OPEN ACCESS – ORIGINAL ARTICLE

Correlation of Tumor-Associated Leukocytes with Prognosis of Colorectal Carcinoma based on Pathologic Stage*

John Anthony Tindoc,[1,2] Emilio Villanueva III,[1,3] Nelson Geraldino[1]

[1]*Department of Laboratories, University of the Philippines – Philippine General Hospital*
[2]*Department of Laboratories, Silliman University Medical Center, Philippines*
[3]*Department of Pathology, University of the Philippines – College of Medicine*

ABSTRACT

Objectives. To perform a pilot study investigating the presence of correlation between the different mean tumor-associated leukocyte counts and the prognosis of colorectal cancer based on pathologic stage.

Methodology. A cross-sectional study, involving colorectal carcinoma cases in the Philippine General Hospital from 2015-2016. Proportional allocation stratified random sampling was done, with pathologic stage (AJCC 7th Edition) as the stratifying variable, collecting a total of 59 samples. Tissue sections from the samples were evaluated for the different tumor-associated lymphocyte counts. Correlation coefficients were computed to determine their correlation with pathologic stage as surrogate marker for prognosis.

Results. Of the myriad populations counted within and around the tumor mass, total lymphocyte, cytotoxic T-cell (CD8+ T-cell), neutrophil, macrophage, and plasma cell populations have significant correlation with pathologic stage as surrogate marker for prognosis of colorectal carcinoma.

Conclusion. The immune system appears to have a significant role in the natural history of colorectal carcinoma. The tumor-infiltrating lymphocytic population and especially the CD8+ T-cell subset, neutrophils, and macrophages are correlated with better prognosis. The same observation can be seen with the peritumoral CD8+ T-cells, neutrophils, macrophages, and plasma cells.

Key words: colorectal adenocarcinoma, tumor-infiltrating lymphocytes, peritumoral leukocytes, prognosis

ISSN 2507-8364 (Online)
Printed in the Philippines.

Received: 2 September 2019.
Accepted: 11 November 2019.
Published online first: 29 November 2019.
https://doi.org/10.21141/PJP.2019.12

Corresponding author: Emilio Q. Villanueva III, MD, MSPH, DPSP
Email: drevilthird@gmail.com

**Presented at the Philippine Society of Pathologists Annual Convention Research Presentation, EDSA Shangri-La, April 20, 2018*

INTRODUCTION

Recently interest on the role of the immune system in influencing prognosis of cancers is on the rise. It has been noted several decades ago that the immune system did have a role with cancer biology but interest receded. Today with the advent of more cancer drugs and the possibility of manipulating the immune system against the tumor cells, interest on the interaction between the immune system and cancer is on the rise. Local data regarding these interactions can provide useful information on the treatment and prognosis of Filipino cancer patients.

Over the course of the medical investigation of cancers, the emphasis has been on the nature of the tumor cells. Rightly so, a veritable caché of inherent characteristics of the rouge cells determines the course of the disease. A lot of therapeutic forays have been based on the results of the studies of the tumor characteristics. The initial chemotherapeutic agents were chosen on their ability to destroy tumor cells with characteristic rapid mitotic capability but with poor genetic proofreading and repair. However, normal cells, likewise, can approach the same velocity of cell replication in times of tissue repair or normal turn-over. This rendered the first chemotherapeutic agents very nonspecific and fraught with a lot of side-effects bringing into limelight the discussion of the dichotomy of quality versus length of life.

Eventually, research opened up the possibility of having therapeutic agents with lesser collateral tissue damage. This was made possible with the discovery of protein markers which are increased or mutated in tumor cells. A degree of greater specificity was achieved by engineering drugs which target the cells that express these markers. Hence, the advent of monoclonal antibodies. As promising as this technology may seem, the cancer cells possess an impeccable resilience by adapting to the treatment modality through myriad means – increase extracellular drug transport, gain of new mutations, and circumvention of apoptosis, among other things.

At the very beginning of the study on cancer biology, an early postulate on the nature of cancer was summarized with the "seed and soil hypothesis." Today, an emerging interest is seen for the study of, not the seed, but of the soil. Cancer, like any disease, is an interplay of the pathogen and the host. It is in this context that we would begin to investigate the host response to cancer.

