Systematic review and meta-analysis of the prognostic impact of lymph node micrometastasis and isolated tumour cells in patients with stage I–IIIA non-small cell lung cancer

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REVIEW

Systematic review and meta-analysis of the prognostic impact of lymph node micrometastasis and isolated tumour cells in patients with stage I–IIIA non-small cell lung cancer

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Systematic review and meta-analysis of the prognostic impact of lymph node micrometastasis and isolated tumour cells in patients with stage I–IIIA non-small cell lung cancer

Lymph node micrometastases could be one of the reasons for the high recurrence rate after complete surgical resection in stage I–IIIA non-small cell lung cancer (NSCLC). The standard evaluation of a single haematoxylin and eosin (H&E) slide of a paraffin-embedded section of a lymph node is insufficient for the detection of micrometastases, and there is a need for additional histopathological evaluation. The association of lymph node micrometastases with survival remains as yet unresolved. The aim of this systematic review and meta-analysis is to investigate if lymph node micrometastases and isolated tumour cells in patients with stage I–IIIA NSCLC, detected with multiple sectioning and/or immunohistochemistry (IHC) and/or reverse transcriptase polymerase chain reaction (RT-PCR), are associated with overall survival (OS) and disease-free survival (DFS) after surgical resection. We performed a meta-analysis of time-to-event outcomes based on 15 articles using ancillary techniques to detect micrometastases. We extracted the OS and DFS every 3–6 months after surgery, for patients with and without occult lymph node micrometastasis, from the survival curves published in each article. These data were used to reconstruct OS and DFS for 'micrometastasis' and 'no micrometastasis' groups. Based on all included studies that used IHC, serial sectioning, or RT-PCR, we found a 5-year OS of 55% (micrometastasis) vs. 75% (no micrometastasis), and a 5-year DFS of 53% (micrometastasis) vs. 75% (no micrometastasis). Patients with stage I–IIIA NSCLC with lymph node micrometastases detected by ancillary histopathological and molecular techniques have a significantly poorer OS and DFS compared to patients without lymph node micrometastases.

Keywords: early stage, meta-analyses, micrometastasis, non-small cell lung cancer, systematic review

Introduction

Worldwide, lung cancer has the highest mortality of all malignancies.[1] In patients with non-small cell lung cancer (NSCLC), pathologic staging is the most important prognostic factor and the nodal stage is the most important determinant for the decision regarding the feasibility of surgical resection and the need for postoperative adjuvant therapy. Patients with suspicious lymph nodes are preoperatively assessed by invasive nodal staging with endosonography (EBUS/EUS). If metastases are not found through this type of conventional cytopathological evaluation, surgical mediastinoscopy with histological assessment should

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ensue. For stage I–II NSCLC and selected locally advanced stage IIIA, curatively-intended surgery remains the gold standard.[2]

However, even after adequate preoperative invasive staging, unexpected lymph node (macro)metastases after surgery are found in more than 10% of patients.[3] Furthermore, the recurrence rate after curatively-intended surgery remains between 18% for stage IA to 70% for stage IIIA.[4–9] Both the high recurrence rate and the high numbers of unforeseen lymph node metastases after surgery raise the question, if early microscopic dissemination in lymph nodes may be missed by conventional histopathological evaluation. According to the American Joint Committee on Cancer, micrometastases are defined as clusters of tumour cells measuring between 0.2 and 2 mm in greatest diameter, and isolated tumour cells (ITC) are defined as single tumour cells or small clusters of cells, smaller than 0.2 mm in greatest diameter.[10] Currently, the standard histopathological method for the evaluation of lymph nodes in NSCLC is the evaluation of only one paraffin-embedded section (4 μm) of the lymph node following haematoxylin and eosin (H&E) staining. With this standard method, only a small part of the lymph node will be evaluated and small metastases in other parts of the lymph node can be missed.[11] For the detection of small micrometastases and ITCs, an additional step in the pathologic evaluation is required. Previous studies have explored different additional evaluation procedures. One of the most important and widely used methods is multiple sectioning through the lymph node.[12] A previous study in patients with breast cancer showed that there is a need for at least six sections to detect more than 90% of small tumour deposits in a lymph node.[13] Other studies, carried out in patients with lung cancer, reported that immunohistochemistry (IHC) or reverse transcriptase polymerase chain reaction (RT-PCR) with different target genes alone, or in combination with multiple sectioning, are reliable methods for the detection of micrometastases/ITCs.[14–16]

The aim of this systematic review and meta-analysis was to investigate whether lymph node micrometastases and ITCs in patients with stage I–IIIA (operable stage) lung cancer, detected with multiple sectioning and/or IHC and/or RT-PCR, have an impact on prognosis.

Methods

SEARCH STRATEGY

We conducted a systematic review and meta-analysis of original articles in accordance with PRISMA guidelines (Preferred Reporting Items for a Systematic Review and Meta-analysis).[17] A systematic search of electronic databases including PubMed, Embase, Web of Science, Cochrane, and Academic Search Premier was undertaken in March 2019. The search was conducted with combinations of synonyms of "micrometastasis", "occult metastasis", and "non-small cell lung cancer" (see supporting information online, Table S1). The reference lists of all included articles and the excluded reviews and/or meta-analyses were hand-searched for additional relevant articles.

