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DISEASES OF THE ESOPHAGUS

Original article

The prognostic impact of occult lymph node metastasis in cancer of the esophagus or esophago-gastric junction: systematic review and meta-analysis

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SUMMARY. **Attempts to define the clinical significance of occult lymph node metastasis have yielded mixed results. We set out to quantify the influence on disease-free survival of occult lymph node metastasis in cases of esophageal or gastro-esophageal cancer previously staged as lymph node-negative by conventional H&E staining. We performed a systematic review and meta-analysis of observational studies published between 1966 and 2006 (identified through Medline and Embase). Twelve suitable cohort studies were identified. These studies suggest there is a significant ($P < 0.001$) association between occult lymph node metastasis and prognosis in cancer of the esophagus or esophago-gastric junction (pooled hazard ratio 3.16 with 95% confidence intervals of 2.25–4.42). We did not demonstrate study quality, number of nodes examined or number of lymph node sections examined to be significant sources of intertrial heterogeneity. Data from observational studies suggest that occult lymph node metastasis is an important prognostic factor in cancer of the esophagus or gastro-esophagus. Meta-analysis using individual patient data can now be justified.**

KEY WORDS: **Ca esophagus, lymph node metastasis, meta-analysis.**

BACKGROUND

Numerous studies attest to the fact that it is possible to detect disseminated tumor cells in lymph nodes that are considered free of metastases using conventional histopathological techniques. Attempts to define the clinical significance of these cells in esophageal cancer has mixed results. We conducted a systematic review of the literature addressing the prognostic significance of occult lymph node metastasis in cancer of the esophagus or gastro-esophagus because it provides higher statistical power and improved precision relative to individual studies. Specifically we set out to quantify the impact on disease-free survival of occult lymph node metastasis in cases staged by conventional means as lymph node metastasis-free (pN0).

Disseminated cancer cells detected by immunochemical means in lymph nodes have been called *inter alia* 'micrometastases', 'subclinical metastases' or 'tumor cell microinvolvement'. The International Union Against Cancer (UICC) has attempted to clarify this terminology by making a distinction between 'micrometastasis' and 'isolated tumor cells'.[1] Micrometastases are defined as being ≤ 2 mm in greatest dimension, in contact with a vessel wall, extravasated, proliferating and usually associated with a stromal reaction. Isolated tumor cells (ITC) in contrast are defined as clusters (< 0.2 mm) or single tumor cells without any of the above characteristics whose presence can only be determined by immunohistochemistry, immunocytochemistry or molecular methods such as flow cytometry or polymerase chain reaction (PCR). Here we have used the term 'occult lymph node metastasis' (OLNM) rather than micrometastasis in order to avoid confusion with the term defined by the UICC and to reflect the range of definitions of 'occult disease' used by studies within this review.

METHODS

Medline and Embase databases (1966 to 1 May 2006) were searched using the following terms: micrometastasis, tumor cell microinvolvement, minimal residual disease, subclinical metastasis, occult metastasis, isolated tumor cells, and esophagus. The

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reference lists of retrieved articles and previous narrative reviews were scanned for other potentially relevant articles. Unpublished or in-press studies known to the authors were also included. Any study reporting the use of immunochemistry to detect metastasis in pN0 lymph nodes of esophageal or gastro-esophageal cancer patients was potentially included. The only additional methodological criterion was that it should also have been possible to reasonably infer from the report that any cases of large lymph node metastases detected by the immunochemical technique, which should have been detectable by conventional staining techniques, were reclassified from pN0 to pN1 and excluded from the survival analysis. No restrictions were placed on the immunochemical method employed. Nor were any restrictions placed based on language of publication, geographic location, use of adjuvant therapies, ethnicity, age, sex or study design (studies in which pN0 patients were a subgroup of the population were included). Where multiple studies were published on the same or overlapping cohorts, only the last published report was included (unless the data was not suitable for meta-analysis in which case the last suitable report was selected). The outcome parameter of interest was disease-free survival; however, if a study contained only 'overall survival' data it was also to be included in the meta-analysis because we thought that overall survival would tend to underestimate a detrimental prognostic effect of OLNM.

