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Peripheral Immune Alterations and Checkpoint Expression in Localized Clear Cell Renal Cell Carcinoma Before and AfterNephrectomy: A Prospective Cohort Study

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Research Article

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Abstract

Background

Clear cell renal cell carcinoma (ccRCC) induces systemic immune alterations that extend beyond the tumor microenvironment. However, the impact of nephrectomy on peripheral immune cell populations and immune checkpoint expression in localized disease remains incompletely understood. This study evaluated changes in circulating leukocytes, inflammatory indices, and immune checkpoint expression before and after nephrectomy in patients with localized ccRCC. Methods: Twenty patients with localized ccRCC undergoing nephrectomy and six age- and sex- matched healthy controls were prospectively enrolled. Peripheral blood samples were collected before surgery and at postoperative follow-up (3–6 months). Multiparametric flow cytometry was used to quantify leukocyte subsets and expression of PD-1, LAG-3, TIM-3, CTLA-4, and PD-L1. The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and systemic immune-inflammation index (SII) were calculated from complete blood counts. Comparisons among groups were performed using the Kruskal-Wallis test followed by Bonferroni correction. Results: Patients with localized ccRCC exhibited significantly increased circulating neutrophils, $CD8^+$ T cell frequencies, and NLR decreased toward control values, indicating partial resolution of tumor-associated systemic inflammation. Monocyte and lymphocyte frequencies, as well as PLR and SII, remained unchanged. Peripheral immune checkpoint profiling demonstrated persistently increased PD-1 and postoperative CTLA-4 expression, together with reduced PD-L1 expression, whereas LAG-3 and TIM-3 expression showed no significant changes. Conclusions: Localized ccRCC is associated with measurable systemic immune dysregulation characterized by neutrophilia, increased $CD8^+$ T cells, elevated NLR, and altered immune checkpoint expression. Although nephrectomy partially reverses inflammatory changes, persistent checkpoint dysregulation suggests incomplete immune restoration. Peripheral immune profiling may represent a minimally invasive approach for monitoring systemic immune status in patients with localized ccRCC.

INTRODUCTION

Renal cell carcinoma (RCC) is the most common type of kidney cancer, accounting for over 3% of all malignancies, with the highest incidence reported in Western countries(1). It is the most frequent solid tumor originating in the kidney, representing nearly 90% of all solid renal tumors(2). RCC exhibits a male predominance with a 1.5:1 male-to-female ratio, and its peak incidence occurs between the ages of 60 and 70 years of age(3). This heterogeneous disease includes multiple histological subtypes, each characterized by distinct pathological and genetic features(4). Approximately 75% of patients diagnosed with RCC present with the clear cell subtype (ccRCC), which typically originates in the tubular epithelium of the kidney and has the potential to metastasize to distant organs(5). Historically, RCC has shown limited responsiveness to conventional cytotoxic chemotherapy. For patients with localized disease, radical nephrectomy remains the standard treatment and offers the best opportunity for long-term disease control(6).

In recent years, ccRCC has been recognized as a highly immunogenic tumor, leading to major advances in the development of immune checkpoint inhibitors (ICI)-based therapies(7). Agents targeting programmed cell death protein-1 (PD-1), its ligand PD-L1, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) have significantly improved survival outcomes in advanced RCC(8). Clinical trials such as CheckMate 214(9) and Keynote 426(10) demonstrated durable clinical responses in a subset of patients treated with immunotherapy-based regimens. However, clinical responses remain highly variable, and not all patients benefit equally from these therapies(11).

This variability has increased interest in identifying accessible and reproducible biomarkers capable of reflecting tumor-associated immune dysregulation. Peripheral blood represents a minimally invasive and dynamic source of immunological information that can be longitudinally monitored during disease progression and treatment(12). Alterations in circulating leukocyte populations and systemic inflammatory markers have been associated with prognosis in both localized and metastatic RCC(13). In clear cell renal cell carcinoma (ccRCC), systemic inflammation reflects the interaction between tumor-driven inflammatory activity and the host antitumor immune response, contributing to disease progression and prognosis(14, 15).. In this context, inflammatory indices such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII) have emerged as accessible blood-based biomarkers that integrate neutrophil- and platelet-mediated protumor inflammation with lymphocyte-dependent immune surveillance(15, 16). Elevated values of these indices have been linked to adverse clinicopathological features, including higher tumor aggressiveness and advanced disease stages(16–18). In particular, an elevated neutrophil-to-lymphocyte ratio (NLR) has consistently been associated with poor clinical outcomes and increased systemic inflammation in RCC patients(19).

