

1 **Lymph node yield after rectal resection in patients treated with**
2 **neoadjuvant radiation for rectal cancer: a systematic review and**
3 **meta-analysis**

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30 radiotherapy, radiochemotherapy, chemotherapy

Summary

Background: The lymph node status (pN) represents a major prognostic factor in colorectal cancer. However, it was demonstrated that neoadjuvant chemoradiotherapy decreases the numbers of lymph nodes in the specimen. The prognostic importance of the number of metastatic lymph nodes in relation to the retrieved remains unclear after radiation. This meta-analysis quantifies the influence of neoadjuvant chemoradiotherapy or radiotherapy on the lymph node yield in rectal cancer patients.

Methods: We performed a systematic review and searched PubMed, Embase and the Cochrane Library without any language restriction from first of January 1980 until 31st March 2015. Two reviewers examined all publications independently and extracted the relevant data if the study assessed lymph node counts or positive lymph node yields of patient who received neoadjuvant treatment compared to patients who did not receive neoadjuvant treatment. Meta-analyses were conducted to quantify the mean difference in lymph node yield.

Results: A total of 34 articles (including 37 datasets) were included in the meta-analyses. Neoadjuvant chemoradiotherapy resulted in a mean reduction of 3.9 lymph nodes (95% CI 3.7 to 4.1) and an average reduction in harvested positive lymph nodes of 0.7 (95% CI 0.2 to 1.2) compared to patients who received no neoadjuvant therapy. Individuals who received neoadjuvant radiotherapy had, in average, 2.1 lymph node less (95% CI 1.7 to 2.5) resected compared to their counterparts who received no neoadjuvant treatment.

Conclusions: Neoadjuvant chemoradiotherapy or radiotherapy only in rectal cancer patients leads to a decrease in lymph node harvest of approximately four and two lymph nodes, respectively. Therefore, it is challenging to reach the requirement of the

57 current guideline of twelve resected lymph nodes. We therefore stress the
58 importance of intensifying all efforts from involved subspecialties (i.e. surgeons and
59 pathologists) to reach the benchmark harvest 12 resected lymph nodes according to
60 current guidelines.

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1. Introduction

With an incidence of 1.4 million cases in 2012, colorectal cancer (CRC) represents one of the most common cancers worldwide [1], second most diagnosed in females and third in males. Despite a decrease over the last centuries - most likely due to enhanced screening, reduced prevalence of risk factors and/or improved treatment [2-4] - the mortality rate with 693'000 deaths worldwide in 2012 remained high [1]. The global burden was estimated to be as high as 14.4 disability-adjusted life years [5], and the lifetime probability of developing invasive CRC in the United States was assessed to be between 4.4 and 4.7% [4].

In rectal cancer, neoadjuvant chemo-radiotherapy (CRT) followed by curative surgery including total mesorectal excision has become standard of care for the International Union against Cancer (UICC) stage II or III [6]. This way a systematic lymphadenectomy can be performed, resulting in the lymph node status (pN) which is defined as the number of tumor-infiltrated lymph nodes [7]. The pN status represents a major prognostic factor and the total number of resected lymph nodes correlates significantly with relapse of disease and overall survival [8-12]. Beside showing an adequate oncologic surgery [13], it additionally represents a quality parameter and plays an essential role in management decisions concerning adjuvant treatment protocols [14]. However the prognostic importance of the number of metastatic lymph nodes in patients who have only a small number of retrieved lymph nodes compared to patients who have several lymph nodes retrieved after radio(chemo)therapy remains not entirely clear.

The current guidelines from the American Joint Committee on Cancer (AJCC) and the UICC defined a minimum of 12 lymph nodes to be examined to reach an appropriate pN staging to avoid understaging [7, 15, 16]. While the extent of tumor

resection in colorectal cancer is well defined and should not differ dependent on the application of a neoadjuvant radio(chemo)therapy [14], the numbers of retrieved lymph nodes varies between reported patient series. Chou et al [17] even demonstrated that only 49% of patients undergoing surgery for colorectal cancer met the suggested standards concerning lymph node yield.

