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Original article

Impact of radiofrequency ablation on PBMC subpopulation in patients with renal cell carcinoma☆

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Abstract

Purpose: With the development of diagnostic techniques, renal cell carcinoma (RCC) is currently diagnosed in earlier stages, allowing the introduction of less invasive techniques in its management. One of the most promising new treatment methods is based on the utilization of high temperature created by radiofrequency current circulating around the needle probe introduced into the tumor. Besides the direct destruction of the cancer tissue, the treatment may induce immunologic reaction to tumor antigens released from destroyed tumor cell. This paper describes changes observed in the peripheral blood lymphocyte population after radiofrequency ablation (RFA) of RCC.

Methods: Blood was tested before, and 2, 4, and 6 weeks after the RFA in 6 patients with RCC for the proportions and numbers of $CD3^+$, $CD3^+HLA\text{-}DR^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD56^+CD16^+$ cells. The blood was stained with fluorochrome-conjugated monoclonal antibodies and percentages of cells expressing various markers were determined by flow cytometry.

Results: In all patients, the changes were most pronounced 2 weeks after the procedure. The proportion of $CD4^+$ and $CD8^+$ lymphocytes were changed. In 1 patient, an increase in both $CD4^+$ and $CD8^+$ cells was observed. In 5 out of 6 patients, the proportion of activated (DR^+) cells was increased over the whole follow-up period with the highest values in the second week after RFA. The percentage of the $CD56^+CD16^+$ was decreased in most of the patients.

Conclusions: Our study confirms that in the majority of patients, RFA of the renal tumors causes significant changes in the proportion of the peripheral immune cells. We suggest that the results presented in this article shows the necessity for further studies.

Keywords: Radiofrequency ablation; Renal cell carcinoma; Tumor antigens; Lymphocytes activation; Immunotherapy

1. Introduction

Renal cell carcinoma (RCC), including all its subtypes, account for 2%–3% of adult neoplasms and about 90% of all primary renal tumors. The progress in diagnostic technologies [ultrasound (USG), computer tomography (CT), and magnetic resonance imaging (MRI)] has led to the increase in T1a tumors (less than 4 cm in diameter) incidence. Partial nephrectomy still remains the most commonly established method of treatment for these lesions. The shift in the staging has stimulated research into the new, minimally invasive methods, based on tumor cell ablation using various procedures such as radiofrequency ablation (RFA), hypothermy (cryoablation), laser-induced thermotherapy, or microwave ablation. Among these, RFA is currently one of the most promising and effective methods. In renal carcinoma, RFA may be used as an alternative to a partial nephrectomy in peripherally located tumors not exceeding 4 cm in diameter, especially in difficult cases such as patient with a single kidney, bilateral tumors, concomitant neoplasms, or serious medical contraindications to the

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Table 1
Patients' characteristics

	Tumor location		Maximum diameter of the tumor (mm)	Tumor of the single kidney	Other tumors in anamnesis, procedures	Concomitant diseases
	Side	Pole				
Patient 1 (P1)	left	upper	35	+	+[a]	+
Patient 2 (P2)	left	central	28	−	−	+
Patient 3 (P3)	left	central	40	+	+[b]	+
Patient 4 (P4)	left	lower	38	−	−	+
Patient 5 (P5)	right	upper	30	−	+[c]	+
Patient 6 (P6)	right	central	12	−	−	+

[a] RCC, right nephrectomy, left adrenalectomy.
[b] RCC, breast carcinoma (right nephrectomy, mastectomy).
[c] Rectal carcinoma (proctectomy).

operation. It is a relatively safe technique with rare, usually insignificant, complications [1–5].

The procedure is based on needle-probe insertion into the tumor and on the generation of a heat zone by the passage of an electric current with 400–500 kHz frequency around it. The probe may be positioned through the skin under imaging control (USG, CT) or laparoscopically. The temperature around the probe exceeds 80°C. At 42–44°C, vascular thrombosis occurs, and at 50°C, cell membranes disintegrate, protein denaturation starts, and the process of coagulative necrosis is induced [1,2,6].

Most publications on RFA efficacy concentrate on the CT or MRI assessment of the local tumor destruction. In patients with liver tumors treated with RFA, together with the local tissue necrosis, a specific inflammatory and immune response was also demonstrated. In the liver biopsies obtained after thermoablation, macrophages, dendritic cells, and T-lymphocyte infiltrates were found [7]. It is possible, that local neoplastic tissue destruction leads to a substantial antigen release and, thus, neoplastic antigen availability for the immune system cells (dendritic cells, macrophages). Moreover, the presentation of the antigens, changed with the high temperature, may lead to T-lymphocyte activation in the peripheral blood, resulting in a specific anti-tumor activity resembling the neoadjuvant therapy effect [6–14]. The role of the cellular immune response in tumor tissue destruction is well documented for RCC. Interleukin-2 and tumor necrosis factor-α immunotherapy activate the immune system and evoke the anti-tumor response. It is currently administered in patients with advanced stage of the disease [15]. High toxicity limits the use of this type of treatment in many patients. Search for new possible mechanisms inducing the anti-tumor immune response continues. The participation of the RFA in specific anti-tumor response induction in patients with liver tumors is far from being complete, and no evidence in the literature shows it has ever been studied in patients with RCC. The evaluation of this response in patients with RCC may lead to a better understanding of the thermoablation effect and improve its efficacy. In addition, detection of new mechanisms of immune system activation can result in the opportunity to use RFA, together with immune therapy, as a new combination therapy in RCC.

The aim of this study was to evaluate the impact of RFA on the peripheral blood lymphocyte subpopulations: T ($CD3^+$), T-helper ($CD4^+$), T-cytotoxic ($CD8^+$), T-activated ($CD3^+HLA\text{-}DR^+$), and natural killer cell (NK) ($CD56^+CD16^+$) in patients with RCC at different time points after the RFA procedure (2, 4, and 8 weeks).

2. Materials and methods

2.1. Patients

The study group included 6 patients (2 females and 4 males) with RCC treated with RFA from January to March 2009 in our department. There were no exclusions, thus everybody who qualified for RFA treatment at that time was enrolled into the study. The mean age was 71 years. The tumors were diagnosed by contrast-enhanced CT. Ultrasound was also performed before the procedure in order to confirm that lesions were visible and accessible. In all cases, tumors were located peripherally and had radiologic features in CT described by Bosniak as characteristic for RCC. Biopsy of the tumor confirmed the presence of RCC (clear type) in all patients (Fuhrman 1 in 4 patients and Fuhrman 2 in 2 patients). In 4 patients, tumors were on the left side, in 2 patients, on the right. Maximum diameter was not longer than 4 cm. In 2 cases, RFA was performed in a single kidney. In both cases, the contralateral kidney had been previously removed due to the RCC (Table 1). The main reason to use RFA in all patients was the presence of medical contraindications to surgical treatment due to numerous concomitant diseases (hypertension, chronic obstructive lung disease, neurological diseases). All patients were informed of the experimental character of the procedure and signed a consent to take part in the study. The protocol was approved by the local ethics committee of the Medical University of Gdansk according to the Helsinki Declaration of 1964.