https://africanjournalofbiomedicalresearch.com/index.php/AJBR
Afr. J. Biomed. Res. Vol. 27 (October 2024); 1140-1143

Research Article

Immunoscore Evaluation in Gastric Adenocarcinoma: Implications for Prognosis and Personalized Immune Therapy

Samyuktha Srinivas[1], Saraswathy Sreeram[1], Hema Kini[1], Shikha Jayasheelan[1], Pooja Kundapur Suresh[1], Jyoti Ramanath Kini[1]

[1]Department of Pathology, Kasturba Medical College Mangalore, Manipal Academy of Higher Education, Karnataka, Manipal, 576 104, India*

Abstract

Gastric Adenocarcinoma has a dismal prognosis, prompting the exploration of novel assessment methods. This retrospective study involved 50 gastrectomy specimens to evaluate the Immunoscore System (IS) as an immune status and prognosis predictor. T-cell densities of CD3+ and CD8+ were measured through immunohistochemistry. Correlations with clinicopathological characteristics and survival time were examined. The study did not reveal significant correlations between Immunoscore, tumor characteristics, and survival time, which may be influenced by the advanced stages of disease and unique gastric carcinoma microenvironment. Future research, encompassing biopsies and digital software, may shed more light on IS in gastric cancers. This study's manual immunoscoring method offers a practical and affordable way to assess the immunological state of tumours in different organs, especially during initial diagnostic biopsies that might guide neoadjuvant treatments. In the future, immunoscoring is expected to be very important in the field of personalised immune therapy.

Keywords: Gastric adenocarcinoma, Immunoscore, Tumor infiltrating lymphocytes, Prognosis, Immune therapy, Histopathology, Clinicopathological characteristics.

Grant Acknowledgement: This project was funded by the Indian Council of Medical Research – Short term studentship (ICMR STS-2020) (Reference ID: 2020-04128).

**Author for correspondence: Dr. Saraswathy Sreeram, Associate Professor, Department of Pathology, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Karnataka, Manipal, 576 104, India. Email: swameeram@gmail.com*

Receiving Date: 22/10/2024 Acceptance Date: 26/10/2024

DOI: https://doi.org/10.53555/AJBR.v27i3.3136

© 2024 The Author(s).
This article has been published under the terms of Creative Commons Attribution-Non-commercial 4.0 International License (CC BY-NC 4.0), which permits non-commercial unrestricted use, distribution, and reproduction in any medium, provided that the following statement is provided. "This article has been published in the African Journal of Biomedical Research"

INTRODUCTION:

Gastric adenocarcinoma (GC) is the second most deadly disease worldwide and the fourth most prevalent cancer overall. It is a very aggressive cancer with a wide impact [1][2]. It is the second most common cause of cancer-related death in India for people between the ages of 15 and 44 [3], frequently detected at advanced clinical stages with a dismal prognosis [4].

Recent developments have highlighted the critical function of tumour infiltrating lymphocytes (TILs), namely CD8+ cytotoxic T cells, in anti-tumor immune responses. [5][6]. The density of TILs, specifically the CD3+/CD8+ T-cell ratio, has emerged as a crucial prognostic marker, surpassing TNM staging in its predictive value [7]. High CD8+ lymphocyte infiltration is associated with improved GC prognosis [8]. When used to measure CD8+ and CD3+ T-cell densities, the Immunoscore System (IS) has shown impressive results in the treatment of colorectal cancer. [9-11].

This study aims to apply the IS concept to predict outcomes for GC patients. The goal is to assess the CD3+/CD8+ lymphocyte ratio in GC in order to determine staging and prognosis. Specific goals include assessing CD3+ and CD8+ lymphocyte densities, exploring associations with demographic and pathological

features, and comparing ratios in tumor centers and invasive margins.