INCLUSION AND EXCLUSION CRITERIA

Comparative studies of any design, published in English, were eligible for inclusion. Editorials, meeting abstracts, case reports, and reviews or meta-analyses were excluded. Studies were considered eligible if they included patients with T1-4, N0-1, or M0 NSCLC, whose lymph nodes underwent additional pathologic analysis for micrometastases with IHC, RT-PCR, or multiple sectioning. Studies were excluded if they included patients with a distant metastasis, known N2 disease, or with experimental methods and/or other methods than IHC, RT-PCR, or serial sectioning to detect micrometastasis. Studies without OS and/or disease-free survival (DFS) as outcome data were also excluded.

STUDY SELECTION

Two investigators (M.H. and J.T.) independently assessed titles and abstracts of all studies found with electronic searches, and relevant articles were selected for full-text review. The full-texts of the selected articles were screened by both investigators for the inclusion- and exclusion-criteria as mentioned earlier. Discrepancies were resolved by discussion and studies were only included if a consensus was reached and both investigators agreed.

QUALITY ASSESSMENT

For the evaluation of the quality of the included studies, we used the Modified Downs and Black checklist.[18] This checklist is useful for all possible study designs and includes 27 items covering a thorough evaluation, including reporting, external and internal validity, risk of bias/confounding, and power of studies. One investigator (M.H.) assessed the study quality according to this checklist and studies were ranked as excellent (26–28), good (20–25), fair (15–19), and poor (<15) (see supporting information online,

Table S2).[18] Only studies with poor quality were rechecked by two other investigators (J.T. and D.C.), and when consensus was reached, studies with poor quality were excluded.

DATA EXTRACTION

Data extraction was performed by one investigator (M.H.) and checked by a second investigator (J.T.). The following items were extracted from each included study: author and publication year, country, study period, design, total number of patients included, pN/cN stage at inclusion, control method, additional pathologic analysis method, target gene/antigen used (if applicable), total number of lymph nodes analysed, total number of lymph node micrometastases, total number of upstaged patients, and median follow-up period.

Survival probabilities for OS and DFS at different timepoints were extracted from published survival curves by one investigator (M.H.). OS probabilities were reported at every 6 months after surgery until 5-year follow-up for both micrometastasis and no micrometastasis. DFS was reported at every 3 months after surgery for the first 2-year follow-up, and at every 6 months after 2 years of follow-up until 5 years follow-up.

DEFINITION OF ITC AND MICROMETASTASIS

For this meta-analysis, we defined ITC and micrometastasis as described in the breast cancer literature. ITC was defined as single tumour cells <0.2 mm and micrometastasis as a cluster of cells between 0.2–2 mm in diameter. This definition does not cover RT-PCR studies; therefore, we considered molecular-positive single cells or clusters as micrometastasis/ITC.

DEFINITION OF UPSTAGING

From all studies, the initial pN-stage was extracted. Patients with a micrometastasis and/or ITC in a previously tumour-free lymph node were upstaged according to the current AJCC 8th edition for NSCLC to N1 or N2.

STATISTICAL ANALYSIS

Based on the data extraction, the number of patients, events, and censoring were reconstructed at each timepoint for each study and each arm. Details about this procedure are provided in online supplementary Appendix S1. Subsequently, a meta-analysis of time-to-event outcomes was performed based on a methodology for pairs of survival curves under heterogeneity. This meta-analysis cannot be cast in the classical meta-analysis where the well-known forest plot is used to illustrate the results of the meta-analysis. A multivariate model for a joint analysis of survival proportions, reported at different times in the different studies, was estimated in order to be able to use all information available in each study in the meta-analysis. Technical details of the model have previously been reported.[19-21] This methodology has previously been applied to a meta-analysis based on the published literature, where data in each study consisted of OS and DFS probabilities at different timepoints in the first 5 years after treatment.[22] By using this methodology, DFS and OS were estimated based on survival probabilities reported for each individual study at 3 and 6 months during the first 5 years after the operation. In this way, all information was used regarding the estimated DFS and OS in each study included in the meta-analysis. With this methodology, studies can also be included in the meta-analysis which did not report the hazard ratio.

Results

RESULTS OF THE SEARCH AND SCREENING

The systematic search resulted in 675 records (Figure 1). After removing duplicates and excluding articles based on title and abstract, 63 full-text articles were screened for inclusion in the meta-analysis. An additional two full-text articles were retrieved from the reference list of other eligible articles and were screened for inclusion. This resulted in the selection of 16 articles.

QUALITY ASSESSMENT

The majority of the included articles were retrospective cohort studies. As mentioned earlier, we used the Modified Downs and Black checklist for the quality assessment.[18] The results are shown in Table 1. None of the included articles had excellent quality. Nine articles (56%) were scored as good quality and six articles (37.5%) were scored as fair quality.[23–37] Only one article (6.25%) had a poor quality score (<14 points), and was therefore excluded from the final meta-analysis.[38]

CHARACTERISTICS OF INCLUDED ARTICLES

After screening and quality assessment, 15 studies with a total of 1893 patients, were included in the

