Microwave ablation enhances the systemic immune response in patients with lung cancer

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Abstract. Microwave ablation (MWA) is a key alternative therapy to conventional surgery for the treatment of lung cancer. In addition to eliminating local tumors, MWA may promote antitumor immunological responses, such as abscopal effects in distant lesions. However, the intensity of MWA is limited and the underlying mechanisms are not well-defined. The present study assessed the impact of MWA on immune cell subsets and cytokines in patients with lung cancer. A total of 45 patients with lung cancer who underwent percutaneous lung tumor MWA were enrolled. Peripheral blood samples were collected before and 24 h after MWA and changes in immune cell subsets [lymphocytes, $CD3^+$, $CD4^+$ and $CD8^+$ T cells, B cells and natural killer (NK) cells] and serum cytokine levels (IL-1β, IL-2, IL-4-6, IL-8, IL-10, IL-12p70, IL-17A and F, IL-22, TNF-α, TNF-β and IFN-γ) were assessed by flow cytometry and ELISA. The number of total lymphocytes, $CD4^+$ T and NK cells in the peripheral blood significantly decreased 24 h after MWA, while number of CD8+ T cells remained stable, leading to a higher proportion of $CD8^+$ T cells. In addition, the serum levels of IL-2, IL-1β, IL-6, IL-12p70, IL-22, TNF-α and IFN-γ were significantly increased 24 h after MWA, indicating a T helper 1 type immune response. The immune response in patients with advanced stage disease was comparable with patients in the early stage group; however, the number of total lymphocytes and $CD3^+$ T cells significantly decreased and the ratio of CD4/CD8 and IL-2 levels significantly increased. The early immune response after MWA may contribute to systemic antitumor immunity in patients with both early and advanced disease. Thus, MWA may exhibit potential as a local therapy and trigger abscopal effects in distant lesions in patients with lung cancer.

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Introduction

Lung cancer is the leading cause of cancer-associated death, with high rates of morbidity and mortality (1,2). At present, surgery is the standard treatment for resectable cancer (3), however, it is associated with numerous complications and limitations, such as tissue damage and extensive thoracic adhesion. Thus, innovative treatments are required to eliminate tumors more effectively (4). Microwave ablation (MWA) is used in the treatment of inoperable early stage and metastatic lung cancer. MWA causes water molecules to vibrate and collide in microwave electromagnetic fields within tumor tissue, resulting in high temperatures and a coagulative necrotic zone that kills the local tumor (5). For local treatment, MWA is precise with minimal invasiveness and few complications (6,7). Notably, the clinical efficacy of MWA is comparable with that of surgery (8).

Unlike conventional surgery, MWA not only kills local tumors but produces abscopal effects in certain cases, resulting in reduction or removal of distant tumor lesions (9). Previous studies have reported that thermal ablation may promote the release of tumor-associated antigens (TAAs) (10,11). These may induce tumor-specific antitumor immune responses, which induce abscopal effects (12,13). Shao *et al* (14) reported that MWA induces abscopal effects and resistance to immunotherapy in a patient with advanced squamous non-small cell lung cancer (NSCLC). Xu *et al* (15) reported spontaneous regression of distant tumor lesions after MWA in patients with lung metastasis of endometrial carcinoma. To the best of our knowledge, however, few cases of MWA-induced abscopal effects have been reported (16), and the specific mechanisms underlying the abscopal effects remain to be elucidated.

In addition to MWA, other local therapies have demonstrated systemic antitumor immune responses, including abscopal effects. Radiofrequency ablation (RFA) reshapes the tumor microenvironment (TME) by promoting the recruitment of $CD8^+$ T/natural killer (NK) cells and suppressing the accumulation of myeloid-derived suppressor

and regulatory T cells (17). Notably, cryoablation induces a strong tumor-specific tumor-infiltrating lymphocyte response, increases $CD8^+$ T cell and granzyme B levels and induces abscopal effects (18). Irreversible electroporation induces abscopal effects by increasing the infiltration of specific T cells and enhancing specific immune memory (19). In addition, both radiotherapy (20) and heavy ion therapy (21) may modulate occurrence of immune-induced abscopal effects.

Research into MWA-induced immune responses is primarily conducted in hepatocellular carcinoma (22) and breast cancer (23). To the best of our knowledge, MWA-induced immune responses in patients with lung cancer have rarely been reported in clinical studies (24,25). Thus, the present study assessed potential changes in immune response after MWA in patients with lung cancer. The present study aimed to provide a novel theoretical basis to study the mechanisms of MWA and occurrence of abscopal effects.

Materials and methods

Study design. Patients admitted to Putuo Hospital, Shanghai University of Traditional Chinese Medicine (Shanghai, China) were recruited. In total, 45 patients with lung cancer who successfully received MWA were involved in the present study between January 2021 and December 2022 (Table I). The inclusion criteria were as follows: i) Lung cancer (either primary or metastatic) diagnosed via histopathology or artificial intelligence-based lung computed tomography (CT) diagnostic system; ii) MWA guided by CT; iii) ≤5 lesions and iv) a maximum tumor diameter ≤3 cm. Patients were excluded according to the following criteria: i) Immunotherapy, chemotherapy, radiotherapy, glucocorticoid or immunosuppressive therapy <3 months before enrollment; ii) serious complications after MWA, such as infection or high levels of bleeding and iii) refusal to participate in the study. Patients with lung cancer were divided into those with early (stage I-II) or advanced stage (stage III-IV) disease (26). The present study was approved by the Ethics Committee of Putuo Hospital, Shanghai University of Traditional Chinese Medicine [Shanghai, China; approval no. PTEC-A-2022-2(S)-1] and all patients provided written informed consent to participate. Peripheral blood samples were collected (prior to intervention and 24 h after MWA) to assess levels of immune cell subsets and cytokines (Fig. 1A).