The tumor milieu is a complex environment. It rivals the complexity of the offending tumor cells. In the background environment, we see the body's response to and how it interprets the rogue cells. The immune system does not appear to be ignorant of the presence of these cancer cells since histologic assessment has seen immune cells infiltrating the tumor environment. Several studies have begun to investigate the significance of these cells in the tumor environment. At the beginning of tumor formation is an accompanying inflammatory response that contributes to a pro-tumorigenic niche.[1,2] This protumorigenic niche is developed over time through repetitive inflammation leading to accumulation of immune cells. Eventually, their tissue repair functions become maladaptive and the excessive response provides a focus of tumor development or metastasis.[1,2] On each of the step of tumor growth and metastatic cascade, bone marrow derived cells have been observed to influence the tumor microenvironment as either susceptible or resistant to tumorigenic growth.[1] Although data on leukocyte infiltration, especially lymphocytic cell line, has initially shown mixed results; it would seem that further studies have revealed that these monotonous mononuclear lymphocytes are composed of distinct populations of cytotoxic and suppressor lymphocytes, among others.[3-5] It is beginning to show that the populations of these cells rather than the general leukocyte population per se influence the prognosis of the patient.[6,7]

As far as colorectal cancers are concerned, there are several studies pointing out the significance of the lymphocytic infiltration with regards to underlying mechanism of tumorigenesis and the prognosis.[8] In fact, tumors arising from microsatellite instabilities tend to create a colorectal carcinoma with a distinctly heavy tumor lymphocyte infiltration and Crohn-like reaction with lymphoid nodules.[9] Several studies have also shown the lymphocytic infiltration on colorectal carcinoma as an independent prognostic factor. Some even find it a stronger prognostic factor than TNM staging.[10] More recent studies have investigated into the subpopulations of these lymphocytes to determine a sharper correlation with prognosis, especially on the T-cell population which is inherently associated with cytotoxic immune response.[6]

A unique subpopulation of cytotoxic lymphocytes are the natural killer cells (NK cells). They differ from the T-cells in that they can attack cells with or without aid of antibodies. They do this by targeting cells with depressed expression of MHC class I molecules. NK cells have been an established arm in immunologic tumor surveillance. In recent studies, NK cell activity was shown to be depressed during post-tumor resection presumably due to "tissue stress."[11]

Macrophages play an active role in tumors. In fact their presence in the stroma of the tumor is dense to the point that they may compose up to 30% of the total tumor mass. These tumor-associated macrophages (TAMs) represent the source of most proteases and cytokines involved in tumor growth. A study on intimate macrophage-tumor cell interaction has been done where it is shown that the TAMs aid during tumor intravasation in the process of metastasis.[11]

The myeloid lineage contributes several granulocytes in the circulating immune system. Of these, neutrophils and eosinophils have been objects of interest as far as their contribution to tumor growth is concerned. Early studies on neutrophils appeared to show a protumorigenic profile wherein they enhance angionesis and support metastatic seeding. Recent studies however begin to show contrasting results wherein tumor infiltrating neutrophils appeared to blunt metastatic colonization of the lung by breast carcinoma. In some other studies, the lymphocyte:neutrophil ratio in the tumor has also been suggested to be of prognostic import.[11]

There is no local data investigating the interplay between tumor cells and immune response. All patient care decisions have been based on studies of foreign populations with the assumption that the results will hold true for Filipinos. Observing the interplay of local cancers and their hosts at the immunologic level can both influence future patient care decision making and lay the ground for future local studies into this subject. This study wants to determine the presence of correlation between the different tumor-associated leukocyte counts and the pathologic stage as surrogate marker for the prognosis of colorectal carcinoma.

METHODOLOGY

This is a pilot study investigating the correlation of the different tumor-associated leukocyte counts with pathologic stage as surrogate marker for prognosis of colorectal carcinoma. The study was carried out as a cross-sectional design, involving colorectal carcinoma cases from the Philippine General Hospital during 2015-2016. There is a total of 230 colorectal carcinoma cases with surgical resection specimens in the records filed by organ-system for the years 2015-2016. The different cases were grouped according to pathologic stage (AJCC 7th edition) with the following count and proportion of the cases in each stratum (Table 1).