Beyond hematologic parameters, peripheral immune checkpoint expression may also reflect systemic immune dysfunction associated with tumor presence. Increased PD-1 expression on circulating immune cells and alterations in T-cell subsets have been reported in RCC patients, suggesting the presence of systemic immune exhaustion and dysregulated antitumor responses(20). Nevertheless, the dynamic changes in peripheral immune cell populations and checkpoint molecule expression before and after nephrectomy remain incompletely understood, particularly in patients with localized ccRCC.

Therefore, the aim of this study was to evaluate nephrectomy-induced alterations in circulating leukocyte subsets, systemic inflammatory indices, and immune checkpoint expression in patients with localized clear cell carcinoma.

MATERIAL AND METHODS

Patients and tissue sampling

This study was conducted at the University Hospital “Dr. José Eleuterio González” between January 2022 and January 2024 and was approved by the Institutional Ethics Committee (Protocol No. IN-23-00002).

A total of 20 patients diagnosed with localized ccRCC scheduled for elective therapeutic nephrectomy were prospectively recruited through the Urology Department. Six age- and sex- matched healthy volunteers without known inflammatory, autoimmune, or malignant diseases were included as controls. Written informed consent was obtained from all participants prior to inclusion.

Patients with metastatic disease, active inflammation, autoimmune disorders, previous immunotherapy, hematologic malignancies, or histological subtypes other than ccRCC were excluded.

Peripheral venous blood samples (4 mL) were collected from patients the day before nephrectomy and after 3–6 months of clinical follow-up. A single peripheral blood sample was obtained from healthy controls at enrollment.

Flow Cytometry

Peripheral blood immune profiling was performed using multiparametric flow cytometry. Briefly, 50 µL of whole blood was incubated with monoclonal antibody panels directed against lymphoid and myeloid markers, followed by an erythrocyte lysis buffer for 20 minutes.

The lymphocyte antibody panel included anti-CD3 (APC-Cy7, clone SK7, Cat. 557832), CD4 (BV605, clone RPA-T4, Cat. 562658), CD8 (FITC, clone RPA-T8, Cat. 555366), PD-1/CD279 (PE, clone MIH4, Cat. 557946), TIM-3/CD366 (AF647, clone 7D3, Cat. 565558), LAG-3/CD223 (PE-CF594, clone T47-530, Cat. 565718), and CTLA-4/CD152 (BV421, clone BNI3, Cat. 562743).The myeloid panel included PD-L1/CD274 (BV421, clone MIH1, Cat. 563738) and the viability dye 7-AAD (Cat. 559925). All antibodies were obtained from BD Biosciencies (Becton Dickinson, Franklin Lakes, NK, USA) and used according to manufacturer recommendations.

Samples were acquired using BD LSRFortessa™ flow cytometry (Becton Dickinson, Franklin Lakes, NJ, USA), collecting a minimum of 100,000 events per sample. $CD4^+$, $CD8^+$, and $CD3^+CD4^-CD8^-$ (double negative) T cell percentages were calculated relative to total $CD3^+$ cells. Expression of immune checkpoint molecules (PD-1, LAG-3, TIM-3, CTLA-4 and PD-L1) was evaluated using mean fluorescence intensity (MFI) within their corresponding gated subsets (Fig. 1).

Inflammatory Index and Hematological Parameters

Routine complete blood count (CBC) analyses were performed at each time point. Absolute neutrophil, lymphocyte, monocyte, and platelet counts were obtained from automated hematologic analysis.

The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic immune-inflammation index (SII) were calculated as follows: NLR = absolute neutrophil count / absolute lymphocyte count; PLR = absolute platelet count / absolute lymphocyte count; and SII = platelet count × absolute neutrophil count / absolute lymphocyte count

Statistical Analysis

Data distribution was assessed using normality tests. The variables were expressed as median ± min-to-max values.. Comparisons among healthy controls, pre-nephrectomy, and post-nephrectomy groups were performed using the Kruskal-Wallis test followed by Bonferroni's multiple comparisons test.

A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA).

Artificial Intelligence Disclosure

Artificial intelligence-based language tools (ChatGPT) were used exclusively for language editing and manuscript organization. No artificial intelligence tool was used for data analysis, statistical calculations, figure generation, or interpretation of scientific results. All content was critically reviewed and approved by the authors.

RESULTS

Patient Characteristics

Baseline clinical and demographic characteristics of patients with localized ccRCC and healthy controls are summarized in Table 1.