CT-guided MWA. Under local anesthesia, CT-guided MWA (ECO-100E; Yigao Microwave Electric Institute) was performed. The microwave antenna was placed in the target lesion and MWA was performed by an experienced respiratory doctor under CT guidance. The ablation parameters were determined by tumor load, with a microwave irradiation frequency of 2,450 MHz and an ablation power of 40-60 W for 2-9 min. Ablation was performed according to the manufacturer's recommendations to generate a safety margin of ≥0.5 cm to achieve complete ablation.

Measurement of immune cell subset and cytokine levels. Briefly, peripheral blood samples were centrifuged at 1,600 x g for 20 min at 4˚C to separate cells and serum. Cells from blood samples were processed for immunophenotype analysis. Cells were incubated with Human TruStain FcX (No. 422302, BioLegend) for 10 min at 4˚C to block the Fc receptors and fixated and permeabilized using a fix and perm buffer for 15 min at 20˚C (No. GAS004, Thermo). Cells were stained with BD Multitest 6-Color TBNK Reagent (No. 644611, BD Biosciences, including CD45 PerCP-Cy5.5, CD3 FITC, CD4 PE-Cy7, CD8 APC-Cy7, CD16 PE, CD56 PE, CD19 APC). BD Multitest 6-Color TBNK dissolved in 1 ml buffered saline, and pipette 20 μl of beforementioned antibodies into 50 μl of blood sample according to the manufacturer's instructions. Incubate for 30 min in the dark at 20˚C. Add 450 μl of 1X BD FACS™ Lysing Solution (No. 349202, BD Biosciences) to the blood sample, and incubate for 30 min in the dark at 20˚C. $CD3^+$ ($CD45^+CD3^+$), $CD4^+$ ($CD3^+CD4^+$) and $CD8^+$ T ($CD3^+CD8^+$), B ($CD3^-CD19^+$) and NK cells ($CD3^-CD16^+CD56^+$) were analyzed by flow cytometry (BD FACSCanto™ II, BD Biosciences), and data analysis was performed using FlowJo software (Tree Star, Inc., Ashland, OR, USA).

Cytokine concentrations in patients were quantified using ELISA kits according to the manufacturer's instructions, and these cytokines included IL-1β (No. 20212400166, Hotgen), IL-2 (cat. no. 20222400096, Hotgen), IL-4 (No. 20222400091, Hotgen), IL-5 (No. 20222400088, Hotgen), IL-6 (No. 20172400287, Hotgen), IL-8 (No. 20212400164, Hotgen), IL-10 (No. 20212400165, Hotgen), IL-12p70 (No. ab213791, Abcam), IL-17A (No. ab216167, Abcam), IL-17F (No. ab309176, Abcam), IL-22 (No. ab216170, Abcam), TNF-α (No. 20212400161, Hotgen), TNF-β (No. ab229202, Hotgen) and IFN-γ (No. ab174443, Abcam). Briefly, serum was added to microplate strips and incubated at 20˚C for 2 h. After a total of three washes with wash buffer, primary antibodies (1:100 dilution) were added and incubated for 2 h at 20˚C. The wash step was then repeated. Finally, after incubation with substrate solution for 30 min at 20˚C, stop solution was added. Wash buffer, substrate solution, primary antibodies and stop solution included in Elisa kit. The levels of cytokines were measured by a fully-automatic chemiluminescence platform (Hotgen c2000; Beijing Hotgen Biotech Co., Ltd.) according to the manufacturer's instructions.

Clinical observation of local effects of MWA. According to expert consensus (27), contrast-enhanced chest CT scans were performed monthly for the first 3 months after MWA. Thereafter, contrast-enhanced chest CT or positron emission tomography (PET)/CT scans and tumor markers were evaluated every 3 months to assess whether complete ablation was achieved. Complete ablation was defined as the absence of focal enhancement signals within or at the periphery of the tumor, confirmed via contrast-enhanced images.

Statistical analysis. Data are presented as the mean ± standard error of the mean of three independent experimental repeats. GraphPad Prism 8 (Dotmatics) was used to analyze the data and generate heatmaps. Differences before and after ablation were assessed using paired Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Phenotypical characterization of peripheral blood mononuclear cells following MWA. Changes in immune cell subsets in peripheral blood of 45 patients with lung cancer were

Table I. Baseline characteristics of the study population.

Characteristic	Value
Median age, years (range)	59 (40-83)
Sex (%)	
Male	25 (55.56)
Female	20 (44.44)
Stage (%)	
I	33 (73.33)
II	0 (0.00)
III	4 (8.89)
IV	8 (17.78)
Number of tumors (%)	
1	22 (48.89)
2	15 (33.33)
≥3	8 (17.78)
Mean diameter of index tumor, cm	1.26±0.92
Previous treatment (%)	
Local ablative therapy	1 (2.22)
Surgical resection	6 (13.33)
None	38 (84.44)

Tumor stage was determined according to the 8th Edition of the American Joint Committee on Cancer Staging Manual (75).