Table 1. The count and proportion of the different colorectal carcinoma cases with surgical resection specimens for the years 2015-2016, and the number of samples for each statum taken for the study

Stage	Sampled Population		Sample	
	Count	Proportion	Count	Proportion
I	29	12.61%	8	13.56%
IIA	63	27.39%	16	27.12%
IIB	0	0.00%	0	0.00%
IIC	5	2.17%	2	3.39%
IIIA	10	4.35%	3	5.08%
IIIB	75	32.61%	18	30.51%
IIIC	29	12.61%	7	11.86%
IVA	13	5.65%	3	5.08%
IVB	6	2.61%	2	3.9%

Table 2. The ranks used in the Spearman rank correlation analysis of TAL counts with 5-yr observed survival rates of the different colorectal cancer stages as surrogate marker for prognosis

Rank	Stage	5-yr survival rate
1	IVB	9.7%
2	IVA	15.2%
3	IIIC	46.9%
4	IIIB	70.3%
5	IIB	71.3%
6	IIC	73.8%
7	IIA	79.7%
8	IIIA	85.4%
9	I	86.3%

The minimum sample was computed using G*Power 3.1 and at least 27 samples is needed for the study to achieve a power of 0.80 and level of significance of 0.10 in detecting presence of correlation with an effect size of ±0.40, i.e., coefficient of $\rho > 0$ (one-tailed). Stratified random sampling was done, with pathologic stage as the stratifying variable. For each stratum, all cases were numbered from 1 to N, and simple random sampling by random number method using the random number generator function of Microsoft Excel was employed. The number of samples taken per stratum was proportional to that of the sampled population of the study (Table 1).

The paraffin blocks and the tissue slides of the samples were retrieved and reassessed. Hematoxylin and eosin staining for the tumor sections were used to assess by light microscopy, the intensity of the following tumor-associated leukocytes (TALs): total lymphocytes, neutrophils, eosinophils, and plasma cells. Additional tissue sections were obtained from the paraffin blocks and were subjected to immunohistochemical staining with CD4, CD8, CD56, and CD68 to assess the population of $CD8^+$ T-cells, helper T-cells ($CD4^+$ T-cells), NK cells, and macrophages, respectively. Tumor-infiltrating leukocyte count were recorded for each TALs by obtaining the average cell count over 10 high power fields on the intratumoral areas more than 1 low power field from the tumor border and with at least 80% tumor within the field. Likewise, peritumoral leukocyte count were recorded for each TALs by obtaining the average cell count over 10 high power fields on the peritumoral border within 1 low power field at the edge of the deepest site of tumor invasion.

Data analysis was done using Stata 15.1. The difference between median tumor-infiltrating and peritumoral counts of the different tumor-associated leukocytes was evaluated using Wilcoxon sign-rank test. Spearman's rho correlation coefficients of the different TAL counts and pathologic stage were computed and presence of correlation is identified when the 90% confidence interval did not intersect with the null value $\rho = 0$. It is noted that the 5-year survival rate of stage IIIA is higher than stage IIA, IIB, and IIC, as well as the 5-year survival rate of stage IIC than IIB. Therefore, for the variable of pathologic stage to be used as a surrogate marker for prognosis, the ordered ranks of the different stages were coded following an increasing 5-year survival rate (Table 2).

This study is limited to samples of patients with colorectal adenocarcinoma in our institution. Histologic variants were not specified. Immunohistochemical stains were done to assess the $CD8^+$ and $CD4^+$ T-cell subpopulations, but without segregation of $CD4^+$ T-cells into Th1 and Th2 helper T-cells. No immunohistochemical staining for B-cells were done. Macrophages were not segregated as well into their M1 and M2 cytokine profiles. Neutrophils were likewise not segregated into their N1 and N2 cytokine phenotypes.

RESULTS

A total of 59 samples were included in the study. The number of samples included per stratum is summarized in Table 1.

There were significant differences between the median TAL counts present infiltrating the tumor from those at the periphery of the tumor. This difference exists across all populations of the enumerated TALs in the study. More TALs are found at the periphery of the tumor than within the tumor parenchyma (Table 3).

Table 3. The difference in counts (per hpf) of tumor-infiltrating leukocytes and peritumoral leukocytes

Leukocyte	Tumor-infiltrating		Peritumoral		p-value*
	Median	(IQR)	Median	(IQR)	
Total Lymphocyte	16.4	(16.1)	31.1	(16.5)	<0.0001
CD4+ T-cell	5.5	(6.5)	15.7	(14.3)	<0.0001
CD8+ T-cell	8.5	(8.8)	25.0	(15.1)	<0.0001
NK Cell	0.3	(0.4)	0.5	(1.1)	<0.0001
Neutrophil	6.2	(8.0)	12.4	(16.6)	<0.0001
Eosinophil	0.2	(0.8)	2.2	(5.1)	<0.0001
Macrophage	7.0	(5.3)	15.2	(18.7)	<0.0001
Plasma Cell	1.7	(3.0)	9.5	(14.1)	<0.0001

*Wilcoxon Sign-Rank Test

Tumor-infiltrating total lymphocyte count has a positive correlation with survival based on pathologic stage. The more lymphocytes seen infiltrating the intratumoral milieu, the better the prognosis the patient has (Table 4 and Figure 1A). Similar observations were made with tumor-infiltrating $CD8^+$ T-cells, neutrophils, and macrophages (Table 4, and Figures 1B to 1D).