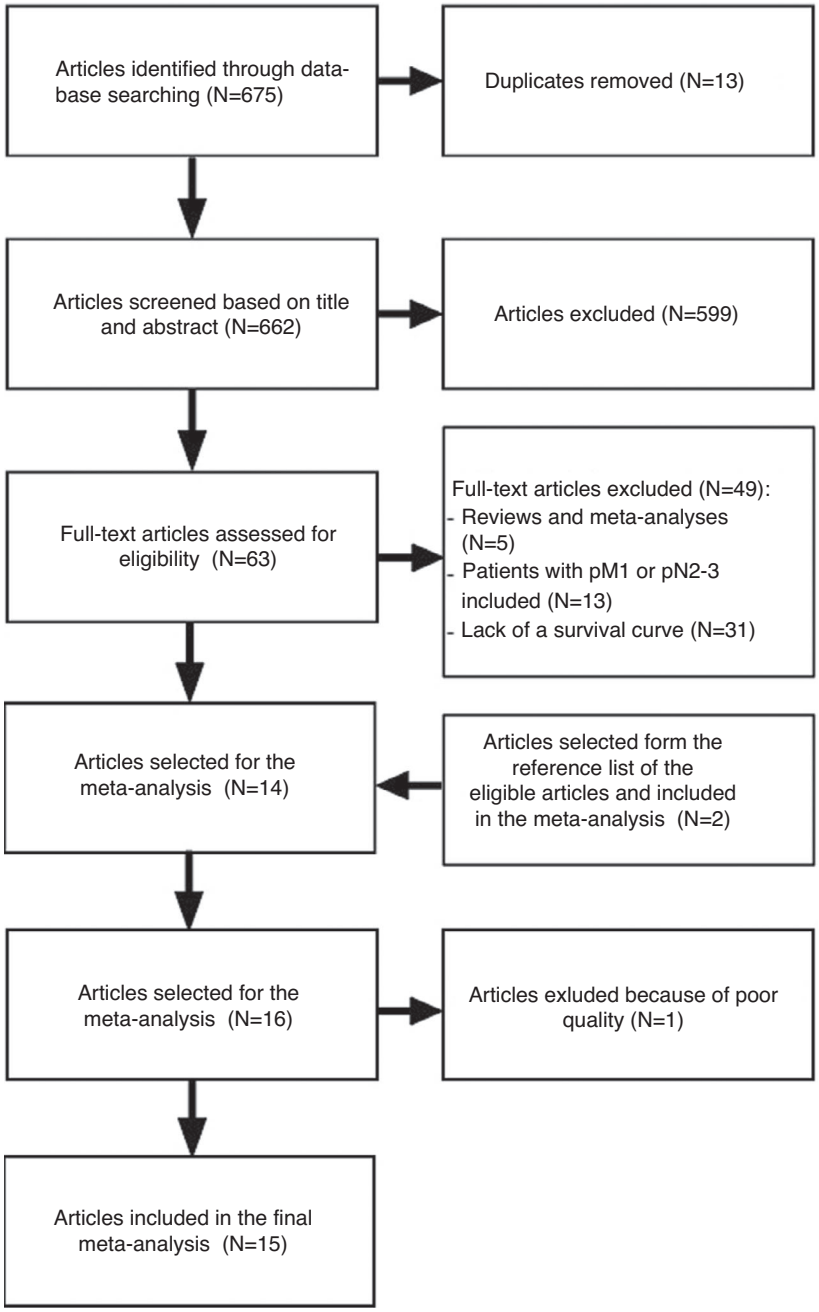

Figure 1. Flow-chart of the search strategy.

final meta-analysis. The study characteristics of each included article are described in Table 2. Of the included studies, nine were from East-Asia, four from Europe, and two from the USA. Of all included patients, 680 had adenocarcinoma, 441 had squamous cell carcinoma, and 772 patients had an NSCLC not-otherwise-specified (NOS), adenosquamous carcinoma or an unknown subtype. In all, 1866 of the included patients had a confirmed pN0 stage with conventional pathologic evaluation and only 27 patients were confirmed as pN1. The conventional pathologic evaluation used was different for some included studies: 13 studies used standard evaluation with one H&E section and two studies used two H&E

Table 1. Modified Downs and Black score per article

Article	Reporting – 11 points	External validity – 3 points	Internal validity; bias – 7 points	Internal validity; confounding (selection bias) – 6 points	Power – 1 point	Total score – maximal score 28	Final score*
Benlloch 2008	8	1	6	4	0	19	Fair
Gu 2002	9	3	7	4	0	23	Good
Gwozdz 2018	8	3	7	4	0	21	Good
Harden 2003	7	2	3	2	0	14	Poor†
Hashimoto 2000	8	3	4	3	0	19	Fair
Kawano 2002	8	1	6	3	0	17	Fair
Kubushock 1999	8	1	6	4	0	19	Fair
Li 2013	10	1	5	4	0	20	Good
Li 2008	7	1	5	4	0	17	Fair
Martin 2016	11	3	6	4	1	25	Good
Maruyama 1997	8	1	7	2	0	18	Fair
Osaki 2002	9	3	7	4	0	23	Good
Rena 2007	9	3	6	3	0	21	Good
Roh 2004	9	2	6	3	0	19	Fair
Rusch 2011	10	3	6	4	1	24	Good
Yasumoto 2003	10	3	6	4	0	23	Good

The quality of the included studies was assessed based on the modified Downs and Black checklist (see supporting information online Table S2 for the full checklist). All included articles were scored for 26 items, max. 11 points for reporting, max. 3 points or external validity, max. 7 points for internal validity and max. 1 point for power; here they are summarised in these categories, the detailed items can be found in the supplementary Table S2.

