

Cancer histology in metastatic lymph node predicts prognosis in patients with node-positive stage IV colorectal cancer

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ABSTRACT

Background: Appropriate prognostic indicators are required for patients with stage IV colorectal cancer (CRC). Lymph node metastasis mainly involves four histological types of CRC. Some metastatic lymph nodes (mLNs) showing cribriform carcinoma are associated with distant metastasis in patients with node-positive CRC and are correlated with recurrence and survival in stage III disease. However, the significance of mLN histology in the prognosis of patients with node-positive stage IV disease remains unclear.

Methods: We enrolled 449 consecutive patients with CRC who underwent primary tumor resection with lymph node dissection between January 2011 and November 2018. This study included 88 patients with node-positive stage IV CRC and synchronous or metachronous distant metastases. We retrospectively investigated the association between cancer histology in the mLNs based on our classification and cancer-specific survival (CSS) in patients with node-positive stage IV CRC.

Results: Kaplan-Meier analysis showed that CSS was better in patients with CRC and all the mLNs showing tubular-type carcinoma. In contrast, patients with at least some mLNs showing poorly differentiated-type carcinoma had poor prognosis. Multivariate analysis showed that “all mLNs showing tubular-type carcinoma” was an independent good prognostic factor for CSS in patients with node-positive stage IV CRC. In addition, “at least some mLNs showing poorly differentiated-type carcinoma” was an independent poor prognostic factor for CSS in patients with node-positive stage IV disease.

Conclusions: The histological type of the mLN may indicate a better or poor prognosis for patients with stage IV CRC.

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Additional Information and
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INTRODUCTION

The prognosis of stage IV colorectal cancer (CRC) has improved with the development of anticancer drugs. Treatment guidelines for stage IV CRC have been published based on the results of clinical trials (*Cervantes et al., 2023; Morris et al., 2023*). Advances in treatment have resulted in prolonged cancer-specific survival (CSS). Optimal selection of a better or poor prognosis group will allow for more effective treatments to be administered. Studies

targeting various predictive factors ([Cheng et al., 2022](#); [Kawamura et al., 2022](#); [Miyakawa et al., 2022](#); [Nicolazzo et al., 2023](#); [Sayagues et al., 2023](#); [Shibutani et al., 2023](#); [Su et al., 2022](#); [Wang et al., 2023](#); [Wei et al., 2023](#); [Wu et al., 2022](#); [Ziranu et al., 2023](#); [Zozaya et al., 2023](#)) have reported the prognosis of patients with stage IV CRC. However, these predictive factors have not been used as clinical criteria. Therefore, it is important to explore more accurate predictors to select better or poor prognostic cases of stage IV CRC.

Cancer histology in CRC lymph node metastasis (LNM) includes four histological types ([Yokoyama et al., 2023](#)), and “some metastatic lymph nodes (mLNs) showing cribriform carcinoma” is associated with distant metastasis in patients with node-positive CRC and is correlated with recurrence and survival in stage III disease ([Yokoyama et al., 2021](#)). The significance of CRC histology in mLNs for patients with stage IV CRC remains unclear. This study explored the significance of cancer histology in mLNs for predicting prognosis in patients with node-positive stage IV CRC.

MATERIALS AND METHODS

Patients

This study retrospectively enrolled 449 consecutive patients with CRC who underwent colectomy, anterior resection, Hartmann’s operation, or abdominoperineal resection with lymphadenectomy between January 2011 and November 2018 at the National Hospital Organization Minami Wakayama Medical Center (Tanabe, Japan). The follow-up period was 5 years. This study was approved by the ethics committee of the National Hospital Organization Minami Wakayama Medical Center (#2023-11). All methods were performed in compliance with the Declaration of Helsinki, the Guidelines for Ethical Principles for Medical Research Involving Human Subjects, and the Ethics Guidelines of the National Hospital Organization Minami Wakayama Medical Center. Informed consent was obtained in the form of opt-out on the web page of the National Hospital Organization Minami Wakayama Medical Center.

Histological analysis

Colorectal and lymph node tissue sections were stained using hematoxylin & eosin stain. The surgical specimens were fixed in a 10% buffered formaldehyde solution, washed under running tap water, dehydrated, and cleared in xylene. Paraffin embedding was performed. Sections of 5 µm were cut and placed on glass slides. The specimens were deparaffinized in xylene and rehydrated in a degressive series of ethanol solutions. The specimens were incubated in hematoxylin solution for 10 min at room temperature (RT, 15–30 °C), rinsed in distilled water, and immersed in acid alcohol for 1 min. The slides were then washed under running tap water for 10 min at 40 °C, incubated in eosin solution for 4 min and rinsed in distilled water for a few seconds. Subsequently, the slides were immersed in 100% ethanol for 2 min at RT and then dehydrated in xylene for 2 min at RT and mounted on cover slides. The slides were then examined under a microscope. Data were collected as previously described in [Yokoyama et al. \(2023\)](#). Specifically, all slides were blindly reviewed twice by three individuals (SY, TW, and SM). Discrepancies regarding the specimens’ observations were discussed to reach a consensus. All images were acquired using an