MATERIALS AND METHODS

This retrospective investigation was carried out in our institution's pathology department. Gastric resection specimens from patients with cancer that were obtained from connected hospitals for histopathological analysis made up the study population. The criteria for inclusion were both partial and total gastrectomies with an adenocarcinoma diagnosis. The following criteria were used to exclude patients: (i) inability to obtain paraffin blocks of the tumour; (ii) adenocarcinoma biopsies; (iii) benign ulcers; (iv) neoadjuvant therapy history; (v) adenocarcinomas of the lower end of the oesophagus and gastroesophageal junction; and (vi) other histological tumour types, such as gastrointestinal stromal tumours and neuroendocrine carcinomas.

Fifty histopathological samples that were obtained between September 2015 and August 2020 were included in the study. A case study form that included the patients' clinical information, histology, and immunohistochemistry results was used to collect the data. Prior to starting the study, the Institutional Ethics Committee gave its approval, and the medical superintendents at each hospital granted access to the patients' records so that pertinent clinical and pathological information could be found. Every piece of information was recorded using case study forms, and any relevant follow-up data was also gathered.

For the purpose of assessing Tumor-Infiltrating Lymphocytes (TILs) at the central tumour (CT) and invasive margin (IM), at least four tumour slides per case were carefully examined, and the relevant blocks were chosen for immunohistochemistry. The selected blocks were subjected to CD3+ and CD8+ antibody immunohistochemical staining. To measure CD3+ and CD8+ T-cell densities, at least four high-power fields in the CT and IM regions were examined. An average value of positive lymphocytes per high-power field for each marker at each location was then determined. Based on the amount of high values present, each category's median value was determined and used as a cutoff to divide the data into two groups: those above and below the median value. The data was then classified into five Intensity Scores (IS): I0, 1, 2, 3, and 4.

During the slide review, histological parameters including tumour type, metastases to lymph nodes, and other observations were noted. Following analysis of the study population data, the grade, stage, and pertinent clinicopathological features of the lesions were correlated with the immunohistochemical results. Descriptive statistics, chi-square tests, the Kaplan-Meier method of survival analysis, and Cox proportion hazard regression were among the statistical techniques used to evaluate the risk ratios, prognostic accuracy, and overall survival (OS) from the date of surgery to the date of death or the last follow-up. The Statistical Package for Social Sciences, version 25.0, was used for all statistical analyses. Focusing on gastric cancer stage as an independent prognostic predictor, a significance level of $p<0.05$ was deemed statistically significant.

RESULTS

There were 50 patients in the study, with a male to female ratio of 3.54:1. The patients were 39 men and 11 women. Among them, 64% were aged over 60 (youngest: 40, oldest: 78). Tumor sizes varied, with 13 tumors measuring <4cm, 27 between 4-8cm, and 10 >8cm. Most were of the intestinal type (62%), followed by mixed (10%) and diffuse (28%). In 78% of instances, there was evidence of lymphovascular invasion, whereas in 36% of cases, there was perineural invasion.

Most of the tumours (88%), invasive in subserosal connective tissue, serosa, and surrounding structures, were categorised as pT3 and pT4. Eighty-four percent of cases had lymph node metastases, and four percent had distant metastases. 40% of patients were in stage IIIA of pathological staging, which extended from IB to stage IV. The Immunoscore categorized 38% with low density, 30% with moderate density, and 32% with high density.

Among the 50 patients, 20 had passed away. Of the 30 remaining, 22 had follow-up data available, while 8 were lost to follow-up. Seven months was the total median survival time (95% CI: 3.689-10.311). Out of the 22 patients, 17 survived for less than 15 months, 3 for 15–30 months, and 2 for more than 30 months. One patient remained alive at 60 months. Immunoscore was grouped into three categories (1, 2, and 3) for correlation with other parameters.

DISCUSSION:

We studied the significant role of Immunoscore (IS) in gastric adenocarcinomas, a relatively unexplored field. We measured the CD3+ and CD8+ T-cell densities in 412 pictures of the Invasive Margins (IM) and the Centre of Tumours (CT) using a thorough analysis. High-density lymphocyte-derived IS showed an interesting inverse connection with tumour stage, indicating that higher Immunoscores are associated with a lower chance of cancer recurrence. This finding underscored the potential of IS as a highly valuable prognostic marker, surpassing the limitations of TNM staging and providing a more comprehensive understanding of prognosis and clinical outcomes in patients with similar stage malignancies [11].

