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RESEARCH ARTICLES

The clinical significance of lymph node micrometastasis in stage I and stage II colorectal cancer

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Abstract
Aim Recent advances in immunohistochemical techniques have made it possible to identify micrometastasis using antibodies to cytokeratins (CK). The aim of the study was to determine the prevalence and prognostic significance of immunohistochemically detected micrometastasis (IHM) in patients with localised colorectal cancer (CRC) (Dukes' A and B). A further aim was to study the prognostic role of histopathological factors such as vascular invasion.
Methods The original histology of 168 consecutive patients with Dukes' A or B tumours who had undergone curative resection was reviewed. Immunohistochemical staining was performed using CK antibodies, AE1/AE3 and MNF116 on all (n=898) lymph nodes. Survival analysis was performed on 105 cases that had been followed up until death or for at least 5 years.
Results IHM were detected in 17.3% of lymph nodes analysed. Adverse outcome (death/local recurrence) was recorded in 8/49 (16%) patients with IHD-positive nodes and in 10/56 (18%) patients negative for IHM. IHM was not associated with adverse outcome on either univariate (p=0.540) or multivariate analyses (p=0.673). There was no correlation of IHM with age, gender, site, size and grade of tumour, depth of tumour invasion or perineural and vascular invasion. Vascular invasion was the only independent prognostic factor identified.
Discussion We have shown that isolated CK-positive epithelioid cells are commonly found in morphologically benign pericolic lymph nodes of patients with localised (Dukes' A or B) CRC. These cells may represent occult micrometastasis but are not clinically significant. Vascular invasion identifies patients with localised CRC likely to develop recurrences or die of disease.

Keywords Colorectal cancer · Micrometastasis · Cytokeratin

Introduction

Colorectal cancer (CRC) is one of the most common malignancies in the western world. It is the second most common cause of cancer-related death and the third most common cancer in the UK. It affects around 30,000 people per annum and the average five-year survival rate is 40% [1]. One major factor that determines the prognosis is Dukes' staging, which is based on the depth of tumour invasion and the lymph node status. The lymph node status determined by the light microscopic examination of conventional haematoxylin and eosin (H&E)-stained sections of lymph nodes, is generally considered the most important prognostic factor in clinical practice and adjuvant chemotherapy is routinely offered to Dukes' C CRC patients [2]. However, there is no uniform consensus as to the role of chemotherapy in Dukes' B colonic cancer.

Dukes' B cancers, comprising 40–50% of colonic cancers, represent a wide spectrum of disease with early penetration of the bowel wall to aggressive tumours with deep penetration of the colonic wall and extensive extramural vascular invasion. The overall 5-year survival rate of Dukes' B cancer patients is 70–80% [3]. Thus, despite having localised disease at operation, a significant proportion

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(20–30%) of patients die from local tumour relapse or distant metastasis. Risk factors that would identify this subgroup of patients with poor outcome would be valuable as more aggressive therapy may be indicated. One such factor could be occult lymph node micrometastasis not detectable by routine histological techniques. Some studies have shown that increasing the yield of lymph nodes [4] with or without clearance technique and combining immunohistochemistry and fat clearance [5] or multiple sectioning of lymph nodes may improve the detection of lymph node micrometastasis [4–6]. Recent advances in immunohistochemical techniques have made it possible to identify micrometastasis including single metastatic cells in lymph nodes using antibodies to cytokeratins (CK). CK-positive dendritic cells have been demonstrated in benign lymph nodes but these cells can generally be easily distinguished from metastatic carcinoma cells by routine morphology [7, 8].

In this study we sought to study the prevalence and prognostic significance of immunohistochemically detected micrometastasis (IHM) in patients with localised CRC (Dukes' A and B), using univariate and multivariate analysis. A further aim was to study the prognostic role of histopathological factors such as vascular invasion.

Patients and methods

Case selection

One hundred and sixty-nine consecutive patients who were pathologically staged as Dukes' A or B (no evidence of lymph node or distant metastasis) following curative colorectal resections between 1992 and 1996 for Dukes' A and B were identified from our pathology database. Patients who had any macroscopic or radiological evidence of distant metastases at the time of surgery were excluded. In each case, all the original H&E stained sections were reviewed by a single pathologist (VIS) to confirm the pathologic stage of the disease. Other factors including age, gender, site, size, grade of the tumour, vascular and perineural invasion were also recorded. One case in which overt lymph node metastasis was identified on review was excluded from the study.

Follow-up

Patients were seen as surgical outpatients at six weeks to check for postoperative complications. Patients were then reviewed at 6 months and at one year and then on a yearly basis for five years. A CT of the thorax and abdomen was arranged at 1 and 2 years postoperatively. Colonoscopy was performed on a 3-yearly basis or within the first year if no colonoscopy was performed preoperatively. Colonoscopy was typically continued until the age of 75.