assessed using flow cytometry before and 24 h after ablation (Fig. 1B). Characteristics of peripheral immune cells before and after ablation (Fig. 1C). Notably, the proportion of $CD8^+$ T cells significantly increased after ablation (23.97±1.18% vs. 25.25±1.27%; Fig. 1D and E) and the CD4/CD8 ratio significantly decreased after ablation (1.95±0.13 vs. 1.74±0.13; Fig. 1F). Moreover, the proportion of $CD3^+$ and $CD4^+$ T, NK and B cells remained stable, with no significant differences before and after ablation (Fig. S1A-D). The proportion of immune cell subsets in the peripheral blood was also analyzed; MWA treatment notably reduced the proportion number of immune cells (Figs. 2A and B and S2). In addition, the number of total lymphocytes was not significantly increased after ablation (1,680.27±78.33 vs. 1,543.63±66.37 cells/μl; Fig. 3A and B), as well as the number of $CD4^+$ T (689.41±37.83 vs. 630.95±37.37 cells/μl; Fig. 3D and E) and NK cells (304.58±22.09 vs. 260.05±16.90 cells/μl; Fig. 3F and G). Notably, the number of $CD3^+$ T cells was markedly decreased, however, this was not significant (1,154.26±60.14 vs. 1,074.53±56.26 cells/μl; Fig. 3C). In addition, the number of circulating B and $CD8^+$ T cells remained stable (Fig. S1E and F), with no significant difference before and after ablation.

MWA induces T helper (Th)1-type immune response. To assess serum cytokine levels after ablation in patients with lung cancer, peripheral Th1 and Th2 cytokines were measured, and the levels of multiple cytokines were altered (Figs. 4 and 5A-C). Levels of Th1 cytokines IL-2 (1.66±0.04 vs. 1.81±0.06 pg/ml; Fig. 5D), IFN-γ (0.93±0.02 vs. 0.99±0.03 pg/ml; Fig. 5E), TNF-α (9.16±0.92 vs. 10.86±1.08 pg/ml; Fig. 5F) and IL-12p70 (1.44±0.04 vs. 1.52±0.05 pg/ml; Fig. 5G) were significantly elevated following ablation, indicative of MWA-activated Th1-type immune response. By contrast, the levels of Th2 cytokines, IL-4 and IL-10, were not significantly changed following ablation compared with before (Fig. S3A and B). In addition, significantly increased levels of proinflammatory cytokines IL-6 (20.73±2.81 vs. 29.54±5.06 pg/ml Fig. 5H), IL-1β (1.16±0.05 vs. 1.39±0.09 pg/ml; Fig. 5I) and IL-22 (1.43±0.06 vs. 1.56±0.07 pg/ml; Fig. 5J) were observed after ablation, compared with before; however, no significant changes were observed in the levels of IL-5, IL-8, IL-17A, IL-17F and TNF-β (Fig. S3C-G).

MWA may induce immune responses in patients with early and advanced stage disease. To assess differences in immune responses after ablation according to tumor stage, patients with lung cancer were divided into early and advanced stage groups. Proportion of $CD8^+$ T cells was significantly increased in patients with advanced stage disease following ablation, compared with before (26.41±2.62% vs. 27.79±2.72%; Fig. 6A), but there were no significant changes in $CD3^+$, $CD4^+$ T, NK and B cells. The overall number of lymphocytes was significantly decreased in patients with advanced stage disease following ablation (1,553.37±131.93 vs. 1,362.18±126.82 cells/μl; Fig. 6B), compared with before. This was markedly decreased in comparison with the early disease stage group, which had no significant difference between before and after ablation (1,726.44±95.29 vs. 1,609.28±75.75 cells/μl). Moreover, the number of $CD3^+$ T cells was significantly reduced in patients with advanced stage disease after ablation compared with before (1,088.07±111.92 vs. 965.64±107.57 cells/μl; Figs. 6B and S1G). The number of $CD4^+$ T cells was notably decreased in patients with both early (720.90±45.35 vs. 662.27±40.79) and advanced (602.97±63.94 vs. 544.83±79.49 cells/μl; Fig. 6B) stage disease following ablation compared with before; however, these results were not statistically significant. Results of the present study also demonstrated that the number of $CD8^+$ T, NK and B cells remained unchanged after ablation (Fig. 6B).

In addition, differences in cytokine levels between groups were assessed. IL-2 levels were significantly higher in patients with advanced stage disease after ablation, compared with before (1.62±0.11 vs. 1.91±0.13; Fig. 6C), while levels remained stable in patients with early stage disease (1.68±0.05 vs. 1.77±0.07 pg/ml; Fig. 6C), with no significant differences demonstrated. There were no significant changes in the levels of IL-4, IL-5, IL-6, TNF-α, IL-1β, IL-8, IL-10, IL-12p70, IL-17A, IL-17F, IL-22 TNF-β or IFN-γ in patients with early or advanced stage disease after ablation, compared with before (Fig. 6C-E).

Discussion

As a minimally invasive therapy, MWA is increasingly used in the treatment of lung cancer (28,29). In addition to elimination of local tumors by MWA, the resulting tumor fragments may serve as *in situ* vaccines that trigger a series of immune responses (30-32). A detailed study of MWA-mediated immunity is essential for combining MWA