The meta-analysis of 22 trials, which concluded that CD3+ and CD8+ T cells improved overall cancer survival (OCS) and overall cancer relapse-free survival (OCRFS), is consistent with our findings. The positive effect of CD3+ and CD8+ TILs on patient survival was highlighted by their respective hazard ratios (HR) of 0.64 and 0.66. Furthermore, the infiltration of CD3+ and CD8+ TILs was found to increase OCRFS in seven trials, underscoring the importance of these immune cells as possible prognostic indicators in gastric cancer [13].

In the contemporary landscape, TNM classification alone has limitations in predicting therapy response and cancer prognosis. This has led to the search for novel prognostic biomarkers that extend beyond conventional pathologic and clinical staging. Immunological markers, such as Tumor-Infiltrating Lymphocytes (TILs), have gained recognition for their role as indicators of anti-tumor responses. Specifically, effector memory T cells have become important agents in promoting long-term immunity against cancer and inhibiting the recurrence of tumours. [14-18].

When it comes to colorectal malignancies in particular, the Immunoscore system—which evaluates the frequency of

immune cell infiltrates in both the CT and IM of tumors—has shown better predictive value than TNM categorization. However, our study ventured into uncharted territory by applying this system to gastric adenocarcinomas. It is interesting to note that there was no significant correlation between our results and the tumor's pathological stage. The analysis may have been biased due to the high percentage of patients with advanced-stage stomach cancer at our centre, which could be the cause of this discrepancy.

Furthermore, we found that there was no significant correlation between IS and a number of histological features, including tumour size, grade, Lauren classification, lymphovascular and perineural invasion, pathological tumour stage, and regional lymph node status. This emphasises how the tumour microenvironment and immunological state in gastric carcinomas differ from those in colorectal cancer, where IS has demonstrated significant importance.

Our study yielded a seven-month median overall survival period, in line with that of specialized centers, reflecting the generally late diagnosis and poor prognosis associated with gastric cancers. Despite the valuable insights gained, our study does possess limitations and offers ample room for further investigation. Factors like tumor microenvironment and geographical variations might have influenced our results. Moreover, the study's reliance on a relatively small cohort of 50 gastrectomy specimens and a lack of complete follow-up data underscore the potential for a larger, more comprehensive study involving biopsies to enhance our understanding of IS's role in gastric cancer.

An international consortium has firmly established the Immunoscore method in colorectal tumours; however, in a resource-poor situation, where digital pathology tools and advanced image analysis are not easily accessible, we have developed a novel approach. Tumour immune cell ratios in the centre of tumours are much higher than those in their margins, as demonstrated by our manual assessment method, which is described in the methods section. This could pave the way for initial diagnostic biopsies in gastric cancer to incorporate immunoscoring, facilitating better planning of neoadjuvant therapies. Refining and validating this cost-effective manual method developed in our study may prove valuable not only in assessing tumour immunological state in different organs as well as in early diagnostic biopsies of different kinds of cancers.

In conclusion, our study provides a novel perspective on the role of Immunoscore in gastric adenocarcinomas. While it may not replace traditional TNM staging, Immunoscore emerges as a promising adjunct, offering a more objective assessment of the immune microenvironment and contributing to the growing field of individualized immune-based cancer therapy. This exploration may potentially open the door to the eventual categorization of tumours using an immune system, emphasizing the dynamic nature of cancer research and the potential for novel approaches to therapy and prognosis prediction.

In conclusion, gastric adenocarcinoma stands as a formidable adversary among malignancies, marked by resistance to conventional chemotherapies and dire prognosis primarily due to late-stage detection, often with metastases that render treatment options ineffective, resulting in high mortality rates. The landscape of cancer therapy is experiencing a transformative shift towards personalization, driven by our growing comprehension of tumor immunity. Immunotherapy is one of the state-of-the-art options, and its inclusion highlights how important the immune milieu is in influencing prognoses and predicting treatment responses. By incorporating immunoscoring into this framework, the difficulties associated with inter-observer variation are lessened and an objective and standardised assessment of immunological state is provided. Immunoscoring won't replace the accepted TNM staging method, but it will undoubtedly be important in the soon-to-come era of tailored immune-based cancer treatments.

