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Review

The impact of methylene blue in colorectal cancer: Systematic review and meta-analysis study

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ABSTRACT

Purpose: In patients with colorectal cancer (CRC), the most important factor to decide the need of adjuvant chemotherapy is the histological lymph node (LN) evaluation. Our work aimed to give a broad view over the use of methylene blue and its consequences in the number of lymph node harvest.
Methods: PUBMED, WEB OF SCIENCE and EMBASE databases were consulted, retrieving clinical trials, which mentioned the used of intra-arterial methylene blue in patients with colorectal cancer.
Results: Eighteen clinical trials analyzing the use of intra-arterial methylene blue in specimens of colorectal cancer were selected. The articles show a statistical difference between the use of methylene blue and the classical dissection in both variable at study. The results of the statistical analysis of the lymph node harvest variable demonstrate a significant statistical difference between the group that received methylene blue injection and the group that underwent conventional dissection. There is a significant statistical difference between the experimental and control groups for the ideal lymph node harvest (lymph node harvest count greater than 12).
Conclusion: The use of intra-arterial methylene blue revealed a high potential for the quantification of lymph nodes, considering the increase of lymph node harvest and the higher percentage of cases with more than 12 lymph nodes count, albeit the high heterogeneity between the studies in terms of reported results. Future investigations with controlled double blinded studies obtaining better categorized results should be conducted in order to better evaluate this technique and compare it to the current paradigm.

1. Introduction

In patients with colorectal cancer (CRC), the decision for or against adjuvant chemotherapy is mainly based on the results of the histological lymph node (LN) evaluation [1]. Adjuvant therapy significantly reduces mortality and the risk of recurrence in stage III (T1–4, N1–2, M0) colorectal cancer relative to surgery alone [2]. Stage III cancers, which are defined by LN metastases, are generally treated with adjuvant chemotherapy [3]. In fact, patients with positive LNs showed a poorer prognosis than patients without metastatic LNs [4]. In Stage I and II the 5-year survival rates are between 82 and 93%, decreasing to 59% in the presence of lymph node metastases (Stage III) [5]. Additionally, it is also known that 20% of stage II cancers show an unexpected aggressive clinical course and these patients benefit of adjuvant therapy [1] Among patients who have intended curative surgery, the relapse rate with local and/or distant metastases are as high as 30% depending on the stage of cancer [6]. The high relapse rate indicates that adequate and accurate lymph node assessment is crucial for histopathological staging, and therefore in prognosis estimation and treatment stratification, in colorectal cancer [6].

In this context, the correct analysis of lymph node status is one of the most important parameters. For an accurate evaluation of lymph node status, the UICC (International Union Against Cancer) recommends examination of a minimum of 12 lymph nodes in colorectal cancer resection specimens, although recommendations published in the past differ considerably in a range from 9 to 18 lymph nodes [3,7]. Despite these recommendations, it has been reported that this minimum number of LNs are not detected or examined in some colorectal specimens [8]. The harvest is especially negatively influenced by neoadjuvant radiochemotherapy in rectal cancer patients [9]. Understaging in colorectal

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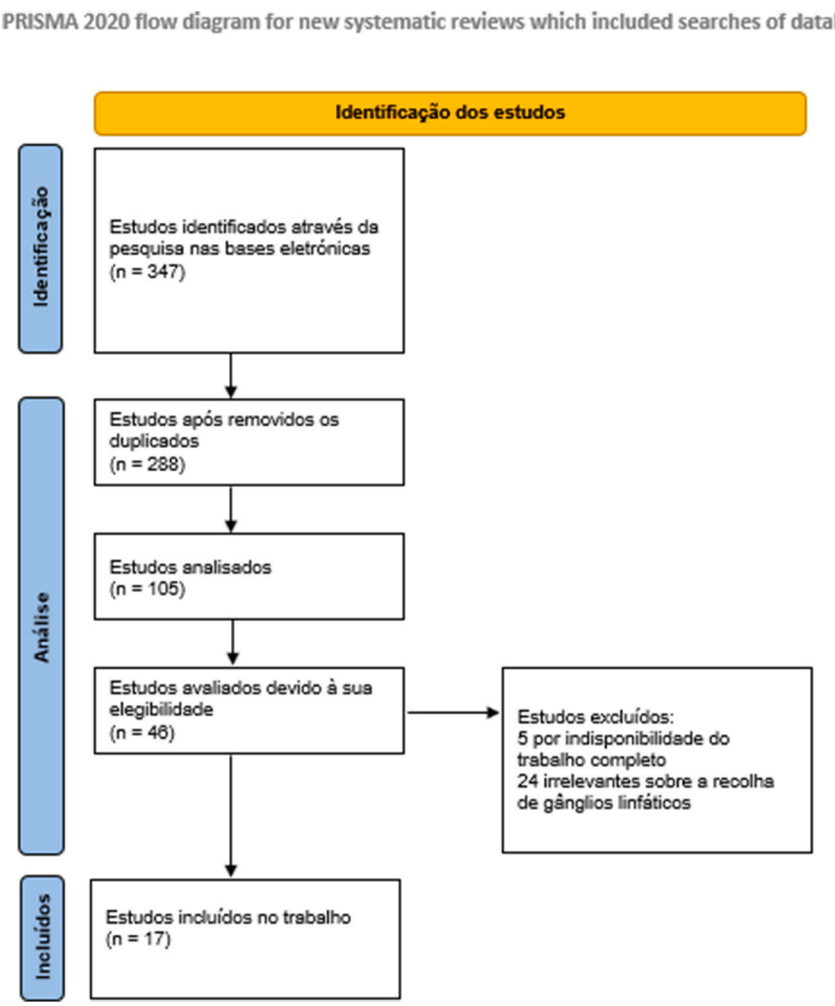

