



van Wyk, H.C., Going, J., Horgan, P. and McMillan, D. C. (2017) The role of perineural invasion in predicting survival in patients with primary operable colorectal cancer: a systematic review. *Critical Reviews in Oncology/Hematology*, 112, pp. 11-20.
(doi:[10.1016/j.critrevonc.2017.02.005](https://doi.org/10.1016/j.critrevonc.2017.02.005))

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Manuscript Number: CROH-D-16-00046R2

Title: The role of perineural invasion in predicting survival in patients with primary operable colorectal cancer: A systematic review

Article Type: Oncology reviews

Keywords: perineural invasion, prognosis, survival, colorectal cancer

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Abstract: Abstract

Perineural invasion is a clear route for cancer cell spread however; the role of nerves in cancer progression is relatively unknown. Recent work would suggest that nerves can actively infiltrate the tumour microenvironment and stimulate cancer cell growth. Therefore, the aim of the present study was to systematically review the identification and associations of perineural invasion and survival in patients with primary operable colorectal cancer.

From initial search results of 912 articles, 38 studies were selected. Using H&E stains; five studies including 1835 patients reported on survival stratified by perineural invasion in colon cancer with weighted average detection rates of 26%; eleven studies including 3837 patients reported on rectal cancer with weighted average detection rates of 25% and; sixteen studies including 9145 patients reported on survival stratified by perineural invasion in colorectal cancer with weighted average detection rates of 17%. Using special techniques (S100), six studies including 1458 patients reported on the identification of perineural invasion in colorectal cancer. In comparison to H&E staining alone, the use of immunohistochemistry with S100 increased the detection of perineural invasion to approximately 70%. However, those studies did not examine the relationship with outcomes, so further research is required to establish the clinical significance of perineural invasion detected by immunohistochemistry.

In conclusion, perineural invasion deserves special attention for improved prognostic stratification in patients with colorectal cancer. Further work is required to standardise pathology assessment and reporting of perineural invasion, in particular its definition, use of special stains and routine inclusion in pathology practice. Reliable assessment is required for investigations into mechanisms of perineural invasion, its role tumour spread and prognostic value.

The role of perineural invasion in predicting survival in patients with primary operable colorectal cancer: A systematic review

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Table of Contents

| | |
|--|----|
| Abstract..... | 2 |
| 1: INTRODUCTION..... | 3 |
| 2: METHODS..... | 4 |
| 3: RESULTS..... | 5 |
| SEARCH RESULTS..... | 5 |
| INTERPRETATION OF RESULTS..... | 5 |
| 3.1 PROSPECTIVE STUDIES USING H&E TO IDENTIFY PERINEURAL INVASION IN PATIENTS WITH COLON, RECTAL AND COLORECTAL CANCER | 6 |
| 3.1.1 COLON CANCER | 6 |
| 3.1.2 RECTAL CANCER | 7 |
| 3.1.3 COLORECTAL CANCER..... | 7 |
| 3.2 STUDIES USING SPECIAL STAINS/ TECHNIQUES TO IDENTIFY PERINEURAL INVASION IN COLORECTAL CANCER | 8 |
| 4. DISCUSSION..... | 10 |
| References | 13 |

Abstract

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In conclusion, perineural invasion deserves special attention for improved prognostic stratification in patients with colorectal cancer. Further work is required to standardise pathology assessment and reporting of perineural invasion, in particular its definition, use of special stains and routine inclusion in pathology practice. Reliable assessment is required for investigations into mechanisms of perineural invasion, its role tumour spread and prognostic value.

1: INTRODUCTION

Colorectal cancer is a major cause of both cancer incidence and mortality [1]. Currently, Tumour-Node-Metastasis (TNM) staging is considered the most robust predictor of outcome of patients with colorectal carcinoma but is less accurate in early stage disease. Thus supplemental risk factors are required to allow selection of patients who may benefit from adjuvant treatment [2].

Currently the indication for adjuvant therapy for patients with stage II disease is based on the presence of at least one of six clinical and pathological high risk factors ;poor differentiation, emergency surgery, fewer than 12 examined lymph nodes, the presence of extramural vascular invasion, perforation or a pT4 tumour [3]. However, additional features, such as perineural invasion and the presence of tumour budding are recognised risk factors that do not yet influence treatment decisions. As such, perineural invasion has been included in the TNM Supplement for colorectal cancer since 2001[4]. Perineural invasion is also identified as a site- specific prognostic factor by the American Joint Committee on Cancer (AJCC) Staging Manual (7th edition) and a high risk factor for recurrence under the National Comprehensive Cancer Network (NCCN) guidelines. Perineural invasion is included in the Royal College of Pathologists dataset (UK) to be reported as microscopic non-core data for colorectal cancer [5].

Metastatic disease is the principle cause of death in colorectal cancer, tumour dissemination via blood and lymphatic vessels are accepted as the dominant routes of malignant spread [6]. However, tumour spread via nerves is plausible as an alternative route of spread and can therefore influence possible treatment prevention.

Perineural invasion has recently emerged as a key pathologic feature of several common solid cancers, including pancreas, prostate, biliary tract, and stomach. Neoplastic cells in perineural spaces may not be removed during tumour resection, and thus may result in local recurrence [7]. Perineural invasion in colorectal cancer has been reported as an independent prognostic factor [8, 9, 10] however, is not always assessed and reported. Problems with the detection of perineural invasion such as the presence of inflammatory cells, mucinous carcinoma and microscopic foci of perineural invasion can hinder consistent reporting. Presently, the clinical significance of perineural invasion remains unclear.

The aim of the present study was therefore to systematically review the identification of perineural invasion and associations with clinopathological features and survival in patients with primary operable colorectal cancer.

2: METHODS

A systematic review of the published literature on perineural invasion in colorectal cancer was undertaken. In addition to methods of assessment, outcomes of interest were relationships with other clinical and pathological factors and cancer outcomes (cancer-specific survival / overall survival).

