factor for the overall survival of the patient. Information concerning the importance of the prognostic IDT BM factor apart from other factors is otherwise not normally given [1, 2, 6, 7, 13,22]; however, it is indicative of the importance of IDT BM detection.

At follow-up biopsy in cytokeratin-positive patients with the A45-B/B3 antibody six revealed progression of the tumor, while five remained tumor-free. Thirteen of the cytokeratin-negative patients were tumor-free and in eight the tumor had progressed. Nine of the cytokeratin-positive patients were tumor-free and 16 experienced tumor progression. Six of the cytokeratin negative patients were tumor-free and in one tumor had progressed. Cytokeratin positivity was by no means an indicator of ensuing tumor progression, which was mainly due to the randomly selected one-time follow-up puncture biopsy during the first to the third postoperative years. A quantitative analysis of the follow-up biopsy and the multiple follow-up biopsies showed predominantly a decrease in IDT BM with no tumor and an increase in IDT BM with tumor progression. Multiple follow-up biopsies at various time intervals have been suggested by some authors as a possible means of monitoring therapy [18, 19,23]. Investigations conducted by others, in addition to our own earlier studies [8, 9,10], show a correlation with clinical developments. However, the number of cases in the group receiving follow-up biopsies was small. Because of the small group size, no statement can be made about the influence of adjuvant treatment. At any rate, the optimization and standardization of detection methods is a decisive requirement for effective monitoring.

In the metastasis stage, we isolated tumor cells with the HEA-125 antibody with the help of MACS and cultivated the viable cells. Cell growth stagnated after approximately 1 week. Data on cultures of tumor cells isolated from venous blood or bone marrow are rather rare; the aforementioned fact has also been observed by other authors [14]. A viable cell culture indicates the proliferative potential of the IDT BM. Atrophy of the isolated tumor cells in culture after some time could perhaps be traced back to the still not fully developed metastatic potential of these cells and the lack of endogenic factors for tumor development [20]. An additional therapeutic option using a cytostatic resistogram for venous blood is desirable but is not feasible for the reasons mentioned above.

Conclusion

Due to the importance of the independent prognostic IDT BM factor, a standardized method should be established at institutions of higher learning for ascertaining the pathological stages. With accumulating experience, a decision should be made as to whether patients who would not normally receive adjuvant additional therapy because of the stage of their tumor should nevertheless be advised to undergo an adjuvant therapy simply because IDT BM have been detected.