Fig. 1. Summary of data collection process.

cancer is suggested to result from an insufficient number of recovered lymph nodes at pathological examination [2]. This hypothesis is known as the stage migration theory [2]. An insufficient assessment of lymph nodes may leave undetected metastases [4]. Patients diagnosed with stage II cancer and with few examined lymph nodes may, in fact, have stage III cancer [2]. This may cause inadequate treatment post-operatively with a detrimental effect on patient outcome [2]. Increasing age, American Society of Anesthesiology grade, and preoperative radiotherapy are found to be factors for reduced lymph node harvest [7]. Transverse colectomy and abdominoperineal resected rectal specimens are the resections that show the lowest numbers of detected lymph nodes [7].

Manual palpation of the surgical specimen is the standard technique used by histopathologists, but this may miss smaller nodes and it is known that nodes smaller than 5 mm in diameter may account for up to half of metastatic nodes present [10]. To overcome this limitation, several techniques to improve the dissection and analysis of a greater number of lymph nodes in surgical specimens of CRC and enhancing the lymph node staging have been developed [11]. Fat-clearing protocols (this technique is time consuming, expensive and involves potentially hazardous substances), compression techniques, and the methylene blue-assisted LN dissection (MBLND) method have been shown to be very effective in enhancing LN detectability [12]. Using MBLND, a past study reported a rate of adequate LN staging of 98% and a mean LN number of 34.9 [12]. For the injection, the main artery (ileocolic, middle colic or inferior mesenteric artery) was identified and the clip or ligature was cut off. The artery was opened longitudinally to facilitate cannulation with a standard 16- or 17-gauge intravenous catheter without a steel mandrin. To seal the catheter in the artery, a clamp was fixed beside the artery in parallel orientation. The success of the gentle injection of 15–20 mL of methylene blue solution (50 mg diluted with 0.9% saline; ratio 1:3) can be observed by instantaneous blue staining of the specimen's serosal layer.

This revision is important because so far there is not any work that compare the efficacy of MBLD compare to the standard method, and the conclusions of this work may change the way the majority of pathologists management the patients with colorectal cancer.

2. Methods

This review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [13,14].

2.1. Eligibility criteria

All studies regarding the use of MBLD for the lymph node harvest in patients with colorectal cancer were considered. Only trials conducted in humans, published in English, reporting original results were selected. Conference abstracts, reviews, commentaries, case reports and book chapters were excluded.

