International Journal of Clinical and Diagnostic Pathology

ISSN (P): 2617-7226
ISSN (E): 2617-7234
www.patholjournal.com
2020; 3(2): 49-53
Received: 28-02-2020
Accepted: 30-03-2020

Betsy Jose
Department of Pathology, Pushpagiri Institute of Medical sciences and Research Institute, Kerala, India

Marjorie Correa
Department of Pathology, St. John's Medical College Hospital, Bangalore, Karnataka, India

Jyothi S Prabhu
Division of Molecular, Medicine, St. John's Research Institute, Bangalore, Karnataka, India

Corresponding Author:
Betsy Jose
Department of Pathology, Pushpagiri Institute of Medical sciences and Research Institute, Kerala, India

Association of tumour infiltrating lymphocytes with clinicopathological prognostic variables in colorectal carcinoma: A study from South India

Betsy Jose, Marjorie Correa and Jyothi S Prabhu

DOI: https://doi.org/10.33545/pathol.2020.v3.i2a.224

Abstract
Introduction: The tumour infiltrating lymphocytes (TIL) have a complimentary role alongside TNM assessment in determining the future management in colorectal carcinoma. The present study aimed at evaluating tumour infiltrating lymphocytes at the invasive front of the tumour and to correlate with other known prognostic variables.
Methods: A total of 76 cases of colorectal adenocarcinoma were selected and TILs were assessed on hematoxylin and eosin (H-E) stained tumour sections based on Klintrup-Makinen (K-M) score; classified cases into high TIL and low TIL groups and correlated with clinicopathological parameters.
Results: Of the 76 cases, 46 cases (60%) belonged to low TIL group and 30 cases (40%) to high TIL group. Statistical analysis showed that density of TILs at the invasive front of the tumour negatively correlated with grade of differentiation (p=0.02), lymphovascular invasion (p=0.03), nodal involvement (p=0.01) and pathological staging (p=0.02).
Conclusion: Tumour infiltrating lymphocytes showed significant association with grade of tumour differentiation, lymphovascular invasion, nodal involvement and stage of tumour.

Keywords: Colorectal cancer, tumour infiltrating lymphocyte, Klintrup-Makinen (K-M) score

Introduction
Colorectal carcinoma (CRC) is one among the leading causes of cancer related mortality and morbidity throughout world and represents a major public health problem [1]. The focus on the tumor microenvironment has recognized inflammatory activity as a critical predictor of disease progression [2]. Tumor-infiltrating lymphocytes (TILs) are an important histopathologic feature of colorectal cancer that has prognostic significance and is often interpreted as the host immune response against tumour development [3]. Although in early years colorectal cancer TILs revolved around their association with cancers showing sporadic or familial microsatellite instability (MSI), recent attention has shifted to the prognostic value of TILs, different scoring methodologies and the ability of TIL scoring to predict response to neoadjuvant therapy in colorectal cancer [4, 5].
The morphological assessment of colorectal TILs and the survival advantage of pronounced lymphocytic infiltration in specimens of large bowel carcinomas have received much attention in the literature [3, 5, 6]. However, the association between morphological assessment of colorectal TILs and other clinicopathological parameters remains unclear. Hence the aim of the study was to assess the TILs based on Haemtoxylin and Eosin (H&E) stained tumour sections and to correlate with other clinicopathological variables

Methods
The study included 76 cases of colorectal resection specimens diagnosed as colorectal adenocarcinoma in the Department of Pathology from January 2013 to December 2016. Patients who had undergone preoperative chemo-radiation for CRC were excluded. This retrospective study was reviewed and approved by the Institutional Ethical Committee. The relevant clinical data were collected from the medical records. Histopathology slides were retrieved from the archives. Standard pathologic analysis was done on all cases by two experienced pathologists who were blinded to the patient details. After the final histopathologic evaluation, the colorectal carcinoma was staged according to the seventh

American Joint Committee on Cancer (AJCC) TNM staging system. The resection specimens were analysed for histopathological prognostic parameters like tumour site, histologic type, histologic grade, lymphovascular invasion, perineural invasion, tumour extension, lymph node involvement, pathological TNM staging and tumour infiltrating lymphocyte.

