

Review

Prognostic Significance of Lymphatic Vessel Invasion and Density in Colorectal Carcinoma: A Systematic Review and Meta-analysis

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Abstract

Background/Aim: This systematic review and meta-analysis evaluated the relationship between lymphatic vessel invasion (LVI) and lymphatic vessel density (LVD) – with specific focus on distinguishing peritumoral from intratumoral LVD, assessed by immunohistochemistry – and survival outcomes in patients with colorectal carcinoma (CRC).

Materials and Methods: Following Cochrane Handbook and PRISMA guidelines, we included original studies evaluating tumor LVD and/or LVI in colorectal cancer using immunohistochemistry and reporting hazard ratios for overall survival (OS) and/or disease/recurrence-free survival (DFS). Data from MEDLINE, Cochrane Library, and PubMed (past 25 years) were extracted, analyzed with Stata, and assessed for heterogeneity and publication bias.

Results: The principal results of the present study were the statistically significant correlations between a) LVI and reduced OS ($p=0.003$), b) high peritumoral LVD and longer DFS ($p=0.044$) and c) high LVD (total) and reduced OS ($p<0.001$). However, no statistically significant correlation was found between peritumoral or intratumoral LVD and OS.

Conclusion: LVI and LVD parameters (peritumoral, intratumoral, total), evaluated using immunohistochemistry, may provide valuable information with respect to the prognosis of patients with CRC.

Keywords: Colorectal carcinomas, lymphatic vessel density, lymphatic vessel invasion, meta-analysis, disease-free survival, overall survival, review.



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Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide and one of the leading causes of cancer-related deaths (1). Although overall CRC mortality continues to decline, CRC is shifting to diagnosis at a younger age of patients, at a more advanced tumor stage, and left-sided tumors (colon/rectum) (2). Stage of CRC at diagnosis is the most crucial predictor of survival with 5-year survival ranging from 91% for localized disease to 14% for distant metastatic disease (2).

Lymph node (LN) metastasis is one of the most important routes of cancer dissemination and a major prognostic factor for survival of CRC patients (3-5). Overall, the 5-year survival rate was 30-60% in CRC patients having LN metastasis whereas LN-negative patients had a 70-80% 5-year survival rate (3).

The lymphatic spread in human cancers including CRC has been frequently associated with increased tumor-associated lymphangiogenesis (proliferation of lymphatic vessels) (5-9). Notably, tumor-associated lymphatic vessels do not only operate as routes for cancer cell dissemination but also facilitate the transport of cancerous antigens to the tumor draining LN in order to initiate anti-neoplastic immunity (8, 9). Tumor cells and non-malignant cells of the tumor microenvironment (pe, macrophages, mast cells, neutrophils) can secrete growth factors [such as vascular endothelial growth factor (VEGF) C/D], which promote lymphangiogenesis through activation of the VEGF-R3 in lymphatic endothelial cells (6-10). Lymphangiogenesis which is expressed as lymphatic vessel density (LVD) and lymphatic vessel invasion (LVI), which is the presence of cancer cells within lymphatic vessels, can be reliably evaluated histologically using immunohistochemistry using antibodies specific for lymphatic endothelial cells (11-13). For example, the monoclonal antibody D2-40 that detects podoplanin, is considered as a specific marker of lymphatic endothelial cells (11). Podoplanin, is a 43-kDa transmembrane glycoprotein that is expressed specifically in lymphatic endothelial cells and is not detected in the blood vasculature (11).

Interestingly, clinico-pathological studies provided evidence that intratumoral and peritumoral LVD may have different relationships with metastatic disease and survival outcomes in some human cancers (5-7, 9, 10). The possibility that intratumoral and peritumoral lymphatic vessels may play different roles in cancer dissemination (6, 7) is supported by experimental evidence in animal models (14). Indeed, Padera *et al.*, used functional assays and immunohistochemistry to examine mouse tumors expressing normal or increased levels of the lymphangiogenic factor VEGF-C and showed that a) functional peritumoral lymphatics alone are sufficient for lymphatic metastasis of cancer cells and b) lymphatic metastasis occurs in the absence of functional intratumoral lymphatic vessels (14).

