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Dynamic Changes of Immune Cells and their Relationship with Prognosis in Late-Stage Gastric Cancer Patients During PD-1 Inhibitor Immunotherapy

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CT participated in conceiving the design of the study and collecting and reviewing the data and coordination of project. LC participated in doing literature review, collecting the data and analysis and in preparing the manuscript. XW helped in critical revision and finalizing the manuscript. All authors read, revised, and approved the final manuscript.

Key words
PD-1 inhibitor, Cancer, Late-stage gastric cancer, Immune cells, Prognosis, Dynamic changes

ABSTRACT

The study aimed to investigate the dynamic changes in immune cells during the programmed death-1 (PD-1) inhibitor therapy in patients with late-stage gastric cancer (GC) and to analyze the correlation between these changes and patient prognosis. One hundred thirty-nine GC patients receiving PD-1 inhibitor therapy were enrolled. Peripheral blood (PB) and tumor tissues were collected before treatment (TB), during treatment (TM), and after treatment (TA). Multiple-parameter flow cytometry and immunohistochemical analysis were employed to analyze changes in immune cells and biomarkers. Correlations between these changes with treatment response, overall survival (OS), and progression-free survival (PFS) were analyzed. T cells at TB, TM, and TA increased sequentially, with remarkably increased counts of helper T (Th), cytotoxic T (Tc), and natural killer (NK) cells at TM to those at TB and TA ($P<0.05$). Programmed death ligand-1 (PD-L1) at TM was elevated to that at TB and TA, and CD8 and CD4 at TM and TA were higher than at TB ($P<0.05$). Multivariate regression analysis (MRA) revealed a positive correlation between the quantity of PD-L1, T, Th, and Tc cells with treatment response. The count of T cells was positively linked with OS, and those of programmed PD-L1 and T cells were positively associated with PFS ($P<0.05$). In patients with late-stage GC undergoing PD-1 inhibitor therapy, the quantities of T, Th, and Tc cells as well as PD-L1 levels were conductive to treatment response and prognosis, providing new biological markers for personalized treatment.

INTRODUCTION

Gastric cancer (GC) is a frequently found tumor in digestive system globally, and treating later-stage GC has long been a challenging issue in the medical field (Machlowska *et al*., 2020). Despite significant progress in treatment modalities such as surgery (Liao *et al*., 2020), radiotherapy (Li *et al*., 2022), and chemotherapy (Zhou *et al*., 2021) over the past few decades, the prognosis for late-stage GC patients remains relatively unfavorable, with survival rates still a cause for concern (Xiang *et al*., 2020). In this context, immunotherapy, as an emerging treatment strategy, especially with the introduction of programmed cell death-1 (PD-1) inhibitors, has brought new hope for late-stage GC patients (Demirtas and Gunduz, 2021).

PD-1 inhibitors, as a revolutionary class of immunotherapeutic drugs, demonstrate outstanding efficacy across various types of cancers by blocking the binding of PD-1 to its ligand PD-L1. This activation of the immune system enables immune cells to recognize and attack cancer cells, showcasing remarkable therapeutic effects (Kong *et al*., 2022). The successful application of this treatment strategy has injected new vitality into the field of cancer therapy, and the significant treatment outcomes have sparked immense interest among scientists and clinicians in the potential of immunotherapy.

PD-1 is an immune checkpoint molecule located on the cell membrane and is widely present on the surfaces of immune cells such as T cells, B cells, and NK cells (Onorati *et al*., 2022). Its primary physiological function is

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to inhibit the activity of immune cells, preventing excessive immune responses to maintain the balance of the immune system. However, in certain situations, cancer cells exploit the binding of PD-1 to its ligand PD-L1 to evade attacks from the immune system, leading to immune escape and promoting cancer development (Jiang *et al*., 2019). The emergence of PD-1 inhibitors has changed this scenario. PD-1 inhibitors can disrupt the binding between PD-1 and PD-L1, relieving immune system suppression and activating the patient's own immune defense mechanisms. This enables the immune system to recognize and destroy tumor cells (Zhang *et al*., 2022). In various types of cancer, PD-1 inhibitors have demonstrated outstanding efficacy in malignancies such as malignant melanoma (Zhang *et al*., 2023), non-small cell lung cancer (Ibusuki *et al*., 2022), lymphoma (Zheng *et al*., 2021), bringing patients longer survival periods and a significant improvement in the quality of life. This successful experience has sparked extensive research into the potential application of PD-1 inhibitors in other types of cancer. It is important to note that, despite the significant achievements of PD-1 inhibitors in various cancers, their therapeutic effects in late-stage gastric cancer patients remain a relatively new and insufficiently explored area.