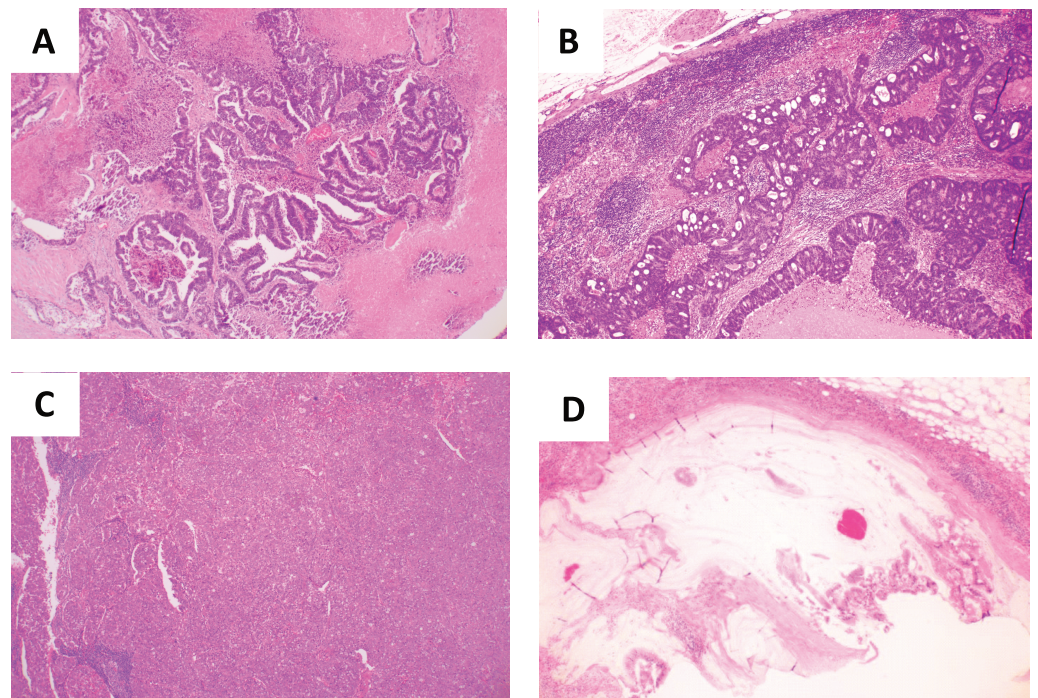


Figure 1 Histologies in metastatic lymph node. (A) Tubular-type carcinoma; (B) cribriform-type carcinoma; (C) poorly differentiated-type carcinoma; (D) mucinous-type carcinoma.

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Olympus CX33 (Olympus, Tokyo, Japan) with an NY1S adaptor (Micronet, Torrance, CA, USA), EOS X9, and the EOS utility software program (Canon, Tokyo, Japan). As previously reported (Yokoyama *et al.*, 2023), the histological classification of mLNs was performed based on the shape of the hollow cavities and cellular polarity. “Tubular-type” cells have an elongated hollow cavity and cancer cell polarity (Fig. 1A). “Cribriform-type” has small rounded cavities and no cancer cell polarity (Fig. 1B). “Poorly differentiated-type” has almost no hollow cavities and no cancer cell polarity (Fig. 1C). “Mucinous-type” contains mucin in the cavity (Fig. 1D).

Statistical analyses

Pearson’s chi-square test was used to compare clinicopathological factors. The Kaplan–Meier method was used to estimate postoperative survival, and the log-rank test was used to determine statistical significance. The Cox proportional hazards model was used to assess the risk ratio with simultaneous contributions from several covariates. A $p < 0.05$ was considered statistically significant. Calculations for the Pearson’s chi-square test, Kaplan–Meier method, log-rank test, and Cox proportional hazards model were performed using the JMP Pro software application (version 14.1.0; SAS Institute, Cary, NC, USA). Schoenfeld residuals were tested to examine the validity of the proportional hazard assumption using the STATA software application (version 13.1.0; StataCorp, College Station, TX, USA).

RESULTS

Patient characteristics

Of 449 patients with CRC, 88 (19.6%) patients with node-positive stage IV CRC who had synchronous ($n = 43$) or metachronous ($n = 45$) distant metastases were included in this study. The mean age of the patients was 71 years (range: 34–90 years). This study included 48 males and 40 females. The resected tumors were located in the colon ($n = 51$) or rectum ($n = 37$). Regarding T stages, 57, 27, and four patients had T3, T4a, and T4b stage tumor, respectively. Patients were divided into four groups based on the classification of histology in mLN as previously reported: tubular-type group ($n = 16$) with all mLN showing tubular carcinoma (Fig. 1A), cribriform-type group ($n = 56$) with some mLN showing cribriform carcinoma (Fig. 1B), poorly differentiated-type group ($n = 15$) with some mLN showing poorly differentiated carcinoma (Fig. 1C), and mucinous-type case ($n = 1$) with some mLN showing mucinous carcinoma (Fig. 1D). Patients with stage III tumor received 5-fluorouracil-based adjuvant chemotherapy before metachronous relapse. Of the 88 patients with stage IV tumors, 22 (25%) patients underwent metastasectomy, including 12 liver resections (six patients with tubular-type and six patients with cribriform-type), six lung resections (two patients with tubular-type, three patients with cribriform-type, and one patient with mucinous-type), three liver and lung resections (one patient with tubular-type and two patients with cribriform-type), one lung resection and splenectomy (one patient with poorly differentiated-type). Two patients experienced local recurrence with distant metastases. The rate of local recurrence was 2.3%. Distant metastatic organs (the number of patients) were liver (30); liver and lung (15); peritoneum (five); lung (four); lung and distant lymph node (four); distant lymph node (three); bone (three); liver and peritoneum (three); liver and distant lymph node (three); lung and peritoneum (three); liver, lung and peritoneum (three); liver, lung and distant lymph node (three); lung and bone (two); lung, distant lymph node and bone (two); peritoneum and spleen (one); peritoneum and distant lymph node (one); liver, peritoneum and distant lymph node (one); lung, peritoneum and distant lymph node (one); and liver, lung, distant lymph node and kidney (one).