Tumour infiltrating lymphocytes (TIL) were detected within the cancer epithelium (intratumoral) and in the stroma (peritumoral) and this study focused on the TILs at the invasive margins of the tumour. Invasive margin was defined as an interface between the host stroma and the invading edge of a tumour. Lymphocytes were the predominant population present in the tumour stroma at the invading margin and were identified on H&E stained tumour sections as small blue mononuclear cell. For estimation of lymphocytic reaction, areas of deepest tumour invasion were selected.

Semi-quantitative H&E assessment based on Klintrup-Makinen (K-M) score was done and the immune infiltrate was scored from 0 to 3. Score 0: No increase in inflammatory cells. Score 1: Patchy increase of inflammatory cells at the invasive margin, but no destruction of invading cancer cell islets. Score 2: Band like infiltrate at the invasive margin with some destruction of cancer cell islets. Score 3: Prominent inflammatory reaction, forming a cup-like zone at the invasive margin, and frequent and invariable destruction of cancer cell islets. Scores were collapsed into low- TIL/ low grade inflammation (score 0 to 1) and high TIL/ high grade inflammation (score 2 to 3) [5, 7]. The statistical analysis was carried out using SPSS version 23.0. Descriptive statistics were reported using mean for the continuous variables, number and percentages for the categorical variables. Chi-square test and Fisher's Exact test were used to test the association between outcome variables with clinicopathological characteristics of the study subjects. P value less than 0.05 was considered statistically significant.

Results

A total of 85 colorectal resection specimens were received at our institute during the study period, of which 76 cases fulfilled the inclusion criteria. The clinicopathological findings of the 76 cases included in the study are summarized in table 1. The study group had patients with age ranging from 24 years to 81 years with a mean age of 55.1 years. Out of the 76 cases 46 were males and 30 were females. The most common symptoms at presentation were rectal bleeding, altered bowel habits and abdominal pain. A few cases presented with mass per abdomen.

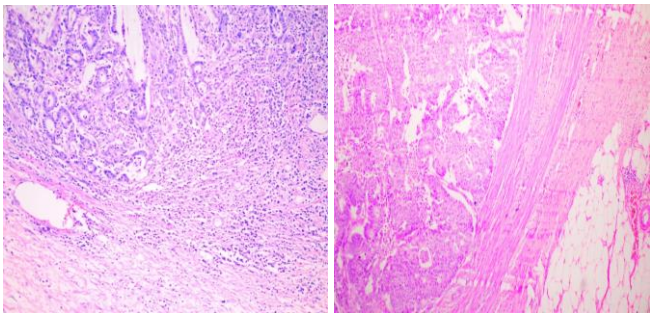
Table 1: Clinicopathological characteristics of 76 patients

Clinicopathologic Variables		Number of Cases	Percentage
1. Age	<50	28	37
	>50	48	63
2. Sex	Male	46	61
	Female	30	39
3. Site	Right	38	50
	Left	38	50
4. Histologic type	Adenocarcinoma NOS	53	70
	Mucinous	18	24
	Signet ring	5	6
5. Lymphovascular invasion	Present	34	45
	Absent	42	55
6. Perineural invasion	Present	7	9
	Absent	69	91
7. Tumour extension	T1	3	4
	T2	11	15
	T3	45	59
	T4	17	22
8. Lymph Node involvement	N0	39	51
	N1	21	28
	N2	16	21
9. Pathological Stage	Stage I	10	13
	Stage II	28	37
	Stage III	33	43
	Stage IV	5	7

Histologically 70% (n=53) of the colorectal carcinomas were adenocarcinoma NOS; of which 58% (n=44) were moderately differentiated; 8% (n=6) were poorly differentiated and 4% (n=3) were well differentiated. Rest of the cases included 24% (n=18) of mucinous carcinoma and 6% (n=5) of signet ring carcinoma and were considered as poorly differentiated. Of the 76 cases, 45% (n=34) cases showed lymphovascular invasion. Perineural invasion was found only in 4 cases of initial pathology reports which when reviewed showed 3 additional cases amounting to a total of 7 cases.