*The final score is classified as: 26–28 = excellent, 20–25 = good, 15–19 = fair, <15 = poor.

†Poor studies were excluded from the final analysis.

sections from the lymph node as control. For the evaluation of lymph node micrometastasis/ITC, 11 studies used IHC in combination with multiple sectioning (3–10 sections) with cytokeratins or BER-EP4 as the antigen, and four studies used RT-PCR with different target genes.

INDIVIDUAL PUBLISHED SURVIVAL DATA FOR THE INCLUDED ARTICLES

The individual survival probabilities for each article included in the meta-analysis are provided in Table 3 for IHC studies and Table 4 for RT-PCR studies. The percentage of detected lymph node micrometastases in each article differed from 9% to 16.8% for RT-PCR studies and from 0.4% to 20.9% for IHC studies. If the N-stage is corrected according to newly-detected micrometastases, the number of upstaged patients was 21.1% to 34.1% for RT-PCR studies, and 9.5% to 70.5% for IHC studies. A total of 14 articles published OS, and seven articles reported on DFS. The 5-year OS in RT-PCR studies for patients with micrometastasis varied between 23.8% to 44% vs. 44.1% to 100% for patients without micrometastasis. The 5-year OS in IHC studies for patients with micrometastasis varied between 21.4% to 74% vs. 61.8% to 90.9% for patients without micrometastasis. The 5-year DFS for patients with micrometastasis varied between 22% to 64.7% vs. between 59% to 92.3% for patients without micrometastasis.

OVERALL SURVIVAL CURVES FOR ALL METHODS

Meta-analysis for OS based on 14 studies was performed. The results are shown in Figure 2. OS for micrometastasis at 1, 2, and 5 years were 90%, 80%,

Table 2. Overview of the included studies

Author and publication year	Country	Total number of patients	Pathologic subtype			Tumour size		pN-stage at inclusion based on single H&E		Method used to exclude macro-metastasis	Method used to detect micro-metastasis	Target gene/ antigen	Outcomes reported
			Adeno-carcinoma	Squamous cell carcinoma	Other*	≤3 cm	>3 cm	N0	N1				
Benlloch 2008	Spain	38	14	14	10	18	20	38	0	H&E (1 section)	RT-PCR	CEACAM PLUNC	OS, DFS
Gu 2002	Japan	49	27	21	1	18	31	49	0	H&E (1 section)	IHC, 10 sections	P53, CK AE1/AE3	OS
Gwozdz 2018	Poland	148	41	94	13	50	98	130†	18†	H&E (2 sections)	IHC, 2 sections	CK AE1/AE3 and Ber-Ep4	OS, DFS
Hashimoto 2000	Japan	31	22	9	0	18	26	22	9	H&E (2 sections)	RT-PCR	P53 and KRAS	OS
Kawano 2002	Japan	49	30	19	0	26	23	49	0	H&E (1 section)	IHC, 3 sections	Ber-Ep4, CK AE1/AE3	OS
Kubushock 1999	Germany	70	–	–	70‡	NR	NR	70	0	H&E (1 section)	IHC, 3 sections	Ber-Ep-4	OS
Li 2013	China	44	26	17	1	18	26	44	0	H&E (1 section)	RT-PCR	Surviviv and livin	OS, DFS
Li 2008	China	89	49	40	0	40	49	89	0	H&E (1 section)	RT-PCR	MUC1	OS
Martin 2016	USA	298	168	101	29	26	15	298	0	H&E (1 section)	IHC and 1 section	CK AE1/AE3	OS, DFS
Maruyama 1997	Japan	44	29	13	2	23	21	44	0	H&E (1 section)	IHC and 2 sections	CK (78.)	OS, DFS
Osaki 2002	Japan	115	78	29	8	62	53	115	0	H&E (1 section)	IHC and 5 sections	CK AE1/AE3	OS
Rena 2007	Italy	87	38	36	13	27	61	87	0	H&E (1 section)	IHC and 5 sections	CK AE1/AE3	DFS
Roh 2004	Korea	35	35	0	0	21	14	35	0	H&E (1 section)	IHC, serial sections‡*	CK AE1/AE3	OS

Table 2. (*Continued*)

Author and publication year	Country	Total number of patients	Pathologic subtype			Tumour size		pN-stage at inclusion based on single H&E		Method used to exclude macro-metastasis	Method used to detect micro-metastasis	Target gene/ antigen	Outcomes reported
			Adeno-carcinoma	Squamous cell carcinoma	Other*	≤3 cm	>3 cm	N0	N1				
Rusch 2011	USA	580	–	–	580‡	NR	NR	580	0	H&E (1 section)	IHC and 1 section	CAM5.2, CK AE1	OS, DFS
Yasumoto 2003	Japan	216	153	48	15	129	87	216	0	H&E (1 section)	IHC and 5 sections	CK AE1/AE3	OS, DFS

Overview of all included studies. The study of Gwozdz *et al.* only included preoperative lymph node samples retrieved during transcervical mediastinal lymphadenectomy. All other studies included lymph nodes retrieved during surgery. We reported the standard control method and the additional test method per study with the target gene or antigen.
NR, not reported; OS, overall survival (5 years); DFS, disease-free survival; CK, cytokeratin.
*Other subtypes include large cell, NOS and adenosquamous carcinoma.
†All studies were conducted postoperatively; only the study of Gwozdz was preoperative, mentioned stage is the c-stage.
‡Subtype not mentioned or unable to extract from article.

and 55%, respectively; while for no micrometastasis this was 98%, 90%, and 75%, respectively.