Studies were identified via a literature search of the electronic databases the US National Library of Medicine (MEDLINE), the Excerpta Medical database (EMBASE), the Cochrane Database of Systematic Reviews (CDSR) and the Database of Abstracts and Reviews (DARE) between 1984 and 2015 using the key words: perineural invasion, nerve, colon/rectal cancer and prognosis (last search was updated on December 3, 2015).

For inclusion, studies had to meet the following criteria: (a) perineural invasion was assessed in surgically resected primary colon and /or rectal tumours, and (b) the relationship between perineural invasion and survival was investigated in primary operable disease, and the results were published as a full paper. Studies that only reported perineural invasion as an incidental finding were excluded.

The title and abstract of each identified study was examined for relevance. Full text was obtained for all potentially relevant studies. Studies that examined the prognostic value of perineural invasion in colon and /or rectal cancer were included while studies relating to duplicate datasets, studies not available in English language and those published only in abstract form were excluded. Studies in which sample size was less than 75 patients and the median/mean follow-up was less than 3 years were also excluded. The bibliographies of all included articles were subsequently hand searched to identify any additional studies. Studies were selected after review by the author (HvW) or if there was doubt with another co-author (DCM).

Study heterogeneity precluded a meaningful meta-analysis and the results of the review are presented in descriptive form with specific reference to definitions, localisation and assessment of perineural invasion and the effects of these on incidence, outcomes, including survival and characteristics of the tumour microenvironment in primary operable colon and /or rectal cancer.

3: RESULTS

SEARCH RESULTS

A total of 912 potentially relevant articles were retrieved by the database search. 853 articles were excluded as they did not meet inclusion criteria (Fig. 1). Cross-referencing resulted in 4 additional articles that fulfilled the eligibility criteria. After exclusion of 25 studies as a result of incidental reporting of perineural invasion, 38 studies were reviewed.

INTERPRETATION OF RESULTS

Several factors influence recognition and interpretation of perineural invasion in colorectal cancer.

I) Currently, there is no concise, accepted definition of perineural invasion in cancer and this prevents consistent prospective reporting of perineural invasion by pathologists. According to Batsakis (1984) [11], perineural invasion is tumour cell invasion in, around, and through nerves. Liebig et al. (2009)[12] has advocated a definition of perineural invasion; that include tumour cells in close proximity to neural structures (involving at least 33% of the neural circumference) or tumour cells within any of the 3 layers of the nerve sheath. In the large bowel, there are no site-specific rules for the identification of perineural invasion as two neural plexuses are located in the submucosa. One plexus lies immediately beneath the muscularis mucosa (Meissner plexus) and the deeper (Auerbach's myenteric plexus). Invasion of Auerbach's plexus is seldom recorded but may be important in terms of research. Fujita (2007), [13] defined perineural invasion as cancer cells inside the perineurium in the Auerbach plexus adjacent to the tumour front. Ueno et al.2013 [14] defined cancer spread along nerves of Auerbach's plexus as intramural perineural invasion and extramural perineural invasion as tumour cells invading or spreading along nerve fascicles external to the muscularis propria. Although perineural invasion has been observed intramurally and extramurally, the incidence and prognostic value based on location relative to the bowel wall have not been clarified. Perineural invasion is mostly reported as extramural (external to the muscularis propria) in the absence of more specific guidance. The AJCC Cancer Staging Manual also does not specify the bowel layer where perineural invasion should be recorded as a site-specific prognostic marker. These definitions and sites have been included in the present review.

II) Since reporting of perineural invasion is not part of standard pathology practice, many studies report perineural invasion as recorded incidentally without systematic prospective reporting or a retrospective review of pathology sections. Only studies were included in which perineural invasion was recorded as part of pathology review or prospectively when

systematically reported as a prospective part of standard pathology practice. Twenty five studies were excluded on this basis.

III) Most studies combine the results of perineural invasion in colonic and rectal cancer, but the colon and the rectum have anatomically different patterns of innervations. Most of the colon is intraperitoneal with no external plexus, while the rectum is largely extraperitoneal and has its own rectal plexus. Therefore, the colon and rectum have different innervation densities and rectal cancer is potentially more able to induce neuroplasticity as a consequence of higher innervation density due to proximity of large-calibre nerve trunks in the mesorectum compared to the mesocolon [15]. As a result perineural invasion was considered in terms of colon cancer, rectal cancer, and both.

IV) Routine practice is to use H&E sections for identification of perineural invasion but some studies utilised immunohistochemistry to improve identification of perineural invasion in colorectal cancer [16]. S100 is a specific marker of neural fibres of the peripheral nervous system and has been considered useful in the identification of perineural invasion by immunohistochemistry. Both H&E and immunohistochemical studies have been included in the present review.

3.1 PROSPECTIVE STUDIES USING H&E TO IDENTIFY PERINEURAL INVASION IN PATIENTS WITH COLON, RECTAL AND COLORECTAL CANCER

3.1.1 COLON CANCER

Five studies including 1835 patients reported on survival stratified by perineural invasion in colon cancer using H&E sections (Table 1). In these studies the weighted average detection rate for perineural invasion was 26%, range 13 - 39%.

Perineural invasion was associated with high tumour stage, poor tumour differentiation and the incidence of metastasis at diagnosis. Perineural invasion was independently associated with poor survival in 3 of 5 studies, 1 of 5 studies confirmed a positive association on univariate analyses only and 1 of 5 studies reported no association with survival (both overall and cancer specific survival). Age, T4 stage, venous invasion and pre-operative CEA levels were also independently associated with poor survival.

Two studies supplied a definition for perineural invasion. All studies reported on extramural perineural invasion.

In summary, with H&E stains, perineural invasion was identified in approximately 26% of patients with primary operable colon cancer. Only extramural perineural invasion was reported and was independently associated with survival.

3.1.2 RECTAL CANCER

Eleven studies including 3837 patients reported on survival stratified by perineural invasion in rectal cancer using H&E sections (Table 2). In these studies the weighted average detection rate for perineural invasion was 25%, range 10 - 38%.

Perineural invasion was significantly associated with high tumour stage, poor tumour differentiation, incidence of metastasis at time of diagnosis, lymphatic and venous invasion.