The number of lymph nodes examined ranged from 1 to 51 with an average of 14. The mean number of lymph nodes harvested from right and left sides of the colon were 16 (range: 1-51) and 12 (range: 1-40) respectively. Fifty one percentage cases showed no nodal involvement while 49% showed lymph node metastases; as detailed in Table 1. The commonest tumour stage was Stage III (n=33; 43%) followed by Stage II tumours (n=28; 37%) and Stage I (n=10; 13%). Only 5 cases were Stage IV (7%) and these presented with metastases to peritoneum, ovary, liver and umbilical skin.

The study population was divided into two groups on the basis of Klintrup-Makinen score with 60% (n=46) of cases showing low TIL score and 40% (n=30) of tumour showing high TIL score as shown in Figure 1.



A. B.

Fig 1: Tumour infiltrating lymphocytes at the invasive front of colorectal carcinoma. A. High grade inflammation (H&E, x100); B. Low grade inflammation (H&E, x100)

The study has found significant association between TIL score and various histopathologic parameters like grade of differentiation, lymphovascular invasion, lymph node involvement and pathological staging. Statistical analysis demonstrated that density of TILs at the invasive front of the tumour were negatively correlated with grade of differentiation (p=0.02), lymphovascular invasion (p=0.03), nodal involvement (p=0.01) and pathological staging (p=0.02).

Seventy nine percentage of tumours with higher grade of differentiation which included poorly differentiated adenocarcinomas, mucinous and signet ring carcinoma showed low grade inflammation at the invasive front of tumour. The presence of lymphovascular invasion was associated more with low grade inflammation than high grade inflammation (73% vs 27%).

Similar observation was noted in parameters like nodal involvement and pathological staging. High grade inflammation was more associated with absence of node involvement (N0- 70%, N1- 10%, N2- 20%) as compared to low grade inflammation. Early stage tumours were noted in high grade inflammation; 70% belonging to either stage I (23%) or Stage II (47%) and rest 30% in stage III/ stage IV. Additionally it was observed that out of the 7 cases of perineural invasion, 6 cases showed low grade inflammation while only one case showed high grade inflammation, though it was statistically not significant.

Overall, low density of TILs was found to be associated with tumour progression and an unfavourable clinical outcome. The correlation between the degree of inflammation and various histopathological parameters are outlined in the table 2.

Follow up data was available for 40 cases of which four cases expired due to immediate post operative complications and three cases of recurrence were noted on follow up for 3 years.

Table 2: Correlation between Tumour infiltrating lymphocytes and clinicopathological parameters in Colorectal Carcinoma

		Low TIL (n=46)	High TIL (n=30)	P value
1. Age	<50 years	18	10	0.61
	>50years	28	20	
2. Sex	Male	29	17	0.58
	Female	17	13	
3. Site	Right	24	14	0.64
	Left	22	16	
4. Histologic differentiation	Well	1	2	0.02
	Moderate	22	22	
	Poor	23	6	
5. Lymphovascular invasion	Present	25	9	0.03
	Absent	21	21	
6. Perineural invasion	Present	6	1	0.15
	Absent	40	29	
7. Tumour extension	T1	1	2	0.43
	T2	5	6	
	T3	28	17	
	T4	12	5	
8. Node involvement	N0	18	21	0.01
	N1	18	3	
	N2	10	6	
9. Pathological Staging	Stage I	3	7	0.02
	Stage II	14	14	
	Stage III	25	8	
	Stage IV	4	1	

Discussion

Tumor-infiltrating lymphocytes serve a significant role in antitumor immune responses and correlation studies between the nature and density of the in situ immune reaction and the signs of tumour dissemination have provided a strong indirect evidence of immune mediated control of colorectal cancer progression [8]. In the present study, semi-quantitative H&E based assessment of tumour infiltrating lymphocytes according to K-M score was done which stratified the study cohort into low grade and high grade inflammation. This study found that high grade inflammation was associated with better differentiated tumours, absence of lymphovascular invasion and lymph node involvement and lower pathological stage of tumour.