OVERALL SURVIVAL CURVES FOR IHC ONLY (IN COMBINATION WITH SERIAL SECTIONING)

A total of 10 studies reported on OS and used only IHC as a detection method for micrometastasis. The OS data estimated with the meta-analysis for micrometastasis and no micrometastasis are shown in Figure 3. OS for micrometastasis at 1, 2, and 5 years was 90%, 78%, and 58%, respectively, and for no micrometastasis was equal to 98%, 90%, and 76%, respectively. Survival for each single study for micrometastasis and no micrometastasis for IHC studies are also reported (see supporting information online, Figures S1 and S2).

DISEASE-FREE SURVIVAL CURVES FOR ALL METHODS

Meta-analysis of eight studies reporting DFS was performed. Only a single study used RT-PCR and seven studies used IHC. Figure 4 shows the results. DFS for micrometastasis at 1, 2, and 5 years was 82%, 75%, 53%, respectively, and for no micrometastasis was 90%, 83%, and 75%, respectively.

Discussion

For this systematic review and meta-analysis we extracted data from 15 studies, with a total of 1893 patients and estimated OS and DFS for patients with stage I–IIIA NSCLC, with and without occult lymph node micrometastasis/ITC after surgical resection. Based on the results of the meta-analysis, patients with micrometastases and/or ITCs compared to those without had poor OS and DFS of 55% vs. 75% and 53% vs. 75%, respectively, regardless of the detection method. The reported OS/DFS in our meta-analysis is very similar, because all patients included in the individual studies were included before the introduction of checkpoint inhibitors and targeted therapies as the standard of care for NSCLC. Before these treatments, the survival for NSCLC after recurrence varied between 6.8 and 9.8 months for local vs. distant recurrence.[4,39] Our analysis includes both RT-PCR studies as well as IHC studies in combination with serial sectioning. As previously reported, we found an OS with RT-PCR for occult micrometastasis/ITC between 23.8–44% and for no occult micrometastasis/ITC between 44.1–100%. We also found an OS

Table 3. Upstaging and survival for micrometastasis (LNMM+) vs. no micrometastasis (LNMM−) in IHC studies

Author and publication year	Definition used	Total LN analysed	Total LNMM+*	Total upstaged patients†	pN-stage of upstaged patients based on LNMM‡			Median follow-up (months)	OS (5 years)		DFS (5 years)	
					N0 to N1mi	N0 to N2mi	N1 to N2mi		LNMM−	LNMM+	LNMM−	LNMM+
Gu 2002	ITC and micrometastasis	474	35 (7.4%)	22 (44.9%)	9	13	–	NR	81.30%	31.80%	–	–
Gwozdz 2018	ITC and micrometastasis	4810	19 (0.4%)	14 (9.5%)	–	7	7	53	61.80%	21.40%	70%	22%
Kawano 2002	ITC and micrometastasis	1820	NR	13 (26.5%)	5	8	–	66.6	84.40%	74%	–	–
Kubushock 1999	ITC	386	NR	11 (15.7%)	NR	NR	–	64	76%	58%	–	–
Martin 2016	ITC and micrometastasis	NR	NR	41 (13.8%)	17	24	–	100.8	66.90%	50%	60%	40%
Maruyama 1997	ITC and micrometastasis	339	71 (20.9%)	31 (70.5%)	19	12	–	48	90.90%	27.80%	92.30%	33.30%
Osaki 2002	ITC and micrometastasis	2432	42 (1.7%)	32 (27.8%)	19	13	–	35.8	78.70%	52.40%	–	–
Rena 2007	ITC and micrometastasis	694	19 (2.7%)	14 (16.1%)	14	–	–	38.6	–	–	64.70%	63.80%
Roh 2004	ITC and micrometastasis	434	24 (5.5%)	14 (40%)	NR	NR	–	NR	71.40%	35.70%	–	–
Rusch 2011	ITC and micrometastasis	NR	NR	130 (22.4%)	NR	NR	–	60	73%	63%	75%	60%
Yasumoto 2003	ITC and micrometastasis	NR	NR	34 (15.7%)	18	16	–	48	85.60%	58.40%	78%	64.70%

Upstaging and survival data from each individual study in which only IHC (in combination with serial sectioning) was used. Reported here are the total number of analysed lymph nodes, total lymph node micrometastasis, total upstaged patients, median follow-up, overall survival, and disease-free survival.

LN, lymph nodes; LNMM, lymph node micrometastasis; OS, overall survival (5 years); DFS, disease-free survival (5 years); NR, not reported; pN1mi, pathologic N1micrometastasis; pN2mi, pathologic N2 micrometastasis.

*Total number of micrometastatic lymph nodes.

†Total number of upstaged patients based on lymph node micrometastasis.

‡Upstaging to pN1 micrometastasis or pN2 micrometastasis according to the additional pathologic evaluation.