Perineural invasion was independently associated with poor survival in 7 of 11 studies, 3 of 11 studies confirmed a positive association on univariate analyses only and 1 of 11 studies reported no association with survival (mostly overall survival). Age, tumour stage, nodal status, venous invasion and CEA levels were also independently associated with poor survival.

In summary, with H&E stains, perineural invasion was identified in approximately 25% of patients with primary operable rectal cancer. Six studies supplied a definition of perineural invasion and only extramural perineural invasion was reported and was independently associated with survival.

3.1.3 COLORECTAL CANCER

Sixteen studies including 9145 patients reported on survival stratified by perineural invasion in colorectal cancer using H&E sections (Table 3). In these studies the weighted average detection rate for perineural invasion was 17%, range 8- 42 %.

Perineural invasion was significantly associated TNM stage, poor tumour differentiation, lymphatic and venous invasion. Perineural invasion was independently associated with poor survival in 13 of 16 studies and 3 of 16 studies confirmed a significant association on univariate analyses only. In 1 of 16 studies no association between perineural invasion and poor survival was found [32]. Gender, grade, T stage, N stage, lymphovascular invasion, tumour budding, tumour necrosis, peritumoral lymphocytic infiltration and CEA levels were also independently associated with poor survival.

Three studies reported on both intra and extramural perineural invasion [13, 14, 42]. Fujita [13] defined perineural invasion as cancer invasion to the Auerbach plexus and reported perineural invasion to be a more important prognostic factor than venous and lymphatic invasion in patients with colorectal cancer. Ueno et al, 2013 [14], defined intramural perineural invasion as cancer spread along the Auerbach plexus and extramural perineural invasion as the histological finding of tumour cells invading or spreading along nerve fascicles external to the muscularis propria. In their study extramural perineural invasion was further examined, depth and incidence (number of foci) were also evaluated. Ueno

further reported that staging could be enhanced by site-specific criteria as well as a grading system based on the location of perineural invasion within the bowel wall. The study by Suzuki (2015) [42] also reported both intra and extramural perineural invasion.

An earlier study by Ueno (2001) [27] reported a three- tier grading system that included intensity and depth of perineural invasion, with an association with survival and local recurrence, independent of TNM stage.

In summary, with H&E stains, perineural invasion was identified in approximately 17% of patients with primary operable colorectal cancer. Six studies supplied a definition for perineural invasion while 3 studies reported on both intramural and extramural invasion however most studies reported only extramural perineural invasion and was independently associated with survival.

3.2 STUDIES USING SPECIAL STAINS/ TECHNIQUES TO IDENTIFY PERINEURAL INVASION IN COLORECTAL CANCER

The identification of perineural invasion using H&E staining varied between 8 % and 42 %. Review of slides reported an improvement of detection of perineural invasion however special stains using immunohistochemistry with anti-S 100 protein has been proposed to improve the identification of perineural invasion [16]. However, S100 is not the only neuronal biomarker used to detect nerves and individual axons in cancer. PGP9.5 has also been used to detect nerves in the cancer microenvironment. PGP9.5, an ubiquitin hydrolase, is widely expressed in neuronal tissues and has been suggested as a neuro endocrine marker [47,48]. However, the prognostic value of this staining has yet to be determined.

Six studies including 1458 patients reported on the identification of perineural invasion in colorectal cancer using special techniques (Table 4). Most were comparative studies that showed an increased in detection of perineural invasion with S100 protein. The detection rates were up to 70% with the use of S100 protein.

Perineural invasion (S100) was significantly associated with the TNM stage, poor tumour differentiation and lymphatic invasion. Only the study by Shimada et al, 2014 (n=184) [46] showed that perineural invasion detected by immunohistochemistry with S100 protein was independently associated with poor survival. The inter-observer assessment showed superior reproducibility in comparison to H&E staining.

In summary, the use of S100 protein increased detection of perineural invasion in patients with primary operable colorectal cancer. Furthermore, results suggested that the use of

immunohistochemistry with S100 protein could be of value in the identification of perineural invasion. Further studies are warranted to assess the effect on outcome.

4. DISCUSSION

In this systematic review of more than thirty studies, perineural invasion was independently prognostic in primary operable colon and rectal cancer. Age, gender, TNM stage, venous invasion, tumour necrosis, peri- tumoural lymphocytic infiltration, tumour budding and CEA levels were also independently associated with poor survival in at least some studies [17, 18, 21, 22, 26, 30, 32, 33, 39].

Perineural invasion was consistently associated with poor differentiation, T stage, incidence of metastasis at time of diagnosis, lymphatic and venous invasion. All of these are established histopathologic factors, indicative of aggressive behavior in colorectal cancer, and therefore suggest that perineural invasion can also be used as surrogate marker for an aggressive phenotype of colorectal cancer.

Thus, even though perineural invasion is not routinely reported, it can play an important role in the stratification of colorectal cancer and consideration should therefore be given to routine assessment of “perineural invasion status” in primary colorectal cancer specimens.

Furthermore, results of the present review suggested that in studies where perineural invasion was well defined, detection rates were higher. However it is not clear whether this increased detection rate was associated with better prediction of outcome. Further studies are warranted to examine the relationship between the assessment of perineural invasion and the prediction of outcome. Interobserver variability in the assessment of perineural invasion still needs to be further evaluated, as little information concerning the reproducibility of perineural invasion exists in the literature [14].

In the present review no difference could be demonstrated between the incidence, association and outcome of perineural invasion in colon and /or rectal cancer despite the anatomic differences in nerve supply. However, a small number of studies reported on colon cancer only and therefore no conclusion could be made..

It was clear that immunohistochemistry can be used to improve detection of perineural invasion with an antibody against S-100 protein with reported incidence of 70 percent, approximately more than 3 times the incidence utilising routine H&E staining [16]. Results from the present review suggested the use of immunohistochemistry in identification of perineural invasion could be useful despite the labour, time, and cost involved. However, further research is required to directly compare the H&E and special stain approaches and the prediction of outcomes in patients with colorectal cancer and the clinical relevance.