Extracted data was entered into a customized data sheet. The data chosen for extraction was limited to those variables where sound scientific rationale for their inclusion existed. The data from each study was reviewed twice to minimize the probability of data-entry error. Quality assessment was performed independently by two of the authors. Our assessment of study quality was based on recently published guidelines for the evaluation of the quality of prognosis studies.[2] In summary, six quality items (study participation, study attrition, prognostic factor measurement, outcome measurement, confounding measurement and analysis) were used. Each quality item was described or 'operationalised' on a quality assessment sheet. For each quality item a score of 'high risk of bias', 'low risk of bias' or 'unclear' was given. If a study scored 'high risk of bias' for any item it was given an overall quality assessment of 'high risk of bias'. The only exception was where there was a high risk of attrition bias. These studies were not given an overall assessment of 'high risk' as discussed in the Cochrane Handbook.[3] Studies which reported overall survival data rather than disease-free survival data were classified as 'high risk' under 'outcome measurement'. The quality assessment data was incorporated into the sensitivity analysis.

The first part of the numerical analysis was to obtain an estimate of the hazard ratio and its standard error for each trial. If a Cox proportional hazards model was used to analyze the data the coefficient for the OLNM positive versus OLNM negative comparison was used as a direct estimate of the hazard ratio. The associated standard error was estimated from the accompanying 95% confidence intervals or *P*-value using conventional methods.[4] When a *P*-value for the log rank statistic, the total number of relapses and/or disease-related deaths among pN0 patients, the number of OLNM positive and the number of OLNM negative cases could be extracted from the text, the methods for indirectly estimating the natural log of the hazard ratio and its variance as described by Parmar *et al*.[5] were used. Where neither of the above approaches could be used to estimate the log hazard ratio and its variance it was possible in all other cases to use the published survival curves to obtain them as per Parmar *et al*.[5]

The meta-analysis of the extracted summary statistics was performed on RevMan version 4.2 software (Copenhagen, Denmark: The Nordic Cochrane Centre, The Cochrane Collaboration, 2003). For the best estimate of prognostic effect, we obtained pooled estimates using a fixed effects model and inverse variance weighting. The chi-squared test for heterogeneity was used with *P*-value of 0.1 rather than 0.05 to determine statistical significance.[6] The I^2 statistic was used to quantify inconsistency across studies. An I^2 value greater than 50% was considered significant heterogeneity. We used subgroup analysis to explore interstudy heterogeneity. Three subgroup analyses with robust clinical justification defined *a priori*. The first was defined by the number of sections examined per lymph node (1 or more than 1); the second was defined by the mean number of lymph nodes examined per case (≤ 20 or > 20) and the third was based on methodological quality (studies at high risk of bias *vs.* the remaining studies). The significance test described by Deeks *et al*.[7] was used to investigate differences between subgroups. We assessed publication bias visually using a funnel plot.

RESULTS

The search strategy yielded 762 articles, of which 14 were non-overlapping and met the inclusion criteria. Two[8,9] of these 14 studies were excluded from the meta-analysis as it was not possible to obtain sufficient data to accurately estimate effect (see addenda for details of excluded studies). The remaining 12 cohort studies[10–21] included information on 741 patients (192 positive for occult lymph node metastases). The prevalence rate of OLNM ranged between 11% and 56%. Key features of the included and excluded studies are compared in Table 1.

Table 1 Characteristics of included and excluded studies

Study	*N*	Positive	Histology	Location	Antibody	Sections	Nodes	Risk of bias
Included studies								
Natsugoe 1998[10]	48	27%	SCC	Es	AE1/AE3	1	24	High
Mueller 2000[11]	75	17%	Adeno	EGJ	AE1/AE3	1	28	High
Heeren 2005[12]	60	30%	Adeno	EGJ	AE1/AE3	> 1	11	High
Matsumoto 2000[13]	59	56%	SCC	Es	AE1/AE3	1	50	Low
Sato 2001[14]	50	40%	SCC	Es	AE1/AE3	1	37	Low
Vazquez 2002[15]	124	11%	Adeno & SCC	Es	AE1/AE3	1	16	Low
Nakamura 2002[16]	53	26%	SCC	Es	AE1/AE3	1	47	Low
Hosch 2000[17]	54	56%	Adeno & SCC	Es	BerEP4	3	17	High
Laso 2004[20]	21	24%	Adeno & SCC	Es	AE1/AE3	1	N/A	High
Komukai 2000[21]	37	37%	SCC	Es	AE1/AE3	5	75	High
MacGuill 2006[18]	146	8%	Adeno & SCC	EGJ & Es	MNF116	1	11	Low
Excluded studies								
Glickman 1999[8]	78	26%	Adeno & SCC	Es	AE1/AE3	5	7	Low
Xiao 2002[9]	42	62%	Adeno & SCC	Es	AE1/AE3	3	9	High

N = number of cases; Positive = % of occult lymph node metastasis positive cases; SCC = squamous cell carcinoma, Adeno = adenocarcinoma; % adjuvant = % of patients who received neo-adjuvant chemoradiotherapy; N/A = not available; EGJ = esophagogastric junction; Es. = esophagus; sections = number of sections examined per block/lymph node; Nodes = mean number of lymph nodes examined per case.