Table 4. Upstaging and survival for occult metastasis vs. no occult metastasis in RT-PCR studies

Author and publication year	Total LN analysed	Total LNMM+*	Total upstaged patients†	pN-stage of upstaged patients based on LNMM‡			Median follow-up (months)	OS (5 years)		DFS (5 years)	
				N0 to N1mi	N0 to N2mi	N1 to N2mi		OM−	OM+	OM−	OM+
Benlloch 2008	344	NR	8 (21.1%)	–	8	–	24	70%	25%	73%	25%
Hashimoto 2000	229	34 (14.8%)	9 (29%)	2	4	3	73	100%	35%	–	–
Li 2013	286	48 (16.8%)	15 (34.1%)	8	7	–	62	75%	44%	59%	22%
Li 2008	402	36 (9.0%)	21 (23.6%)	–	21	–	43	44.10%	23.80%	–	–

Upstaging and survival data form each individual study in which only RT-PCR was used to detect occult metastasis. Reported here are the total number of analysed lymph nodes, total lymph node micrometastasis, total upstaged patients, median follow-up, overall survival, and disease-free survival.

LN, lymph nodes; LNMM, lymph node micrometastasis; OS, overall survival (5 years); DFS, disease-free survival (5 years); NR, not reported; pN1mi, pathologic N1micrometastasis; pN2mi, pathologic N2 micrometastasis.

*Total number of micrometastatic lymph nodes.

†Total number of upstaged patients based on lymph node micrometastasis.

‡Upstaging to pN1 micrometastasis or pN2 micrometastasis according to the additional pathologic evaluation.

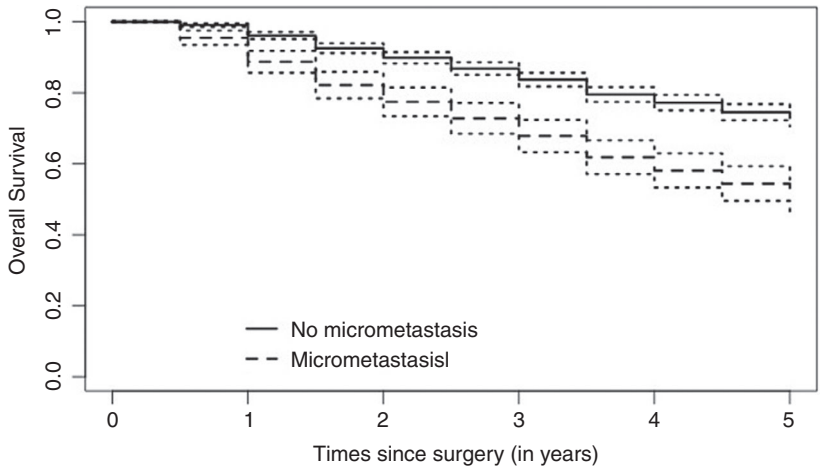

Figure 2. Overall survival along with the 95% confidence interval estimated with the multivariate model for patients with and without micrometastasis. Both RT-PCR and/or IHC studies are included.

with IHC for occult micrometastasis/ITC between 21.4–63% and for no occult micrometastasis/ITC between 61.8–90.9%.

The difference in prognosis between patients with and without lymph node micrometastasis emphasises the importance of the N-status. Current routine care, which is the evaluation of a single H&E slide, is not accurate enough for the detection of small micrometastases and/or ITCs. Occult micrometastases in lymph nodes, which are deemed tumour-free on routine examination, are easily missed and some patients with operable NSCLC could therefore be understaged after surgery.

This meta-analysis showed that between 0.4–20.9% of routinely assessed negative lymph nodes contained micrometastasis or ITC after additional IHC or RT-PCR. None of the included studies investigated the role of micrometastasis found during routine practice with one H&E slide, without additional methods. Two of the included studies used only one

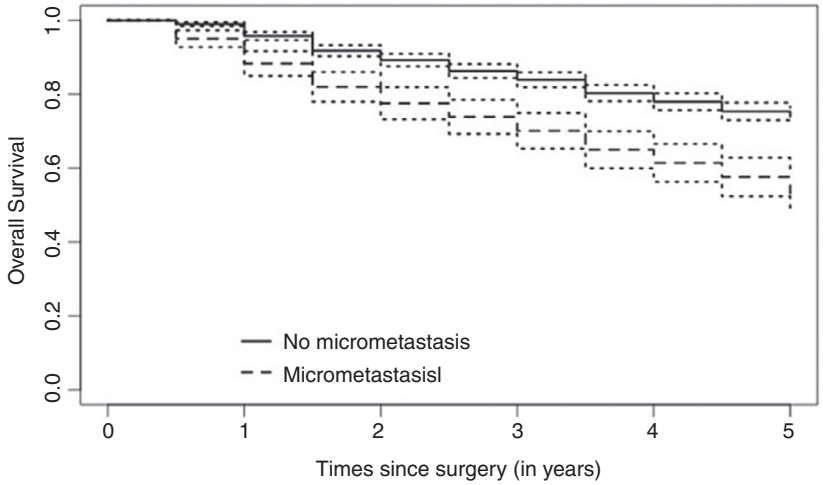

Figure 3. Overall survival along with the 95% confidence interval estimated with the multivariate model for patients with and without micrometastasis. Only studies that used IHC are included.