The association of perineural invasion with histopathologic factors, indicative of tumour spread (lymphatic invasion and venous invasion) suggest these factors can all be part of

steps in the metastatic processes that include vascular emboli, lymphatic invasion, and perineural invasion (collectively referred to as VELIPI (Pages et al.2008) [10]. Tumour progression still remains the main cause of death for patients with colorectal cancer however; the mechanisms that produce metastases remain poorly understood and further studies are required to clarify the association between VELIPI (collective) and cancer progression..

Nerves involved in perineural invasion may be considered passive as they provide a channel for cancer cell dissemination. However, recently the infiltration of the tumour microenvironment by nerves has provided evidence that there is an active process involved termed, tumour neurogenesis and has been associated with cancer progression [7]. Infiltrating nerve fibres can stimulate tumour growth and dissemination, while tumour cells can drive nerve outgrowth in a cross-talk that contributes to tumour progression [49].

Cancer cell growth can be stimulated by receptors for autonomic neurotransmitters through activation of the corresponding signalling pathways [50]. Autonomic neurotransmitters can also stimulate endothelial cells, immune cells, and fibroblasts thus having an impact on the tumour microenvironment [51]. Moreover, it has recently been reported that nerves in prostate and gastric tumours, release neurotransmitters directly into the vicinity of cancer cells to stimulate survival, proliferation, and the ability to spread [52,53].

The effect of receptors for autonomic neurotransmitters is not yet known in colorectal cancer although the microenvironment of colorectal cancer is rich in autonomic nerve fibres. Furthermore, the potential impact of nerves on colorectal cancer progression has not been reported and therefore it is still to be determined whether a similar nerve dependent tumour growth takes place [49].

Previously, a potential explanation for the strong association between colorectal cancer liver metastases and perineural invasion was that the liver is richly innervated by autonomic nerve fibres and those sympathetic fibre innervations in the liver may share a preganglionic origin with the sympathetic nerves that innervate the colon and rectum [54]. However, despite the findings of perineural invasion as a strong independent prognostic factor in colorectal cancer; the role of neurogenesis in colorectal cancer still remains unclear [55].

Recently Knijn et al. 2016, carried out a systematic review and meta-analysis of the prognostic value of perineural invasion in patients with colorectal cancer and they concluded that "perineural invasion should be one of the features in the standardised reporting of CRC and might be considered a high-risk feature" [56].

However, unlike the present review they did not consider the importance of (I) the variable definition of perineural invasion in colorectal cancer (II) retrospective nature of most studies in the literature (III) the importance of site in colon and rectum (IV) the different staining approaches to the detection of perineural invasion and their impact on the relationship with survival.

Consideration of these, results in a more limited conclusion that results of the present review suggest that perineural invasion has promise as an independent prognostic factor in patients with colorectal cancer. However, further research is required to (a) devise an optimal method for detection of perineural invasion, (b) to establish prognostic value of the optimal method and (c) for this methodology to be externally validated.

References

1. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin* 2014; 64:104–17.
2. Horgan PG, McMillan DC. Surgeons and selection of adjuvant therapy for node - negative colonic cancer. *British Journal of Surgery* 2010; 97:1459-60.
3. Benson AB, Schrag D, Somerfield MR, Cohen A M, Figueredo AT, Flynn PJ, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. *Journal of Clinical Oncology* 2004; 22: 3408-19.
4. Compton CC. Colorectal carcinoma: diagnostic, prognostic, and molecular features. *Modern pathology* 2003; 16: 376-88.
5. Loughrey MB, Quirke P, Shepherd NA. Standards and datasets for reporting cancers Dataset for colorectal cancer histopathology reports July 2014.
6. Valastyan S, Weinberg RA. 2011. Tumor metastasis: molecular insights and evolving paradigms. *Cell* 2011; 147: 275-292.
7. Marchesi F, Piemonti L, Mantovani A, Allavena P. Molecular mechanisms of perineural invasion, a forgotten pathway of dissemination and metastasis. *Cytokine Growth Factor Rev* 2010; 21: 77-82.
8. Betge, J. Langner, C. Vascular Invasion, Perineural Invasion, and Tumour Budding: Predictors of Outcome in Colorectal Cancer. *Acta Gastro-Enterologica Belgica* 2011; 74: 516-29.
9. Liebig C, Ayala G, Wilks J, Verstovsek G, Liu H, Agarwal N, et al. Perineural invasion is an independent predictor of outcome in colorectal cancer. *Journal of Clinical Oncology*. 2009; 27:5131-7.
10. Pagès F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. *New England journal of medicine* 2005; 353: 2654-66.
11. Batsakis J. Nerves and neurotropic carcinomas. *The Annals of otology, rhinology, and laryngology*. 1984; 94(4 Pt 1):426-7.
12. Liebig C, Ayala G, Wilks JA, Berger DH, Albo D. Perineural invasion in cancer. *Cancer* 2009; 115:3379-91.
13. Fujita, S., Nakanisi, Y., Taniguchi, H., Yamamoto, S., Akasu, T., Moriya, Y. & Shimoda, T. Cancer invasion to auerbach's plexus is an important prognostic factor in patients with pT3-pT4 colorectal cancer. *Diseases of the Colon & Rectum* 2007; 50: 1860-66.
14. Ueno H, Shirouzu K, Eishi Y, Yamada K, Kusumi T, Kushima R, et al. Characterization of perineural invasion as a component of colorectal cancer staging. *The American journal of surgical pathology* 2013; 37:1542-9.