The introduction of PD-1 inhibitors has provided a new therapeutic option for late-stage GC patients, who face a highly invasive and refractory form of cancer. However, compared to other types of cancer, research on the dynamic changes in immune cells and their relationship with prognosis during PD-1 inhibitor therapy in late-stage GC patients is still limited (Liang *et al*., 2022). The inadequacy in this research area restricts our comprehensive understanding of the true impact of PD-1 inhibitors in the treatment of late-stage GC, affecting their optimization and personalized application in clinical practice.

Therefore, this work was dedicated to delving into the dynamic changes in immune cells during PD-1 inhibitor therapy in late-stage GC patients, aiming to unveil the specific mechanisms of action of this treatment strategy in late-stage GC. By comprehensively and systematically analyzing changes in the quantity, activity, and functionality of different cell types within the immune system, it was hoped to provide more precise grounds for the personalized treatment of late-stage GC patients and open up new avenues for the application of immunotherapy in late-stage GC. The results acquired in this work would not only enhance our understanding of the mechanisms behind PD-1 inhibitor therapy but also bring new hope and possibilities for treating late-stage GC patients.

MATERIALS AND METHODS

Subjects

This work focused on 139 GC patients admitted to The Changan Hospital from March 2021 to April 2023, including 83 male and 56 female patients. They were 30~76 years, averaged as (61.05±12.18) years old. Patients with late-stage GC, undergoing PD-1 inhibitor therapy, 18 years old or above, with good overall physical fitness and those who signed informed consent were included in this study. Patients who received prior immunotherapy or chemotherapy, with severe immunological disorders, other severe comorbidities, pregnancy and lactation, short life expectancy were excluded.

Treatment protocol using PD-1 inhibitor

In this work, all late-stage GC patients were subjected to PD-1 inhibitor therapy. PD-1 inhibitors, including but not limited to nivolumab (Bristol Myers Squibb, USA) and pembrolizumab (Merck, USA), were administered.

The standard dosage for nivolumab was once every two weeks, with each dose being 240 mg. The standard dosage for pembrolizumab was once every three weeks, with each dose being 200 mg.

Observation indicators

The dynamic changes of PB immune cells, including T cells, NK cells, antigen-presenting cells, before treatment (TB), during treatment (TM), and after treatment (TA) were observed. The distribution and expression levels of PD-L1, CD8, and CD4 in tissue slices were observed, and quantitative analysis was performed. Moreover, clinical responses such as CR, PR, SD, PD, and survival statuses like OS and PFS during the treatment process of patients were recorded.

Statistical analysis

Data were analyzed using SPSS 19.0. Continuous variables were expressed as mean ± standard deviation. For variables following a normal distribution, the t-test was employed for intergroup comparisons. Categorical variables were presented as percentages (%), and intergroup comparisons were performed using the x^2 test. Multivariate regression analysis was employed to explore the relationship between dynamic changes in immune cells and patient prognosis. $P<0.05$ was considered statistically significant.

RESULTS

Figure 1 shows the trend of changes in immune cells in late-stage GC patients before, during, and after PD-1 inhibitor treatment. It is evident that the levels of T cells

increased sequentially at TB, TM, and TA, with TM and TA being higher than TB, and TA being higher than TM (all P<0.05). As for Th cells, Tc cells, and NK cells, their levels at TM were remarkably and significantly higher in contrast to those at TB and TA.

In Figure 2, the trend of changes in immune cell levels in late-stage GC patients before, during, and after PD-L1 inhibitor treatment is presented. Regarding PD-L1, it was noticeable that the PD-L1 level at TM was much higher in comparison to that at TB and TA, showing visible differences (P<0.05). Regarding CD8 and CD4, the levels of cells at TA and TM were greatly and significantly elevated when comparing to those at TB.