While neoadjuvant CRT and radiotherapy (RT) only has been shown to induce shrinkage of the tumour and improved local control [18-20], several studies demonstrated a decrease of the number of lymph nodes examined in these pre-treated mesorectal specimens [21-26].

Given the importance of the lymph node status and its implication on outcome we performed the first series of meta-analyses to quantify the influence of preoperative CRT and RT in rectal cancer patients on number of retrieved lymph nodes in the specimens.

2. Methods

2.1. Search Strategy and inclusion criteria

For this systematic review we adhered to the Meta-analysis of Observational Studies (MOOSE) guidelines [27] and the Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) statement [28] (appendix). The study protocol for this systematic review and meta-analysis can be reviewed in the appendix. In brief, Pubmed, EMBASE and the Cochrane Library were systematically searched for relevant studies from January 1, 1980 until March 31, 2015, without language restrictions. We used the MeSH terms and keywords “lymph node”, and “lymph node ratio”. These terms were combined with “colorectal cancer”, “rectal cancer”, “rectal carcinoma”, and “rectal resection”. This search was then again combined with the MeSH terms and keywords “preoperative therapy”, “preoperative radiation”, “preoperative chemoradiotherapy”, “preoperative radiotherapy”, “preoperative radiochemotherapy”, “neoadjuvant therapy”, “neoadjuvant radiation”, neoadjuvant chemoradiotherapy”, “neoadjuvant radiotherapy”, and “neoadjuvant radiochemotherapy”. Regardless of study type, publications were eligible for inclusion if they compared quantitative data on lymph node harvest in resected specimen of human rectal cancer patients between individuals who received preoperative RT only or CRT *versus* patients who received no neoadjuvant therapy.

In the first step, all identified titles and abstracts were examined by two independent reviewers (RM and BS). In the second step, the same two reviewers independently examined the full text of potentially relevant articles. The eligibility of the studies was assessed using the study protocol (appendix). In case of disagreement, a third reviewer (RR) was consulted and the respective article discussed until consensus was reached.

2.2. Data extraction and quality assessment

The following relevant information was extracted from all included publications: study design, period of subject enrolment, country of enrolment, neoadjuvant therapy (i.e. CRT or RT only), dose of RT, if a total mesorectal excision (TME) was performed, the time which passed between the last neoadjuvant therapy and the operation, total number of patients with neoadjuvant therapy, and total number of patients without neoadjuvant therapy. The effect measure was the mean difference of lymph node yield between patients who received neoadjuvant RT only or CRT compared to patients who received no neoadjuvant therapy. A secondary outcome was to compare the number of positive lymph nodes among patients who received neoadjuvant RT only or CRT compared to patients who received no neoadjuvant therapy. Therefore, the following data was extracted if available: mean number of lymph nodes examined (including standard deviation (SD) or 95% confidence interval (CI); stratified by neoadjuvant treatment vs. no treatment), median or mean number of positive lymph nodes examined (including SD or 95% CI; stratified by neoadjuvant treatment vs. no treatment). In case no means or standard deviations were reported, available information on medians, inter quartile ranges, ranges and confidence intervals were used to estimate these quantities [29, 30]. All data were independently extracted in duplicate by two authors (RM and BS) and compared for consistency. Inspired by the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) approach [31], we developed a panel of criteria to assess the quality of the included studies. Our criteria focus on study design, characteristics of neoadjuvant therapy, and potential bias. With regard to study design, a study was assigned two points if patients were randomly allocated to either neoadjuvant therapy or no adjuvant therapy. One point was given for retrospective studies which matched the patients. Simple retrospective studies or cohort studies received zero points. With

regard to study characteristics we assigned one point if the radiation dose was stated, one point was given if TME was mentioned and a further point was assigned if the appropriate time between neoadjuvant therapy and the operation had passed. We defined the appropriate time as 4-8 weeks after treatment for long course radiation (40-65 Gy) [19] and maximum 7 days for short course radiation (25 Gy) [6]. Finally, for some studies a point was subtracted if there was evidence for a potential bias (e.g. "all patients enrolled from 1988-1997 did not receive neoadjuvant therapy; all patients from 1997-2001 received neoadjuvant treatment [32]. Publications with a total score of four to five points were considered to be of high quality; two to three points indicating moderate quality, whereas scores from minus one up two one indicate low quality. The quality assessment is descriptive and hence no studies were excluded due to a low quality score.