Table 2 *P*-value associated with significance test described by Deeks *et al.*[7]

	No. of studies	HR (95% CI)	*P*-value
No. of nodes			
> 20	7	3.45 (2.02, 5.88)	0.099
< 20	4	2.96 (1.89, 6.65)	
No. of sections			
1	9	2.68 (1.85, 3.89)	0.079
> 1	3	5.23 (2.61, 10.47)	
Risk of bias			
high	7	4.25 (2.54, 7.11)	0.089
low	5	2.52 (1.61, 3.94)	

A fixed effect meta-analysis produced a pooled hazard ratio for disease relapse in positive patients relative to negative patients of 3.16 (95% CI 2.25–4.42). The *P*-value obtained from the fixed effect meta-analysis of overall effect was < 0.00001. When the seven trials judged to be at high risk of bias were excluded from the meta-analsysis, the hazard ratio was reduced to 2.52 (1.61–3.94).

Neither the chi-squared test ($P = 0.65$) or a visual assessment of overlap of confidence intervals on the Forest plot suggested significant intertrial heterogeneity (Fig. 1). Significance testing using the method described by Deeks *et al.*[7] did not demonstrate that any of the subgroup factors (number of sections examined per node/paraffin block, mean number of lymph nodes examined per case or methodological quality) were significant sources of heterogeneity (Table 2).

In order to assess the sensitivity of the study we reanalyzed the data using a random effects model instead of a fixed effect model (the random effects model pooled hazard ratio was 3.16 with 95% CI of 2.25–4.42). Then we re-examined the data following exclusion of studies deemed to be at high risk

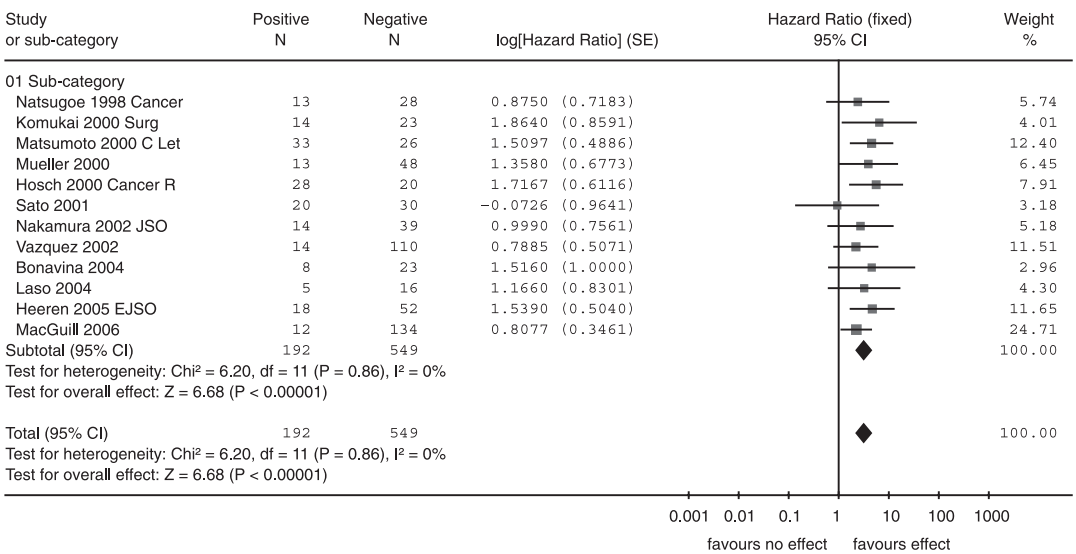

Fig. 1 Forest plot for survival and details of studies.