Histological types in mLN and clinicopathological factors in patients with node-positive stage IV CRC

The clinicopathological factors were compared among patients with different histological types in mLN. The cases of poorly differentiated adenocarcinoma in the primary tumor were significantly more, with poorly differentiated carcinoma in the mLN. Cribriform-type carcinomas in mLN was significantly associated with lower lymphatic permeation (Table 1).

Comparison of CSS times among histological types of mLN in patients with node-positive stage IV CRC

To assess the impact of the histological types of mLN on CSS in patients with node-positive stage IV CRC, the tubular, cribriform, or poorly differentiated-types were compared. All

Table 1 Histological types in metastatic lymph node and clinicopathological factors.

Variables	Histological types in metastatic lymph node			
	Tubular-type <i>n</i> = 16	Cribriform-type <i>n</i> = 56	Poorly differentiated-type <i>n</i> = 15	<i>p</i> -value
Gender (male/female)	10/6	28/28	9/6	0.5959
Age (>70 vs. ≤70)	9/7	32/24	11/4	0.4162
Tumor site (rectum/colon)	10/6	24/32	3/12	0.0905
Primary tumor differentiation (por/others)	0/16	1/55	6/9	<0.0001
Tumor depth (T4 /T3)	2/14	21/35	8/7	0.0917
Lymphatic permeation (presence/absence)	15/1	47/9	14/1	0.0465
Venous permeation (presence/absence)	8/8	36/20	11/4	0.3130
Number of mLNs (>3/≤3)	10/6	28/28	11/4	0.2422
Distant metastasis (synchronous/metachronous)	9/7	26/30	7/8	0.6690
PS ECOG (Grade 0/1/2/3/4)	16/0/0/0/0	56/0/0/0/0	15/0/0/0/0	–
5-year follow-up rate (%)	100	100	100	–

Note:

Tubular-type in mLNs, all mLNs showing tubular carcinoma; Cribriform type in mLNs, some mLNs showing cribriform carcinoma; Poorly differentiated-type in mLNs, some mLNs showing poorly differentiated carcinoma; PS, performance status; ECOG, Eastern Cooperative Oncology Group.

patients were followed up for 5 years. There were no missing data or outliers. No adjustments were made to the Kaplan-Meier analysis, and the prognosis was in the following order: tubular-type, cribriform-type, and poorly differentiated-type in patients with node-positive stage IV CRC. Kaplan-Meier analysis of CSS showed that the cribriform-type group had a significantly shorter CSS ($p = 0.0003$) than the tubular-type group, and that the poorly differentiated-type group had a significantly shorter CSS ($p = 0.0006$) than the cribriform-type group (Fig. 2).

The association between clinicopathological factors and CSS times in patients with node-positive stage IV CRC

A Cox proportional hazards model was employed to explore factors associated with CSS in patients with node-positive stage IV disease. All patients were followed up for 5 years. There are no missing data or outliers. No adjustments were made to the Cox proportional hazards model. Before the Cox proportional hazards model, the proportional hazard assumption, Schoenfeld residuals, were tested for each factor, including sex (male vs. female) ($p = 0.7901$), age (>70 vs. ≤70) ($p = 0.3568$), tumor site (rectum vs. colon) ($p = 0.1998$), primary tumor differentiation (poor vs. others) ($p = 0.9920$), tumor depth (T4 vs. T3) ($p = 0.2618$), lymphatic permeation (presence) ($p = 0.6739$), venous permeation (presence) ($p = 0.2287$), number of mLNs (>3 vs. ≤3) ($p = 0.0572$), synchronous metastasis (vs. metachronous) ($p = 0.1407$), tubular-type in mLNs (vs. other types in mLNs) ($p = 0.7331$), and poorly differentiated-type in mLNs (vs. other types in mLNs) ($p = 0.8719$). Because the p -value for the cribriform-type in mLNs (vs. other types in mLNs) was 0.0418, the cribriform-type in mLNs (vs. other types in mLNs) was not analyzed in the Cox proportional hazards model. Age >70 years (hazard ratio (HR): 1.669), poorly differentiated adenocarcinoma in the primary tumor (HR: 2.352), T4 stage