2.3. Statistical analysis

Meta-analyses were performed with the use of R software, version 4.3-2. (www.r-project.org) using the *meta* package [33]. Differences in number of resected lymph nodes and positive lymph nodes between the respective study arms were subsumed as pooled mean differences (MDs) with 95% confidence intervals (CIs) based on fixed and random effects models [34, 35].

In addition, 95% prediction intervals were reported. Prediction intervals estimate the range of effects (mean differences) expected to be observed in future individual studies within the same population as defined by the studies included in the meta-analysis. Complementary effect heterogeneity assessments were performed using forest plots and the inconsistency statistics (I^2). Conclusions regarding evidence of effects were based on confidence and prediction interval limits rather than on statistical tests.

180 In order to allow for a meaningful assessment of effect heterogeneity, supporting
181 subgroup meta-analyses (e.g. stratified by treatment duration) were only conducted if
182 data from at least three independent studies were available.

183 Potential for publication bias was assessed using funnel plots and related symmetry
184 statistics, following the Cochrane recommendations on testing for funnel plot
185 asymmetry [36].

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3. Results

After removing duplicates, our computer-aided search yielded 2388 publications from Pubmed, Embase, and the Cochrane Library (figure 1). Based on the titles and abstracts 109 articles were classified as potentially relevant. From these studies 75 were excluded after consulting the full text resulting in 34 articles (including a total of 37 datasets) met our inclusion criteria and were therefore included in our meta-analysis (figure 1).

The characteristics of the included studies are described in table 1. In short, most of the included papers (n=29) were simple retrospective studies [21, 24, 25, 32, 37-61], while two retrospective studies used a matched case-control design [62, 63]. Furthermore, two included papers present a randomised controlled trial [64, 65] and one was a prospective cohort study [66]. Patient enrollment dates back until 1980 [38]; all studies were published after the millennium. According to our quality grading, four studies were of high quality (4 or 5 points) [62-65]. Seventeen studies had a score of 2 and 3 points indicating moderate quality, while the remaining studies (n=13) had lower quality scores (for more details on potential bias see appendix).

The majority of the retrieved studies (n=28) assessed the difference in lymph node yield between neoadjuvant CRT and no neoadjuvant therapy. Nine out of these 28 studies presented also data about positive resected lymph nodes. Six publications presented the lymph node yield between neoadjuvant RT and no neoadjuvant treatment. From those six studies only one publication presented data about positive resected lymph nodes. Therefore no meta-analysis for this group was performed.

Forest plot on the effect of CRT and RT only on the number of resected lymph nodes and number of resected positive lymph nodes are presented in figure 2-4. For the two meta-analyses including at least 10 studies (Figures 2 and 4), neither visual