2.2. Information sources

Studies were identified by searching the electronic databases

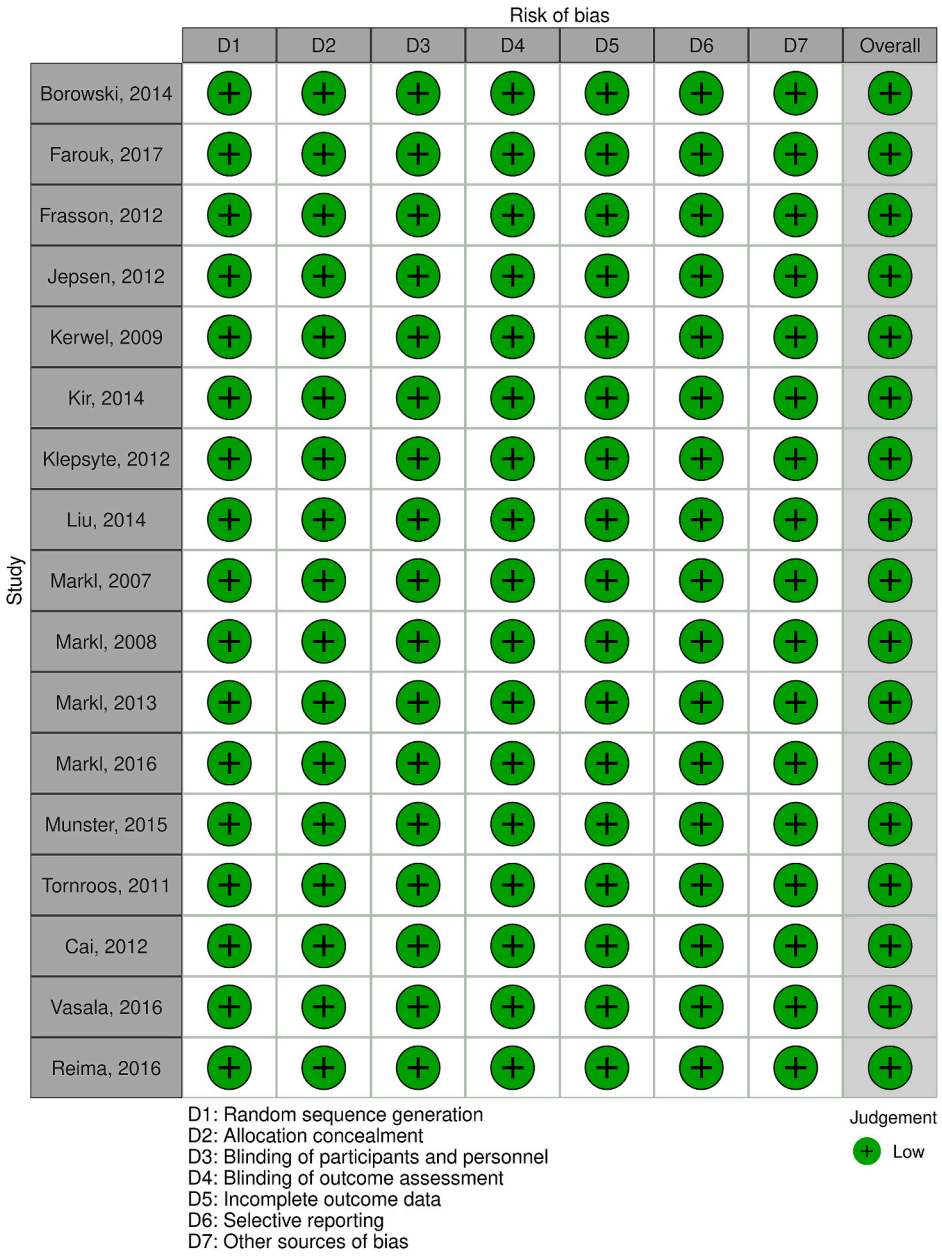

Fig. 2. Summary of the results of the quality of studies.

PUBMED, WEB OF SCIENCE and EMBASE. This search was last conducted by the authors on 23th of may 2023.

2.3. Search

The following setup of search terms was used for PUBMED: "("colorectal cancer"[MeSH Terms] OR "colorectal cancer"[All Fields]) AND ("lymph node"[MeSH Terms] OR "lymph node"[All Fields] AND ("methylene blue"[All Fields] OR "methylene blue"[MeSH Terms]").

The following setups of search terms were used for EMBASE: "methylene blue" and "colorectal cancer" and "lymph node".

The following setups of search terms were used for WEB OF SCIENCE: "methylene blue" and "colorectal cancer" and "lymph node".

2.4. Study selection

The authors performed an eligibility assessment. In case of questionable eligibility, the results were discussed among all authors. All trials were included, regardless of the existence and type of a comparative group. The primary outcome measure was the impact of the intra-arterial MBLD on lymph node harvest. Articles that did use MBLD pared with other surgical procedures to identify sentinel lymph node were excluded. Articles that used MBLD peri-tumoral were also excluded. Articles not in english were also excluded.

2.5. Data collection process

We developed a data extraction sheet with the descripted data of each report, adding new parameters throughout the analysis as soon as new data was found. All data extracted by the authors was reviewed twice to avoid errors. In cases of uncertain validity, the results were discussed among all authors. Studies from the same research group or group of authors were carefully analyzed to avoid double counting the same data.

Table 1
Summary of demographic and clinical information of the included studies.