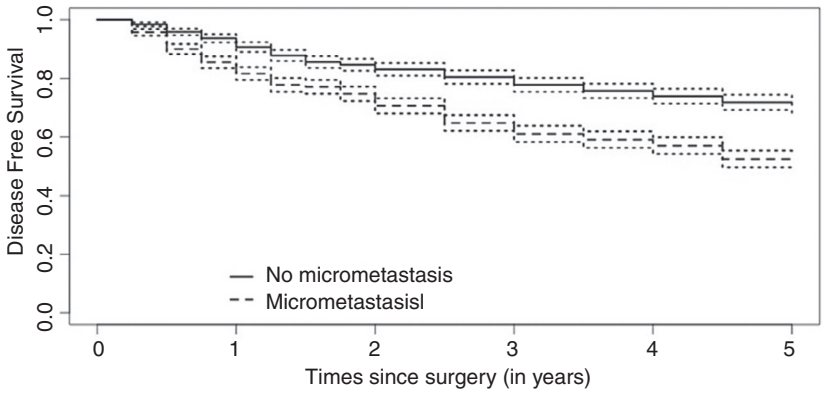

Figure 4. Disease-free survival along with the 95% confidence interval estimated with the multivariate model with and without micrometastasis. Both RT-PCR and/or IHC studies are included.

additional section with IHC (not H&E) and found significantly worse survival.[31,36] As an example, one of the studies reported an OS for patients with N2 micrometastasis/ITC of 50% compared to patients with an N0 of 66.9%.[31] Based on these studies, we expect that if a micrometastasis is found during routine care, it still predicts a worse survival compared to patients with N0. Several studies compared an N1/N2 macrometastasis with an N1/N2 micrometastasis. The study of Maruyama *et al.* found an OS for N1 macrometastasis of 30% compared to N1 micrometastasis/ITC of 83%; likewise, the study of Osaki *et al.* reported an OS for N1 macrometastasis of 50.8% compared to 62.3% for N1 micrometastasis/ITC.[33,40] There were no differences in survival between N2 macrometastasis and N2 micrometastasis/ITC. Based on these studies, we recommend that regardless of the detection method a micrometastasis/ITC should be specifically mentioned in pathology reports if it is found in routine clinical practice, because a micrometastasis/ITC predicts a worse survival compared to N0, and a N1 micrometastasis/ITC predicts a better survival than an N1 macrometastasis.

Based on our results, pathology laboratories may consider implementing a more sensitive method, such as serial sectioning with H&E and/or IHC, as the standard diagnostic method for the evaluation of lymph nodes in patients with NSCLC. Even though serial sectioning and IHC seem to be effective and the most widely accepted method in most of the studies, there is not an accepted standard of how many levels are required to detect all the occult micrometastases.

The number of sections varied between 1 and 10 in the included studies. One previous study showed in patients with breast cancer that the percentage of detected micrometastases was increased with the number of sections compared to standard care, and reached a plateau after six sections with the detection of more than 90% of occult micrometastases.[13]

The implementation of these additional detection methods is important to estimate the survival for these patients with early-stage NSCLC more precisely, and it is also important in the decision for adjuvant or neoadjuvant therapy. Currently, postoperative adjuvant therapy is not recommended for patients with micrometastases/ITCs, but the high recurrence rate in early-stage NSCLC after surgery, which varies between 18–70%, emphasises that there is a need for a more accurate decision model that includes more factors than only the current N-stage based on macrometastases in lymph nodes.[4–9] Lymph node micrometastasis might be one of these factors and could help clinicians in decision making.

The meta-analysis performed in this study was based on a new statistical methodology implemented for the first time in this area. We included a study only if it presented a representative survival curve with OS or DFS data, as described in the inclusion criteria. The advantage of this method is that studies with a single arm can also be included in the meta-analysis. We did not select the studies to perform a traditional meta-analysis with pooled hazard ratios, because we expected to miss studies due to the use of different prognostic factors in their regression model. As an example, of the included studies 14 reported the OS. Of these, only 10 studies reported an adjusted hazard ratio with different risk factors in their Cox model. Only three studies used the same risk factors and the covariates such as the proportion of the tumour size or proportion of the pathologic subtype were not reported at the study level, and therefore we were not able to conduct a traditional meta-analysis or a meta-regression analysis by factor. Studies that did not include a hazard ratio were scored as "fair quality" based on our Downs and Black checklist and their results might include some bias/confounding.

Previous meta-analyses have also shown a significantly poorer OS and/or DFS for patients with micrometastasis compared to no micrometastasis in lymph nodes.[41–43] The analysis by He *et al.* included 18 studies with a total of 1951 patients. They found a hazard ratio of 2.22 (1.87–2.64) for OS and 2.4 (1.71–3.36) for DFS and concluded that the prognosis of patients with micrometastases in lymph nodes was worse compared to patients without lymph node micrometastasis. Similar results were shown by Deng *et al.*[42] and Jeong *et al.*[43] Only Marchevsky *et al.* showed a similar prognosis for micrometastasis vs. no micrometastasis.[44] However, this meta-analysis included a relatively small number of 835 patients and compared studies that used IHC as the detection method for micrometastases.

In the current meta-analysis we found differences in percentages of micrometastasis, upstaging, and survival rates between each individual study. A possible explanation could be the heterogeneity of the study population. Almost all studies included different pT-stages, such as T3 and T4 tumours, but the survival was not separately reported for different T-stages. Based on international therapeutic guidelines such as the ESMO guideline, T3–T4 tumours are considered locally advanced, but are still considered for curatively intended surgical resection along with T1–T2 tumours and are therefore not excluded from our analysis. Another explanation of different rates could be the use of different specimen grossing protocols, and whether the lymph node is sectioned before embedding and whether the lymph node is fragmented.[12,45] Especially if a lymph node is fragmented or sectioned before embedding, the rate of metastasis detection will increase. However, none of the included studies mentioned their protocols or the integrity of the lymph nodes they used.

