Bone marrow micrometastasis is associated with both disease recurrence and poor survival in surgical patients with non-small-cell lung cancer: a meta-analysis

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Abstract

OBJECTIVES: We performed a meta-analysis in order to determine whether the molecular tumour cell detection of either micrometastasis or isolated tumour cells in the bone marrow micrometastasis is indicative of a high risk of both disease recurrence and poor survival in the setting of node-negative non-small-cell lung cancer (NSCLC).

METHODS: Before beginning this study, a rigorous protocol was established in accordance with the recommendations of the Cochrane Collaboration. A systematic literature search of Medline, Embase, the Cochrane Library and the Web of Science was conducted in order to identify studies regarding the prognostic value of molecular tumour cell detection in the bone marrow of node-negative NSCLC. Any study describing the use of both immunohistochemistry and flow cytometry to detect bone marrow metastasis was selected. We extracted the associated 95% confidence intervals (CIs) and hazard ratios (HRs) from the included studies and performed meta-analyses on overall survival and either disease-free survival (DFS) or disease-free recurrence. Meanwhile, we compared the occurrence of bone marrow micrometastasis among different pathological types and different stages of disease.

RESULTS: Eleven studies with a cumulative sample size of 2159 patients were included in our analysis. Our meta-analyses revealed that the occurrence of bone marrow micrometastasis was not related to patient pathological types and stages in cancers ranging from adenocarcinoma and squamous cell carcinoma [relative risk (RR): 0.92; 95% CI: 0.78–1.08; P = 0.29], stages I and II (RR: 0.88; 95% CI: 0.67–1.17; P = 0.39), stages II and III (RR: 0.98; 95% CI: 0.73–1.31; P = 0.89) and stages I and III (RR: 0.84; 95% CI: 0.68–1.05; P = 0.13). However, molecular tumour cell detection within the bone marrow was associated with both poor OS (HR: 1.84; 95% CI: 1.41–2.40; P < 0.00001) and poor DFS (HR: 1.75; 95% CI: 1.18–2.60; P = 0.005). Our subgroup analyses indicated that the presence of bone marrow micrometastasis was not a significant prognostic factor with respect to DFS at stage I (HR: 2.35; 95% CI: 0.67–8.25; P = 0.18).

CONCLUSIONS: The molecular detection of isolated tumour cell in the bone marrow is associated with both poor survival and an increased rate of recurrence in patients with node-negative NSCLC; this approach may result in the development of a new metastatic cascade concept and the development of novel approaches to cancer therapy.

Keywords: Bone marrow micrometastasis • Non-small-cell lung cancer • Minimal residual disease • Subclinical metastasis • Occult metastasis • Isolated tumour cells

INTRODUCTION

Lung cancer remains the leading cause of cancer-related death, and was responsible for 19.4% of the total number of cancer deaths worldwide in 2012 [1]. Surgical resection is the treatment of choice for early-stage non-small-cell lung cancer (NSCLC). Non-small-cell lung cancer accounts for ~80% of all cases of lung cancer, including adenocarcinoma and squamous cell carcinoma.

In theory, patients with localized NSCLC who present without either lymph node or distant metastases may be cured via surgical resection alone. However, a significant proportion of patients undergoing potentially curative resections subsequently relapse in spite of potentially curative surgery, which is indicative of the presence of early disseminated disease that was not apparent at the time of primary treatment. The postoperative recurrence rates in early stage NSCLC range from 22 to 38% [2], and many patients ultimately die from disease recurrence. Because of the strong prognostic relevance of bone marrow micrometastasis in the setting of NSCLC, either micrometastasis or isolated tumour cells...
within the bone marrow that are not detected via conventional histopathological examinations with eosin and haematoxylin staining are believed to be a signal of systemic tumour distant metastasis in patients with a history of cancer. Therefore, the detection of occult metastasis may help to identify those patients with bone marrow micrometastasis who are at high risk for tumour recurrence and who may also benefit from adjuvant therapy. However, the prognostic value of occult metastasis in patients with bone marrow micrometastasis remains uncertain because of the inconsistent results reported by the available studies [3-11].

In order to clarify this issue, we performed a meta-analysis of several studies that evaluated the prognostic significance of occult metastasis in the bone marrow. This article is the first meta-analysis to report on the prognosis of NSCLC with bone marrow micrometastasis.

MATERIALS AND METHODS

A systematic literature search of Medline, EMBASE, the Cochrane Library and the Web of Science was conducted. The search terms were as follows: micrometastasis, minimal residual disease, subclinical metastasis, occult metastasis, isolated tumour cells, bone marrow micrometastasis, lung cancer and NSCLC. We also reviewed the reference lists of relevant articles and review articles. No language restrictions or time limits were applied to the initial search. All of the patients provided informed consent for surgery, and bone-marrow aspiration was performed on the operating table before surgery, from the posterior iliac crest or rib. None of the patients described in the included literature received either preoperative chemotherapy or radiotherapy. Preoperative staging included the performance of computed tomography (CT) of the brain, chest and liver, as well as a bone scan, a position emission computed tomography (PET-CT) scan or a CT-PET scan, without evidence of systemic metastasis.