Table 4. Correlation coefficients of tumor associated leukocyte counts (per hpf) with good prognosis (increasing 5-year survival rates)

Leukocyte	Tumor-infiltrating		Peritumoral	
	p	[90% CI]	*p*	[90% CI]
Total Lymphocyte	*0.405	[0.207, 0.571]	*0.368	[0.165, 0.541]
CD4+ T-cell	0.127	[-0.092, 0.334]	-0.092	[-0.302, 0.127]
CD8+ T-cell	*0.419	[0.223, 0.582]	*0.324	[0.116, 0.505]
NK Cell	0.134	[-0.084, 0.341]	0.177	[-0.040, 0.379]
Neutrophil	*0.398	[0.199, 0.566]	*0.344	[0.138, 0.522]
Eosinophil	0.015	[-0.202, 0.231]	0.192	[-0.026, 0.392]
Macrophage	*0.372	[0.169, 0.545]	*0.481	[0.295, 0.631]
Plasma Cell	0.157	[-0.062, 0.361]	*0.586	[0.423, 0.712]

*Presence of correlation, ρ>0

At the peritumoral border of the deepest site of invasion of the tumor, presence of positive correlation with prognosis can be seen with total lymphocyte count, $CD8^+$ T-cells, neutrophils, macrophages, and plasma cells (Table 4 and Figures 2 to 3A). The more total lymphocytes, $CD8^+$ T-cells, neutrophils, macrophages and plasma cells seen bordering the deepest site of tumor invasion, the better the prognosis of the patient. It is noted that it is the abundance of plasma cells at the peritumoral border that is correlated with prognosis rather than plasma cells infiltrating the tumor. Further investigation on the peritumoral plasma cells showed that there is presence of correlation between peritumoral plasma cells and the depth of invasion (T criterion of TNM), ρ=-0.432, 90% CI [-0.593, -0.238]. There is a decrease in the peritumoral plasma cell count as the depth of invasion increases (Figure 3B).

The rest of the cellular population within and around the tumor infiltrate show a trend of decreasing counts as the prognosis and stage worsens; however, there is not enough evidence to show the presence of correlation.

DISCUSSION

It is interesting to note that the inflammatory response is starkly more prominent at the periphery of the tumor than within the tumor parenchyma. Inflammation is a response to proteins or markers that lead to the activation of the inflammatory cascade. As to why the response is much more prominent at the periphery is not fully understood. This study only corroborates that observation.

It is understood by previous studies that the degree of lymphocytic infiltration can have positive or negative correlation with the prognosis of certain tumors. The direction of the correlation however has been uncertain.