When the N-stage was corrected according to IHC and/or RT-PCR, the upstaging range varied between the 9.5% up to 70.5%. The lowest percentage of 9.5% was reported in the study of Gwozdz *et al.*, but this study only included preoperative lymph node samples from a transcervical mediastinal lymphadenectomy.[25] They probably included smaller lymph node samples than in the studies using lymph nodes retrieved during surgery. The highest percentage of 70.5% was reported in the study of Maruyama *et al.*; in that study they only included 44 patients, which is a small group and the study was performed in 1997.[40] An explanation for the high rate of upstaging could be centre-specific, as larger metastases could have been missed due to the method of initial evaluation, or it could be that CAM 5.2 is a more sensitive antigen to detect these micrometastases.

This meta-analysis has some limitations. First, due to a lack of data we could not compare survival data for pN1mi vs. pN2mi and between different T-stages. Based on previous studies, we expect an inferior survival for patients with pN2mi compared to patients with pN1mi.[24,25,40,44] It was also unclear if the patients received adjuvant therapy after surgery and

what the effect of such therapy was on their survival; thus, an analysis of, or correction for, the benefit of adjuvant therapy was not possible.

Second, we could not determine what the best method for evaluation of lymph node micrometastasis and ITC is in patients with early and locally advanced stage NSCLC. Most of the included studies used a combination of serial sectioning with IHC and some studies used RT-PCR. The limitations regarding the detection methods (RT-PCR and IHC) are separately summarised in supplementary Tables S3 and S4.

A further important limitation of our meta-analysis was the use of divergent definitions for ITC and micrometastasis in different studies. For our meta-analysis we used the same definition as currently employed in breast cancer: ITC were defined as single tumour cells or nests with a diameter of <0.2 mm and micrometastasis as a cluster of cells between 0.2–2 mm. This definition does not cover RT-PCR studies, but we considered molecular-detected cells as ITC/micrometastasis. Overall, in only 3 out of 11 included studies using IHC, the exact same definition as in breast cancer was used to define micrometastasis. In the remaining eight studies, micrometastases were defined as single cells or clusters of cells missed by routine histopathological evaluation. As a consequence of this definition, missed macrometastases larger than 2 mm could be misclassified as micrometastases. We therefore assume that we may have underestimated survival for patients with micrometastases in our meta-analysis.

Another limitation of this meta-analysis is the possibility of selection bias. This is due to the fact that we excluded studies with a lack of survival curves, since this was necessary to estimate the OS and DFS, as shown in the Results (Figures 2–4).

Furthermore, we expect that we may have introduced reporting bias, as published studies are more likely to show a survival difference between micrometastasis and no micrometastasis, because studies with a lack of significant results are less frequently published. As an example, we counted the studies with, versus those without, a difference in survival between patients with and without micrometastasis, and from the 65 records we previously screened in full-text, only a total of eight studies did not find any difference in survival.[14,34,46–52]

Future prospects

This meta-analysis included 14 studies in which only lymph nodes retrieved during surgery were used in the detection of micrometastasis. Only one study, of Gwozdz *et al.*, included lymph nodes procured during mediastinoscopy, which also showed a significant difference in survival. With the ongoing trend towards neoadjuvant therapy in early-stage lung cancer, it will be increasingly important to detect occult lymph node micrometastasis prior to surgery. Thus, we think in the future there will be a need for larger and prospective studies, especially those investigating lymph node samples obtained before resection of the primary tumour. These analyses could help clinicians in decision making for neoadjuvant therapy, and even in the choice for surgery when pN2 micrometastases are found. We also think that there is a need for studies exploring which pathologic method is the best to evaluate lymph node micrometastasis and ITC in the context of NSCLC, as this important question could not be answered in this meta-analysis. There is also a need for prospective trials in patients with lung cancer with (neo)adjuvant therapy based on pN-micrometastasis-status to investigate the role of systemic therapy in these patients.

Conclusion

This meta-analysis based on 15 studies and 1893 patients shows poorer OS and DFS in patients with stage IA–IIIA NSCLC with lymph node micrometastasis/ITC compared to patients without lymph node micrometastasis/ITC.

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Author Contributions

M. Hüyük: Design and conceptualisation, investigation, writing original draft. M. Fiocco: Methodology, Analysis of data, writing, review, and editing. P.E. Postmus: Supervision, writing, review, and editing. D. Cohen: Design and conceptualisation, supervision, writing, review, and editing. J.H. von der Thüsen: Design and conceptualisation, supervision, writing original draft, review, and editing.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Data Availability Statement

Data sharing not applicable – no new data generated.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Data reconstruction.

Figure S1 Results of the meta-analysis: Overall survival curves in years after surgery for patients with micrometastasis. Only IHC studies are included.

Figure S2 Results of the meta-analysis: Overall survival curves in years after surgery for patients without micrometastasis. Only IHC studies are included.

Table S1 Search strategy.

Table S2 Modified Downs and black checklist.

Table S3 Limitations of IHC in combination with serial sectioning studies.

Table S4 Limitations of RT-PCR studies.