inspection of funnel plots nor Egger's test indicated presence of publication bias (linear regression test of funnel plot asymmetry: $p=0.8$ for both assessments). The fixed effect model indicated that patients who receive neoadjuvant CRT yield 3.9 less lymph nodes compared to patients who receive no neoadjuvant treatment (95% CI -4.1 to -3.7, figure 2). The result based on a random effect model is similar (mean difference -3.9; 95% CI -4.5 to -3.2). The prediction interval suggests for future studies expectable differences in the mean number of resected lymph nodes between -6.9 and -0.8. This fairly large range in expected mean differences is due to the observed high heterogeneity of effects across the included studies (inconsistency index $I^2=79\%$). For RT only individuals who received preoperative treatment had in average 2.1 lymph node less resected compared to their counterparts who received no neoadjuvant treatment (fixed effect model: 95% CI -2.5 to -1.7; random effects model: 95% CI -2.5 to -1.7, figure 3). Both prediction interval (-1.5 to -2.8) and heterogeneity index ($I^2=7\%$) indicate consistency of effects across the included studies. Furthermore, our meta-analysis indicates that individuals who receive neoadjuvant CRT had in average less positive lymph nodes compared to patients who received no neoadjuvant treatment before operation (fixed effect model: mean difference -0.7; 95% CI -0.9 to -0.5; random effects model: mean difference -0.7; 95% CI -1.2 to -0.2, figure 4). Nevertheless, given this relatively small average difference and the high observed effect heterogeneity ($I^2=80\%$), the relatively wide prediction interval (-2.8 to +0.9) indicates that future studies may produce results that are inconsistent with the estimated overall effect. Subgroup analysis examining the effect of short course and long course radiation revealed no difference compared to the merged results (Table 2 and appendix). [The effect of lymph node retrieval on the overall survival was assessed by only nine studies in a highly heterogeneous way. Therefore these findings are only mentioned exploratory within the discussion.](#)

Discussion

This systematic review and meta-analysis included 37 datasets from 34 studies and analysed the influence of a neoadjuvant CRT or RT only on total and metastatic lymph-node harvest in rectal cancer specimens. We clearly identified a mean decrease of resected lymph nodes of 3.9 in the group of CRT and a mean decrease of 2.1 in the RT only group. In addition we demonstrated that neoadjuvant CRT lead to a significant decrease of positive lymph nodes (mean 0.7) as compared to patients who received no CRT before operation. For RT only no meta-analysis on positive lymph nodes could be performed as we identified only one study which presented data. In short, Marijnen et al., [64] found a mean of 1.6 (standard deviation 3.6) in patients who received neoadjuvant radiotherapy and 1.9 positive lymph nodes (SD 4.1; $p=0.11$) without neoadjuvant treatment.

The lymph node harvest is influenced by several factors including patients' anatomical and physiological factors the extent and technique of surgical dissection, and the pathologist's workup [26, 67, 68]. We present now clear evidence that neoadjuvant CRT and RT only decreases the number of harvested lymph nodes. The vast majority of studies found a negative mean difference, indicating that individuals who received neoadjuvant treatment had a lower lymph node yield. Only Amajoyi et al. [52] (impact of RCT on total lymph node harvest in patients above 65 years) and Taflampas et al [41] (impact of long course RCT on tumour infiltrated lymph node harvest) found a nonsignificant increase while McFadden et al. [55] and Kim et al. [58] reveal a mean difference of exactly zero. Interestingly, each separate study which could be included in the meta-analysis on the effect of RT only revealed a significant negative mean difference, indicating that less lymph nodes are harvested after preoperative radiation. Nevertheless, our analyses found a stronger effect for

CRT with a total reduction of approximately 4 lymph nodes compared to a reduction of roughly 2 lymph nodes when using preoperative RT only. However, as these results are from different studies, it is difficult to draw a clear conclusion. Bujko and colleagues [69] studied the difference of preoperative short course RT compared to conventional CRT while Bosset and colleagues [18] compared neoadjuvant long course RT and CRT. Both could not show any difference in long term disease free-survival and overall survival, respectively. Furthermore, the meta-analysis assessing the effect of RT only on lymph node yield was dominated by three large studies (from a total of six studies) [39, 51, 64]. However, our analyses showed no indication of heterogeneity (Figure 3).

While chemotherapy in the context of a neoadjuvant CRT has the effect of radio sensitising [70, 71], the mechanism leading to a reduced lymph node yield is a radiotherapy-induced shrinkage and fibrosis, as well as lymphocyte depletion, atrophy of the stroma and replacement by adipocytes [72, 73]. We believe that these changes make lymph nodes difficult to detect and unrecognizable during pathological examination.