Immunohistochemistry

All lymph nodes were immunostained with a low-molecular-weight CK cocktail (DAKO, AE1/AE3, 1:100, 30 min, protease 1 pretreatment, 12 min) and pan-cytokeratin (DAKO, MNF116, 1:100, 30 min, protease pretreatment, 12 min) on a Nexus autostainer using a Ventana detection system. The morphology of the immunoreactive cells was evaluated and their number scored as 0, absent, rare, <1%; 1+, 1–5%; 2+, 6–10%; 3+, >10%.

Outcome analysis

Adverse outcome was defined as CRC-related death and local or distant recurrence. All cases included for adverse outcome analysis had at least 5 years of follow-up (mean 45 months, median 48 months). Sixty-three patients were excluded from adverse outcome analysis: 12 patients who died within 60 days postoperatively due to postoperative complications, 41 patients who had pre/postoperative chemo-radiotherapy, 8 patients in whom lymph node histology was not available for review and 2 with less than 5 years' follow-up. The chemo-radiotherapy patients were excluded as it may have affected the natural course of the disease. One hundred and five patients were analysed after all exclusions. Survival and disease recurrence data was retrieved from hospital case notes, cancer registry and primary care physicians.

Differences in adverse outcome between subjects positive and negative for IHM were analysed by Cox regression analysis using univariate and multivariate analyses. All the histopathological factors were entered into Cox regression analyses to assess an independent prognostic value. Statistical analysis was performed using SPSS 10 (Statistical Package for Social Sciences, Chicago, IL, USA).

Results

Eight hundred and ninety-eight lymph nodes were identified in the 168 resection specimens of Dukes' A and B CRCs. IHM, generally as single epithelioid cells or as small clusters of 2–5 cells in the subcapsular sinus (Fig. 1), were present in 155 (17.3%) lymph nodes (range 1–10, median 2) from 65 (43.2%) patients (7/28 Dukes' A, 58/140 Dukes' B). The number of CK immunoreactive cells varied from 1+ to 3+.

Adverse events

Among 105 patients analysed for survival statistics, there were 15 cancer-related deaths (14 Dukes' B and 1 Dukes' A) and 3 (all Dukes' B) recurrences. Fourteen of the deaths and 2 recurrences were within 5 years and one death and one recurrence each occurred after 6 years.

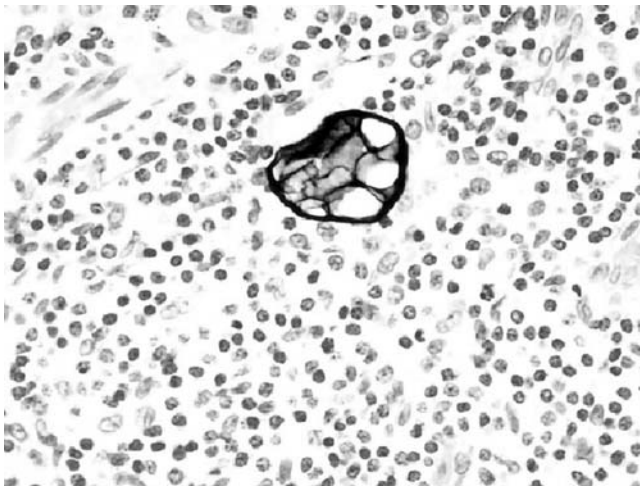

Fig. 1 A cluster of CK-positive cells (IHM) in a morphologically benign lymph node (AE1/AE3)

Data analyses

Among 105 patients analysed for survival statistics, 82 were Dukes' B and 23 were Dukes' A. IHM was present in 49 patients (47%). Adverse outcome was recorded in eight (16%) of these 49 patients in comparison with 10 (18%) of 56 patients without IHM ($p>0.05$). All 8 patients were Dukes' B. Among the 10 patients with adverse outcome but no IHM, 9 were Dukes' B patients and 1 was Dukes' A. IHM was not associated with adverse outcome on either univariate ($p=0.540$) or multivariate analyses ($p=0.673$). There was no correlation of IHM with age, gender, site, size and grade of tumour, depth of tumour invasion or perineural and vascular invasion.

Other prognostic factors

Vascular invasion was seen in 15 cases, of which 13 had extramural and 2 had submucosal vein invasion. Seven patients with vascular invasion died of CRC and one patient developed recurrence. Vascular invasion was an independent prognostic factor by univariate and multivariate analysis ($p=0.0001$, Tables 1 and 2). The combination of vascular invasion and/or IHM identified 14 of 18 patients (78%) who had adverse outcome (86% of 15 cases of cancer related death). Increasing depth of tumour invasion (T stage) showed a trend towards poorer prognosis, however this trend was not statistically significant (Table 3). Dukes' stage was a significant factor on univariate analysis ($p=0.03$) but not on multivariate analysis. Age, gender, site, size and grade of tumour and perineural invasion were not statistically significant (Table 2).