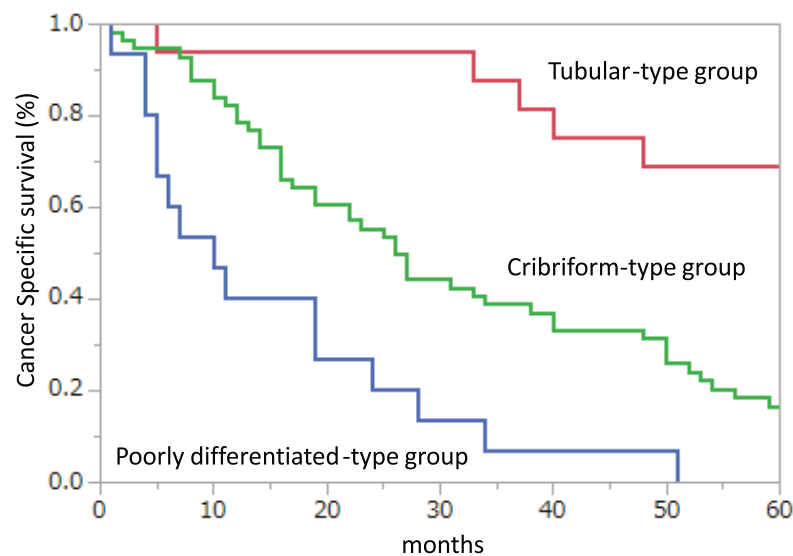


Figure 2 Kaplan-Meier plots of cancer-specific survival (CSS) in node-positive disease stage IV patients. CSS when all metastatic lymph nodes (mLNs) showed tubular carcinoma (Tubular-type group), some mLNs showed cribriform carcinoma (Cribriform-type group), and some mLNs showed poorly differentiated carcinoma (Poorly differentiated-type group).

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Table 2 Univariate analyses for cancer-specific survival of patients with node-positive stage IV colorectal.

Variables	Univariate analysis		
	HR	95% CI	p-value
Gender (male vs. female)	1.229	[0.758–1.994]	0.4023
Age (>70 vs. ≤70)	1.669	[1.009–2.762]	0.0461
Tumor site (rectum vs. colon)	0.524	[0.316–0.867]	0.0120
Primary tumor differentiation (por vs. others)	2.352	[1.068–5.178]	0.0337
Tumor depth (T4 vs. T3)	1.810	[1.100–2.976]	0.0195
Lymphatic permeation (presence)	2.340	[1.0083–5.4330]	0.0478
Venous permeation (presence)	1.186	[0.713–1.973]	0.5101
Number of mLNs (>3 vs. ≤3)	1.190	[0.731–1.937]	0.4842
Synchronous metastasis (vs. metachronous)	1.421	[0.875–2.308]	0.1561
Tubular-type in mLNs (vs. other types in mLNs)	0.196	[0.078–0.492]	0.0005
Poorly differentiated-type in mLNs (vs. other types in mLNs)	3.554	[1.955–6.463]	<0.0001

Note:

HR, hazard ratio; CI, confidence interval; mLN, metastatic lymph node; Tubular-type in mLNs, all mLNs showing tubular carcinoma; Cribriform type in mLNs, some mLNs showing cribriform carcinoma; Poorly differentiated-type in mLNs, some mLNs showing poorly differentiated carcinoma.

(HR: 1.810), lymphatic permeation (HR: 2.340), and some mLNs showing poorly differentiated carcinoma (HR: 3.554) were significantly associated with shorter CSS. The tumor site (HR: 0.524) and all mLNs showing tubular carcinoma (HR: 0.196) were significantly associated with longer CSS (Table 2).

Table 3 Multivariate analyses, including tubular-type in mLN, for cancer-specific survival of patients with node-positive stage IV colorectal cancer.

Variable	Multivariate analysis		
	HR	95% CI	p-value
Age (>70 vs. ≤70)	1.798	[1.074–3.011]	0.0257
Tumor site (rectum vs. colon)	0.652	[0.381–1.114]	0.1179
Primary tumor differentiation (por vs. others)	1.445	[0.629–3.322]	0.3856
Tumor depth (T4 vs. T3)	1.281	[0.753–2.178]	0.3601
Lymphatic permeation (presence)	2.711	[1.134–6.481]	0.0250
Tubular-type in mLN (vs. other types in mLN)	0.196	[0.077–0.502]	0.0007

Note:

HR, hazard ratio; CI, confidence interval; mLN, metastatic lymph node; Tubular-type in mLN, all mLN showing tubular carcinoma.

Table 4 Multivariate analyses, including poorly differentiated-type in mLN, for cancer-specific survival of patients with node-positive stage IV colorectal cancer.

Variable	Multivariate analysis		
	HR	95% CI	p-value
Age (>70 vs. ≤70)	1.806	[1.077–3.029]	0.0251
Tumor site (rectum vs. colon)	0.680	[0.394–1.172]	0.1651
Primary tumor differentiation (por vs. others)	1.048	[0.409–2.686]	0.9229
Tumor depth (T4 vs. T3)	1.432	[0.829–2.476]	0.1980
Lymphatic permeation (presence)	1.988	[0.838–4.714]	0.1187
Poorly differentiated-type in mLN (vs. other types in mLN)	2.525	[1.209–5.271]	0.0136

Note:

HR, hazard ratio; CI, confidence interval; mLN, metastatic lymph node; Poorly differentiated-type in mLN, some mLN showing poorly differentiated carcinoma.

All mLN showing tubular-type carcinoma predict better CSS in patients with node-positive stage IV CRC

Multivariate analyses of six parameters with significant differences in the univariate analysis, including age, tumor site, primary tumor differentiation, tumor depth, lymphatic permeation, and all mLN showing tubular carcinoma, revealed that age, lymphatic permeation, and all mLN showing tubular carcinoma were independent prognostic factors for CSS (Table 3). The HR for age, lymphatic permeation, and all mLN with tubular carcinoma were 1.798, 2.711, and 0.196, respectively. Therefore, only “all mLN showing tubular carcinoma” was an independent “better prognostic factor” in patients with node-positive stage IV CRC.