Table 1
Population Clinical Characteristics

Variable	Parameter	Controls (N = 10)	ccRCC (N = 20)	P-value
Age (years)	Mean (± S.D.)	60.8 (± 6.0)	59.4 (± 8.0)	0.61*
	Min.	51	44	
	Max.	72	71	
Sex, N (%)	Female	6 (60%)	6 (30%)	NA
	Male	4 (40%)	14 (70%)	
pTNM, N	Stage 1	NA	6 (30%)	NA
	Stage 2		2 (10%)	
	Stage 3		10 (50%)	
	Stage 4		2 (10%)	
Nuclear Grade, N (%)	Grade 2	NA	6 (30%)	NA
	Grade 3		9 (45%)	
	Grade 4		5 (25%)	
Tumor Size (cm)	Mean (± S.D.)	NA	8.2 (± 3.9)	NA
Necrosis, N(%)	Area > 50%	NA	2 (10%)	NA
	Area < 50%		18 (90%)	
*Controls vs RCC, unpaired t-test, NLR: neutrophil-to-lymphocyte ratio				

Alterations in peripheral leukocyte composition in ccRCC patients

To evaluate systemic immune alterations induced with nephrectomy in localized ccRCC patients, the peripheral blood leukocyte populations were analyzed before and after the procedure and compared with healthy controls.

Patients with ccRCC prior to nephrectomy showed increased circulating neutrophil percentages compared with healthy controls (Fig. 2A). Following nephrectomy, neutrophil levels decreased toward control values, suggesting partial reversal of tumor-associated systemic inflammatory bias after tumor removal.

In contrast, no significant differences were observed in circulating monocyte frequencies among groups (Fig. 2B). Similarly, lymphocyte percentages remained relatively stable between controls and patients before and after surgery (Fig. 2C).

To further characterize the systemic immune dysregulation, we quantified the circulating T-cell subsets and inflammatory indices in the same groups of participants.

The frequency of $CD4^+$ T cells did not differ significantly among groups (Fig. 3A). In contrast, circulating $CD8^+$ T cells frequencies were significantly increased in pre-nephrectomy patients compared with healthy controls (Fig. 3B). Following tumor removal, $CD8^+$ T-cell levels decreased toward baseline values, suggesting a partial normalization of immune activation following tumor removal.

No statistically significant differences were detected in double-negative ($CD3^+CD4^-CD8^-$) T cell frequencies between groups (Fig. 3C), suggesting that this T-cell subset is not a major contributor to the systemic immune alterations seen in ccRCC at this disease stage.

Next, we calculated the inflammatory indices NLR, PLR and SII. The value for NLR was significantly elevated in patients before nephrectomy and decreased following surgery (Fig. 4A). No significant differences were observed for PLR and SII among the groups of participants (Figs. 4B and 4C).

Collectively, these findings suggest that the systemic inflammatory bias associated with localized ccRCC decreased or partially reversed following therapeutic nephrectomy.

Immune checkpoint molecule expression in peripheral leukocytes

Given the great clinical success for the use of checkpoint inhibitors in the treatment of RCC, we sought to evaluate the expression of immune checkpoint markers in peripheral blood leukocytes using multiparametric flow cytometry. In this context, we evaluated the mean fluorescence expression for the markers PD-1, LAG-3, TIM-3, CTLA-4, and PD-L1 among the group of participants (Fig. 5).

Following therapeutic nephrectomy, we found increased expression of markers PD-1 and CTLA-4 (Figs. 5A and 5D) along decreased expression of PD-L1 (Fig. 5E) relative to values expressed in healthy control participants.

In contrast, the markers LAG-3 and TIM-3 showed unchanged levels of expression other than increased interindividual variability (Figs. 5B and 5C).

Collectively, these findings demonstrate that dynamic alterations in peripheral immune function permeate to peripheral circulating leukocytes, affecting its numbers but also the expression of functional costimulatory markers.

DISCUSSION

In this study, we evaluated systemic immune alterations in patients with localized clear cell renal cell carcinoma (ccRCC) before and after nephrectomy. Our findings demonstrate that localized ccRCC is associated with significant alterations in circulating leukocyte populations, inflammatory biomarkers, T-cell subsets, and immune checkpoint expression. The most consistent alterations were preoperative

neutrophilia, and persistently elevated neutrophil-to-lymphocyte ratio (NLR), increased $CD8^+$ T-cell percentage, and dysregulated expression of markers PD-1, PD-L1, and CTLA-4 following therapeutic nephrectomy. Given the lack of clinical comorbidities in the group of participants, the only source of inflammatory bias in these patients was the primary tumor, as previously suggested in the literature (21–23).

The preoperative neutrophilia observed in our cohort is consistent with previous studies linking ccRCC with systemic inflammatory response and adverse clinical outcomes. Elevated NLR has been widely associated with poor prognosis in RCC and is considered a surrogate marker of tumor-driven inflammatory activity(24). The reduction in neutrophil percentages following nephrectomy further supports the concept that the primary tumor contributes directly to systemic immune perturbation and that peripheral inflammatory parameters may reflect tumor burden. Interestingly, although NLR differed significantly among groups, PLR and SII did not show significant changes, suggesting that NLR may be a more sensitive indicator of systemic immune perturbation in localized disease.