Guidelines define a minimum of 12 lymph nodes to be examined in both colon and rectal cancer specimens to reach an appropriate pN staging to avoid understaging [7, 15, 16]. Despite increasing yields observed over time [74], patients with rectal cancer and older patients who had distally located, early colon cancer were less likely to meet the benchmark harvest of 12 lymph nodes [17]. The application of a neoadjuvant treatment will therefore decrease this number additionally. Therefore all involved personnel should intensify their effort to acquire at least 12 lymph nodes. In the studies included in our meta-analyses nine investigated the relation of a guideline conform lymph node harvest on survival. McFadden [55], Damin et al. [48], Roullier et al. [40], Ha et al. [25], Doll et al. [32], Klos et al. [43] and La Torre et al. [53] failed

to demonstrate a significant association between a cut off level of 12 lymph nodes on overall and/or disease free survival when comparing patients who received neoadjuvant CRT. Persiani and colleagues [60] even tried to determine a new cut-off value for the number of retrieved lymph nodes to estimate differences in overall survival. However, they found no statistically significant difference in survival between subgroups. Kim HJ et al. [58] found an increased 3 year disease free survival when less than 12 lymph nodes were retrieved but solitarily among patients with good tumor response following neoadjuvant CRT. As a consequence some authors suggest that a lymph node harvest of twelve or more lymph nodes should not be considered for cancer-specific prediction in neoadjuvant treated patients and other parameters beside pN status, such as lymph node ratio should be further evaluated to give a more accurate prediction of prognosis [53, 61, 76, 77].

Subgroup analyses differentiating between long course and short course therapy revealed no clinical relevant difference in the estimated pooled effects. However, in these sub analyses, only a few studies were excluded from the respective overall meta-analyses; hence it is difficult to draw a definite conclusion. The meta-analyses comparing outcomes between neoadjuvant CRT and no neoadjuvant therapy revealed substantial effect heterogeneity, i.e. high inconsistency indices and wide prediction intervals. These findings caution that the pooled averages and associated confidence intervals may not well represent expectable effects in the entire underlying population.

The most important limitation of the presented evidence is that most of the included studies were, according to our rating score, of moderate or low quality. This finding is mainly due to the reason that most of the included data derived from retrospective studies. These studies are prone to introduce bias. For example, Doll and colleagues [32] stated that the standard procedure in the time period from 1988 until 1997 was to

give no neoadjuvant treatment while all patients selected from 1997 until 2001 received neoadjuvant CRT. This approach most certainly introduces a bias as for example different surgeons and pathologists are present, and methodologies improve over time. Other studies mentioned that only patients with five or more lymph nodes were included [57] or that a second examination was performed in case that less than twelve lymph nodes were retrieved [40]. However, none of these biases should favor decrease lymph node retrieval in patients who retrieve neoadjuvant therapy. A further limitation of our study is that we only have merged data and no individual patient data (IPD). Conducting a IPD meta-analysis would result most likely in more comprehensive results; nevertheless receiving these data has several obstacles [78].

In conclusion, we demonstrated that (i) neoadjuvant CRT and RT only in rectal cancer patients leads to an average decrease in lymph node harvest of approximately four and two lymph nodes, respectively; and (ii) that neoadjuvant CRT resulted in a pooled mean difference of positive lymph node yield of not more than 1.2, possibly only 0.2. Although the current recommendation in colorectal cancer surgery suggests the analysis of at least 12 lymph nodes, our result demonstrate that a considerable number of studies did not reach this aim after surgery Therefore we recommend that all involved subspecialities (i.e. surgeons and pathologists) intensify their efforts to reach the benchmark harvest of 12 resected lymph nodes according to current guidelines.

Authors Contribution

RM, RR and BS designed the study; RM and BS produced literature search and data collection; RM, BS, and RR conducted the data comparison, BS, RM, TS and RR

340 analysed and interpreted the data; RM and BS wrote the first draft of the manuscript;
341 TS and RR revised the manuscript. All authors read and approved the final version of
342 the manuscript.