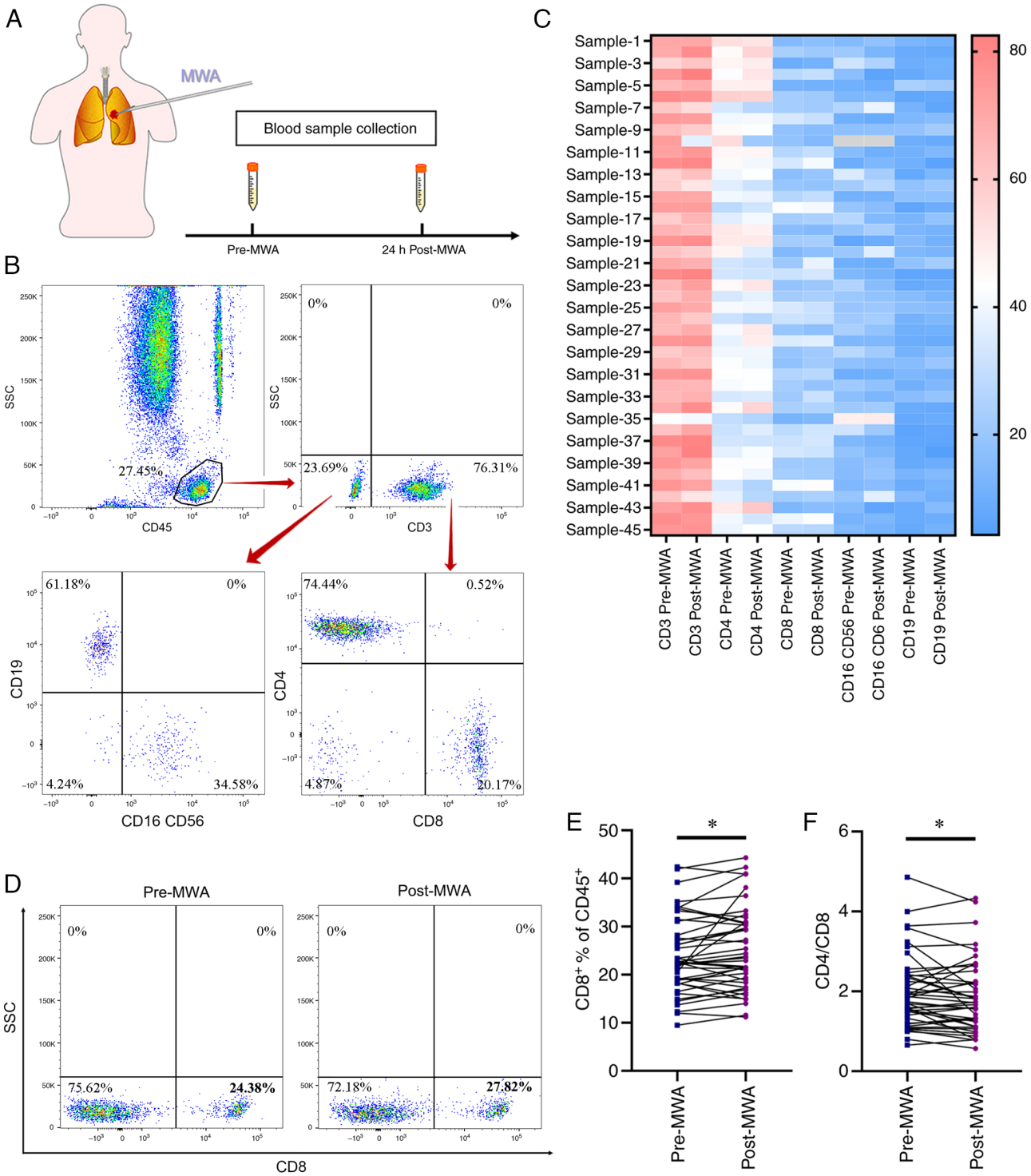

Figure 1. Proportion of peripheral lymphocytes in patients treated with MWA. (A) Study design schematic. (B) Immune cell expression was detected using flow cytometry. (C) Heatmap of changes in the proportions of peripheral $CD3^+$, $CD4^+$ and $CD8^+$ T, NK and B cell subsets before and after ablation. Red, up-; blue, downregulation. (D) Flow cytometry analysis of $CD8^+$ T cells in peripheral blood before and after ablation. (E) Increased proportions of $CD8^+$ and (F) decreased ratio of CD4/CD8 in patients treated with MWA. $^*P<0.05$. MWA, microwave ablation; NK, natural killer; SSC, side scatter.

with immunotherapy in future (33,34). In the present study, MWA induced Th1-type immune response and significantly increased the proportion of the peripheral $CD8^+$ T cell subset. However, the number of peripheral total lymphocytes and $CD4^+$ T and NK cells in patients with lung cancer decreased 24 h after ablation. To the best of our knowledge, these results have not been reported (24). Patients with advanced stage disease had increased levels of $CD8^+$ T cells and IL-2 and decreased levels of total lymphocytes and $CD3^+$ T cells, compared with patients with early stage disease; however, the majority of parameters were comparable between groups. These results suggested that the stage of disease may not impact the MWA-induced immune response.