In addition to hematologic alterations, patients with ccRCC demonstrated increased circulating CD8 + T cell frequencies prior to nephrectomy, while CD4 + and double-negative T cell populations remained relatively stable. Following surgery, CD8 + T cell levels decreased toward control values, suggesting partial reversal of systemic immune activation after tumor removal. These findings are consistent with prior evidence showing alterations in circulating T-cell subsets in RCC patients, particularly within the cytotoxic T-cell compartment. Together, these observations support the notion that localized ccRCC induces measurable systemic immune activation even in the absence of metastatic disease(25).

Peripheral immune checkpoint profiling revealed a distinct pattern of immune dysregulation characterized by elevated PD-1 expression, reduced PD-L1 expression, stable LAG-3 and TIM-3 levels, and increased postoperative CTLA-4 expression. Interestingly, these findings differ from what is commonly described within the tumor microenvironment, where checkpoint molecule expression is frequently upregulated and associated with aggressive disease behavior. This discrepancy suggests that peripheral immune alterations may not directly mirror intratumoral immunopathology and highlights the complexity of systemic immune regulation in ccRCC(26).

Our observation of increased PD-1 expression is consistent with previous studies reporting elevated PD-1 levels expression in circulating immune cells from RCC patients(20). However, unlike previous reports describing rapid postoperative normalization, PD-1 levels remained elevated after nephrectomy in our cohort. This persistent elevation may reflect prolonged immune exhaustion, residual systemic inflammation or delayed immune recovery following surgery.

In contrast, LAG-3 and TIM-3 expressions remained unchanged between groups. Similar findings have been reported in localized ccRCC, where these checkpoint pathways appear to be more prominent within tumor-infiltrating lymphocytes than in peripheral circulation(27). These results suggest that not all inhibitory immune pathways are equally represented systematically during early-stage disease and reinforce the importance of distinguishing between peripheral and intratumoral immune profiles.

Interestingly, CTLA-4 expression increased after nephrectomy. Although the biological significance of this finding remains uncertain, it may represent a compensatory regulatory response accompanying immune re-equilibration after tumor removal. Similar postoperative modulation of immune checkpoint pathways has been described in RCC and other solid tumors, suggesting that immune recovery following surgery may involve activation of inhibitory regulatory mechanisms(28).

This study has several limitations. First, the sample size was relatively small, which may limit statistical power and generalizability. Second, this was a single-center study restricted to patients with localized ccRCC. Third, immune profiling was limited to peripheral blood and was not correlated with tumor tissue immunophenotyping or functional immune assays. Finally, postoperative immune monitoring was limited to a single follow-up time point.

Despite these limitations, this study provides evidence that peripheral immune profiling may represent a useful minimally invasive approach for evaluating systemic immune alterations in localized ccRCC and monitoring immune changes following nephrectomy. Future studies integrating peripheral and tumor immune profiling in larger cohorts will be necessary to better define the clinical relevance of these immune alterations and their potential role as biomarkers of disease activity and treatment response.

CONCLUSION

In patients with localized clear cell renal cell carcinoma treated with nephrectomy, there is a persistent systemic immune dysregulation comprising neutrophilia, CD8 + lymphocytosis and expression changes in the costimulatory markers PD-1, PD-L1, and CTLA-4 in peripheral leukocytes, supporting a potential value for peripheral immune profiling in postoperative following patients with localized ccRCC.

Abbreviations

- **CBC** – Complete Blood Count
- **ccRCC** – Clear Cell Renal Cell Carcinoma
- **CTLA-4** – Cytotoxic T-Lymphocyte-Associated Protein 4
- **FMO** – Fluorescence Minus One
- **ICI** – Immune Checkpoint Inhibitor
- **LAG-3** – Lymphocyte Activation Gene 3
- **MFI** – Mean Fluorescence Intensity
- **NLR** – Neutrophil-to-Lymphocyte Ratio
- **PD-1** – Programmed Cell Death Protein 1
- **PD-L1** – Programmed Death Ligand 1
- **PLR** – Platelet-to-Lymphocyte Ratio
- **RCC** – Renal Cell Carcinoma

- **SII** – Systemic Immune-Inflammation Index
- **TIM-3** – T-cell Immunoglobulin and Mucin Domain-containing Protein 3

Declarations

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This research received no specific grant from any funding agency

Author Contribution

RGGo: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, writing—original draft.MMT: Conceptualization, Flow cytometry methodology, supervision, writing—review & editing.RGGa: Pathological analysis, methodology, writing—review & editing.RJD: Patient recruitment, surgery, investigation, data collection.PAMM: Patient recruitment, surgery, investigation, data collection.EDCD: Investigation, data collection, writing—review & editing.NLL: Investigation, writing—review & editing.HETR: Investigation, patient recruitment, writing—review & editing.AGG: Conceptualization, supervision, project administration, validation, writing—review & editing.urology.hu.uanl@gmail.com

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The authors have no acknowledgment to declare.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of the University Hospital "Dr. José Eleuterio González", Autonomous University of Nuevo León (Protocol No. IN-23-00002). Written informed consent was obtained from all participants before enrollment.