The relationship of LVD with survival endpoints [overall survival (OS) and disease-free survival (DFS)] and various clinico-pathological parameters in CRC was analyzed in previous original studies with some of them using the distinction between peritumoral and intratumoral LVD and with inconsistent results regarding the relationship between LVD and survival outcomes (15-27). A metaanalysis published in 2013 provided evidence that LVD (evaluated using immunohistochemistry) has prognostic significance in CRC but no prognostic information was reported on the basis of the distinction between peritumoral and intratumoral LVD (28).

However, the presence of LVI has been associated with an increased risk of LN metastasis, recurrence and reduced survival outcomes in several cancers (29-31). In CRC, LVI was significantly correlated with LN metastasis in some studies and inconsistent results were reported concerning the relationship between LVI and survival parameters (12, 32-37). A systematic review without meta-analysis published in 2014 summarized the variability of the prognostic significance of LVI (evaluated using routine hematoxylin-eosin stain or immunohistochemistry) in node negative operable CRC (38).

The aforementioned data indicate that the prognostic significance of LVI and LVD in CRC, especially with focus on the distinction between peritumoral and intratumoral LVD,

needs further investigation. Thus, the purpose of this systematic review and meta-analysis was to investigate the possible relationship of LVI and LVD (peritumoral, intratumoral, total), evaluated using immunohistochemistry, with survival outcomes in patients with CRC.

Materials and Methods

This systematic review was conducted in accordance with the methodologies outlined in the Cochrane Handbook for Systematic Reviews (39) and the PRISMA guidelines (40).

Study eligibility criteria. The inclusion criteria generated to filter the studies included in the present meta-analysis were: 1) original full research article that evaluated tumor LVD and/or LVI, 2) results referred to OS and/or DFS, 3) HR and 95% CI mentioned or indirectly calculated through given data and 4) immunohistochemistry using antibodies against podoplanin (D2-40 *etc.*) for the detection of lymphatic endothelial cells (11, 13). The anti-podoplanin antibodies were selected a) because they were the preferred immunohistochemical markers in the vast majority of original research studies including 12/13 studies reviewed in the reference 5 and in all the recent original research studies concerning LVD and LVI in CRC (16-20, 25, 32, 34) and b) in order to maintain statistical homogeneity. Both prospective and retrospective studies were eligible. Case reports, animal studies, *in vitro* studies, reviews and meta-analyses were excluded.

Information sources. The electronic databases MEDLINE, Cochrane Library and PubMed were used and studies from the past 25 years were selected through a search conducted up to July 31, 2025.

Search strategy. The search strategy included the terms “colorectal AND lymphatics”, “colorectal AND lymphatic vessel invasion”, “colorectal AND LVD”, “gastrointestinal AND LVD”, “colorectal AND lymphatic vessel invasion”, “colorectal AND LVI”. Only studies written in English language were included.

Selection process-data items – data collection – bias assessment. Two authors (DV & AM) performed the full text screening and independently extracted the data from all eligible studies including the name of the first author, publication year, geographic location, sample size, sex, method of immunohistochemistry (antibodies, hot-spots, magnification field), LVD (peritumoral and intratumoral), LVI, follow up period and survival/recurrence data. For any missing or unclear information, the authors were contacted. All extracted information was stored in an Excel file (Microsoft, Redmond, WA, USA) and was checked for accuracy by one more author (GN).

Quantitative synthesis and analysis. Summary mean differences (MDs) and HRs, along with the corresponding 95% CI, were calculated for the OS and the DFS. Comparisons included participants with high *versus* low peritumoral LVD, high *versus* low intratumoral LVD, high *versus* low LVD, and as well as presence *versus* absence of LVI. For continuous outcomes, summary MDs were calculated by pooling study-specific estimates using either fixed- or random-effects models (41), depending on the level of observed heterogeneity. For time-to-event outcomes, pooled HRs were similarly estimated using appropriate models based on heterogeneity levels. The presence of heterogeneity was assessed using Cochran’s Q statistic and quantified with the I^2 index (42). A p -value <0.10 in the forest plots was considered indicative of significant heterogeneity. Publication bias was assessed using funnel plots and Egger’s regression test when ten or more studies were available for a given analysis (39). All statistical analyses were conducted using Stata (version 14; StataCorp, College Station, TX, USA).