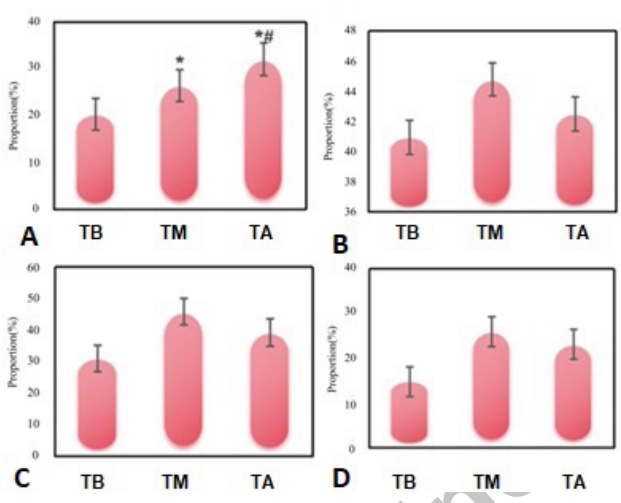

Fig. 1. Changes in proportion of immune cells at TB (before treatment), TM (during treatment) and TA (after treatment). A, T cells; B, helper T (Th), cytotoxic T (Tc), and natural killer (NK) cells; (*) and (#), a great difference with P<0.05 to levels at TB and TM, respectively.

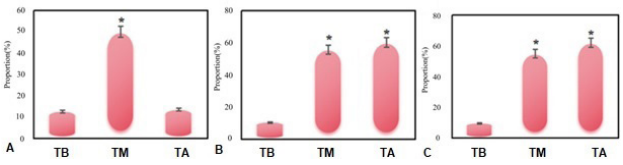

Fig. 2. Changes in programmed death ligand-1 (PD-L1), CD8, and CD4expression at TB, TM, and TA. A, PD-L1; B, CD8; C, CD4; (*), a remarkable difference with P<0.05 to the level at TB.

Table I shows the relationship between PD-L1, T cells, Th cells, Tc cells, and NK cells and treatment response, OS and PFS of patients in this work. MRA indicated an obviously positive correlation between PD-L1 and treatment response, meaning that patients with high PD-L1 expression experienced better treatment responses (β=-0.25, P<0.05). The quantities of T cells, Th cells, and Tc cells were positively linked with treatment response, suggesting that patients with higher numbers of these three cell subtypes exhibited better treatment responses (T cells: β=0.20, P<0.05; Th cells: β=0.18, P<0.05; Tc cells: β=0.25, P<0.05).

Table I. Effect of PD-L1 on immune cells in gastric cancer patients with therapeutic effect, overall survival and progression-free survival.

Indicators	β coefficient	t-value	P-value
Treatment response			
PD-L1	0.25	2.45	0.018*
T cells	0.20	2.28	0.025*
Th cells	0.18	2.01	0.049*
Tc cells	0.25	1.76	0.048*
NK cells	0.39	0.88	0.382
Overall survival			
PD-L1	0.22	1.98	0.052
T cells	0.27	2.34	0.025*
Th cells	0.11	1.05	0.298
Tc cells	0.21	1.86	0.076
NK cells	0.08	0.72	0.477
Progression-free survival			
PD-L1	0.21	2.68	0.013*
T cells	0.32	2.15	0.036*
Th cells	0.29	1.22	0.053
Tc cells	0.14	1.29	0.202
NK cells	0.12	1.08	0.267

For abbreviations, see legends of Figure 1 and 2.

The relationship between PD-L1, T cells, Th cells, Tc cells, NK cells, and OS in Table I shows that MRA revealed a positive connection between the quantity of T cells and OS, indicating that patients with a higher number of T cells experience longer OS (β=0.27, P<0.05). In addition, the MRA results indicated that the quantity of T cells was positively linked to PFS, suggesting that patients with a higher number of T cells experience longer PFS (Table I). PD-L1 exhibited a positive association with PFS, indicating that patients with high PD-L1 expression have a longer PFS.

DISCUSSION

In recent years, immunotherapy, as an innovative approach to cancer treatment, particularly with the

introduction of PD-1 inhibitors, has brought new hope for late-stage GC patients (Li *et al*., 2022). However, there is still a need for a thorough exploration of the dynamic changes in immune cells during PD-1 inhibitor treatment and their potential relationship with patient prognosis. Therefore, this study analyzes the dynamic changes in immune cells during PD-1 inhibitor treatment in late-stage GC patients and explores the potential connections between these changes and patient prognosis.