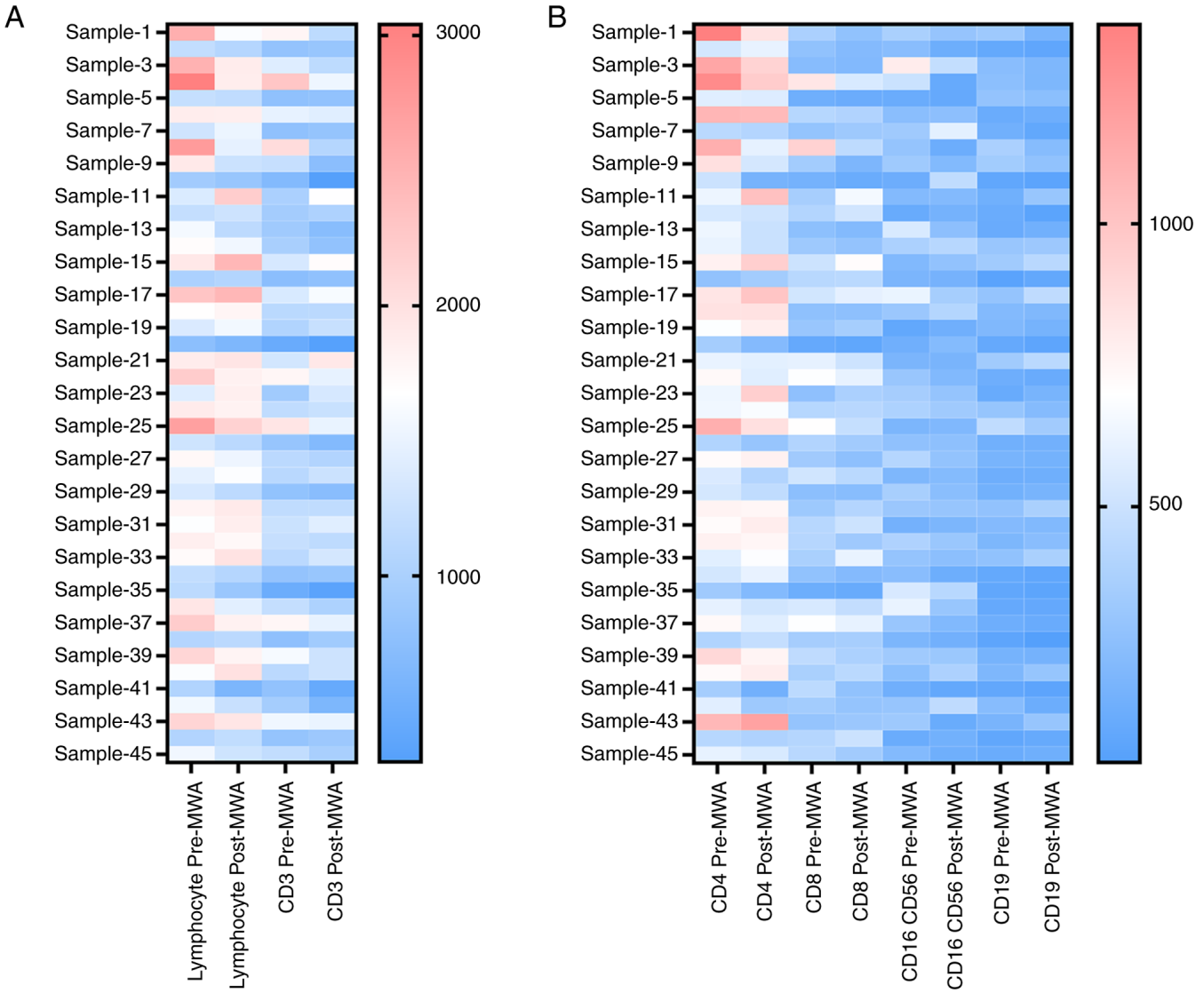

Figure 2. Number of peripheral lymphocytes in patients treated with MWA. Heatmap of changes in number of (A) peripheral total lymphocytes, including $CD3^+$, (B) $CD4^+$, $CD8^+$ T and NK and B cell subsets before and after ablation. Red, up-; blue, downregulation. MWA, microwave ablation; NK, natural killer.

$CD8^+$ T cells are immune cells that directly kill tumors. Notably, these may provide an important immune basis for abscopal effects or for the combination of MWA with immune checkpoint inhibitors in the treatment of cancer (31,35). Levels of $CD8^+$ T cells increased and CD4/CD8 ratio decreased 24 h after ablation. By contrast, the proportions of $CD3^+$ and $CD4^+$ T, NK and B cells remained unchanged and these results were comparable with those reported in a previous study (24). Moreover, results of the present study demonstrated that MWA decreased the number of peripheral immune cells, including total lymphocytes and $CD4^+$ T and NK cells; however, there were insignificant changes in number of $CD8^+$ T cells. Notably, these data were not consistent with other reports: Zhou *et al* (23) reported that $CD8^+$ T and NK cells are notably activated in patients with breast cancer 1 week after ablation. In addition, Wu *et al* (36) reported that the proportions of $CD3^+$ and $CD4^+$ T cell subsets and the CD4/CD8 ratio in the peripheral blood of patients with thyroid cancer, is significantly increased at 1 day and 2 weeks after ablation. Leuchte *et al* (22) reported that the proportion of $CD3^+$ T cells in patients with hepatocellular carcinoma is markedly increased 1 week after ablation. Thus, MWA may lead to different immune cell responses in patients with different tumors. In addition, the time after ablation is an important factor affecting the immune response (36). Further investigation with increased sample sizes is required to assess the response of immune cells.

The underlying mechanism of the MWA-induced systemic immune response is unclear. In the present study, the levels of Th1-type cytokines, such as IL-2, IFN-γ and TNF-α, were significantly elevated 24 h after ablation, while the levels of Th2-type-related cytokines, such as IL-4, IL-5 and IL-10, remained unchanged, Notably, these results were consistent with those of previous studies (25,37,38). IL-2 stimulates T cell proliferation and differentiation and enhances the function of cytotoxic T lymphocytes (39). Zhang *et al* (24) reported that levels of IL-2 notably decreased and the levels of TNF-α do not change in the peripheral blood 1 month after ablation in patients with lung cancer. In addition, Xu *et al* (25) reported that the levels of IL-2 and IFN-γ in the peripheral blood of patients with lung cancer markedly decrease 48 h after MWA