15. Liebl F., Demir IE, Rosenberg R, Boldis A, Yildiz E, Kujundzic K, et al . The Severity of Neural Invasion Is Associated with Shortened Survival in Colon Cancer. *Clinical Cancer Research* 2013; 19: 50-61.
16. Bellis D, Marci V, Monga G. Light microscopic and immunohistochemical evaluation of vascular and neural invasion in colorectal cancer. *Pathol Res Pract*, 1993; 89: 443-7.
17. Wied U, Nilsson T, Knudsen J, Sprechler M, Johansen A. Postoperative survival of patients with potentially curable cancer of the colon. *Diseases of the Colon & Rectum*. 1985;28:333-5
18. Quah H-M, Chou JF, Gonen M, Shia J, Schrag D, Landmann RG, et al. Identification of patients with high-risk stage II colon cancer for adjuvant therapy. *Diseases of the Colon & Rectum*. 2008; 51:503-7.
19. Poeschl EM, Pollheimer MJ, Kornprat P, Lindtner RA, Schlemmer A, Rehak P, et al. Perineural invasion: correlation with aggressive phenotype and independent prognostic variable in both colon and rectum cancer. *Journal of Clinical Oncology* 2010; 28:e358-e60.
20. Canney AL, Kevans D, Wang LM, Hyland JM, Mulcahy HE, O'Donoghue DP, et al. Stage II colonic adenocarcinoma: a detailed study of pT4N0 with emphasis on peritoneal involvement and the role of tumour budding. *Histopathology* 2012; 61:488-96.
21. Knudsen J, Nilsson T, Sprechler M, Johansen A, Christensen N. Venous and nerve invasion as prognostic factors in postoperative survival of patients with resectable cancer of the rectum. *Diseases of the Colon & Rectum* 1983; 26:613-7.
22. Bentzen SM, Balslev I, Pedersen M, Teglbjaerg P, Hanberg-Soerensen F, Bone J, et al. A regression analysis of prognostic factors after resection of Dukes' B and C carcinoma of the rectum and rectosigmoid. Does post-operative radiotherapy change the prognosis? *British journal of cancer* 1988; 58:195-201.
23. Horn A, Dahl O, Morild I. Venous and neural invasion as predictors of recurrence in rectal adenocarcinoma. *Diseases of the colon & rectum* 1991; 34:798-804.
24. Shirouzu K, Isomoto I, Morodomi T, Araki Y, Hoshiko M, Akagi Y, et al. Clinicopathologic study of perineural invasion in rectal cancer. *The Kurume medical journal* 1992; 39:41-9.
25. Shirouzu K, Isomoto H, Kakegawa T. Prognostic evaluation of perineural invasion in rectal cancer. *The American journal of surgery* 1993; 165:233-7.
26. Bognel C, Rekacewicz C, Mankarios H, Pignon J, Elias D, Duvillard P, et al. Prognostic value of neural invasion in rectal carcinoma: a multivariate analysis on 339 patients with curative resection. *European Journal of Cancer* 1995; 31:894-8.

27. Ueno H, Hase K, Mochizuki H. Criteria for extramural perineural invasion as a prognostic factor in rectal cancer. *British Journal of Surgery* 2001; 88: 994-1000.
28. Rullier A, Laurent C, Vendrely V, Le Bail B, Bioulac-Sage P, Rullier E. Impact of colloid response on survival after preoperative radiotherapy in locally advanced rectal carcinoma. *The American journal of surgical pathology* 2005; 29:602-6.
29. Peng J, Sheng W, Huang D, Venook A P, Xu Y, Guan Z, Cai S. Perineural Invasion in pT3N0 Rectal Cancer The Incidence and Its Prognostic Effect. *Cancer* 2011; 117: 1415-21.
30. Lee JH, Jang HS, Kim J-G, Cho HM, Shim BY, Oh ST, et al. Lymphovascular invasion is a significant prognosticator in rectal cancer patients who receive preoperative chemoradiotherapy followed by total mesorectal excision. *Annals of surgical oncology* 2012;19:1213-21.
31. Krasna MJ, Flancbaum L, Cody RP, Shneibaum S, Ari GB. Vascular and neural invasion in colorectal carcinoma. Incidence and prognostic significance. *Cancer* 1988;61:1018-23.
32. Mulcahy HE, Toner M, Patchett SE, Daly L, O'Donoghue DP. Identifying stage B colorectal cancer patients at high risk of tumor recurrence and death. *Diseases of the colon & rectum* 1997;40:326-31.
33. Oñate-Ocaña LF, Montesdeoca R, López-Graniel CM, Aiello-Crocifoglio V, Mondragón-Sánchez R, Cortina-Borja M, et al. Identification of patients with high-risk lymph node-negative colorectal cancer and potential benefit from adjuvant chemotherapy. *Japanese journal of clinical oncology* 2004;34:323-8.
34. Tsai HL, Chu KS, Huang YH, Su YC, Wu JY, Kuo CH, et al. Predictive factors of early relapse in UICC stage I–III colorectal cancer patients after curative resection. *Journal of surgical oncology* 2009;100:736-43.
35. Huh JW, Kim HR, Kim YJ. Prognostic value of perineural invasion in patients with stage II colorectal cancer. *Annals of surgical oncology* 2010;17:2066-72.
36. Kang H, Min BS, Lee KY, Kim NK, Kim SN, Choi J, et al. Loss of E-cadherin and MUC2 expressions correlated with poor survival in patients with stages II and III colorectal carcinoma. *Annals of surgical oncology* 2011;18:711-9.
37. Allard M-A, Bachet JB, Beauchet A, Julie C, Malafosse R, Penna C, et al. Linear quantification of lymphoid infiltration of the tumor margin: a reproducible method, developed with colorectal cancer tissues, for assessing a highly variable prognostic factor. *Diagn Pathol.* 2012;7:156-67.
38. Huh JW, Lee JH, Kim HR, Kim YJ. Prognostic significance of lymphovascular or perineural invasion in patients with locally advanced colorectal cancer. *The American Journal of Surgery* 2013;206:758-63.