Some mLN showing poorly differentiated-type carcinoma predict poor CSS in patients with node-positive stage IV CRC

Multivariate analyses of six parameters with significant differences in the univariate analysis, including age, tumor site, primary tumor differentiation, tumor depth, lymphatic permeation, and some mLN showing poorly differentiated carcinoma, showed that age

and some mLNs showing poorly differentiated carcinoma were independent prognostic factors for CSS (Table 4). The HR for age and some mLNs with poorly differentiated carcinoma were 1.806 and 2.525, respectively.

DISCUSSION

Establishing an accurate indicator of the prognosis of patients with stage IV CRC will enhance the selection of appropriate treatments for CRC. To improve the prognosis of stage IV CRC, it is necessary to first extract a better prognosis group based on current treatment, including surgery, chemotherapy, and radiotherapy, so that patients can choose the best treatment from the currently available treatment option for longer survival. However, it is necessary to consider treatment policies for the poor prognosis group, and novel treatments for this group need to be developed. In the current study, tubular-type carcinomas in the mLN had a better prognosis. In contrast, poorly differentiated carcinoma in the mLN had a poor prognosis. The tubular-type group may have a long-term prognosis with current chemotherapy and surgery. In contrast, the poorly differentiated-type group is not likely to achieve long-term survival with current therapies. Therefore, changes in current chemotherapy regimens, indications for immune checkpoint inhibitors, introduction of chemotherapy for other cancers, or development of novel anticancer agents may be needed to improve the prognosis of the poorly differentiated-type group. Although further studies are required, our classification system may guide treatment decisions for patients with different prognoses.

In the good prognosis group with stage IV CRC, the goal was to achieve complete long-term regression. Recently, the use of chemotherapy in patients with stage IV CRC has progressed significantly. Some cases achieve complete response with multidisciplinary therapy including chemotherapy (Aomatsu et al., 2021; Arata et al., 2016; Baik et al., 2021; Baimas-George et al., 2018; Bulajic et al., 2019; Dang et al., 2020; Higgins et al., 2021; Lara-Morga et al., 2023; Li et al., 2021; Tokuhara et al., 2019; Yoshida et al., 2017). Currently, we do not fully understand which cases lead to a complete response. If a group with a high possibility of complete remission can be selected, a more effective treatment can be provided. In this study, of the 18 patients with tubular-type carcinoma of the mLN, 11 had recurrence-free survival for 5 years. Current treatments, including surgery and chemotherapy, for the tubular-type group may lead to complete regression. Our classification, based on the shape of the hollow and cancer cell polarity in the mLN, has the potential to provide a better understanding of cancer malignancy and therapeutic susceptibility.

A prognostic prediction has been performed using LNM as an indicator of stage IV CRC. Positive LNM (Ishihara et al., 2014; Kuo et al., 2021), the number of positive lymph nodes (Han et al., 2018, 2020; Li et al., 2017), and the ratio of the number of positive lymph nodes to the total number of retrieved lymph nodes (lymph node ratio) (Ahmed et al., 2016; Fu et al., 2015; Jiang et al., 2019; Ozawa et al., 2015) have been reported as prognostic indicators. However, these are not sufficient to classify the malignant phenotypes of individual cases; thus, more accurate indicators are required. Cancer histology in the mLN

using conventional pathological classification has been performed for stage III CRC but not for stage IV CRC (Hirayama et al., 2018; Takahashi, Mori & Yasuno, 2000). In this study, we investigated the prognosis of patients with stage IV CRC using a classification system based on the shape of the hollow cavity and cancer cell polarity. “All mLNs showing tubular carcinoma” was an independent good prognostic factor, and “some mLNs showing poorly differentiated carcinoma” was an independent poor prognostic factor for CSS in patients with node-positive stage IV CRC. Our classification of cancer histology in the mLN may distinguish between patients with stage IV CRC with good or poor prognosis and allow individualized treatment plans.

In the present study, the survival rate was different between the tubular-type and poorly differentiated-type groups. Cancer cell biology should be considered in further interpretation. LNM is the metastatic front. Therefore, LNM histology may indicate characteristics of metastatic cancer cells. Our classification, based on cancer cell polarity, mainly refers to epithelial or mesenchymal cell types. Poorly differentiated-type histology in mLN may be more mesenchymal “in the metastasis front” than tubular-type histology, resulting in poor prognosis. Moreover, in a clinical setting, poorly differentiated carcinomas in the mLN may be resistant to current chemotherapy. Further investigation of the cellular mechanism in LNM is required to address the malignant properties of poorly differentiated carcinomas in mLN.

We have previously reported an association between cribriform-type carcinoma in LNM and poor survival (Yokoyama et al., 2021, 2023). In this study, cribriform-type mLNs (vs. other types in mLNs) were not analyzed in the Cox proportional hazards model because the p -value for cribriform-type mLNs (vs. other types in mLNs) was 0.0418. In the current study, the percentages of patients with all tubular-type, some cribriform-type carcinoma, and some poorly differentiated-type carcinoma in LNM in stage IV CRC were 18.4%, 64.4%, and 17.2%, respectively. Other types in mLNs include tubular and poorly differentiated-types in mLNs. Therefore, during the first half of the observation period, the poorly differentiated-type group showed more reduced numbers than the cribriform-type group. In contrast, during the latter half, the tubular-type group maintained the number better than the cribriform-type group (Fig. 2). This may explain why the proportional assumption was not met in cribriform-type in mLNs (vs. other types in mLNs).