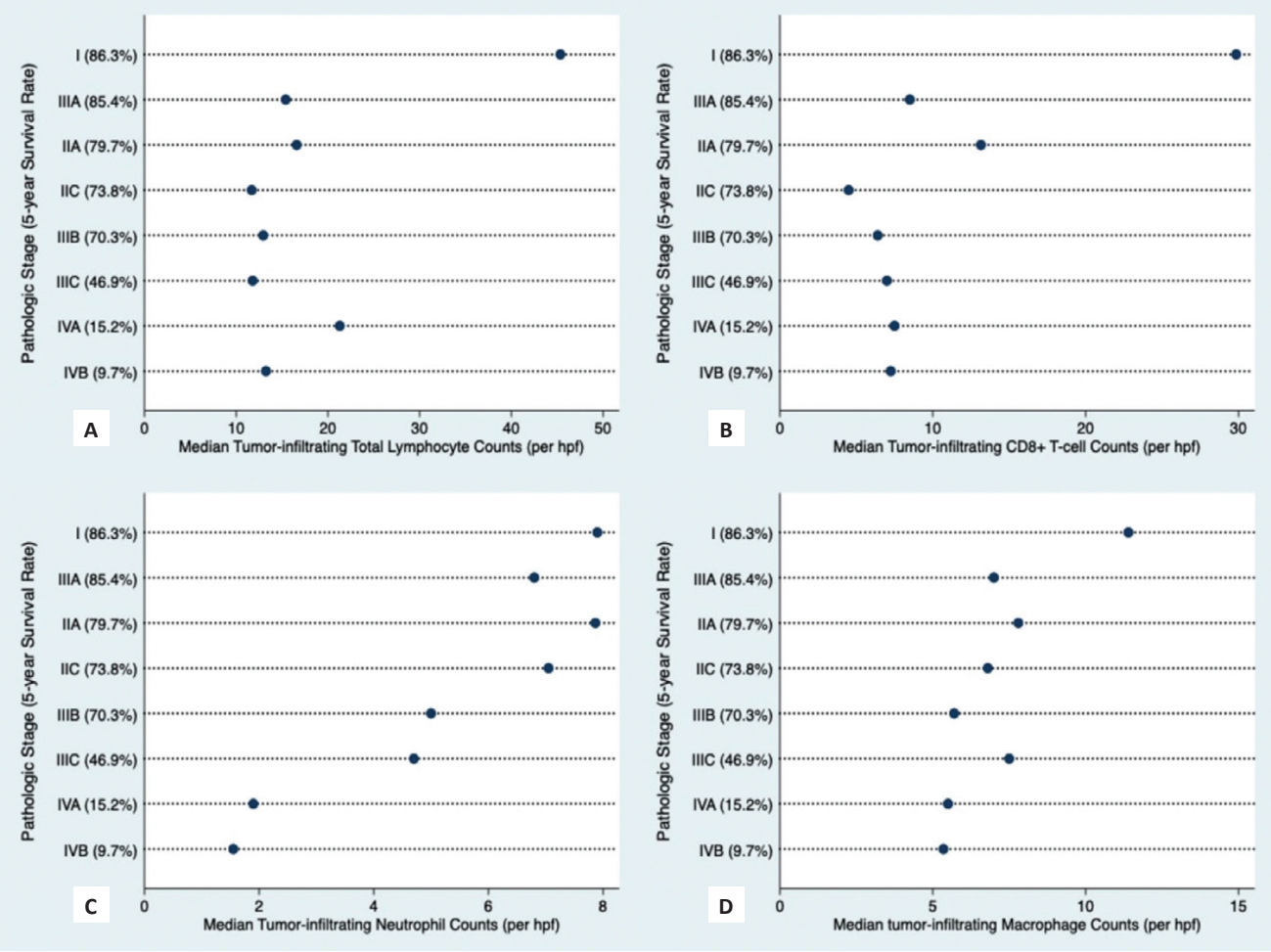

Figure 1. Median tumor-infiltrating leukocyte counts (per hpf) of different 5-year survival rates of the different pathologic stages: **(A)** total lymphocyte, **(B)** CD8+ T-cell, **(C)** neutrophil, and **(D)** macrophage.