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interests

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Figures

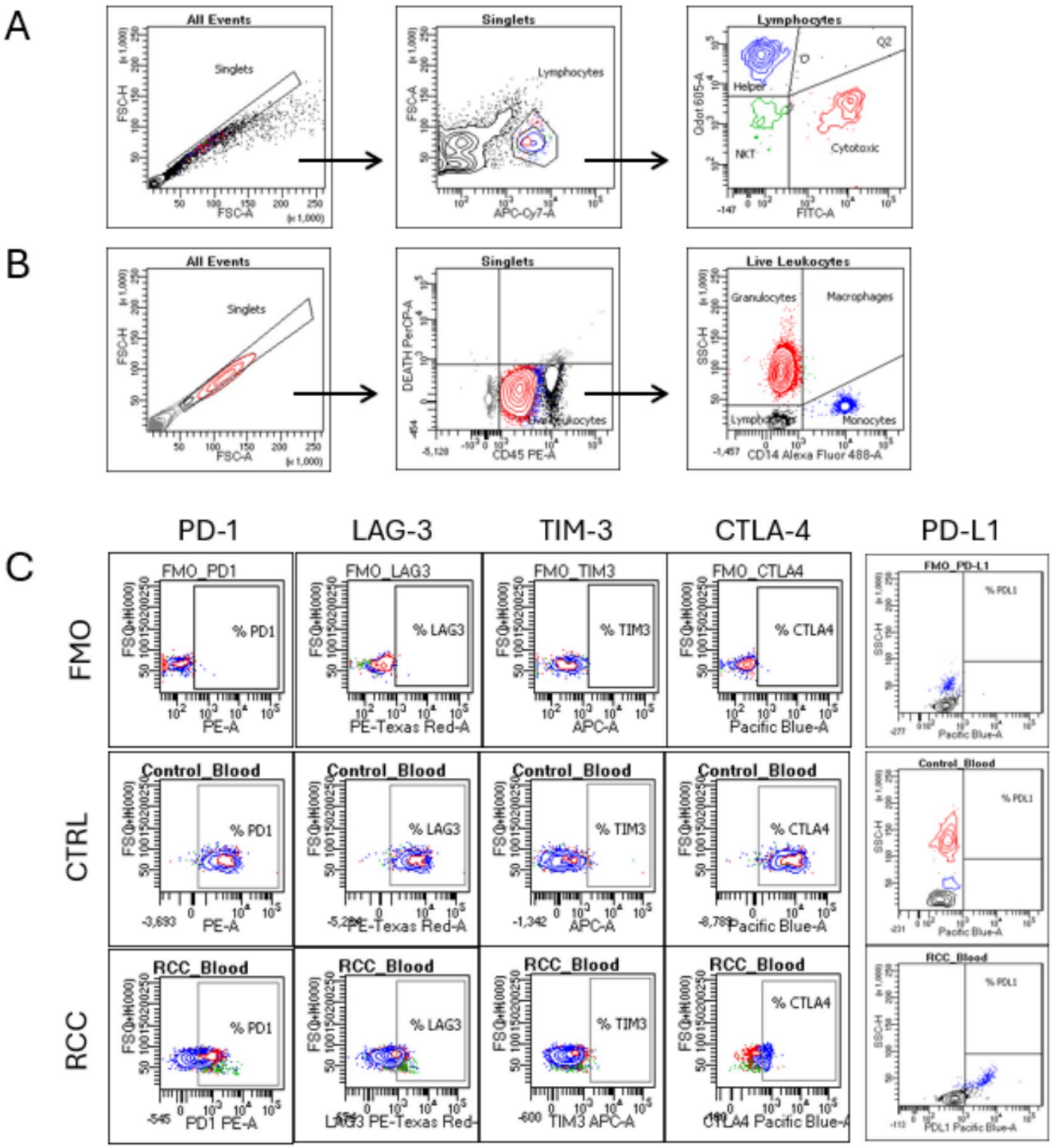


Figure 1

Representative flow cytometry gating strategy and immune checkpoint expression in peripheral blood lymphocytes. Gating for lymphocytes (A), leukocytes (B), and fluorescence expression for costimulatory markers (C). Fluorescence minus-one (FMO), healthy controls (CTRL), and patients with localized clear cell renal cell carcinoma (RCC).