Results

Study selection and population characteristics. The systematic search of the electronic databases (Medline, PubMed) identified a total of 88 studies, 39 of which were selected for full text screening. Eight studies were considered eligible for data extraction and meta-analysis

according to our eligibility criteria (12, 20-25, 33). Figure 1 shows the flow chart of the study selection process. Table I presents the characteristics of the included studies. Table II presents the evaluation results from the authors using the Downs and Black checklist. Following PRISMA guidelines, our systematic review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) - registration number 1156925.

Study outcomes. All included studies reported data on a range of outcomes. Quantitative synthesis was feasible for five comparisons: four of them pertained to OS, including comparisons of high *versus* low peritumoral LVD and high *versus* low intratumoral LVD, both evaluated as continuous outcomes based on differences in the mean duration of survival in months, as well as comparisons of high *versus* low total LVD and presence *versus* absence of LVI, both analyzed as time-to-event outcomes. An additional analysis was conducted for DFS, comparing high *versus* low total LVD, also as a time-to-event outcome.

Two studies reported data on OS for participants with and without LVI, analyzed as a time-to-event outcome. The pooled analysis demonstrated a statistically significant association, with participants presenting LVI exhibiting more than a twofold higher hazard of death, reflecting poorer overall survival, compared to those without LVI (summary HR=2.14; 95%CI=1.30-3.51; p -value=0.003; Figure 2). No heterogeneity was observed across the included studies ($I^2=0\%$).

Five studies reported results on the duration of DFS, expressed in months, for participants with high peritumoral LVD compared to those with low peritumoral LVD. High peritumoral LVD was significantly correlated with longer DFS (summary MD: 3.28; 95%CI=0.10-6.46; p -value=0.044; Figure 3). No heterogeneity was observed across the included studies ($I^2=0\%$).

Two studies reported data on OS for participants with high *versus* low LVD (total), analyzed as a time-to-event outcome. The pooled analysis demonstrated a statistically significant association, with participants in the high-LVD group exhibiting more than a threefold higher hazard of

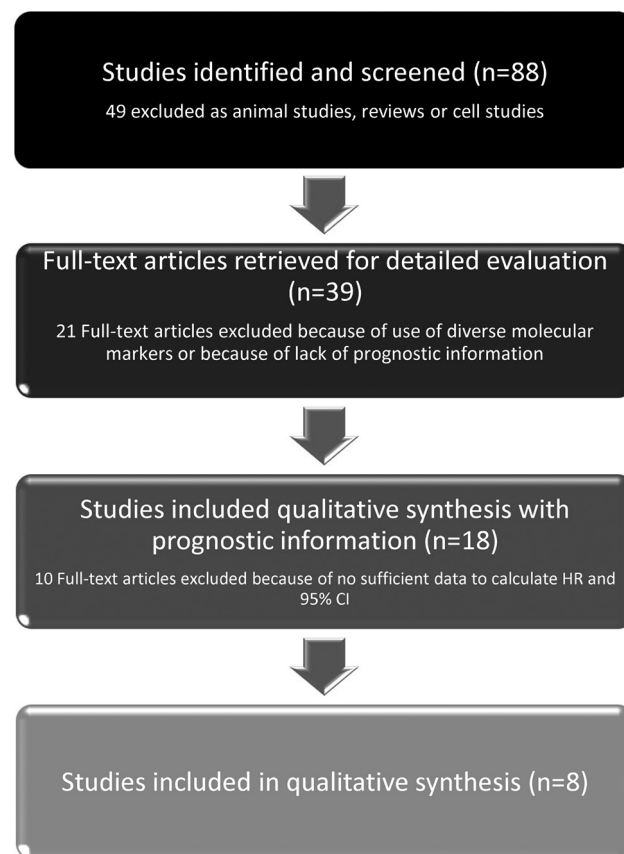


Figure 1. Flow chart.

death – indicating substantially poorer overall survival – compared to those in the low-LVD group (summary HR=3.38; 95%CI=2.11-5.41; p -value <0.001; Figure 4). No heterogeneity was observed across the included studies ($I^2=0\%$).