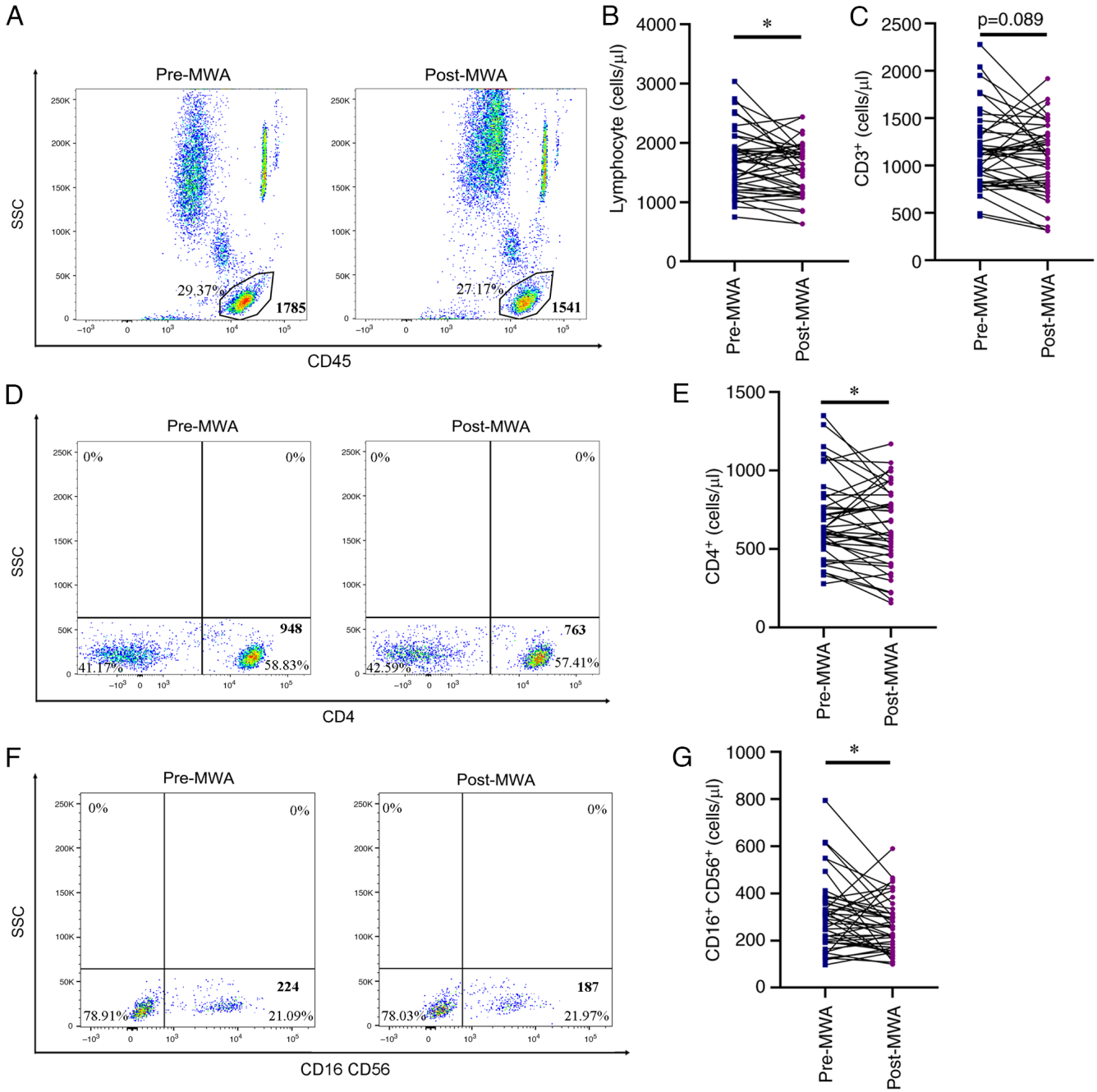

Figure 3. Number of immune cells in peripheral blood is regulated after MWA. (A) Flow cytometry analysis of (B) number of total lymphocytes in peripheral blood before and after ablation. (C) Characterization of the changes in number of $CD3^+$ T cells in peripheral blood before and after ablation. (D) Flow cytometry analysis of (E) $CD4^+$ T cells in peripheral blood before and after ablation. (F) Flow cytometry analysis of (G) NK cells in peripheral blood before and after ablation. *P<0.05. MWA, microwave ablation; NK, natural killer; SSC, side scatter.

and increase after 1 month, while TNF-α levels were not significantly altered. The present study demonstrated that the levels of the Th1-type cytokine IL-12p70 were markedly elevated following ablation. IL-12p70 has been reported to independently induce the differentiation and proliferation of Th1 cells and promote proliferation and killing effects of T and NK cells, especially production of IFN-γ and the formation of cytotoxic T cells (40). Zhao *et al* (41) reported that IL-12p70 and IL-12p40 levels increase significantly 24 h following MWA in patients with hepatocellular carcinoma. In summary, MWA may induce Th1-type immune responses with antitumor effects (42), and these responses may be time-dependent.

In the present study, levels of cytokines were assessed after ablation; levels of proinflammatory cytokines IL-1β, IL-6 and IL-22 were increased in the peripheral blood after ablation. These increases may be associated with tissue repair and the acute phase response (43-45). Notably, levels of IL-8, IL-17A, IL-17F and TNF-β were not significantly changed. IL-1β is considered to serve a key role in initiation of adaptive anti-tumor responses, promoting Th1-type immune responses and activating dendritic cells and cytotoxic T lymphocytes (46). IL-6 serves a key role in T cell proliferation and survival (47) and is also a target of NF-κB. The simultaneous activation of NF-κB and STAT3 in non-immune cells triggers activation of