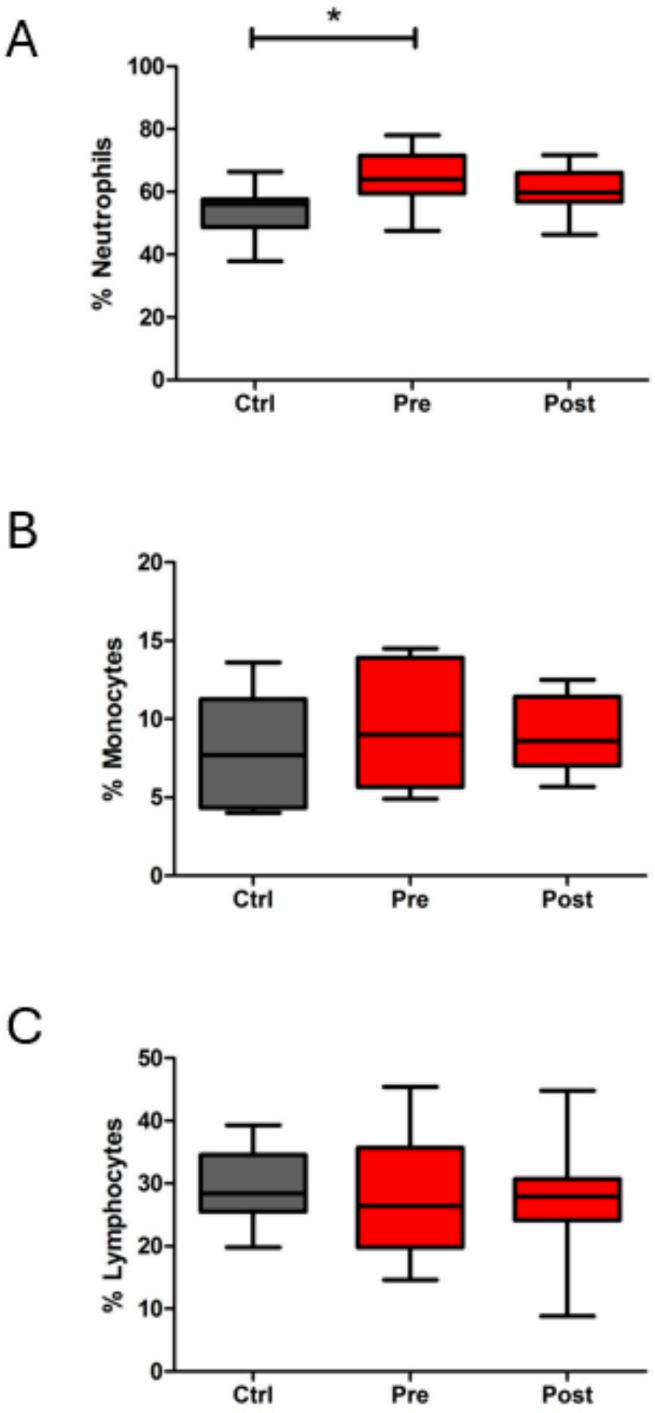


Figure 2

Peripheral blood leukocyte percentages in healthy controls and renal cell carcinoma patients (RCC) . All percentages were relative to CD45: neutrophils (A), monocytes (B), and lymphocytes (C) in healthy controls (Ctrl) and patients before (Pre) and after (Post) therapeutic nephrectomy. Data are presented as median ± min-to-max values. Statistical comparisons were performed using the Kruskal–Wallis test followed with Bonferroni. Healthy controls: *n* = 6; RCC patients: *n*= 20. *P < 0.05.