PD-1 is an immune checkpoint protein, and its expression is closely associated with the development of cancer (Ai *et al*., 2020). PD-1 primarily exists on the surface of T cells and functions to regulate the immune system, preventing excessive activation that could lead to autoimmune reactions (Rotte, 2019). However, in the cancer environment, tumor cells exploit this regulatory mechanism of PD-1 to evade attacks from the immune system (Liu *et al*., 2021). This work unveiled that T cells gradually increased at TB, TM, and TA, with Th cells, Tc cells, and NK cells at TM sharply higher to that at TB and TA ($P<0.05$). This suggests that PD-1 inhibitor treatment exhibits a significant immune activation effect by influencing T cells, Th cells, Tc cells, and NK cells. However, the dynamic changes in immune cells during treatment indicate potential adaptive immune responses and regulatory mechanisms. PD-1 inhibitors can block the interaction between PD-1 and PD-L1, allowing T cells to regain their activity by no longer being suppressed by PD-L1. This leads to an increase in their quantity, enabling more effective recognition and attack against cancer cells (Budimir *et al*., 2022). Immunohistochemistry revealed that the PD-L1 level at TM was much higher in contrast to at TB and TA ($P<0.05$). Under normal circumstances, PD-L1 inhibits the activity of T cells by binding with PD-1, preventing the immune system from attacking its own tissues (Jung and Antonia, 2018). In this work, the elevated PD-L1 level reflected the reduction of PD-L1 binding to PD-1 due to PD-1 inhibitors, thereby activating the activity of T cells. The research also confirms a positive correlation between PD-L1 levels, T cell levels, and patient treatment response, as well as the same correlation between T cell levels and OS ($P<0.05$). Studies have signified that high levels of tumor-infiltrating T cells are often associated with a favorable prognosis, and ample T cell infiltration may reflect a vigorous immune response against cancer (Roderburg *et al*., 2020).

During the mid-term of treatment, there was a slight increase in Th cells, implying a potential adaptive immune response (Hu *et al*., 2021). This response may exert a regulatory role during the treatment process. It has been reported that Th cells play a crucial role in promoting and regulating immune responses within the immune system (Walker, 2022). They activate other immune cells, such as B cells and Tc cells, and modulate the balance of immune responses to combat infection and tumor development. The results of this study confirm a positive correlation between Th cells and treatment response, indicating that patients with a higher quantity of Th cells exhibit better treatment responses. The significant increase in Tc cells during treatment underscores the importance of Tc cells in the anti-cancer antibody response. Studies have confirmed that Tc cells possess the ability to directly attack and eliminate abnormal cells, such as cancer cells, within the immune system (Yamaguchi *et al*., 2019). The results of this study also confirm a positive correlation between Tc cells and treatment response, indicating that patients with a higher quantity of Tc cells exhibit better treatment responses. However, as treatment progresses, Tc cell levels slightly decrease, which may be a result of adaptive adjustments within the immune system to the treatment. This also suggests the need for further attention to the dynamic changes of Tc cells during the continuous course of treatment to better understand the effectiveness of immunotherapy.

NK cells are an essential type of lymphocyte in the immune system, possessing the ability to directly attack tumor cells and infected cells (Wu *et al*., 2020). In contrast to T cells, this work observed a visible increase in NK cells during treatment, indicating that PD-1 inhibitors can effectively stimulate the activity of NK cells, enhancing their ability to attack cancer cells and providing robust support for the success of the treatment. The dynamic changes in immune cells suggest potential adaptive immune responses and regulatory mechanisms during the treatment process, emphasizing the need for further research to guide personalized treatment strategies.

CONCLUSION

In PD-1 inhibitor treatment for late-stage GC patients, the quantities of T cells, Th cells, Tc cells, and the level of PD-L1 are positively linked with treatment response and prognosis. These findings provided crucial clues for a better understanding of the mechanisms of PD-1 inhibitor treatment and the development of more effective therapeutic strategies. Although the study describes changes in the overall levels of immune cells, a more detailed analysis of different subgroups may be more insightful. Future research could delve deeper into exploring the dynamic changes of subgroups such as CD4+ T cells, CD8+ T cells, and regulatory T cells during the treatment process.

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IRB approval

This study was approved by the Advanced Studies Research Board of Shaanxi Cancer Hospital, Xi 'an 710061, Shaanxi Province, China.

Ethical approval

The study was carried out in compliance with guidelines issued by Ethical Review Board Committee of Shaanxi Cancer Hospital, China. The official letter would be available on fair request to corresponding author.

This study obtained approval from the relevant ethics committee, ensuring that the participation of all patients in the research was based on informed consent. The research process strictly adhered to the principles of the World Medical Association Declaration of Helsinki and complied with relevant ethical regulations (Ballantyne and Eriksson, 2019).

Statement of conflict of interest

The authors have declared no conflict of interest.

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