References

1. Broll R, Lembcke K, Stock C, Zingler M, Duchrow M, Schimmelpennig H, Strik M, Müller G, Kujat P, Bruch H-P (1996) Tumorzelldissemination in das Knochenmark und in die Peritonealhöhle – Eine immunzytochemische Untersuchung an Patienten mit einem Magen- oder kolorektalen Karzinom. Langenbecks Arch Chir 381:51–58
2. Broll R, Schauer V, Schimmelpennig H, Strik M, Woltmann A Best R, Bruch HP, Duchrow M (1997) Prognostic relevance of occult tumor cells in lymph nodes of colorectal carcinomas – an immuno-histochemical study. Dis Colon Rectum 40:1465–1471
3. Cox DR (1972) Regression models and life tables. J R Statist Soc B34:187–220
4. Funke I, Schlimok G, Bock B, Schweiberer B, Riethmüller G (1989) Micrometastasis: characterization and tumorgenicity of disseminated tumor cells in human bone marrow. In: Osborn M, Weber K (eds) Cytoskeletal proteins in tumor diagnosis. Cold Spring Harbor Laboratory, Cold Spring Harbor, pp 241–244
5. Funke I, Schraut W (1998) Meta-analyses of studies on bone marrow micrometastases: an independent prognostic impact remains to be substantiated. J Clin Oncol 16: 557–566
6. Juhl H, Kalthoff H, Krüger U, Henne-Bruns D, Kremer B (1995) Immunozytologischer Nachweis mikrometastatischer Zellen bei Patienten mit gastrointestinalen Tumoren. Zentralbl Chir 120:116–122
7. Juhl H, Stritzel M, Wroblewski A, Henne-Bruns D, Kremer B, Schmiegel WH, Neumaier M, Wagener C, Schreiber HW, Kalthoff H (1994) Immunocytological detection of micrometastatic cells: comparative evaluation of findings in the peritoneal cavity and the bone marrow of gastric, colorectal and pancreatic cancer patients. Int J Cancer 57:3030–3035
8. Leinung S, Schönfelder A, Schönfelder M (1998) Cytokeratinpositive Zellen im Knochenmark bei Patienten mit gastralen und colorectalen Carcinomen: Korrelation mit konventionellen Prognosekriterien und dem klinischen Verlauf. Zentralbl Chir 123:520–524
9. Leinung S, Petzold A, Weiss C-L, Braukhoff M, Schönfelder M, Steuber J, Taha HMM, Esele E (1998) The biological significance of free tumor cells in a comparative study of gastric, colorectal and breast cancer using a CK II and a 17-1-A antibody. J Cancer Res Clin Oncol (Suppl) 124:142
10. Leinung S, Schönfelder A, Schönfelder M (1996) Experience in the immunocytochemical detection of micrometastatic tumor cells in human bone marrow of gastric and colorectal cancer patients. J Hematother 5: 574
11. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R (1982) The catalogue of human cytokeratins pattern of expression in normal epithelia, tumors and cultured cells. Cell 31:11–24
12. Oruzio D, Hempel D, Schlimok G (1997) "Knochenmarksmikrometastasen" epithelialer Tumoren-Nachweis und klinische Bedeutung. Onkologe 3:66–72
13. Pantel K, von Knebel Doeberitz M, Izbicki JR, Riethmüller G (1997) Disseminierte Tumorzellen: Diagnostik, prognostische Relevanz, Phänotypisierung und therapeutische Strategien. Chirurg 68:1241–1250
14. Pantel K, Riethmüller G (1994) Detection, characterization and antibody therapy of minimal residual epithelial cancer. Oncology 11:4–9
15. Pantel K, Riethmüller G (1996) Micrometastasis detection and treatment with antibodies. Curr Top Microbiol Immunol 213 (III):1–18
16. Pantel K, Schlimok G, Angstwurm M, Weckermann D, Schmaus W, Gath H, Passlick B, Izbicki JR, Riethmüller G (1995) Methodological analysis of immunocytochemical screening for disseminated epithelial tumor cells in bone marrow. J Hematother 3:165–173
17. Riethmüller G, Johnson J (1992) Monoclonal antibodies in the detection and therapy of micrometastatic epithelial cancer. Curr Opin Immunol 4:647–655
18. Riethmüller G, Holz E, Schlimok G, Schmiegel W, Raab R, Höffken K, Gruber R, Funke I, Pichlmaier H, Hirche H, Buggisch P, Witte J, Pichlmayr R (1998) Monoclonal antibody therapy for resected Dukes's C colorectal cancer: seven-year outcome of a multicenter randomized trial. J Clin Oncol 16:1788–1794
19. Riethmüller G, Schneider-Gädicke, Schlimok G, Schmiegel W, Raab R, Höffken K, Gruber R, Pichelmaier H, Hirche H, Pichelmayr R, Buggisch P, Witte J, the German Cancer Aid 17–1A Study Group (1994) Randomised trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. The Lancet 343:1177–1183
20. Schirren J (1995) Kommentar auf Anforderung der Schriftleitung. Chirurg 8:785–786
21. Schlimok G, Funke I, Bock B, Schweiberer B, Witte J, Riethmüller G (1990) Epithelial tumor cells in bone marrow of patients with colorectal cancer: immunocytochemical detection, phenotypic characterization and prognostic significance. J Clin Oncol 8:831–837
22. Schlimok G, Funke I, Holzmann B, Göttlinger G, Schmidt G, Häuser H, Swierkot S, Warnecke HH, Schneider B, Koprowski H, Riethmüller G (1987) Micrometastatic cancer cells in bone marrow: in vitro detection with anti-cytokeratin and in vivo labeling with anti-17-1A monoclonal antibodies. Proc Natl Acad Sci USA 84:8672–8676
23. Schlimok G, Riethmüller G (1995) Monoclonal antibodies in the therapy of minimally residual solid tumors. Onkologie 18:403
24. Soeth E, Röder C, Juhl H, Kruger U, Kremer B, Kalthoff H (1996) The detection of disseminated tumor cells in bone marrow from colorectal-cancer patients by a cytokeratin-20-specific nested reverse transcriptase-polymerase chain reaction is related to the stage of disease. Int J Cancer 69:278–282

25. Soeth E, Vogler I, Röder C, Juhl H, Marxsen J, Kruger U, Henne-Bruns D, Kremer B, Kalthoff H (1997) Comparative analysis of bone marrow and venous blood isolated from gastrointestinal cancer patients for the disseminated tumor cells using reverse transcription PCR. Cancer Res 57:3106–3110
26. UICC (1993) TNM Klassifikation maligner Tumoren, 3rd edn. Springer, Berlin Heidelberg New York
27. UICC (1998) TNM Klassifikation maligner Tumoren, 4th edn. In: Hermanek P, Wagner G, Wittekind C (eds) Springer, Berlin Heidelberg New York
28. von Knebel Doeberitz M, Gebert J, Herfarth Ch (1996) Der Einfluß molekularer Diagnostikverfahren auf die chirurgische Therapie chirurgischer Erkrankungen. Chirurg 67:967
29. Zippelius A, Kufer P, Honold G (1997) Limitations of reverse transcriptase-polymerase chain reaction analyses for detection of micrometastatic epithelial cancer cells in bone marrow. J Clin Oncol 15:2701