Finally, two studies reported results on the duration of OS, measured as months, for participants with high peritumoral LVD compared to those with low peritumoral LVD, and moreover, for participants with high intratumoral LVD compared to those with low intratumoral LVD. None of the quantitative syntheses revealed statistically significant differences among the groups under examination (Peritumoral LVD: summary MD: 3.88; 95%CI=-1.45 to 9.21; p -value=0.154; Figure 5; Intratumoral LVD: summary MD: 0.44; 95%CI=-3.71 to 4.60; p -value=0.834;

Table I. Characteristics of eligible studies included in the systematic review and meta-analysis.

First author	Year	Country	Number of patients	Antibody type	Hotspots selected	Magnification field	Cut-off value	Reference
LIANG	2006	Japan	87	Podoplanin	5	×400	7.3	21
SAAD	2006	USA	90	D2-40	3	×400	Mean	22
MATSUMOTO	2007	Japan	106	Podoplanin	3	×200	Mean	24
YAN	2008	China	132	D2-40	5	×200	5	23
LIN	2010	China	81	D2-40	2	×200	Mean	33
VAN-WYK	2015	UK	75	D2-40	NR	NR	Mean	12
LV	2016	China	72	D2-40	10	×400	Mean	20
ZHANG	2020	China	120	Podoplanin	3	×200	Mean	25

NR: Not reported.

Figure 6). No heterogeneity was observed across the included studies ($I^2=0\%$).

None of the quantitative syntheses included ten or more studies; therefore, assessment of publication bias was not performed due to insufficient number of studies for a reliable evaluation.

Discussion

The current meta-analysis provides novel findings and useful insights with respect to the biological behavior of CRC by examining the prognostic value of LVI and LVD (peritumoral, intratumoral, total), evaluated using immunohistochemistry for reliable identification of the lymphatic endothelial cells (11, 13).

The present study revealed a statistically significant correlation between LVI and reduced OS (summary HR=2.14; 95%CI=1.30-3.51; $p=0.003$). The low heterogeneity ($I^2=0\%$) observed further strengthens the reliability of our result. Our robust finding is consistent with previous studies showing that LVI represents a major feature of biological aggressiveness in various carcinomas (pe, gastric and breast carcinomas) (29-31, 43, 44). Indeed, LVI in breast carcinomas was correlated with more aggressive clinicopathological features, (such as higher histological grade, axillary LN metastasis and higher T staging) and was an independent negative prognostic factor associated with local recurrence, distant metastasis and worse DFS and OS (31). However, LVI was

Table II. Quality assessment of included studies using the downs and black checklist.

Study (Reference)	MA	VD
Liang, 2006 (21)	16	17
Saad, 2006 (22)	18	19
Yan, 2008 (23)	22	22
Lin, 2010 (33)	20	20
Zhang, 2020 (25)	20	19
Matsumoto 2015 (24)	18	19
LV 2016 (20)	19	20
Van-Wyk 2015 (12)	21	20

MA: Marini Aikaterini; VD: Varvarousis Dimitrios.

significantly correlated with LN metastasis and was an independent negative prognostic factor for both DFS and disease-specific survival in gastric carcinomas (43). Moreover, LVI was an independent prognostic factor, which tended to worsen the prognosis, especially in patients with advanced gastric carcinomas and LN metastases (44). On the other hand, the presence of lymphatic invasion was not significantly correlated with OS or DFS, in a study of 636 patients with stage II CRC (45). Interestingly, in that study, both OS and DFS were significantly lower in stage II CRC patients who were positive (vs negative) for venous invasion (45).

The present study examined separately the relationship of LVD parameters (peritumoral, intratumoral and total LVD) with survival outcomes.

First, a statistically significant correlation was found between high peritumoral LVD and longer DFS ($p=0.044$).