Study	Country	Sample Size		Gender (M |F)		Mean age (years)	
		Experimental	Control	Experimental	Control	Experimental	Control
Cai et al., 2012	China	20	20	14 | 6	13 | 7	58.9 ± 17.8	64.9 ± 7.4
Farouk et al., 2017	Egypt	40	30	26 | 14	18 | 12	52.35 ± 14.84	50.60 ± 12.62
Frasson et al., 2012	Spain	34	473	16 | 18	251 | 222	69.2 ± 10.3	70.5 ± 11.6
Jepsen et al., 2012	Denmark	234	194	1 : 1.1	1 : 1	69.9 ± 10.9	69.6 ± 10.5
Kerwel et al., 2009	Germany	25	25	18 | 7	15 | 10	61.3 ± 16	64.9 ± 13
Kir et al., 2014	Turkey	73	107	42 | 31	61 | 46	–	–
Klepsyte et al., 2012	Lithuania	20	20	11 | 9	12 | 8	60 ± 9	65 ± 12
Liu et al., 2014	China	66	65	32 | 34	38 | 27	–	–
Markl et al., 2007	Germany	12	12	1 : 0.85	1 : 1	57 ± 16	64 ± 16
Markl et al., 2008	Germany	29	30	22 | 7	16 | 14	66 ± 14	72 ± 9
Markl et al., 2013	Germany	669	663	1 : 0.6	1 : 0.7	68 ± 12	68 ± 9
Markl et al., 2016	Germany	292	233	1 : 0.62	1 : 0.76	67 ± 12	67 ± 12
Munster et al., 2015	Germany	21	54	1.62 : 1	2.15 : 1	66.2 ± 4.2	66.8 ± 4
Reima et al., 2016	Estonia	130	136	67 | 63	67| 69	71 ± 20	72 ± 20
Tornroos et al., 2011	Sweden	16	16	7 | 9	10 | 6	73.5 ± 20	69.5 ± 20
Vasala et al., 2016	India	30	30	–	–	–	–
Borowski et al., 2014	UK	50	50	28 | 22	29 | 21	69 ± 34	71 ± 20

2.6. Data items

From each study, we extracted the following data items: (1) participant groups [country, sample size, mean age and gender ratio]; and (2) main outcome measures [lymph nodes count and cases with <12 LN harvest].

2.7. Risk of bias in individual studies

To establish the risk of bias of the eligible studies, the authors determined the quality of each study using the Cochrane "Risk of bias" tool, as described in Chapter 8 of the Cochrane Handbook (version 5 or later) [15].

2.8. Synthesis of results

In order to extract data regarding the outcome variable LN count, we focused our attention on the number of lymph nodes harvested after the injection of MBLD in the intervention group or after the classical dissection in the control group. We selected the mean and range values for LN count in both groups.

Concerning the outcome variable cases with <12 LN harvest we extracted the cases where LN count were less than 12 nodes.

3. Results

3.1. Study selection

Fig. 1 shows the flow diagram representative of the process of study selection. We retrieved 347 potentially relevant reports from our electronic searches. From these, 46 studies were elected to be included in the review after reading the abstract and removing duplicates. From those, 3 articles were discarded due to full text unavailability, as well as 2 meta-analysis and 5 reviews. Twenty-one studies did not meet the inclusion criteria (see Fig. 2).

3.2. Study characteristics

All studies included in the meta-analysis involved the use of methylene blue in patients with colorectal cancer and were published between 2007 and 2017.

We analyzed five randomized controlled trials: two were performed in China [16,17], one in Germany [18], one in Estonia [19] and the other in United Kingdom [10]. Twelve remaining studies included in our search were all clinical trials performed in Germany [7,9,12,20,21], Egypt [22], Sweeden [2], India [23], Denmark [3], Lithuania [24], Spain [4] and Turkey [8].

Table 1 shows a summary of the studies included in the meta-analysis and the general characteristics of the included articles. The mean age of the participants for each study ranges between 50,60 and 73,5 years of age (3 articles didn't report mean age [8,17,23]). The sample size ranges between 12 and 669; predominance in male subjects can be observed in most studies, except for one report [3].

Table 2 shows the inclusion and exclusion criteria used by each study. All studies included participants undergoing elective surgery for colorectal cancer with curative intent. One study included colorectal cancer specimens from emergency procedure [3]. One study included patients with elective surgery with curative intent after receiving neo-adjuvant therapy [22]. Five studies included patients with rectal cancer [7,8,21,22,24]. Six studies didn't have any exclusion criteria [3,7,9,10, 21,22].

3.3. Risk of bias within studies

Table 4 represent the quality of the results based on the Cochrane "Risk of bias" tool, as described in Chapter 8 of the Cochrane Handbook (version 5 or later) [15]. Based on the results, we considered that there isn't no risk of bias in the articles.