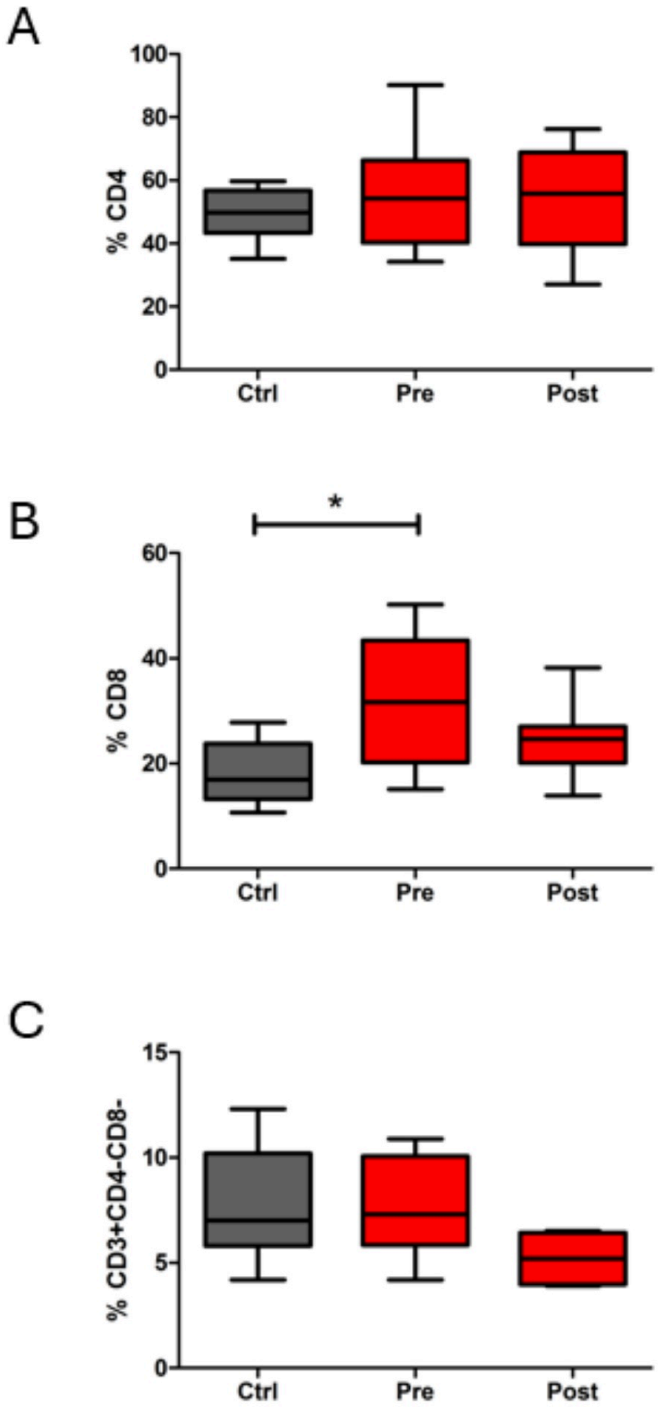


Figure 3

Frequency of peripheral T-cell subsets in healthy controls and renal cell carcinoma patients (RCC). All percentages were relative to CD3: T CD4+ (A), T CD8+ (B), and T CD4-CD8- (C) cell percentages in healthy controls (Ctrl) and patients before (Pre) and after (Post) therapeutic nephrectomy. Data are presented as median ± min-to-max values. Statistical comparisons were performed using the Kruskal–Wallis test followed with Bonferroni. Healthy controls: *n* = 6; RCC patients: *n*= 20. *P < 0.05.