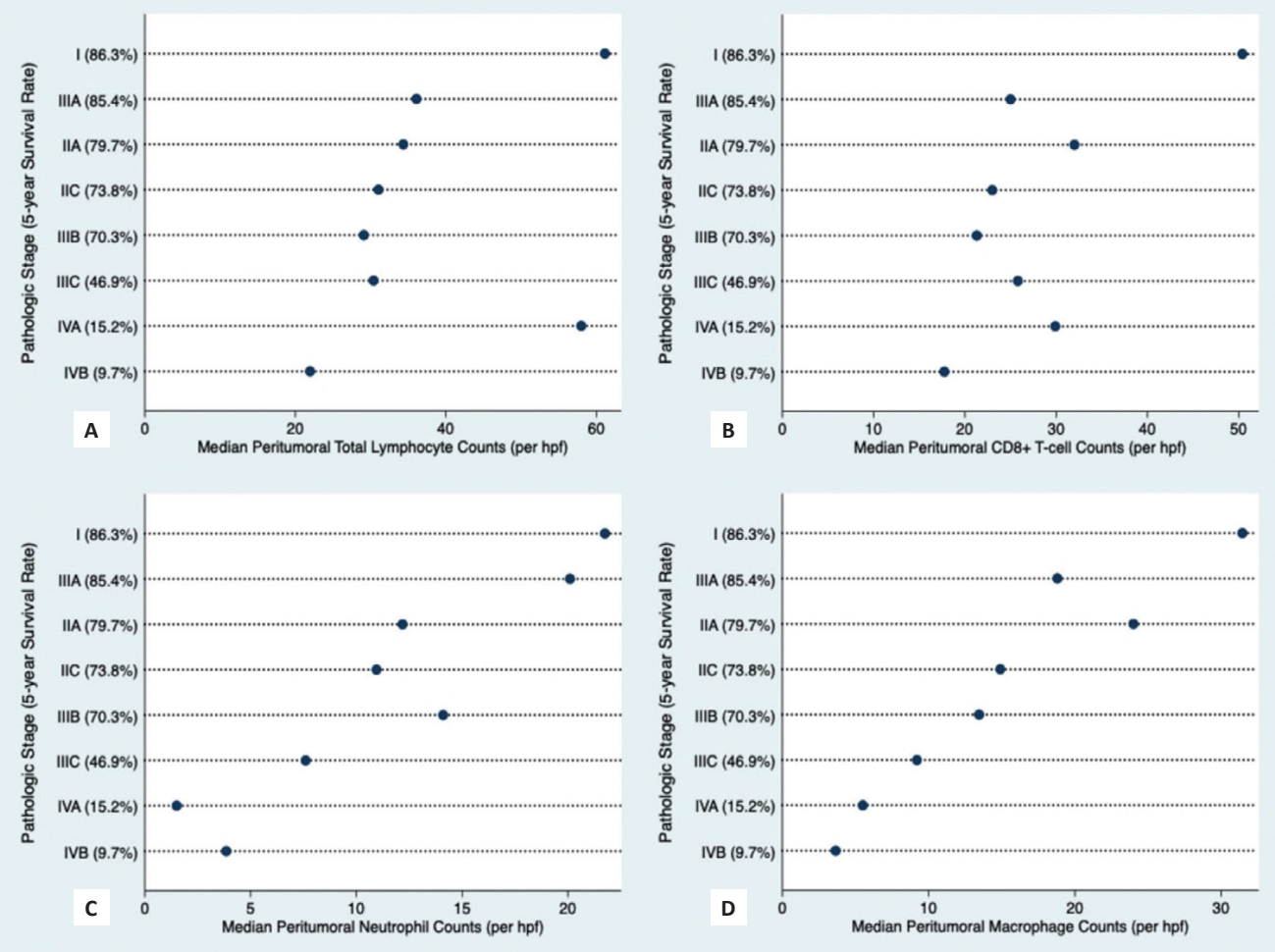

Figure 2. Median peritumoral leukocyte counts (per hpf) of different 5-year survival rates of the different pathologic stages: **(A)** total lymphocyte, **(B)** CD8+ T-cell, **(C)** neutrophil, and **(D)** macrophage.

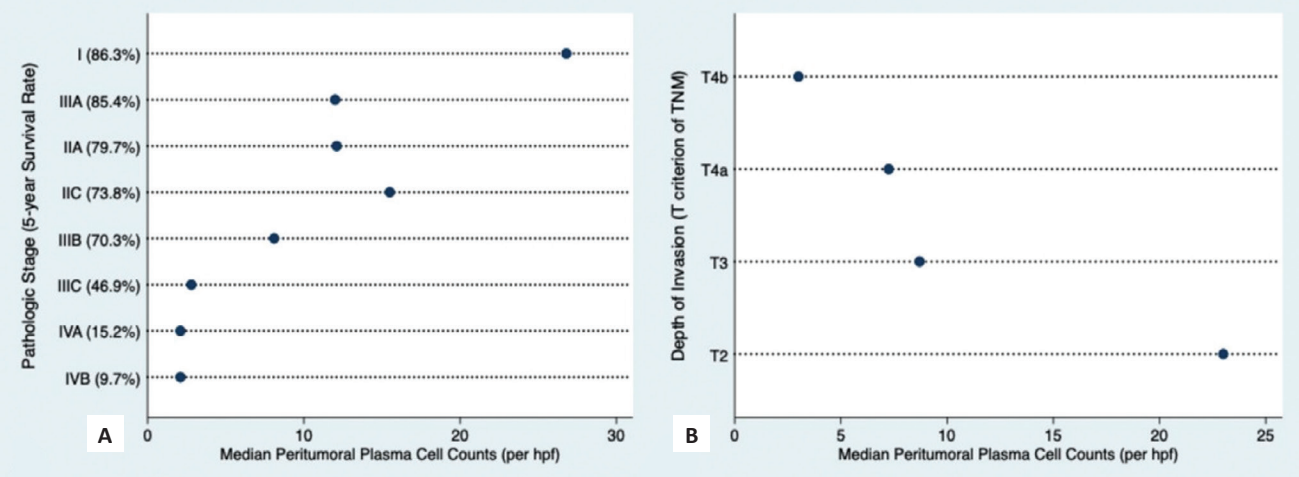

Figure 3. Median peritumoral plasma cell counts (per hpf) of different: **(A)** 5-year survival rates of the different pathologic stages, **(B)** depth of invasion (T criterion of TNM).