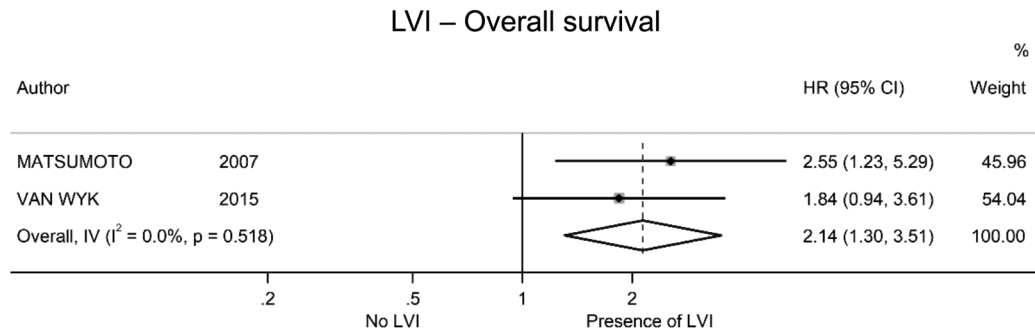


Figure 2. Forest plot of the meta-analysis of overall survival in participants with lymphovascular invasion (LVI) compared to those without LVI. CI: Confidence interval; HR: hazard ratio.

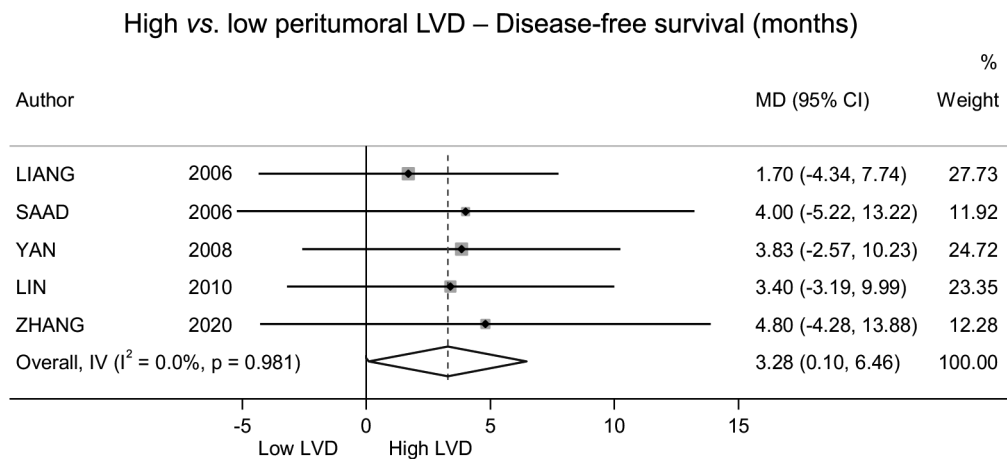


Figure 3. Forest plot of the meta-analysis of disease-free survival in participants with high peritumoral LVD compared to participants with low peritumoral LVD. CI: Confidence interval; LVD: lymphatic vessel density.

This can be related to findings in a large cohort of CRC (838 patients), showing that the peritumoral LVD in the primary tumor, assessed with immunohistochemistry using the antibody D2-40 (anti-podoplanin), was significantly decreased in patients with distant metastasis (46). The authors suggested that distant metastasis in patients with CRC is a consequence of decreased D2-40 positive peritumoral lymphatic vessels and decreased cytotoxic lymphocytes (46). Moreover, a study in mice showed that photodynamic therapy-mediated damage to the tumor-associated lymphatic vessels in breast tumors impairs the development of anti-neoplastic immunity (47). A possible explanation of the aforementioned data is that elevated

peritumoral LVD may increase anti-tumor immune response by facilitating the transport of cancerous antigens alone or antigen-presenting cells (*i.e.*, dendritic cells) to the tumor draining LN in order to initiate anti-neoplastic immunity (8, 9). However, since lymphatic vessels may also facilitate cancer metastatic spread whether the final effect of tumor-associated lymphatic vessels is promotion of cancer metastasis or enhancement of anti-neoplastic immunity may depend on organ and tissue-specific differences of the lymphatic vasculature (8, 9, 48-50).