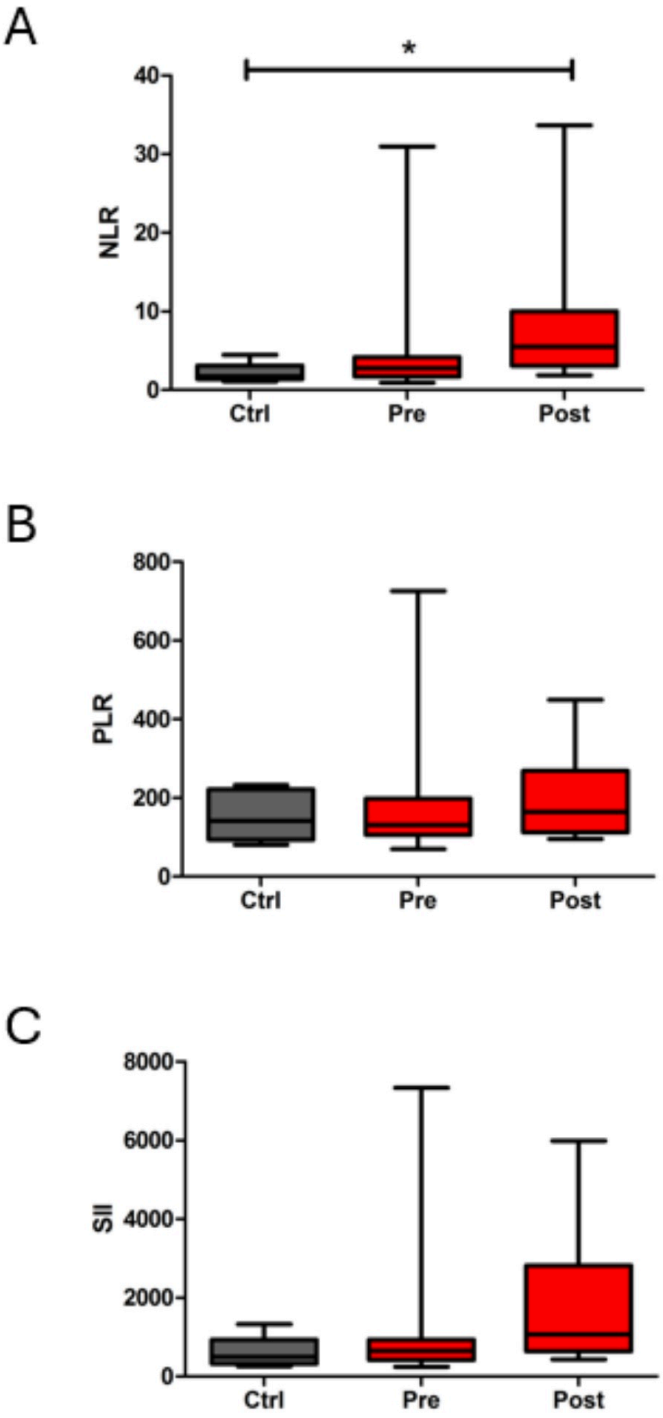


Figure 4

Inflammatory indices in healthy controls and renal cell carcinoma patients. The figure shows the values for NLR (A)„ PLR (B), and SII (C) in healthy controls (Ctrl) and patients before (Pre) and after (Post) therapeutic nephrectomy. Data are presented as median ± min-to-max values. Statistical comparisons were performed using the Kruskal–Wallis test followed with Bonferroni. Healthy controls: *n*= 6; RCC patients: *n* = 20. *P < 0.05.

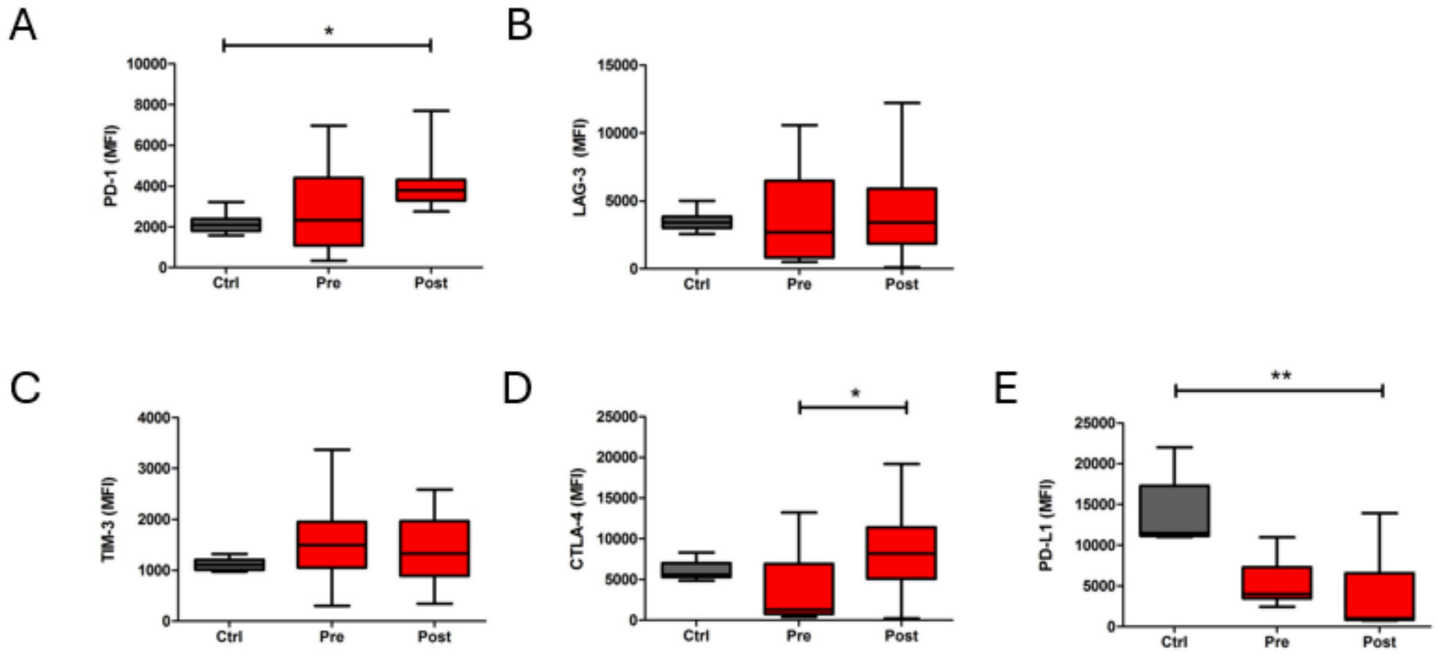


Figure 5

Expression for immune checkpoint markers in peripheral blood lymphocytes obtained from healthy control subjects and RCC patients. We analyzed the markers PD-1 (A), LAG-3 (B), TIM-3 (C), and CTLA-4 (D) within CD3+ lymphocytes compartment. Complementally, the markers PD-L1 (E) were evaluated within the CD14+ cells. MFI, mean fluorescence intensity. Evaluations were performed in healthy controls (Ctrl) and patients before (Pre) and after (Post) therapeutic nephrectomy. Data are presented as median ± min-to-max values. Statistical comparisons were performed using the Kruskal–Wallis test followed with Bonferroni. Healthy controls: n = 6; RCC patients: n = 20. *P < 0.05; **P < 0.01.