It is noted that cancer cells can recruit lymphocytes from the peripheral circulation into the cancer stroma to help promote its growth. It is in this context that increased densities of tumor infiltrating lymphocytes can prove disadvantageous to the host.[11] However there is an alternative view proposed and well-supported that teaches that the lymphocytes are actually sent by the immune system to target and dispatch of the aberrant tumor cells.[11] This study supports the latter in that the correlative evidence points towards the view that cancers with lower stages and better prognoses have higher amounts of tumor infiltrating lymphocytes.

Lymphocytic populations despite their monotonous histologies are actually diverse. Subpopulations of the lymphocytes are categorized based on some antigens they display. In this study interest is focused on two subpopulations of T lymphocytes: $CD4^+$ and the $CD8^+$ T-cells. $CD8^+$ T-cells are known for their function to destroy cells harboring pathogenic particles within the cytosol. It is because of this function that they are called cytotoxic T-cells. The evidence in this study points to a positive correlation of the infiltrating $CD8^+$ T-cells with the increasing survival rates or good prognosis of colorectal cancer patients. $CD4^+$ T-cell populations, on the other hand, have proven to be inconclusive and their association with the tumor is nebulous. This may be attributed to the fact that even this subpopulation can still be categorized into regulatory T-cells (Treg cells) and helper T-cells (Th cells). These two appear to have opposite effects on tumor progression. Treg cells are involved in blunting the activities of the $CD8^+$ T-cells and are primarily involved in promoting peripheral immunotolerance – a mechanism which may be hijacked by the tumor. Th cells on the other hand secrete cytokines that promote the activation of $CD8^+$ T-cells and would most likely help in limiting tumor growth. Differentiating the two populations would require more than labeling of the CD4 antigen.

TAMs likewise have been associated with both tumor growth and anti-tumor activities. This study shows however that TAMs around the tumor border and within the tumor are positively correlated with better prognosis. The disparity seen together with other studies may be attributable to macrophages having at least 2 subpopulations depending on the cytokine profile. M1 macrophages are known for their antitumor properties and with their capacity to help present tumor proteins to Th cells and also help in killing tumors by antibody-dependent cellular cytotoxicity. M2 macrophages on the other hand may be pro-angiogenic and promote tumor progression.

Formation of extrafollicular lymphoid structures can occur in areas surrounding tumor. These often produce a type of plasma cells that are short-lived and that do not acquire the ability to migrate to distant sites. These short-lived plasma cells can produce antibodies of presumably lower affinity and thus still help in antibody-dependent cell mediated cytotoxicity. This study supports the view that plasma cells provide antitumor effects as postulated. The evidence shows a positive correlation between peritumoral plasma cells and increasing 5-year survival rates. Also, it is noted that there is a negative correlation between peritumoral plasma cell counts and the depth of tumor invasion – as peritumoral plasma cell counts decreases, the depth of tumor invasion increases.

Neutrophils are the predominant granulocytic population in the body. They play a vital role in the early response and defense against invading microorganisms. Like the macrophages, the neutrophils can have different phenotypes that polarize their response to tumors.[12] This is the likely reason why there are divergent findings on the tumorigenic versus antitumor effects of neutrophils. Suffice it to say that at least two publications support the antitumorigenic effects of neutrophils by blunting the metastatic colonization of renal cell carcinoma on the lung[13] and by promoting cell death of disseminated breast cancer cells in the premetastatic lung.[14]

For other cellular constituents of the inflammatory infiltrate, the trends hinted of a decreasing numerical trend as prognosis worsens but do not have statistical significance.