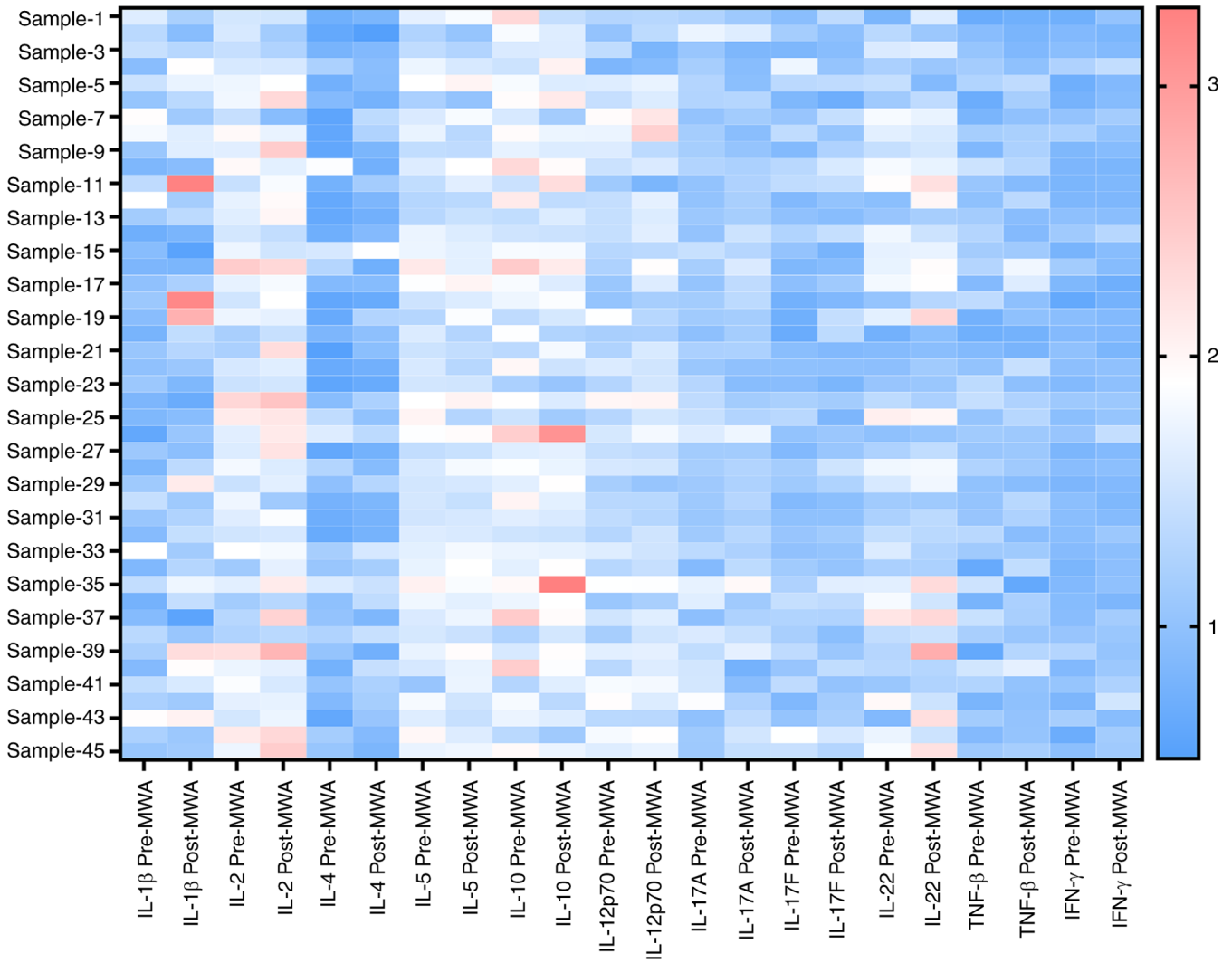

Figure 4. Heatmap of MWA-induced changes in levels of peripheral cytokines. Red, up-; blue, downregulation. MWA, microwave ablation.

the IL-6/STAT3 axis, which is closely associated with tumor development. IL-6 possesses both pro- and anti-inflammatory properties; thus, the ultimate impact of the immune response generated by IL-6 remains unclear (43). IL-22, expressed in activated T cells (48), exhibits dual functions in tumorigenesis. Notably, IL-22 has protective effects in the short term, whilst uncontrolled IL-22 activity promotes cancer growth (49). The cytokines IL-1β, IL-6 and IL-22 can be produced by macrophages, an important type of antigen-presenting cell (50-52). We hypothesized that tumor cell fragments produced by MWA may activate macrophages, potentially inducing specific antitumor immunity, which is consistent with a previous study (53). Further assessment of the specific roles of IL-1β, IL-6 and IL-22 in antitumor activity and changes in cytokine levels at different time points are required to determine the long-term effects of MWA on immune response. IL-8 is a key chemokine for chemotaxis of polymorphonuclear leukocytes and monocytes/macrophages (54), and to evaluate chemotaxis of immune cells in the short term after ablation, IL-8 levels were measured. The levels of IL-8 were not significantly changed after ablation, while Zhao *et al* (41) reported that IL-8 levels notably increase 24 h after MWA in patients with hepatocellular carcinoma. The cytokines IL-17A and IL-17F are produced by differentiated Th17 cells (55,56). Zhou *et al* (57) reported that Th17 cells in patients with hepatocellular carcinoma are regulated 24 h after MWA, and the present study demonstrated that IL-17A and IL-17F levels did not change significantly after ablation. Th17 cells were not activated 24 h after MWA and these results were consistent with those obtained by Xu *et al* (25). TNF-β is an important Th1-type cytokine that is mainly produced by activated T cells (58). MWA did not affect levels of TNF-β in the peripheral blood; however, a Th1-type immune response was induced. Collectively, these results suggest that different tumor types induce different cytokine profiles following ablation.