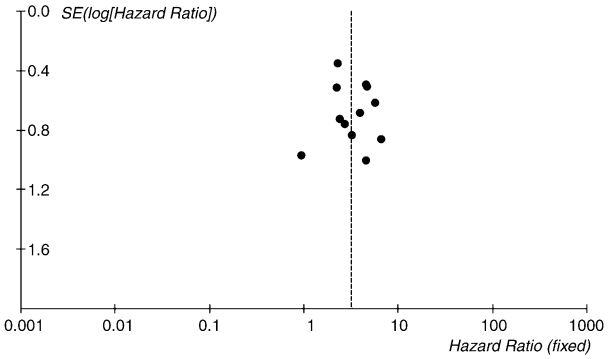

Fig. 2 Funnel plot for all studies.

of bias. Additionally we reanalyzed the data after inputing a reasonable range of values for missing data from excluded studies. These changes did not materially change the results of the meta-analysis. The funnel plot did not suggest marked publication bias (Fig. 2).

DISCUSSION

In summary, the results of this meta-analysis suggest that occult lymph node metastasis has a significant detrimental impact on prognosis in node-negative cancers of the esophagus or gastro-esophagus. The sensitivity analysis showed that the results are not meaningfully affected by variation in the number of sections examined per node or block, the mean number of lymph nodes examined, the choice of the statistical method and the exclusion of trials of poorer quality. Sensitivity analysis also indicated that publication bias is unlikely to have distorted the findings of the meta-analysis.

As with any meta-analysis of observational studies this review comes with a number of caveats that we acknowledge. Since the analysis comprises non-randomised trials, there are a potentially limitless number of unknown confounding factors which could cause selection bias. We attempted to address some of them through sensitivity analyses. An important element of our overall quality assessment was the quality of procedures for controlling for differences between the OLNM positive and negative groups. We classified trials, which did not compare their OLNM positive and negative groups with respect to some key pathological parameters (specifically T-staging and tumor grade), as high risk studies. Additionally two trials were classified as high risk despite reporting a comparison of the pathological characteristics of their two groups because they reported significant differences between the two comparison groups. When investigating intertrial heterogeneity, we sought to avoid 'data-dredging' by strictly limiting the number of parameters selected for analysis *a priori*. Consequently it was not possible to examine all plausible sources of intertrial heterogeneity. The parameters selected for the sensitivity analysis were those which we deemed to have the most robust scientific rationale and potential clinical value: number of nodes examined, number of sections per node/block examined and methodological quality.

Summary statistics from each trial were extracted using standard methodology.[5] Clearly such an approach will never be as complete or secure as collecting individual patient data and has a number of intrinsic sources of error. The smaller the number of events (meaning the total number of relapses and/or disease-related deaths in this study population) the greater is the probability of a significant difference between the estimated log hazard ratio and variance and their true values. Relative to most meta-analyses of large intervention trials, the number of patients and relapses within the cohorts in this study were small. The methods described by Parmar *et al.*,[5] are intended for use with *P*-values quoted for two-sided log rank statistics. We thought it a safe assumption that all statistical tests were two-tailed unless it was clearly stated in the text that they were not. However, Williamson and colleagues[22] state that the method of Parmar *et al.*[5] for estimating the variance of the log hazard ratio from a survival curve tends to underestimate the truth, and consequently results in the trial are being given too much weight in a meta-analysis.

For these reasons and in order to conform with good practice guidelines for the reporting of meta-analyses of observational studies,[23,24] we have sought to avoid giving our quantitative estimate of effect undue prominence and have concentrated instead on analyzing sensitivity and demonstrating the robustness of our findings. However, the sensitivity analysis is itself not without limitations. Care must be taken in the interpretation of the chi-squared test, since it has low power in the context of a meta-analysis of studies with small sample size. This means that our non-significant result should not be taken as evidence of no heterogeneity and is also why we chose a *P*-value of 0.10, rather than the conventional level of 0.05, to judge the statistical significance of this test.[6]

The results of this study are of clinical relevance for a number of reasons. Here we provide a more objective appraisal of the evidence than traditional narrative reviews and a more precise estimate of the prognostic effect of occult lymph node metastases than is currently available. In so doing we hope to have better informed the on-going debate regarding their prognostic significance. The considerable expense of resources necessary to perform an individual patient data meta-analysis of this same question

can now be justified. The data presented here may help in planning future clinical trials examining whether the presence or absence of occult lymph node metastasis should guide decisions concerning new or existing adjuvant therapies of esophageal or gastro-esophageal cancers. The data from observational studies suggests that occult lymph node metastasis has a clinically significant detrimental effect on disease-free survival in node-negative cancer of the esophagus or gastro-esophagus.

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