Before the beginning of this study, a rigorous protocol was established in accordance with the recommendations of the Cochrane Collaboration. The abstracts of the citations identified by our search were then scrutinized by two observers in order to determine their eligibility for inclusion in the meta-analysis. We included studies that evaluated the relationship between bone marrow micrometastasis and the overall survival (OS), the disease-free survival (DFS) or the recurrence-free survival of the patients with node-negative bone marrow micrometastasis. The detection techniques utilized may have included any form of immunohistochemistry and flow cytometry. The exclusion criteria included the following: letters to the editor, reviews, case reports and clinical trials that did not evaluate the relationship between bone marrow micrometastasis and OS, DFS or recurrence-free survival.

In this study, the term bone marrow micrometastasis (BMM) refers to either a single group of cells or several small groups of cells that have left the primary tumour and may be detected in the bone marrow (BM). Data from eligible trials were entered into a computerized spreadsheet for analysis.

STATISTICAL ANALYSIS

The synchronized extraction results were pooled statistically as effect estimates in the meta-analyses. Hazard ratios (HRs) and their corresponding SEs were extracted for the individual time-to-event outcome parameters of each primary study. If an HR and its associated standard error (SE) or CIs were not reported, we approximated the HR by using the statistical data provided in the article [12, 13].

Extracted HRs and SEs were pooled for analysis, using the generic inverse-variance method available in the Review Manager Software, 5.2.9 (Cochrane Collaboration, Oxford, UK). Statistical heterogeneity among the trials was evaluated using both Cochran’s Q test ($\chi^2$ test) and the Higgins $I^2$ statistic in order to determine the percentage of total variations among the studies resulting from heterogeneity. If the $I^2$ statistic was ≤50%, a fixed effects model was used to pool the studies; if not, a random effects model was used.

RESULTS

The characteristics of the included trials

A total of 11 studies were included [3-11, 14, 15]. All eligible studies were published between 1995 and 2011 (Tables 1 and 2). A PRISMA flowchart (Fig. 1) includes the details of the literature search for this systematic review.

The impact of bone marrow micrometastasis status on overall survival

The impact of BMM status on OS was evaluated, using the generic inverse-variance method available in the Review Manager Software, in seven studies. The analysis determined that the presence of isolated tumour cells in the BM correlated significantly with short OS among patients with BM micrometastasis (HR: 1.84; 95% CI: 1.41-2.40; $P < 0.00001$). There was no evidence of statistical heterogeneity ($I^2 = 42\%$, $\chi^2 = 10.30$, df = 6, $P = 0.11$) (Fig. 2).

The impact of bone marrow micrometastasis status on disease-free recurrence

Eight studies reported on the incidence of disease-free recurrence. The meta-analyses revealed the prognostic significance of the presence of occult metastasis in the BM regarding DFS (HR: 1.75; 95% CI: 1.18-2.60; $P = 0.005$). Statistical heterogeneity was detected ($I^2 = 60\%$, $\chi^2 = 17.31$, df = 7, $P = 0.02$); the random effects model was used. (Fig. 3A) Two studies were included in the subgroup analyses. The subgroup analyses that enrolled patients with stage I NSCLC demonstrated that tumour cell detection in the BM was not associated with DFS (Fig. 3B) (HR: 2.35; 95% CI: 0.67-8.25; $P = 0.18$). Statistical heterogeneity was noted, as $I^2 > 50\%$; the random effects model was used.

The occurrence of bone marrow micrometastasis between adenocarcinoma and squamous cell carcinoma

Six studies reported on the occurrence of BMM between adenocarcinoma and squamous cell carcinoma. The analysis found that there was no significant difference between adenocarcinoma and squamous cell carcinoma in the positive rate of BMM (RR: 0.92; 95% CI: 0.78-1.08; $P = 0.29$) (Fig. 4). There was no evidence of statistical heterogeneity ($I^2 = 26\%$, $\chi^2 = 6.75$, df = 5, $P = 0.24$).
Bone marrow micrometastasis in different stages

The meta-analyses confirmed that the occurrence of BMM was not related to patient stages, between stage I and II (RR: 0.88; 95% CI: 0.67–1.17; P = 0.39) (Fig. 5A), stages II and III (RR: 0.98; 95% CI: 0.73–1.31; P = 0.89) (Fig. 5B) and stages I and III (RR: 0.84; 95% CI: 0.68–1.05; P = 0.13) (Fig. 5C). There was no evidence of statistical heterogeneity.
This systematic review and meta-analysis yielded two important findings. First, the molecular detection of tumour cells in the BM was associated with both poor OS and an increased risk of disease recurrence in patients with NSCLC. Secondly, we observed that the presence of BMM in NSCLC is not associated with pathologic-al types and tumour staging.