Table 1 Multivariate analysis of prognostic factors

Vascular invasion	$p=0.0001$
T-stage	$p=0.364$
Dukes stage	$p=0.193$
CK-positive	$p=0.670$
Perineural invasion	$p=0.915$

Table 2 Univariate analysis of histopathological and other factors and its impact on survival

Age (median age 78; range 48–100)	$p=0.972$
Sex (male 50; female 55)	$p=0.245$
Grade	$p=0.717$
Size	$p=0.306$
Site (right 29; left 76)	$p=0.408$
T stage	$p=0.095$
Dukes' stage (A 23; B82)	$p=0.03$
Perineural invasion (12 positive)	$p=0.132$
Vascular invasion (15 positive)	$p=0.0001$
CK positivity	$p=0.540$

Discussion

The pathological stage of CRC using conventional histology is widely used to determine the need for adjuvant chemotherapy [2]. The five-year survival rate of Dukes' C patients is 30–40% and adjuvant chemotherapy has been shown to reduce recurrence and improve survival in these patients [9]. However, the role of adjuvant chemotherapy in Dukes' B patients is controversial, but a proportion (20–30%) of these patients will have an adverse outcome following a curative resection [10]. Hence, whilst it may not be appropriate to subject all patients with Dukes' B cancer to adjuvant chemotherapy, it would be beneficial to identify those at risk of developing an adverse outcome. It has been suggested that immunohistochemical detection of occult lymph node micrometastasis may identify a subset of patients with localised CRC who have a poorer prognosis and may benefit from adjuvant chemotherapy.

The reported incidence of IHM in morphologically benign regional lymph nodes from colorectal resections of patients with localised CRC varies from 25 to 68% [5, 11–18]. In our study, IHM was noted in 43.2% of patients (25% Dukes' A, 48% Dukes' B and 10% in CRC patients with history of preoperative chemo-radiotherapy). IHM was generally seen as either single scattered isolated cells or as small clusters of 2–5 cells in the subcapsular sinuses.

There is conflicting evidence in the literature as to the clinical significance of IHM in morphologically benign lymph nodes retrieved from resections for localised CRC. Several studies have concluded that micrometastasis in pericolic lymph nodes detected by anti-CK antibodies in patients with localised CRC has no influence on prognosis [11, 13, 14, 16, 18]. However, other studies found IHM to correlate significantly with poorer prognosis and recommended routine CK staining of morphologically benign

Table 3 Relationship of depth of invasion and adverse outcome

T-stage	No adverse event	Adverse outcome	Total	Adverse outcome (%)
T0	1	0	1	0
T1	3	0	3	0
T2	18	1	19	5
T3	59	15	74	20
T4	6	2	8	25

pericolic lymph nodes in patients with Dukes' B CRC [12, 15, 17].

In our study, IHM was not associated with a poorer prognosis by univariate or multivariate analyses. Eighteen percent of patients without IHM had an adverse outcome compared to 16% of such patients with IHM. IHM did not correlate with age, gender, site, size and grade of the tumour, depth of tumour invasion or perineural and vascular invasion. Among the tumour characteristics, vascular invasion was the only independent prognostic factor identified. The combination of vascular invasion and/or IHM identified 78% cases of localised CRC with adverse outcome (86% of 15 cases of cancer-related death). This finding is similar to that reported in a study by Greenson et al. in which this combination identified 100% of cases who died of their disease [12]. However, in contrast to their study, we did not find IHM to be statistically significant. Other studies that had found IHM to be a significant prognostic factor in localised CRC had not included vascular invasion as a covariate in the multivariate analysis [15, 17].

An inverse correlation of IHM in lymph nodes with adverse outcome has been noted in patients with breast cancer [19, 20]. It has been hypothesised that micrometastatic nodal disease induces systemic immune surveillance that is protective at low metastatic burden but defeated at some yet to be defined threshold. The precise nature of the CK-positive isolated cells observed in lymph nodes of patients with localised CRC is uncertain [21, 22]. They may be cancer cells that are either not viable or incapable of proliferating at metastatic sites. On the other hand, they could be epithelioid histiocytes that have adsorbed CK antigen shed by the primary tumour into the lymphatics. In either case, our study suggests that these cells are clinically not significant and that upstaging Dukes' A or B patients based on IHM may not be justified.

In the published literature, there is considerable variation in the assessment and prognostic relevance of colorectal micrometastases. This was emphasised in the review by Doekhie et al. in 2006, which concluded that a more standardised approach may determine whether detection of micrometastases can reliably identify high-risk node-positive patients [20]. Similarly, a review by Nicastri et al. in 2007 identified 19 publications that evaluated the clinical significance of micrometastatic CRC by various methods, including immunohistochemistry (n=13) and reverse transcription-polymerase chain reaction (RT-PCR) (n=6) [25]. Significant limitations in methodology were again identified, including inconsistent histological definitions of micrometastatic disease, poor sampling because of an inadequate number of lymph nodes or number of sections per lymph nodes analysed. The methods of analysis were not standardised in terms of the antibodies used and the markers for RT-PCR. Studies were also under-powered because of a small sample size.

Conclusion

We have shown that isolated CK-positive epithelioid cells are commonly found in morphologically benign pericolic lymph nodes of patients with localised (Dukes' A or B) CRC. These cells may represent occult micrometastasis but we have not shown a clinical significance. Vascular invasion identifies patients with localised CRC likely to develop recurrences or die of disease. More work is required to standardise testing.

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