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344 **Conflict of interest statement**

345 The authors declare that no conflict of interest exists.

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Figure 1 Study selection

Figure 2 Meta-analysis comparing lymph node yield in patients with neoadjuvant chemo-radiotherapy versus patient without neoadjuvant therapy

† Patients who are under 65 years. ‡ Patients who are above 65 years. ⋈ Pathologic workup: fat clearance ⋈ Pathologic workup: acetone compression ⋈ Short course radio-chemotherapy (25 Gy) ⋈ Long course radio-chemotherapy (50.4 Gy)

Figure 3 Meta-analysis comparing lymph node yield in patients with neoadjuvant radiotherapy versus patient without neoadjuvant therapy

Figure 4 Meta-analysis comparing assessment of positive lymph nodes yield in patients with neoadjuvant chemo-radiotherapy versus patient without neoadjuvant therapy

⋈ Short course radio-chemotherapy (25 Gy) ⋈ Long course radio-chemotherapy (50.4 Gy)

Table 1: Characteristics of studies examining the association between neoadjuvant treatment and lymph node yield

	General information							Information about quality rating			
	Study design	Period of subject enrolment	Country of subject enrolment	Neo-adjuvant therapy	Dose of radiotherapy (in Gy)	Total mesorectal excision (TME)	Time between last neoadjuvant therapy and operation	Study design (Randomization=2 Matching=1 Retrospective or cohort study=0)	Characteristics of neoadjuvant therapy (Dose stated=1 TME=1 appropriate time of OP=1)	Potential Bias (Indication=-1)	Total points
Ahn et al (2012) [47]	RS	2005-2008	South-Korea	CRTX	50.4	yes	6-8 w	0	3	-1	2
Amajoyi et al (2013) [52]	RS	1990-2010	USA	CRTX	45	yes	N.S.	0	2	-1	1
Chandrasinghe et al (2014) [57]*	RS	1997-2007	Sri Lanka	CRTX	N.S.	N.S.	N.S.	0	0	-1	-1
Damin et al (2012) [48]	RS	2005-2010	Brazil	CRTX	50	yes	6-8 w	0	3	0	3
De la Fuente et al (2009) [24]*	RS	1997-2006	USA	CRTX	50.4	yes	N.S.	0	2	0	0
Deodhar et al (2012) [49]	RS	2010-2011	India	CRTX	N.S.	N.S.	N.S.	0	0	0	0
Doll et al (2008) [32]	RS	1988-2001	Germany	CRTX	45	yes	4-5 w	0	3	-1	1
Edler et al (2007) [39]	RS	1991-1997	Sweden / Denmark	RTX	5x5	N.S.	N.S.	0	1	0	1
Gehoff et al (2012) [50]*	RS	2006-2009	Germany	CRTX	50	yes	N.S.	0	2	0	2
Good et al (2011) [45]	RS	2005-2010	Ireland	RTX	N.S.	N.S.	6-8 w	0	1	0	1
Ha et al (2010) [25]	RS	2002-2006	South-Korea	CRTX	45 + 5.4 boost	yes	4-8 w	0	3	0	3
Kang et al (2012) [62]	RS MC	1997-2008	Korea	CRTX	45 + 5.4-9 boost	yes	6-8 w	1	3	0	4
Kim et al (2015) [58]	RS	2004-2012	South-Korea	CRTX	45 - 50	N.S.	6-8 w	0	2	0	2
Kim et al (2015) [63]	RS MC	N.S.	South-Korea	CRTX	50	yes	6-8 w	1	3	0	4
Klos et al (2010) [43]	RS	2000-2008	USA	CRTX	45/50.4	N.S.	4-8 w	0	2	0	2
La Torre et al (2013) [53]	RS	2003-2011	Italy	CRTX	45 + boost 10	yes	N.S.	0	2	0	2
Le et al (2012) [51]	RS	1988-2006	USA	RTX	N.S.	N.S.	N.S.	0	0	0	0
Lykke et al (2013) [54]*	RS	2001-2011	Denmark	CRTX	N.S.	N.S.	N.S.	0	0	0	0
Marijnen et al (2001) [64]*	RCT	1996-1999	Netherlands	RTX	5x5	yes	≤10 d	2	2	0	4
Maschuw et al (2006) [37]*	RS	1997-2002	Germany	RTX	5x5	yes	N.S.	0	2	0	2
Maurer et al (2012) [66]*	CS	2007-2010	Germany / Switzerland	CRTX	50	yes	6 w	0	3	0	3
McFadden et al (2013) [55]*	RS	1995-2005	USA	CRTX	N.S.	N.S.	N.S.	0	0	0	0
Morcos et al (2010) [44]*	RS	2003-2008	Jordan	CRTX	45	yes	6-8 w	0	3	0	3
Okada et al (2014) [59]	RS	1991-2010	Japan	CRTX	40 or 45	yes	6-8 w	0	3	0	3
Park et al (2015) [61]	RS	2000-2009	South-Korea	CRTX	50	yes	6-8 w	0	3	0	3
Persiani et al (2014) [60]*	RS	1992-2011	Italy	CRTX	50-55	yes	>8 weeks	0	2	-1	1
Rivard et al (2011) [46]	RS	2006-2008	Canada	RTX	N.S.	N.S.	N.S.	0	0	0	0
Rullier et al (2008) [40]	RS	1994-2004	France	CRTX	45	yes	6 w	0	3	-1	2
Scabini et al (2013) [56]	RS	2005-2012	Italy	CRTX	45	yes	8 w	0	3	0	3
Sermier et al (2006) [38]	RS	1980-2004	Switzerland	CRTX	45-50	yes	49(4-229)d	0	2	-1	1
Taflampas et al (2009) [41]	RS	1995-2007	Greece	CRTX (long course and short course)	long course: 50.4 Short course: 5x5	yes	long course: 4-6w short course: 3-4d	0	3	0	3