39. Lee S-J, Choi SY, Kim W-J, Ji M, Lee T-G, Son B-R, et al. Combined aberrant expression of E-cadherin and S100A4, but not beta-catenin is associated with disease-free survival and overall survival in colorectal cancer patients. *Diagn Pathol.* 2013;8:99.
40. Ueno H, Shirouzu K, Shimazaki H, Kawachi H, Eishi Y, Ajioka Y, et al. Histogenesis and prognostic value of myenteric spread in colorectal cancer: a Japanese multi-institutional study. *Journal of gastroenterology* 2014;49:400-7.
41. Yun J-A, Kim S-H, Hong HK, Yun SH, Kim HC, Chun H-K, et al. Loss of E-Cadherin expression is associated with a poor prognosis in stage III colorectal cancer. *Oncology* 2014;86:318-28.
42. Suzuki T, Suwa K, Ogawa M, Eto K, Kawahara H, Fujita T, et al. Adjuvant chemotherapy for the perineural invasion of colorectal cancer. *Journal of Surgical Research* 2015; 199: 84-89.
43. Matsushima T, Mori M, Kido A, Adachi Y, Sugimachi K. Preoperative estimation of neural invasion in rectal carcinoma. *Oncology reports.* 1998;5:73-9.
44. Ueno H, Hase K, Hashiguchi Y, Ishiguro M, Kajiwara Y, Shimazaki H, et al. Growth pattern in the muscular layer reflects the biological behaviour of colorectal cancer. *Colorectal Disease.* 2009;11:951-9.
45. White M, Foulis A, Smith G, Horgan P, Roxburgh C. The role of S100 staining in the pathological assessment of perineural invasion in rectal cancer. *Colorectal disease.* 2014; 16:71-2.
46. Shimada Y, Kido T, Kameyama H, Nakano M, Yagi R, Tajima Y, et al. Clinical significance of perineural invasion diagnosed by immunohistochemistry with anti-S100 antibody in Stage I-III colorectal cancer. *Surgery today.* today 2015;45:1493-500.
47. Yamazaki T, Hibi K, Takase T, Tezel E, Nakayama H, Kasai Y, et al. PGP9.5 as a marker for invasive colorectal cancer. *Clinical Cancer Research* 2002;8:192-5.
48. Mizukami H, Shirahata A, Goto T, Sakata M, Saito M, Ishibashi K, et al. PGP9.5 methylation as a marker for metastatic colorectal cancer. *Anticancer Res* 2008;;28:2697-700.
49. Jobling P, Pundavela J, Oliveira SM, Roselli S, Walker MM, Hondermarck H. Nerve–Cancer Cell Cross-talk: A Novel Promoter of Tumor Progression. *Cancer research* 2015; 75:1777-81.
50. Kim-Fuchs C, Le CP, Pimentel MA, Shackelford D, Ferrari D, Angst E, et al. Chronic stress accelerates pancreatic cancer growth and invasion: a critical role for beta-adrenergic signalling in the pancreatic microenvironment. *Brain Behav Immun* 2014;40:40–7.

51. Li ZJ, Cho CH. Neurotransmitters, more than meets the eye—neurotransmitters and their perspectives in cancer development and therapy. *Eur J Pharmacol* 2011;667:17–22.
52. Magnon C, Hall SJ, Lin J, Xue X, Gerber L, Freedland SJ, et al. Autonomic nerve development contributes to prostate cancer progression. *Science* 2013;341:1236361.
53. Zhao CM, Hayakawa Y, Kodama Y, Muthupalani S, Westphalen CB, Andersen GT, et al. Denervation suppresses gastric tumorigenesis. *Sci Transl Med* 2014;6:250ra115.
54. McCuskey RS. Anatomy of efferent hepatic nerves. *The anatomical record Part A, Discoveries in molecular, cellular, and evolutionary biology.* 2004;280: 821-26.
55. Albo D, Akay CL, Marshall CL, Wilks JA, Verstovsek G, Liu H, et al. Neurogenesis in colorectal cancer is a marker of aggressive tumor behavior and poor outcomes. *Cancer.* 2011;117:4834-45.
56. Knijn N, Mogk SC, Teerenstra S, Simmer F, Nagtegaal ID. Perineural Invasion Is a Strong Prognostic Factor in Colorectal Cancer: A Systematic Review. *The American journal of surgical pathology.* 2016; 40:103-112.

Table Legend

Table 1: Relationship between perineural invasion (PNI) and survival in colon cancer.

Table 2: Relationship between perineural invasion and survival in rectal cancer

Table 3: Relationship between perineural invasion and survival in colorectal cancer.

Table 4: Studies using special stains in the detection of perineural invasion in colorectal cancer

Table 1: Relationship between perineural invasion and survival in colon cancer.

| Study | Year | Number (N) | Site | Follow up | Stage | Invasion (%) | Method | Definition | Outcome measure | Results |
|--------------|------|------------|-------|-----------|-------|--------------|----------------------------|---|---|---|
| Weid [17] | 1984 | 442 | Colon | 5 year | I-III | 39 | Routine H&E | Not mentioned | Overall survival (OS) | Significantly associated with OS P=0.05 (age & venous invasion independent factors) |
| Quah [18] | 2007 | 448 | Colon | 5 year | II | 14 | Standard routine reporting | None | Disease specific survival (DSS) | Independent prognostic factor DSS P=0.04,HR also Stage T4 & pre-op CEA levels |
| Poeschl [19] | 2010 | 195 | Colon | 45 months | I-IV | 13 | Review | None | Cancer specific survival (CSS), Progression free survival (PFS) | Independent prognostic factor for both CSS p=0.002, PFS p<0.001 |
| Canney [20] | 2012 | 77 | Colon | 5 year | II | 22 | Review | Perineural, epineural, myenteric plexus | Overall survival | Independent prognostic factor OS p=0.02 |
| Liebl [15] | 2013 | 673 | Colon | 64 months | All | 31 | Routine 8 slides | 4 categories | Overall survival | Not independent prognostic factor |

Table 2: Relationship between perineural invasion and survival in rectal cancer.