3.4. Results of individual studies

Table 3 and Table 4 represents a summary of the main outcomes extracted of the included studies. The study conducted by Markl et al., 2013 [9] presented with the largest proportion of participants in experimental and control group (669 and 663 respectively) in contrast with the other study conducted by the same author 2007 [7] (12 and 12 respectively). All studies were performed by general surgery or oncological surgery. The respective investigators excluded cases where there was no adequate insertion of the methylene blue dye in the part and, in rectal parts, adequate TME was guaranteed in patients with rectal cancer.

The analysis of the results concerning the lymph node harvest shows that the cases with methylene blue injection in Mark 2016 et al. [12] had a bigger mean lymph node harvest in comparison with the means at group of participants in control group (40 and 14), in contrast with the study by Kir et al. [8] that shows the smaller difference of the means of lymph nodes count between the group with methylene blue injection and classical dissection (24.48 and 21.49). The maximum mean value of lymph node harvest is 47.9 in Frasson et al. [4] in contrast with the study conducted by Liu et al. [17] that have the minimum value of lymph node

Table 2
Summary of the inclusion and exclusion criteria of the included studies.

Study	Inclusion criteria	Exclusion criteria
Cai, 2012 et al.	• 18–80 years of age; •Endoscopic biopsy confirmed; •Performance status of 0–1 on the Eastern Cooperative Oncology Group scale; •Good compliance; •Able to tolerate radical resection; •Adequate hematologic function [white blood cell (WBC) count >4000/mL, absolute neutrophil count >1500/mL, platelet count >100 000/mL, and hemoglobin >10 g/dL]; •Normal hepatic function [bilirubin <1.5 the upper-normal limits (UNL) and alanine amino-transferase or aspartate amino-transferase <2.5 UNL]; •Normal renal function (creatinine <1.5 mg/dL)	•Clinical stage IV CRC according to the American Joint Committee on Cancer (AJCC); •Patients received chemotherapy, radiotherapy or biological therapy prior to surgery; •Previous abdominal surgery; •Significant neurological or mental disorder
Farouk et al., 2017	•Patients with elective surgery for resectable rectal cancer with intent to cure after receiving neoadjuvant chemoradiotherapy	•None
Frasson et al., 2012	•Patient undergoing elective surgery for colon cancer with curative intention	•Patients with palliative resection
Jepsen et al., 2012	•Primary colorectal cancer specimens, from elective or emergency procedures	•None
Kerwel et all, 2009	•Patients undergoing elective surgery with intent to cure	•Patients receiving palliative resections •Surgery for a locoregional recurrence •Emergency surgery
Kir et al., 2014	•Curative resection of any part of the colon or upper rectum for histologically proven adenocarcinoma	•Patients with palliative treatment •Emergency resections •Neoadjuvant treatment
Klepsyte et al., 2012	•Patients underwent conventional rectal resection with total mesorectal excision (TME) and coloanal anastomosis for middle and low rectal cancer performed by the same surgeon	•Preoperative long-course radiotherapy •Patients with distant metastases
Liu et al., 2014	•Patients underwent elective radical surgeries	•Palliative resection •Surgery for recurrence •Emergency surgery
Markl et al., 2007	•Patients with upper rectal cancer	•None
Mark et al.l, 2008	•Curative resection of any part of the colon and the upper rectum for histologically proven or suspected malignancies	•Palliative and emergency resections
Markl et al., 2013	•Histologically proven primary colorectal cancer •Negative resection margins	•None
Markl et al., 2016	•Node positive colorectal cancers with or without neoadjuvant therapy were included; •Cases without LN metastases that were classified as pN1c according to the seventh edition of the UICC TNM classification	•Positive resection margins •Death within 2 months after the operation
Munster et al., 2015	•Patients with s with a rectal carcinoma at a distance of less than 12 cm from the anal verge, after low anterior or abdominoperineal rectal cancer resection by the open approach (with and without prior neoadjuvant RCT)	•None
Reima et al., 2016	•Pathologically confirmed CRC	•Resection with palliative intent
Tornroos et al., 2011	•Patients undergoing intended curative surgery for colorectal cancer at the Departments of Surgery at Norrkoping County Hospital and Linkoping University Hospital, Sweden	•Concomitant inflammatory bowel disease •Massively invasive tumors necessitating a surgical resection technique, rendering subsequent specimen staining impossible
Vasala et al., 2016	•Resected specimens of histologically proven carcinoma involving the colon or rectum, operated at the hospital during 1 year period	•Emergency •Nonelective resections and specimens from patients who received preoperative radiotherapy •Patients with concomitant inflammatory bowel disease
Borowski et al., 2014	•Patients undergoing planned colorectal resection of a suspected cancer following multidisciplinary team discussion	•None

Table 3
Summary of the lymph node harvest outcome extracted of the included studies.