Limitations of the present study include the small number of patients and its retrospective design. To address the significance of mLN histology in stage IV CRC, further investigations, including retrospective studies with large sample sizes, long-term follow-up, and prospective clinical trials, are required.

CONCLUSIONS

This study demonstrated that tubular-type cancer histology in LNM from CRC indicates better prognostic properties. In contrast, the poorly differentiated-type in LNM suggests a poor prognosis in patients with node-positive stage IV CRC. Further examination of cancer histology in LNM in node-positive stage IV CRC might provide clues for appropriate treatment of stage IV CRC.

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ADDITIONAL INFORMATION AND DECLARATIONS

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The authors received no funding for this work.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Shozo Yokoyama conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Takashi Watanabe performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Shuichi Matsumura performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Masato Tamiya performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Shotaro Nagano performed the experiments, authored or reviewed drafts of the article, and approved the final draft.
- Yuya Hori performed the experiments, authored or reviewed drafts of the article, and approved the final draft.

Human Ethics

The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):

The ethics committee of the National Hospital Organization Minami Wakayama Medical Center approved the study (#2023-11).

Data Availability

The following information was supplied regarding data availability:

The raw measurements are available in the [Supplemental File](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.17702#supplemental-information>.

REFERENCES

- Ahmed S, Leis A, Chandra-Kanthan S, Fields A, Zaidi A, Abbas T, Le D, Reeder B, Pahwa P. 2016. Regional lymph nodes status and ratio of metastatic to examined lymph nodes correlate with survival in stage IV colorectal cancer. *Annals of Surgical Oncology* 23:2287–2294 DOI [10.1245/s10434-016-5200-9](https://doi.org/10.1245/s10434-016-5200-9).

- Aomatsu N, Maeda K, Uchima Y, Matsutani S, Tsujio G, Miyamoto H, Okada T, Kurihara S, Nishii T, Tachimori A, Ikeda K, Takeuchi K. 2021. Multiple stage IV colorectal cancers in a patient who received multidisciplinary treatment, including chemotherapy and Japanese Kampo medicine: a case report. *Neuropeptides* 88:102160 DOI 10.1016/j.npep.2021.102160.
- Arata R, Itamoto T, Ikeda S, Nakahara H, Oshita A, Shinozaki K, Nishisaka T. 2016. Pathological complete response after neoadjuvant chemotherapy for rectal cancer with synchronous multiple liver metastases: a report of an unusual case. *Surgical Case Reports* 2(1):106 DOI 10.1186/s40792-016-0231-9.
- Baik H, Lee HJ, Park J, Park HY, Park J, Lee S, Bae KB. 2021. Complete response of MSI-high metastatic colon cancer following treatment with regorafenib: a case report. *Molecular and Clinical Oncology* 15(5):243 DOI 10.3892/mco.2021.2405.
- Baimas-George M, Baker E, Kamionek M, Salmon JS, Sastry A, Levi D, Vrochides D. 2018. A complete pathological response to pembrolizumab following ex vivo liver resection in a patient with colorectal liver metastases. *Chemotherapy* 63(2):90–94 DOI 10.1159/000487814.
- Bulajic P, Bidzic N, Djordjevic V, Ceranic M, Betaasarić D, Pesic V, Djordjevic-Pesic J. 2019. Impact of genetics on neoadjuvant therapy with complete pathological response in metastatic colorectal cancer: case report and review of the literature. *Balkan Journal of Medical Genetics* 22(1):75–80 DOI 10.2478/bjmg-2019-0004.
- Cervantes A, Adam R, Roselló S, Arnold D, Normanno N, Taïeb J, Seligmann J, De Baere T, Osterlund P, Yoshino T, Martinelli E. 2023. Metastatic colorectal cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Annals of Oncology* 34(1):10–32 DOI 10.1016/j.annonc.2022.10.003.
- Cheng P, Chen H, Huang F, Li J, Liu H, Zheng Z, Lu Z. 2022. Nomograms predicting cancer-specific survival for stage IV colorectal cancer with synchronous lung metastases. *Scientific Reports* 12(1):13952 DOI 10.1038/s41598-022-18258-w.
- Dang Y, Gao H, Huang S, Qi T. 2020. Clinical complete regression after local radiotherapy combined with chemotherapy for stage IV rectal cancer: a case report. *Molecular and Clinical Oncology* 13(2):186–190 DOI 10.3892/mco.2020.2049.
- Fu J, Jiang M, Tan Y, Yang J, Wu L, Feng L, Zheng S, Yuan Y. 2015. Synchronous resectable metastatic colorectal cancer: lymph node involvement predicts poor outcome. *Medicine (Baltimore)* 94(30):e1215 DOI 10.1097/MD.0000000000001215.
- Han EC, Kwon YH, Park KJ, Jeong SY, Kang SB, Oh JH, Heo SC, Seoul Colorectal G. 2018. Significance of lymph node metastasis in the survival of stage IV colorectal cancer by hematogenous metastasis. *Annals of Surgical Treatment and Research* 95(4):201–212 DOI 10.4174/ast.2018.95.4.201.
- Han L, Mo S, Xiang W, Li Q, Wang R, Xu Y, Dai W, Cai G. 2020. Prognostic accuracy of different lymph node staging system in predicting overall survival in stage IV colon cancer. *International Journal of Colorectal Disease* 35(2):317–322 DOI 10.1007/s00384-019-03486-w.
- Higgins MJ, Mulsow J, Staunton O, Aird J, Cronin C, Dunne MT, Burke J, Shields C, Faul E, McCawley N, Toomey D, Conneely J, O'Neill B. 2021. Pragmatic use of short-course radiotherapy, chemotherapy and surgery for stage IV rectal cancer with locally advanced or symptomatic primary tumours. *Journal of Medical Imaging and Radiation Oncology* 65(7):940–950 DOI 10.1111/1754-9485.13312.
- Hirayama K, Sudoh M, Shimizu H, Furuya S, Akazawa Y, Iino H, Shiraishi K, Akaike H, Hosomura N, Kawaguchi Y, Amemiya H, Kawaida H, Inoue S, Kono H, Fujii H, Ichikawa D. 2018. Clinical impact of histological heterogeneity in the metastatic lymph nodes of patients with colorectal cancer. *Anticancer Research* 38(8):4797–4803 DOI 10.21873/anticancer.12789.