This paradigm change may also signal the arrival of an immune-based cancer categorization system in the future, demonstrating the dynamic character of cancer research and its capacity to reshape prognostic and treatment strategies. Our study indicates that immunoscore may not wield as pronounced an influence in gastric carcinoma as it does in colorectal cancers. Nevertheless, we advocate for a more extensive, longitudinally tracked cohort study to substantiate our findings and to facilitate the evolution of a validated outcome.

Our research has introduced a pragmatic, straightforward, and cost-effective method for computing immunoscores to assess tumor immune status in carcinomas. Particularly notable is its applicability in resource-constrained settings, where advanced digital pathology is unavailable. The proposed methodology remains open to further validation and holds promise for broader implementation in the realm of initial diagnostic endoscopic biopsies for various cancer types. The present study highlights the dynamic nature of cancer treatment approaches and highlights the significant influence that immunoscoring may have on the direction of cancer treatment in the future.

REFERENCES:

1. Carcas LP. Gastric cancer review. J Carcinog. 2014; 13:14.
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61(2):69–90.
3. Servarayan Murugesan C ,Manickavasagam K, Chandramohan A, Jebaraj A, Jameel ARA, Jain MS, Venkataraman J. Gastric cancer in India: epidemiology and standard of treatment. Updates Surg 2018;70(2):233-39.
4. Bang YJ, Kim YW, Yang HK, Chung HC, Park YK, Lee KH, et al. Adjuvant capecitabine and oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC): A phase 3 open-label, randomised controlled trial. Lancet. 2012; 379:315–21.
5. Ohtani H. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer. Cancer Immun. 2007;7:4.
6. Salama P, Phillips M, Grieu F, et al. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol. 2009;27(2):186–92
7. Koch M, Beckhove P, Op Den Winkel J, et al. Tumor infiltrating T lymphocytes in colorectal cancer: tumor-selective activation and cytotoxic activity in situ. Ann Surg. 2006;244(6):986–92

8. Galon J, Pages F, Marincola FM, et al. Cancer classification using the Immunoscore: a worldwide task force. J Transl Med. 2012;10:205.
9. Galon J, Mlecnik B, Bindea G, et al. Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. J Pathol. 2014;232(2):199–209.
10. Anitei MG, Zeitoun G, Mlecnik B, et al. Prognostic and predictive values of the immunoscore in patients with rectal cancer. Clin Cancer Res. 2014;20(7):1891–9.
11. Lee JS, Won HS, Sun S, Hong JH, Ko YH. Prognostic role of tumor-infiltrating lymphocytes in gastric cancer: A systematic review and meta-analysis. Medicine (Baltimore). 2018;97(32):11769.
12. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin. 2017;67(2):93-9.
13. Trabelsi M, Farah F, Zouari B, Jaafoura HM, Kharrat M. An Immunoscore System Based On CD3+ And CD8+ Infiltrating Lymphocytes Densities To Predict The Outcome Of Patients With Colorectal Adenocarcinoma. Dove Press journal. 2019;12: 8663-73.
14. Nagtegaal ID, Quirke P, Schmoll HJ. Has the new TNM classification for colorectal cancer improved care? Nat Rev Clin Oncol. 2011;9:119–23.
15. Diederichsen AC, Hjelmborg J, Christensen PB, et al. Prognostic value of the CD4+/CD8+ ratio of tumour infiltrating lymphocytes in colorectal cancer and HLA-DR expression on tumour cells. Cancer Immunol Immunother. 2003;52(7):423–428.
16. Ohtani H. Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer. Cancer Immun. 2007;7:4.
17. Sallusto F, Geginat J, Lanzavecchia A. Central memory and effector memory T cell subsets: function, generation, and maintenance. Ann Rev Immunol. 2004; 22:745-63.
18. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science. 2006; 313(5795):1960-4.