Non-small-cell lung cancer remains a leading cause of death worldwide. In most cases, the only chance of a cure comes with the resection of localized disease; however, a significant proportion of patients who undergo potentially curative resections subsequently relapse in spite of potentially curative surgery, which is indicative of the presence of early disseminated disease that was not apparent at the time of primary treatment. It is likely that this group of patients was understaged, most likely because of the presence of occult metastasis in the BM at the time of the initial surgery. Recently, BMM has gained increasing clinical interest in the setting of specific tumours. It refers to either a single group of cells or several small groups of cells that have left the primary tumour and may be detected in the bone marrow. The detection of a single disseminated tumour cell in the BM as a subclinical indicator of tumour dissemination was pioneered by Neville’s group in patients with breast cancer [16], and was subsequently confirmed for various tumour types by several laboratories. In order to improve the survival rates of patients, the identification of individual cancer patients at high risk for developing tumour relapse in spite of complete surgical resection is one of the primary goals in clinical oncology research. Meanwhile, the detection of these isolated tumour cells in distant organs such as the BM may be important in the identification of patients at high risk for disease progression and death, and may also indicate the need for the development of additional therapeutic approaches.

In this study, attempts were made to closely follow the recommendations presented by the Cochrane Collaboration, whenever possible. We prespecified a rigorous study protocol and searched several electronic databases, international conference abstracts and reference research for relevant trials, without restrictions on language. Eleven studies that were published worldwide from 1995 through 2011 were included in the analyses.

In spite of the amount of studies that have been conducted, as well as the well-known risks for patients with BMM, the prognostic value of BMM among patients with NSCLC remains uncertain because of the inconsistent results reported by the available studies. Therefore, we undertook a pooled analysis of all studies that noted strong evidence of the independent, adverse prognostic impact of BMM on both OS and DFS at the time of the initial diagnosis of operable NSCLC.

Micrometastatic disease is the primary cause of mortality from solid tumours. The molecular detection of tumour cells in the BM of patients with node-negative NSCLC most often results in the following three outcomes: death, dormancy or proliferation [17]. Most metastatic cells either die in this hostile microenvironment [18] or balance their rate of growth with their rate of death [19]. Others adapt by responding to stimuli from the microenvironment. The poor prognostic impact of BMM on both OS and DFS most likely results from the survival of tumour cells within the BM that forego dormancy altogether and begin to proliferate as soon as they arrive. Alternately, some may begin to proliferate later, following an unknown event that enables them to overcome inhibitors at the metastasis site [17]. Therefore, these cells are viable tumour cells rather than tumour cells with a limited life span [8].

However, our subgroup analyses demonstrated that the presence of BMM did not have significant prognostic value with respect to DFS in the setting of either stage I or stage II (early stage) disease. A plausible explanation for this failure to demonstrate a significant
relationship between micrometastasis and either disease recurrence or distant metastasis is that the presence of tumour cells in the BM does not always directly reflect the presence of haematogenous metastasis among patients with early stage (stage I or II) disease. Most of cells may be in either a non-proliferative state or a dormant state. This hypothesis was supported by the findings of Pantel et al. [20], who found that many tumour cells in the BM are dormant cells, and that the BM is a reservoir for these tumour cells. The mechanisms of dormancy are unknown. Several models of dormancy have been proposed. First, before the necessity of new blood vessel formation, the cells that survive in the metastatic microenvironment may remain in a non-proliferative state [21]. Secondly, their dormancy may most likely occur as a result of signals that they receive from interactions with components of their microenvironment, as well as with circulating factors [22]. Other factors may be involved in these signalling processes via growth factor receptors.

The second finding of this study was that no significant difference was observed in the frequency of tumour cells in the BM among different groups of patients, in terms of either the tumour's histological type or its pathological stage. The tumour cells were detected with nearly equal frequencies among stages I and II, stages II and III, stages I and III, and were also noted at equal frequencies as adenocarcinoma and squamous cell carcinoma, in particular. Our findings are consistent with the experimental results described in the literature [4, 23]; however, the mechanism underlying this phenomenon remains unclear.

BMM is not detected by a conventional histopathological examination; therefore, the use of additional BM analysis techniques may improve the positive rates of patients with BMM. Moreover, by monitoring the micrometastatic cancer cells in the BM, it may be possible to determine which patients may benefit from adjuvant chemotherapy, as patients with a minimal amount of residual tumour may respond better to chemotherapy. Numerous trials, including two randomized trials [24, 25] of induction chemotherapy for stage IIIA NSCLC, have demonstrated that it was feasible and yielded both higher response rates and improved survival.

This study was limited because of its sample size. Although we contacted each author in order to supplement our data, the number of included studies was insufficient to perform some of the preplanned subgroup analyses. That we could not identify the effect modifiers may be attributable to our study's low statistical power.
Occult metastasis to the BM is indicative of a poor prognosis among patients of NSCLC. Therefore, BMM may represent an important new prognostic factor in lung cancer, and may greatly affect the selection of patients who may benefit from adjuvant therapy, as well as the selection of patients with advanced disease who may benefit from surgery. This approach allows for improved staging accuracy and may therefore allow for the formulation of truly homogeneous treatment groups in future clinical trials. Moreover, we should research whether BM metastasis is indicative of a high risk of disease-free recurrence, poor OS and cancer-specific survival in patients with NSCLC in different pathological types and cancer stages in our future clinical trial, such as adenocarcinoma, squamous cell carcinoma, stage I, stage II, stage III, respectively.

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**REFERENCES**