Wang et al (2009) [42]*	RS	1998-2007	China	CRTX	45-65	yes	3 w - 3 m	0	2	0	2
Wichmann et al (2002) [21]*	RS	1996-2001	Germany	CRTX	45	yes	4-6 w	0	3	0	3
Wijesuriya et al (2005) [65]	RCT	N.S.	Sri Lanka	CRTX	45	yes	4-6 w	2	3	0	5

RS=retrospective study ; RS MC=Retrostpective study with matched cases; RCT=Radomised controlled trial; CS=Cohort study CRTX=Neoadjuvant chemoradiotherapy; RTX=Neoadjuvant chemotherapy; N.S.= Not stated; d=day; w=week; m=month; TME=Total mesorectal excision; OP=Operation; * = studies where standard deviations and/or means were derived from reported standard errors, ranges or quartiles.

Table 2: Results of subgroup analysis examining the effect of neoadjuvant short course and long course radiation.

	Lymph node yield: CRT vs. no CRT			Lymph node yield: RT vs. no RT			Positive lymph node yield CRT vs. no CRT					
	n	mean difference random effects model (95% CI) [95% prediction interval]	<i>I</i> ²	n	mean difference random effects model (95% CI) [95% prediction interval]	<i>I</i> ²	n	mean difference random effects model (95% CI) [95% prediction interval]	<i>I</i> ²	n	mean difference random effects model (95% CI) [95% prediction interval]	<i>I</i> ²
Overall	31	-3.85 (-4.52 to -3.18) [-6.91 to -0.79]	78.6	6	-2.10 (-2.49 to -1.72) [-2.75 to -1.46]	6.5	10	-0.70 (-1.17 to -0.22) [-2.28 to +0.88]	80.2	1	-	-
Only long course therapy	26*	-4.13(-5.00 to -3.27) [-8.01 to -0.26]	76.8	0*	-	-	9	-0.70 (-1.22 to -0.18) [-2.40 to +1.00]	82.4	0	-	-
Only short course therapy	1*	-	-	3*	-2.06 (-2.54 to -1.58) [-5.19 to +1.07]	0.0	1	-	-	1	-	-

*Studies which did not specify if the treatment regime were not categorised as long- or short- course therapy. CRT=neoadjuvant chemoradiotherapy; RT=neoadjuvant radiotherapy