| Study | Year | N | Site | Follow up | Stage | Neo adjuvant | Invasion (%) | Method | Definition | Outcome measure | Result |
|---------------|------|-----|--------|-----------|--------|--------------|--------------|-----------------|--|-------------------------|--|
| Knudsen [21] | 1983 | 682 | Rectal | 5 year | I-III | No | 35 | Routine H&E | Tumour cells outside muscle wall | 5 year survival | Independent prognostic factor also venous invasion |
| Bentzen [22] | 1988 | 468 | Rectal | 5 year | II-III | No | Stage II 17 | Not mentioned | None | Overall survival | Independent prognostic factor OS p=0.004 |
| | | | | | | | Stage III 38 | Not mentioned | None | Overall survival | Independent prognostic factor OS p=0.003 also venous invasion, CEA (elevated) and tumour diameter |
| Horn [23] | 1990 | 254 | Rectal | 5 year | I-III | Yes | | Routine | In and outside of bowel wall | 5 year survival | Worse 5 yr survival rates also venous invasion |
| Shirozou [24] | 1992 | 501 | Rectal | 8 year | I-III | Unknown | 20 | Routine | Cancer cells inside perineum extramural (Auerbach rare) | 5 year, 8 year survival | Independent prognostic factor p<0.005 |
| Shirozou [25] | 1993 | 373 | Rectal | 8 year | I-III | Unknown | 10 | Routine | Extramural | 8 year survival | Worse 8yr survival rates p<0.001 |
| Bognel [26] | 1995 | 339 | Rectal | 5 year | I-III | | 34 | H&E and saffron | Endoneural or perineural invasion | Overall survival | Independent prognostic factor OS p<0.001 also age, distance from anal verge, number of N+ nodes & tumour penetration |
| Ueno [27] | 2001 | 364 | Rectal | 5 year | II-III | No | 14 | H&E | Extramural 3 Grade system based on intensity and depth of invasion | 5 year survival | Independent prognostic factor |

| | | | | | | | | | | | |
|--------------|------|-----|--------|-----------|--------|-----|----------------------------|----------------|---|--|--|
| Rullier [28] | 2005 | 200 | Rectal | 48 months | II-III | Yes | 15.5 | Routine | None | Overall survival , Disease free survival | Significantly associated with OS p=0.04,DFS p=0.001 (univariate analysis) |
| Poeschl [19] | 2010 | 155 | Rectum | 45 months | I-IV | No | 18 | Not mentioned | None | Cancer specific survival, Progression free survival | Independent prognostic factor for both CSS p=0.04, PFS p=0.05 |
| Peng [29] | 2011 | 173 | Rectal | 49 months | II | No | Review 24 Original 8 | Review | 2 groups: SS-PNI :surround sheath TS-PNI : through sheath | Overall survival, Disease free survival | No significant association with survival only with local recurrence |
| Lee [30] | 2012 | 328 | Rectal | 5 year | I-III | Yes | 20 | Routine slides | None | Overall survival, Disease free survival | Independent prognostic factor for DFS p=0.046 also lymphovascular invasion (both DFS,OS) |

Table 3: Relationship between perineural invasion and survival in colorectal cancer.

| Study | Year | Number | Site | Follow up | Stage | Invasion (%) | Method | Definition | Outcome measure | Results |
|---------------|------|--------|------------|-----------|--------|--------------|------------------------------|--|--|--|
| Krasna [31] | 1988 | 77 | Colorectal | 6 year | I-III | 14 | Routine H&E reviewed | Endoneural or perineural invasion | Overall survival | Worse 3 yr survival rates |
| Mulcachy [32] | 1997 | 117 | Colorectal | 8.2 year | II | 8 | Routine reviewed 8 variables | None | 5 year survival | Independent prognostic factor p=0.03 also tumour necrosis |
| Ocana [33] | 2004 | 124 | Colorectal | 11.7 year | I-II | 42 | Review Prospective review | None | Cancer specific survival | Independent prognostic factor, CSS p=0.027 also gender, Tstage, grade and CEA levels |
| Pages [10] | 2005 | 959 | Colorectal | | I-IV | 10 | H&E Reassessed | None | Disease free survival , overall survival | Significantly associated with OS, DFS, (univariate analysis) |
| Fujita [13] | 2007 | 509 | Colorectal | 5 year | II-III | 26 | Review | Extramural intramural - Auerbach plexus | Disease free survival | Independent prognostic factor DFS p<0.0001 also T3-4 stage, N stage and cancer site) |
| Tsai [34] | 2008 | 259 | Colorectal | 32 months | II | 31 | Routine reporting | Cancer cells observed extraneurally | Overall survival | No association with OS |
| Liebig [9] | 2009 | 249 | Colorectal | 5 year | I-IV | 22 | Review | Tumour cells close to neural structures 33% of nerve circumference | Overall survival, Disease free survival | Independent prognostic factor OS p=0.03; DFS p=0.02 |
| Huh [35] | 2010 | 341 | Colorectal | | II | 17 | Routine review | None | Overall survival, Disease free survival | Significantly associated with DFS p= 0.036 (univariate analysis) |
| Kang [36] | 2011 | 229 | Colorectal | | II-III | 21 | Review of pathology | None | Overall survival | Independent prognostic factor OS Stage II&III combined, P=0.013 and |

| | | | | | | | | | | |
|-------------|------|-----------------|------------|-----------|--------|------------------|----------------------------|---|---|--|
| | | | | | | | | | | Stage III, p<0.001 |
| Allard [37] | 2012 | 117 | Colorectal | 61 months | II-III | 21 | Routine Reviewed slides | None | Disease free survival | Significantly associated with DFS p<0.01 (univariate analysis) |
| Huh [38] | 2013 | 1437 | Colorectal | 56 months | II-III | 19 | Routine | None | Overall survival, Disease free survival | Independent prognostic factor for OS and DFS p<0.001 (both) also LVI |
| Lee [39] | 2013 | 333 | Colorectal | 5 year | I-IV | 8 | H&E S100A4 | None | Overall survival, Disease free survival | Independent prognostic factor for OS p=0.010 also stage, tumour budding and peritumoral lymphocytic infiltration |
| Ueno [14] | 2013 | Cohort 1:962 | Colorectal | 5 year | I-III | 16 | H&E | Intramural Extramural PN Grading system | Disease free survival | Independent prognostic factor for DFS p<0.0001 |
| Ueno [40] | 2014 | 2845 | Colorectal | 5 year | | 18 | H&E Reviewed | Myenteric spread | Overall survival, Disease free survival | Independent prognostic factor for DFS p=0.0016 |
| Yun [41] | 2014 | 409 | Colorectal | 5 year | III | 16 | Reviewed | None | Overall survival, Disease free survival | Independent prognostic factor for DFS p=0.002 |
| Suzuki [42] | 2015 | 178 | Colorectal | 5 year | I-III | Not mentioned | Reviewed | Cancer cells in perineurium (Auerbach, Meissner plexus), or in peripheral nerves (intramural or extramural). | Overall survival, Disease free survival | Independent prognostic factor for OS in Stage III p=0.023 |