Study	Experimental Group			Control group		
	Mean	Standard Deviation	N	Mean	Standard Deviation	N
Markl et al., 2016	40	20	292	14	5	233
Munster et al., 2015	32,8	13,56	21	25,6	10,84	33
Kir et al., 2014	24,48	12,99	73	21,49	13,76	107
Farouk et al., 2017	17,52	6,2	40	14,57	2,34	30
Kerwel et al., 2009	30	14	25	17	11	25
Klepsyte et al., 2012	18	5	20	14	6	20
Markl et al., 2007	27	7	12	14	4	12
Markl et al., 2008	35	18	30	17	10	30
Markl et al.l, 2013	34	17	669	13	5	663
Liu et al., 2014	23,2	4,7	66	11,7	3,4	65
Frasson et al., 2012	47,9	17,8	34	21,9	10,8	473
Cai et al., 2012	23,8	8,4	20	12,2	3,2	20
Vasala et al., 2016	22	9	26	17	8	26
Reima et al., 2016	27	4	130	16	3	136
Markl et al., 2016	40	20	292	14	5	233

(11.7). Reima et al. [19] had the lower standard deviation in experimental and control group (4 and 3 respectively). Fig. 3 shows the statistical analysis of the results concerning to lymph node harvest variable. Markl et al. [9,12] have the bigger weight in the analysis that show a significative statistical difference ($p < 0.05$) between the methylene blue injection and the classical dissection group, however the heterogeneity is 95%.

As we can see in Table 4, in Markl et al., 2007 [7] exist the bigger percentage of cases that the lymph node harvest was inferior than 12 nodes (58.3% of the control group participants had a lymph node count

Table 4
Summary of the optimal lymph node harvest outcome extracted of the included studies.

Study	Experimental Group			Control Group		
	n	%	N	n	%	N
Markl et al., 2016	4	1	292	71	30	233
Farouk et al., 2017	5	12,5	40	11	36,7	30
Kerwel et al., 2009	0	0	25	7	28	25
Borowski et al., 2014	1	2	50	8	16	50
Markl et al., 2007	0	0	12	7	58.3	12
Markl et al., 2008	1	3.3	30	8	26.7	30
Markl et al., 2013	14	2	669	251	38	663
Liu et al., 2014	0	0	66	21	32,3	65
Frasson et al., 2012	0	0	34	76	16,1	473
Tornroos et al., 2011	0	0	20	0	0	17
Jepsen et al., 2012	3	1	234	13	7	194
Cai et al., 2012	1	5	20	10	50	20
Vasala et al., 2016	2	7.7	26	6	23	26

<12). In all participants in Tornroos et al. [2] the investigators can count more than 12 lymph nodes. Fig. 4 represent the statistical analysis and shows that Markl 2007 [7] and Cai 2012 [16] had the lower weight in the result of the analysis. There is a statistical difference between the experimental and control group about the optimal lymph node harvest (lymph node harvest count superior than 12) with 41% of heterogeneity.

4. Discussion

Our work aimed to give a broad view over the use of methylene blue in colorectal cancer and its importance in lymph node harvest and consequently in optimal lymph node harvest.

The most important predictive factor now considered is outcome prediction based on tumor stage as expressed by the American Joint Committee on Cancer (AJCC)/Union for International Cancer Control (UICC) tumor node metastasis (TNM) system [25]. Neoadjuvant chemotherapy and total mesorectal/mesocolon excision have improved local control in patients with colorectal cancer. Insufficient lymph node harvest is an indication for expensive chemotherapy with known side effects. The primary criteria to adjuvant therapy is the existence of regional lymph node metastases [26–28]. So, for that reason, the number of lymph nodes harvest is the main prognostic factor in colorectal cancer in lymph node stage and for that we need to have the bigger number of lymph node to determine with precision the stage of the disease and initiate the best postoperative care. For example, numerous studies have conclusively shown a linear relationship between the quantity of LNs analyzed and increased five-year survival rates in T3N0 colon [29–31]. Other study show that patients with localized stage of colorectal cancer had a 5-year relative survival rate of 90.1%, while those with regional metastasis to surrounding organs or LNs had a 5-year relative survival rate of 69.2% [32].

Actually, at least 12 lymph nodes should be found, per the recommendations of the American Joint Committee on Cancer, for a more precise diagnosis [33]. The total number of extracted lymph nodes that are available for histological analysis is necessary for an accurate assessment of nodal status. Since the suggested minimum number of 12 lymph nodes is frequently not reached, the variability in the number of retrieved lymph nodes continues to be a significant challenge in patient management [20].