- Ishihara S, Hayama T, Yamada H, Nozawa K, Matsuda K, Miyata H, Yoneyama S, Tanaka T, Tanaka J, Kiyomatsu T, Kawai K, Nozawa H, Kanazawa T, Kazama S, Yamaguchi H, Sunami E, Kitayama J, Hashiguchi Y, Sugihara K, Watanabe T. 2014. Prognostic impact of primary tumor resection and lymph node dissection in stage IV colorectal cancer with unresectable metastasis: a propensity score analysis in a multicenter retrospective study. *Annals of Surgical Oncology* 21(9):2949–2955 DOI 10.1245/s10434-014-3719-1.
- Jiang C, Wang F, Guo G, Dong J, Liu S, He W, Zhang B, Xia L. 2019. Metastatic lymph node ratio as a prognostic indicator in patients with stage IV colon cancer undergoing resection. *Journal of Cancer* 10(11):2534–2540 DOI 10.7150/jca.29216.
- Kawamura H, Honda M, Takano Y, Kinuta S, Kamiga T, Saji S, Kono K. 2022. Prognostic role of carcinoembryonic antigen and carbohydrate antigen 19-9 in stage IV colorectal cancer. *Anticancer Research* 42(8):3921–3928 DOI 10.21873/anticancer.15886.
- Kuo YT, Tsai WS, Hung HY, Hsieh PS, Chiang SF, Lai CC, Chern YJ, Hsu YJ, You JF. 2021. Prognostic value of regional lymph node involvement in patients with metastatic colorectal cancer: palliative versus curative resection. *World Journal of Surgical Oncology* 19(1):150 DOI 10.1186/s12957-021-02260-z.
- Lara-Morga C, Palka-Kotlowska M, Custodio-Cabello S, Pacheco-Barcia V, Cabezon-Gutierrez L. 2023. Complete response to third-line treatment with trifluridine/tipiracil (TAS-102) in stage IV colon adenocarcinoma. *Exploration of Targeted Anti-Tumor Therapy* 4:307–315 DOI 10.37349/etat.2023.00136.
- Li Q, Wang C, Li Y, Li X, Xu Y, Cai G, Lian P, Cai S. 2017. Lymph node status as a prognostic factor after palliative resection of primary tumor for patients with metastatic colorectal cancer. *Oncotarget* 8(29):48333–48342 DOI 10.18632/oncotarget.15696.
- Li R, Wang Q, Zhang B, Yuan Y, Xie W, Huang X, Zhou C, Zhang S, Niu S, Chang H, Chen D, Miao H, Zeng ZF, Xiao W, Gao Y. 2021. Neoadjuvant chemotherapy and radiotherapy followed by resection/ablation in stage IV rectal cancer patients with potentially resectable metastases. *BMC Cancer* 21(1):1333 DOI 10.1186/s12885-021-09089-5.
- Miyakawa T, Kawamura H, Honda M, Takano Y, Kinuta S, Kamiga T, Yamazaki S, Muto A, Shiraso S, Yamashita N, Iwao T, Kono K, Konno S. 2022. Impact of histological subtype on prognosis in stage IV colorectal cancer: a population-based cohort study. *PLOS ONE* 17(3):e0264652 DOI 10.1371/journal.pone.0264652.
- Morris VK, Kennedy EB, Baxter NN, Benson AB III, Cercek A, Cho M, Ciombor KK, Cremolini C, Davis A, Deming DA, Fakih MG, Gholami S, Hong TS, Jaiyesimi I, Klute K, Lieu C, Sanoff H, Strickler JH, White S, Willis JA, Eng C. 2023. Treatment of metastatic colorectal cancer: ASCO guideline. *Journal of Clinical Oncology* 41(3):678–700 DOI 10.1200/JCO.22.01690.
- Nicolazzo C, Magri V, Marino L, Belardinilli F, Di Nicolantonio F, De Renzi G, Caponnetto S, De Meo M, Giannini G, Santini D, Cortesi E, Gazzaniga P. 2023. Genomic landscape and survival analysis of ctDNA “neo-RAS wild-type” patients with originally RAS mutant metastatic colorectal cancer. *Frontiers in Oncology* 13:1160673 DOI 10.3389/fonc.2023.1160673.
- Ozawa T, Ishihara S, Nishikawa T, Tanaka T, Tanaka J, Kiyomatsu T, Hata K, Kawai K, Nozawa H, Kanazawa T, Kazama S, Yamaguchi H, Sunami E, Kitayama J, Watanabe T. 2015. Prognostic significance of the lymph node ratio in stage IV colorectal cancer patients who have undergone curative resection. *Annals of Surgical Oncology* 22(5):1513–1519 DOI 10.1245/s10434-014-4184-6.
- Sayagues JM, Montero JC, Jimenez-Perez A, Del Carmen S, Rodriguez M, Vidal Tocino R, Montero E, Sanz J, Abad M. 2023. Analysis of circulating tumor dna in synchronous metastatic