Second, no statistically significant correlation was detected between intratumoral LVD and OS. Our finding may be consistent with previous data showing that

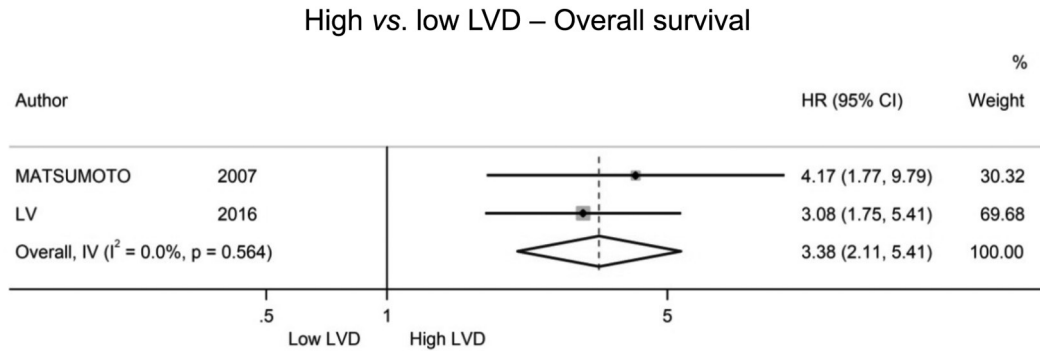


Figure 4. Forest plot of the meta-analysis of overall survival in participants with high LVD compared to participants with low LVD. CI: Confidence interval; LVD: lymphatic vessel density.

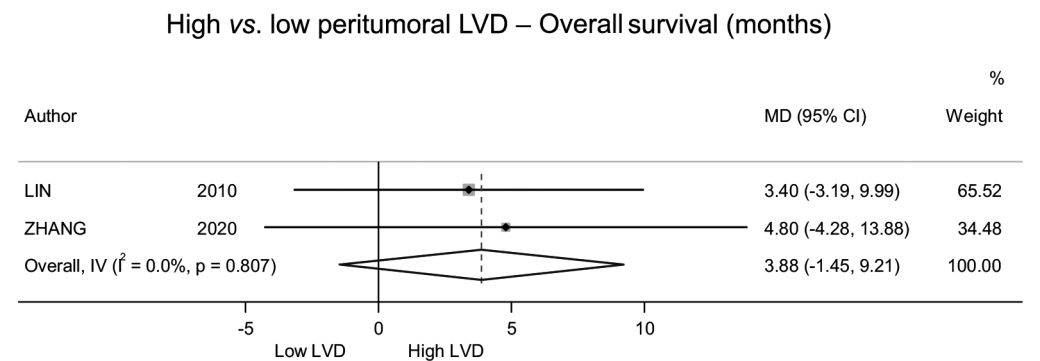


Figure 5. Forest plot of the meta-analysis of overall survival in participants with high peritumoral lymphatic vessel density (LVD) compared to participants with low peritumoral LVD. CI: Confidence interval.

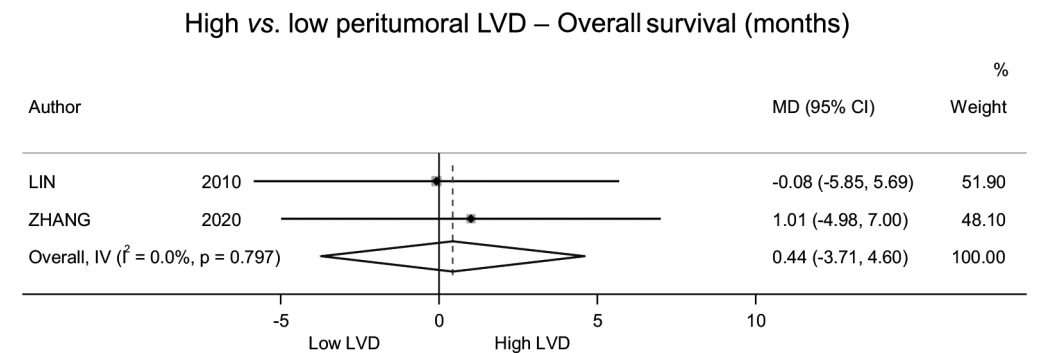


Figure 6. Forest plot of the meta-analysis of overall survival in participants with high intratumoral lymphatic vessel density (LVD) compared to participants with low intratumoral LVD. CI: Confidence interval.

intratumoral LVD had no prognostic impact in patients with stage I CRC (15). However, another study reported that the survival rate of patients with CRC with low

intratumoral LVD was significantly higher than that of patients with CRC with high intratumoral LVD (5-year survival rate: 66.9% vs. 50%, $p=0.036$) (25).