The first World Congress of Gastroenterology recommendation for lymph node examination was made in 1990 [34]. The National Cancer Institute's guidelines for colon and rectal cancer surgery were published in 2000 [35]. In Reima et al. [19] the staining procedure enabled to locate ≥12 lymph nodes in 86% of the patients, which can be considered highly significant improvement. Staining has increased lymph node count to a roughly similar degree in other randomized trials [10,17,20]. Higher lymph node numbers and increased survival have been linked in some studies [29,36]. As a result of more precise nodal staging, this may be attributed to more effective lymphadenectomy and appropriate adjuvant therapy. Tumor biology can potentially have an impact on lymph node numbers. Larger lymph nodes, which are easier to spot, may be present in patients with greater immune responses to cancer. The NCCN guidelines advise examining not only 12 lymph nodes, but as many as possible [37].

The quality of the lymph node yield can be impacted by a number of variables, including age, tumor location, obesity, immunological response, neoadjuvant therapy, surgical technique, and effective dissection procedures [23]. According to some authors, one of the primary reasons for understaging in colorectal cancer is a lack of lymph nodes that have been identified and inspected [12]. Initially the surgeons only used the classical dissection but some authors show that is ineffective and insufficient for a proper evaluation as can be evaluated in the studies of Markl 2007 where we see 58.3% of the participants in classical dissection didn't have a minimum of 12 lymph nodes [7].

For this reason, there was a need to develop more effective techniques for counting lymph nodes and one of this technique is the intra-arterial injection of methylene blue in the specimens extracted in patients with colorectal cancer. We conducted this study to analyze the

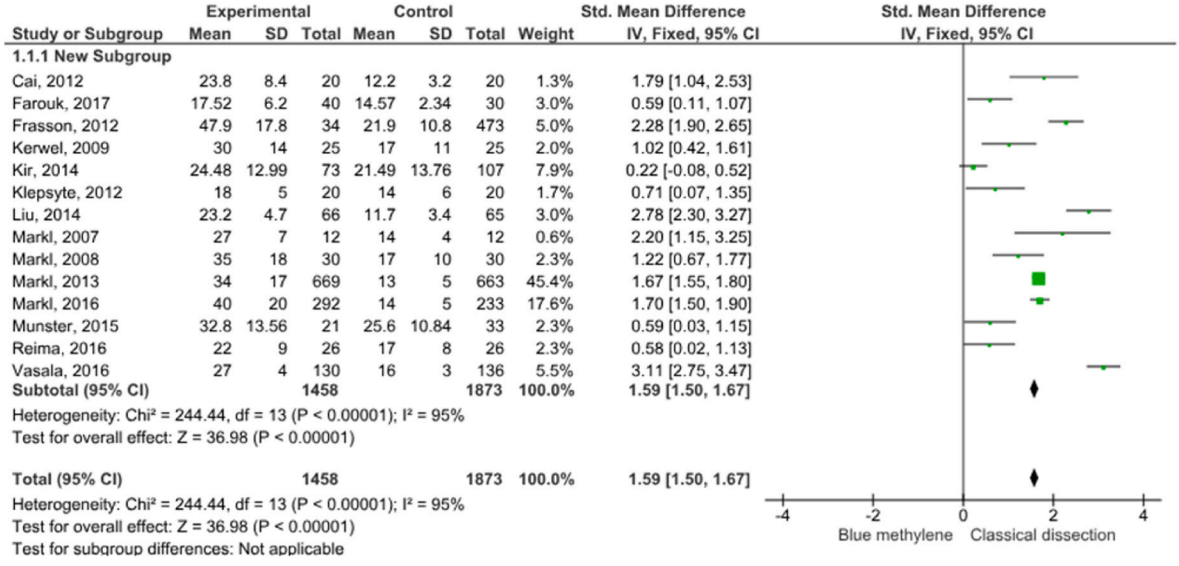

Fig. 3. Statistical analysis of the results concerning to lymph node harvest variable.