The prognostic value of TIL assessment in CRC by K-M score has been validated in many studies. Huh *et al* evaluated the prognostic significance of TIL in 546 patients who underwent colectomy for CRC and found that high grade inflammation significantly correlated with better differentiated tumours and absence of perineural invasion. Though no statistically significant association between perineural invasion and TIL was obtained in the present study, it was noted that lower incidence of perineural invasion was seen in tumours with high grade inflammation. Huh *et al* further demonstrated that TIL was an independent prognostic factor for overall survival [9].

Richards *et al* assessed the peritumoral inflammatory response in CRC and found 48% to be K-M low grade and 52% to be K-M high grade and found significant correlation between K-M grade and tumour extension, nodal involvement, TNM stage and vascular invasion which was similar to the present study [10]. Richards *et al* added that a strong inflammatory cell infiltrate at the invasive margin of tumour confers a distinct survival advantage for patients with primary operable cancer [10]. Similar studies by Jakubowska *et al* have demonstrated that stromal TILs in the invasive tumour front negatively correlated with lymphovascular invasion, perineural invasion and lymph node involvement [11].

A large population based case control study by Rozek *et al* demonstrated that TIL status was highly associated with stage and grade of the tumour while TIL status had no association with age, sex, ethinicity or tumour site which corresponds with present study [12]. A study on Indian cohort by Mallick *et al* noted that low TIL correlated with presence of lymphovascular invasion, lymph node metastasis and metastasis in more than five lymph nodes. The present study in addition proposed the association of TIL with differentiation grade of the tumour which was not analysed by Mallick and collaegues [13].

Multiple studies have stated the clinical utility of local inflammatory response and found it to be an important indicator of prognosis in all areas of tumour microenvironment. Many published reports on lymphocyte subtyping by immunohistochemistry has found a direct correlation between the densities of T lymphocyte subpopulations and improved survival in CRC, supporting a major role of T-cell-mediated immunity in repressing tumor progression of CRC [14,15]. The present study has not attempted to subtype lymphocytes for multiple reasons as there are many supporting evidence for the prognostic value of a generalised tumour lymphocytic infiltrate and that the type and location of lymphocytes are subordinate to the density of infiltration [14, 16, 17].

The heterogeneity in a single tumor section was a problem. The International TILs Working Group in Breast cancer recommended that different regions should be evaluated and that the average density of TILs should be taken into account [18]. In the present study, a global assesment of the tumour section was done by two pathologists and an average infiltrate score based on all available material was reported.

Though the present study has not used any automated imaging software programs for TIL assessment, many researchers utilize it to confirm the validity and objectivity of the evaluation of TILs by an observer. But Teng *et al.* opined that the evaluation of TILs by observers was superior to the evaluation of TILs using an automated software program, as automated imaging software programs distinguished cells based on a different depth of color without considering the shape, features, or positions [19]

A limited cohort number and TIL assessment restricted to the invasive margin were a few limitations in this study. The intratumoral lymphocytes were not considered as the tumour border was felt to represent a critical interface between pro and anti-tumor factors [20].

Conclusion

This is one among the few studies from India which showed that tumour infiltrating lymphocytes in colorectal carcinoma strongly associated with grade of differentiation, lymphovascular invasion, lymph node metastases and pathological stage of tumour. Further larger studies are warranted for the incorporation of such simple and reproducible assessments of TIL into the existing pathological staging system of colorectal carcinoma.