Third, a statistically significant correlation was observed between high LVD (total) and reduced OS ($p < 0.001$). Our finding can be paralleled with those of a previous meta-analysis published in 2013 which revealed statistically significant correlation between high LVD and reduced DFS whereas no significant association was observed between LVD and OS (28).

Taken together, our present and the aforementioned previous results (15, 25, 28) reveal variability concerning the prognostic impact of LVD in CRC. This could result, at least in part, from the separate assessment of the relationship of LVD parameters (peritumoral, intratumoral and total LVD) with different survival endpoints such as OS and DFS, which measure different survival outcomes (51). Thus, it is suggested to analyze all LVD parameters (peritumoral, intratumoral and total LVD) with major survival outcomes such as OS and DFS in large series of CRC in order to obtain more accurate information concerning the prognostic impact of LVD parameters.

The heterogeneity observed across the included studies of the present metanalysis was low ($I^2 = 0\%$ for both OS and DFS analyses), suggesting that the pooled effects are relatively robust despite the limited study numbers. Nonetheless, several limitations exist in the present review. First, the small number of studies eligible for quantitative synthesis may limit the generalization of these findings. Second, although anti-podoplanin antibodies (D2-40) are considered to be reliable immunohistochemical markers for lymphatic endothelial cells (11, 13), variability in immunohistochemistry protocols and LVD counting including the use of various cut-off values in order to define high and low LVD levels may have introduced bias. Third, differences in sample size and geographic location may have introduced bias into the results. Finally, short and varied follow-up periods could hinder long-term outcome evaluations and heterogeneity of CRC stages may have impact on survival outcomes. Despite the aforementioned limitations, the present investigation provides additional information when compared to the previous meta-analysis of 2013, which was based on LVD data (28). Indeed, the present metanalysis incorporates a) three studies

published after 2013, b) studies not only focusing on LVD but also on LVI, both evaluated using immunohistochemical methods and c) prognostic information based on the distinction between peritumoral and intratumoral LVD. Future large prospective studies with standardized methods for the immunohistochemical identification of the lymphatic endothelial cells and additional approaches (*i.e.*, digital pathology to reduce observer variability) are needed to confirm the current findings and further explore the relationships of LVI and LVD with clinico-pathological parameters and survival outcomes in CRC.

Conclusion

The major conclusions of the present systematic review and metanalysis are that LVI and LVD parameters (peritumoral, intratumoral and total), evaluated using immunohistochemistry, may provide valuable information with respect to the prognosis of CRC. These findings underscore the need for further research to refine the clinical utility of immunohistochemical markers of lymphatic endothelial cells for the assessment of LVI and LVD in order to enhance personalized treatment strategies for patients with CRC.

Conflicts of Interest

Dimitrios Varvarousis, Aikaterini Marini, Georgios Dritsos, Kalliopi Iliou, Theocharis Chatzoglou, Alexandra Barbouti, Panagiotis Kitsoulis, Panagiotis Kanavaros have no conflicts of interest that could be perceived as prejudicing the impartiality of the research reported.

Authors' Contributions

Varvarousis Dimitrios N: Writing – review & editing, Writing – original draft, Validation, Methodology, Conceptualization. Kanavaros Panagiotis E: Writing – review & editing, Supervision, Conceptualization. Kitsoulis Panagiotis V: Writing – review & editing. Marini Aikaterini A: Writing – review & editing, Validation,

Investigation. Barbouti Alexandra: Writing – review & editing, Methodology, Data curation. Ntritsos Georgios: Methodology, Formal analysis. Kalliopi Iliou: Writing – review & editing, Data curation. Theocharis Chatzoglou: Writing – review & editing, Data curation.

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Artificial Intelligence (AI) Disclosure

No artificial intelligence (AI) tools, including large language models or machine learning software, were used in the preparation, analysis or presentation of this manuscript.

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