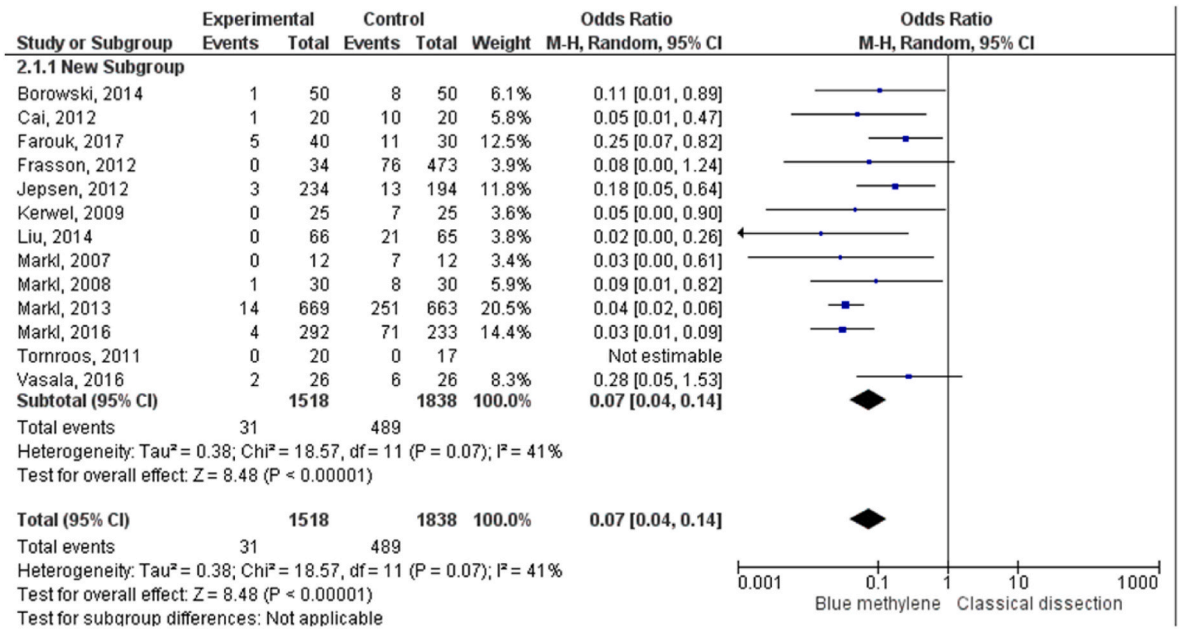

Fig. 4. Statistical analysis of the results concerning to optimal lymph node harvest variable.

sensibility of methylene blue in comparison with classical dissection which concerns to lymph node harvest.

Markl et al. were the first to suggest injecting methylene blue solution into one of the local arteries to enhance the lymph node harvest during the pathological evaluation of colorectal cancer specimens [7]. Accounting for the results previously described, we can see that report information regarding the same issue presents a pattern between most works. The results from the studies are consensual in terms of the number of lymph node harvest and the optimal lymph node harvest, which, when taking into account, the use of methylene blue may direct to possible advantages of this technique over classical dissection.

By analyzing the "number of lymph node harvest" we can see that the data is concordant, revealing a tendency to harvest more lymph nodes with methylene blue. In fact, all studies show that exists a difference between these two techniques and we find a significative statistical difference between the use of methylene blue and the more traditional procedure.

Additionally, the studies show that using methylene blue help the pathologist to identify more easier the lymph nodes in specimens and for that reason there is a significative statistical difference between the two procedures. In most of studies in the group of methylene blue the investigators had no difficulty to identify more than 12 lymph nodes per patient, but in control group, using the classical dissection, there is a bigger number of participants that the pathologist can't find the necessary number of lymph nodes to consider the harvest as optimal.

So, methylene blue ex vivo intra-arterial injection resulted in a much higher overall lymph node yield and a significantly lower percentage of cases with fewer than the specified minimum required of evaluated nodes.

5. Limitation

In both of the study variables exist a high percentage of heterogeneity of the results in the articles included. We supposed that exist this variability because the studies have different proportions of participants and the number of lymph node harvest is affected by that and can impact the value of heterogeneity. However, even with these values all works that exist in literature support the hypothesis that using intra-arterial methylene blue is more effective than classical dissection.

6. Conclusion

In summary the use of methylene blue in patients with colorectal cancer can improve the lymph node harvest and with that the patients have a better classification of their disease by improving the pathological classification of the tumor. In addition, under staging will be reduced and adjuvant treatments will be avoided since with the use of methylene blue it is usually possible to identify more than 12 nodes per operative specimen. In conclusion, the method presented here is easy, cost-effective and accessible, and it should to be simple replicable in other institutions, especially where insufficient nodal harvest are difficult, but it should also be applied as standard practice in other hospitals for all resections performed on patients with colorectal cancer who have curative intent.

Still, more randomized and prospective studies are needed to reduce the heterogeneity of results that still exist around this theme.

Funding

None.

Availability of data and material

All materials are available for review upon request.

Code availability

All employed software codes and applications are available for review upon request.

CRediT authorship contribution statement

Alexandre Carvalho: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nuno Gonçalves:** Validation, Supervision, Resources, Investigation, Formal analysis. **Pedro Teixeira:** Visualization, Validation, Supervision, Methodology, Formal analysis. **André Goulart:** Writing – review & editing, Visualization, Validation, Formal analysis. **Pedro Leão:** Writing – review & editing, Visualization, Validation, Supervision, Project

administration, Conceptualization.

Declaration of competing interest

The authors declare that no conflict of interest exists.

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