References

1. Favoriti P, Carbone G, Greco M, Pirozzi F, Pirozzi RE, Corcione F. Worldwide burden of colorectal cancer: a review. Updates in surgery. 2016; 68(1):7-11.
2. Deschoolmeester V, Baay M, Van Marck E, Weyler J, Vermeulen P, Lardon F *et al*. Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. BMC immunology. 2010; 11(1):19.
3. Idos GE, Kwok J, Bonthala N, Kysh L, Gruber SB, Qu C. The Prognostic Implications of Tumor Infiltrating Lymphocytes in Colorectal Cancer: A Systematic Review and Meta-Analysis. Scientific Reports. 2020; 10(1):1-4.
4. Smyrk TC, Watson P, Kaul K, Lynch HT. Tumor-infiltrating lymphocytes are a marker for microsatellite instability in colorectal carcinoma. Cancer. 2001; 91(12):2417-22.
5. Hendry S, Salgado R, Gevaert T, Russell PA, John T, Thapa B *et al*. Assessing tumor infiltrating lymphocytes in solid tumors: a practical review for pathologists and proposal for a standardized method from the International Immuno-Oncology Biomarkers Working Group: Part 2: TILs in melanoma, gastrointestinal tract carcinomas, non-small cell lung carcinoma and mesothelioma, endometrial and ovarian carcinomas, squamous cell carcinoma of the head and neck, genitourinary carcinomas, and primary brain tumors. Advances in anatomic pathology. 2017; 24(6):311.
6. Jass JR. Lymphocytic infiltration and survival in rectal cancer. Journal of clinical pathology. 1986; 39(6):585-9.
7. Klintrup K, Mäkinen JM, Kauppila S, Väre PO, Melkko J, Tuominen H *et al*. Inflammation and prognosis in colorectal cancer. European journal of cancer. 2005; 41(17):2645-54.
8. Pagès F, Galon J, Fridman WH. The essential role of the in situ immune reaction in human colorectal cancer. Journal of leukocyte biology. 2008; 84(4):981-7.
9. Huh JW, Lee JH, Kim HR. Prognostic significance of tumor-infiltrating lymphocytes for patients with colorectal cancer. Archives of surgery. 2012; 147(4):366-72.
10. Richards CH, Flegg KM, Roxburgh CS, Going JJ,

Mohammed Z, Horgan PG *et al*. The relationships between cellular components of the peritumoural inflammatory response, clinicopathological characteristics and survival in patients with primary operable colorectal cancer. British journal of cancer. 2012; 106(12):2010-5.
11. Jakubowska K, Kisielewski W, Kańczuga-Koda L, Koda M, Famulski W. Stromal and intraepithelial tumor-infiltrating lymphocytes in colorectal carcinoma. Oncology letters. 2017; 14(6):6421-32.
12. Rozek LS, Schmit SL, Greenson JK, Tomsho LP, Rennert HS, Rennert G *et al*. Tumor-infiltrating lymphocytes, Crohn's-like lymphoid reaction, and survival from colorectal cancer. JNCI: Journal of the National Cancer Institute, 2016, 108(8).
13. Debjani Mallick, Sayan Kundu, Sudipta Chakrabarti, Prosun Gayen. Association of tumour infiltrating lymphocytes with conventional clinicopathological prognostic factors of colorectal adenocarcinoma: A study from eastern India. Med Pulse International Journal of Pathology. 2019; 12(2):106-112.
14. Richards CH, Roxburgh CS, Powell AG, Foulis AK, Horgan PG, McMillan DC. The clinical utility of the local inflammatory response in colorectal cancer. European journal of cancer. 2014; 50(2):309-19.
15. Huang CY, Chiang SF, Ke TW, Chen TW, You YS, Chen WT *et al*. Clinical significance of programmed death 1 ligand-1 (CD274/PD-L1) and intra-tumoral CD8+ T-cell infiltration in stage II–III colorectal cancer. Scientific reports. 2018; 8(1):1-0.
16. Ogino S, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K *et al*. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. Clinical Cancer Research. 2009; 15(20):6412-20.
17. Roxburgh CS, McMillan DC. The role of the in situ local inflammatory response in predicting recurrence and survival in patients with primary operable colorectal cancer. Cancer treatment reviews. 2012; 38(5):451-66.
18. Salgado R, Denkert C, Demaria S, Sirtaine N, Klauschen F, Pruneri G *et al*. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. Annals of oncology. 2015; 26(2):259-71.
19. Teng F, Mu D, Meng X, Kong L, Zhu H, Liu S *et al*. Tumor infiltrating lymphocytes (TILs) before and after neoadjuvant chemoradiotherapy and its clinical utility for rectal cancer. American journal of cancer research. 2015; 5(6):2064.
20. Zlobec I, Lugli A. Invasive front of colorectal cancer: dynamic interface of pro-/anti-tumor factors. World journal of gastroenterology: WJG. 2009; 15(47):5898.