- colorectal cancer at diagnosis predicts overall patient survival. *International Journal of Molecular Sciences* **24**:8438 DOI 10.3390/ijms24098438.
- Shibutani M, Kashiwagi S, Fukuoka T, Iseki Y, Kasashima H, Maeda K. 2023.** The significance of the D-dimer level as a prognostic marker for survival and treatment outcomes in patients with stage IV colorectal cancer. *In Vivo* **37**(1):440–444 DOI 10.21873/invivo.13097.
- Su YT, Chen JW, Chang SC, Jiang JK, Huang SC. 2022.** The clinical experience of the prognosis in opposite CEA and image change after therapy in stage IV colorectal cancer. *Scientific Reports* **12**(1):20075 DOI 10.1038/s41598-022-24187-5.
- Takahashi K, Mori T, Yasuno M. 2000.** Histologic grade of metastatic lymph node and prognosis of rectal cancer. *Diseases of the Colon & Rectum* **43**(10):S40–S46 DOI 10.1007/BF02237225.
- Tokuhara K, Yamamoto N, Hishikawa H, Yoshioka K. 2019.** Peritoneal dissemination of ascending colon cancer demonstrating relapse-free survival for 40 months with panitumumab monotherapy: a case report. *International Journal of Surgery Case Reports* **59**:41–45 DOI 10.1016/j.ijscr.2019.05.001.
- Wang T, Tang Y, Pan W, Yan B, Hao Y, Zeng Y, Chen Z, Lan J, Zhao S, Deng C, Zheng H, Yan J. 2023.** Patient-derived tumor organoids can predict the progression-free survival of patients with stage IV colorectal cancer after surgery. *Diseases of the Colon & Rectum* **66**(5):733–743 DOI 10.1097/DCR.0000000000002511.
- Wei M, Su J, Zhang J, Liu S, Ma J, Meng XP. 2023.** Construction of a DDR-related signature for predicting of prognosis in metastatic colorectal carcinoma. *Frontiers in Oncology* **13**:1043160 DOI 10.3389/fonc.2023.1043160.
- Wu B, Yang J, Qin Z, Yang H, Shao J, Shang Y. 2022.** Prognosis prediction of stage IV colorectal cancer patients by mRNA transcriptional profile. *Cancer Medicine* **11**(24):4900–4912 DOI 10.1002/cam4.4824.
- Yokoyama S, Fujita Y, Matsumura S, Yoshimura T, Kinoshita I, Watanabe T, Tabata H, Tsuji T, Ozawa S, Tamaki T, Nakatani Y, Oka M. 2021.** Cribriform carcinoma in the lymph nodes is associated with distant metastasis, recurrence, and survival among patients with node-positive colorectal cancer. *British Journal of Surgery* **108**(3):e111–e112 DOI 10.1093/bjs/znaa123.
- Yokoyama S, Watanabe T, Fujita Y, Matsumura S, Ueda K, Nagano S, Kinoshita I, Murakami D, Tabata H, Tsuji T, Ozawa S, Tamaki T, Nakatani Y, Oka M. 2023.** Histology of metastatic colorectal cancer in a lymph node. *PLOS ONE* **18**(4):e0284536 DOI 10.1371/journal.pone.0284536.
- Yoshida Y, Naito M, Yamada T, Aisu N, Kojima D, Mera T, Tanaka T, Naito K, Yasumoto K, Kamigaki T, Gotoh S, Kodama S, Yamashita Y, Hasegawa S. 2017.** Clinical study on the medical value of combination therapy involving adoptive immunotherapy and chemotherapy for stage IV colorectal cancer (COMVI study). *Anticancer Research* **37**(7):3941–3946 DOI 10.21873/anticancer.11777.
- Ziranu P, Aimola V, Pretta A, Dubois M, Murru R, Liscia N, Cau F, Persano M, Deias G, Palmas E, Loi F, Migliari M, Pusceddu V, Puzzone M, Lai E, Cascinu S, Faa G, Scartozzi M. 2023.** New horizons in metastatic colorectal cancer: prognostic role of CD44 expression. *Cancers (Basel)* **15**(4):1212 DOI 10.3390/cancers15041212.
- Zozaya G, Cienfuegos JA, Marti-Cruchaga P, Hernandez-Lizoain JL, Baixauli J, Pardo F, Robledano R, Rodriguez J, Arbea L, Rotellar F. 2023.** Impact of perineural invasion on the outcome of patients with synchronous colorectal liver metastases treated with neoadjuvant chemotherapy and surgery. *Clinical and Translational Oncology* **25**(8):2523–2531 DOI 10.1007/s12094-023-03138-0.