CONCLUSION

The study demonstrates what has long been held in suspicion to be true: that there is a significant role played by the immune system in tumor progression. The intent of the study is to see if there is a correlation between the quantity and density of inflammatory cells with the colorectal cancer prognosis. True enough, some degree of understanding is elucidated in this study. There are mostly inconclusive trends, showing decreasing TAL cell counts as the prognosis worsens, seen with most inflammatory cells. However, four particular cell populations show a presence of positive correlation with prognosis: tumor-infiltrating and peritumoral $CD8^+$ T-cells, neutrophils, and macrophages; and, peritumoral plasma cells. The tumor-infiltrating and peritumoral $CD8^+$ T-cells are correlated with better prognosis. Similarly, the tumor-infiltrating and peritumoral neutrophils, and macrophages; and, peritumoral plasma cells are correlated with a better prognosis. The greater the quantity of these cells within the tumor, and at the peritumoral border of the deepest point of invasion, the better the prognosis of the colorectal carcinoma patient.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria. All authors have equally contributed to this work, proofread and approved the manuscript for publication.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

REFERENCES

1. Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. Nat Rev Cancer. 2009;9(4):239-52. PMID: 19279573. PMCID: PMC3251309. https://doi.org/10.1038/nrc2618.
2. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008;454(7203):428-35. PMID: 18650913. https://doi.org/10.1038/nature07201.
3. Fridman WH, Pagès F, Sautès-Fridman C, Galon J. The immune contexture in human tumours: impact on clinical outcome. Nat Rev Cancer. 2012;12(4):298-306. PMID: 22419253. https://doi.org/10.1038/nrc3245.
4. DeNardo DG, Andreu P, Coussens LM. Interactions between lymphocytes and myeloid cells regulate pro- versus anti-tumor immunity. Cancer Metastasis

Rev. 2010;29(2):309-16. PMID: 20405169. PMCID: PMC2865635. https://doi.org/10.1007/s10555-010-9223-6.

5. Kovacsovics-Bankowski M, Chisholm L, Vercellini J, et al. Detailed characterization of tumor infiltrating lymphocytes in two distinct human solid malignancies show phenotypic similarities. J Immunother Cancer. 2014;2(1):38. PMID: 25436113. PMCID: PMC4247679. https://doi.org/10.1186/s40425-014-0038-9.
6. Reissfelder C, Stamova S, Gossmann C, et al. Tumor-specific cytotoxic T lymphocyte activity determines colorectal cancer patient prognosis. J Clin Invest. 2015;125(2):739-51. PMID: 25562322. PMCID: PMC4319435. https://doi.org/10.1172/JCI74894.
7. Huh JW, Lee JH, Kim HR. Prognostic significance of tumor-infiltrating lymphocytes for patients with colorectal cancer. Arch Surg. 2012;147(4): 366-72. PMID: 22508783. https://doi.org/10.1001/archsurg.2012.35.
8. Tougeron D, Fauquembergue E, Rouquette A, et al. Tumor-infiltrating lymphocytes in colorectal cancers with microsatellite instability are correlated with the number and spectrum of frameshift mutations. Mod Pathol. 2009;22(9):1186-95. PMID: 19503063. https://doi.org/10.1038/modpathol.2009.80.
9. 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. PMID: 11413533.
10. Ropponen KM, Eskelinen MJ, Lipponen PK, Alhava E, Kosma V-M. Prognostic value of tumour-infiltrating lymphocytes (TILs) in colorectal cancer. J Pathol 1997;182(3):318-24. PMID: 9349235. https://doi.org/10.1002/(SICI)1096-9896(199707)182:3<318::AID-PATH862>3.0.CO;2-6.
11. Mendelsohn J, Howley P, Israel M, Gray J, Thompson C. The Molecular Basis of Cancer, 4th ed. Elsevier; 2014.
12. Fridlender ZG, Sun J, Kim S, et al. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. Cancer Cell. 2009;16(3): 183-94. PMID: 19732719. PMCID: PMC2754404. https://doi.org/10.1016/j.ccr.2009.06.017.
13. López-Lago MA, Posner S, Thodima VJ, Molina AM, Motzer RJ, Chaganti RS. Neutrophil chemokines secreted by tumor cells mount a lung antimetastatic response during renal cell carcinoma progression. Oncogene. 2013;32(14):1752-60. PMID: 22665059. PMCID: PMC3435490. https://doi.org/10.1038/onc.2012.201.
14. Granot Z, Henke E, Comen EA, King TA, Norton L, Benezra R. Tumor entrained neutrophils inhibit seeding in the premetastatic lung. Cancer Cell. 2011;20(3):300-14. PMID: 21907922. PMCID: PMC3172582. https://doi.org/10.1016/j.ccr.2011.08.012.