Table 4: Studies using special stains for detection of perineural invasion in colorectal cancer.

| Study | Year | N | Site | Stage | Method | Invasion (%) | Outcome | Agreement (K) | Results |
|-----------------|------|-----|------------|-------|-----------|--|---|-----------------------------------|--|
| Bellis [16] | 1993 | 160 | Colorectal | | Anti-S100 | 14% H&E 70% IHC | Not mentioned | - | Recommended routine use |
| Matsushima [43] | 1998 | 128 | Rectal | | S100 | 30% | Not mentioned | - | Worst prognosis |
| Ueno [44] | 2009 | 994 | Colorectal | I-III | Anti-S100 | PNI more often mode of H spread (horizontal) | Not mentioned | - | PNI in myenteric plexus predominant |
| Ueno [14] | 2013 | 50 | Colorectal | | Glut 1 | 40% | Not mentioned | - | - |
| White [45] | 2013 | 44 | Rectal | I-IV | S100 | 17% H&E 31% REVIEW 60% IHC | Not mentioned e | ICC H&E (0.94) ICC S100 (0.72) | - |
| Shimada [46] | 2014 | 184 | Colorectal | I-III | S100 | 33% (H&E) 61% (IHC) | Overall survival, Recurrence free survival | ICC H&E (0.47) ICC S100 (0.77) | Independent prognostic factor for OS and RFS |

Figure

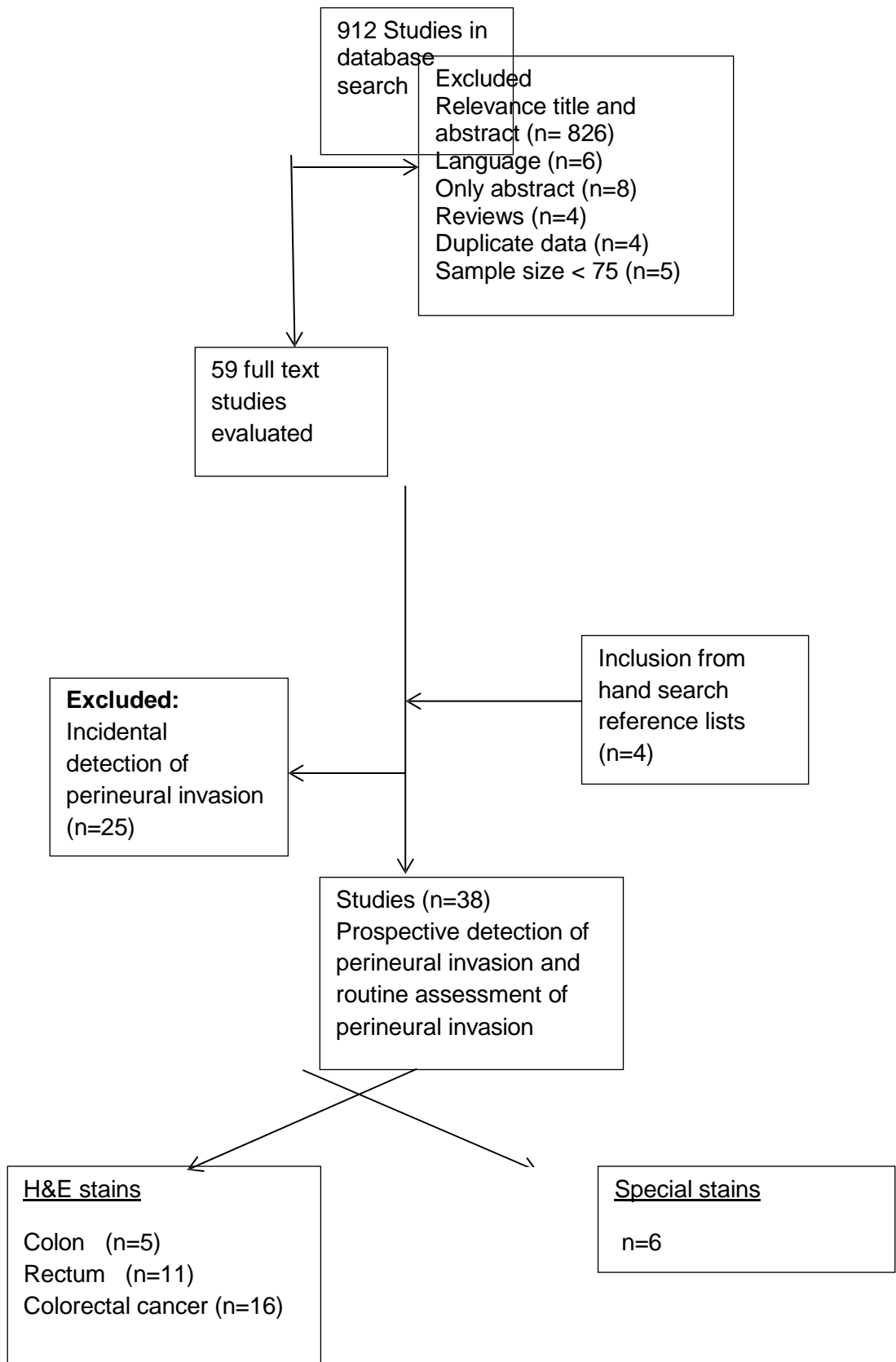


Figure 1 Flow chart of study selection process