It is crucial to understand the effects of MWA on immunity in patients with early or advanced stage lung cancer. However, to the best of our knowledge, there are no data describing the immune response following ablation in patients with different stages of lung cancer. In the present study, differences in immune response were investigated after ablation in patients with different stages of disease. The overall effects of MWA were comparable between patients; however, MWA decreased the number of total lymphocytes and $CD3^+$ T cells and increased the proportion of $CD8^+$ T cells, in patients with advanced stage disease;

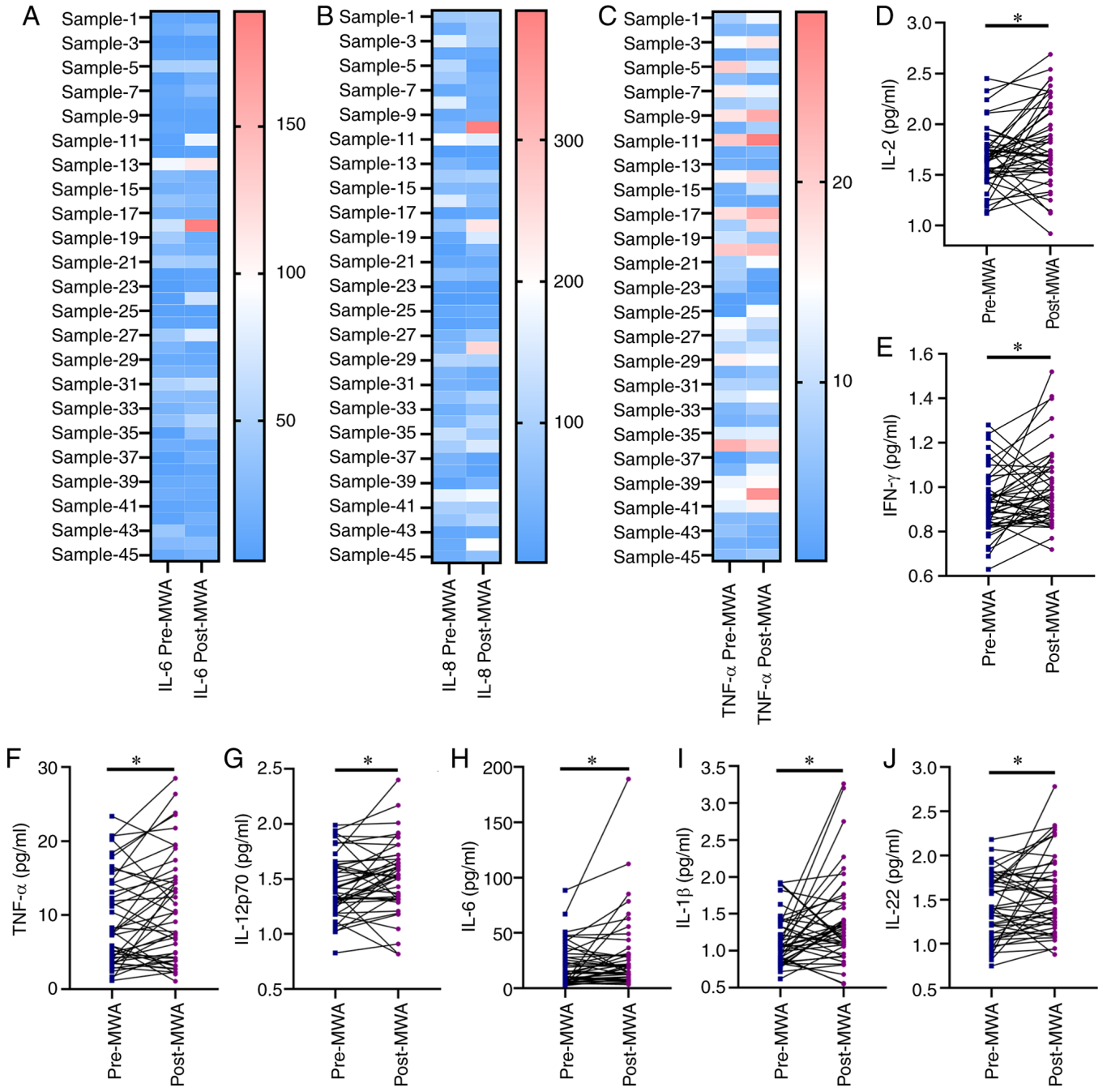

Figure 5. Changes in levels of peripheral cytokines induced by MWA. Heatmap of MWA-induced changes in levels of (A) IL-6, (B) IL-8 and (C) TNF-α peripheral cytokines. Changes in levels of (D) IL-2, (E) IFN-γ, (F) TNF-α, (G) IL-12p70, (H) IL-6, (I) IL-1β and (J) IL-22 peripheral cytokines following ablation. *P<0.05. MWA, microwave ablation.

these results were consistent with those of a previous study (59). These results indicated that decreased levels of total lymphocytes may be reflected by reduced levels of $CD3^+$, but not $CD8^+$, T cells in patients with advanced stage disease. Moreover, levels of NK cells were markedly reduced in patients with advanced stage disease; however, these results were not significant. Thus, further investigation with increased sample sizes is required. Furthermore, the number of $CD4^+$ T cells decreased in both groups after ablation; however, these results were not significant. Thus, the effects of MWA on $CD4^+$ T cells was independent of disease stage. Gabrielson *et al* (60) reported that high levels of $CD3^+$ and $CD8^+$ T cell infiltration are associated with prolonged relapse-free survival, and the function of immune cells is associated with numerous factors, such as surface costimulatory molecules (61). Therefore, further preclinical studies are required to determine the association between circulating and infiltrating immune cells and the differences in immune cell function after MWA.

In the present study, analysis of cytokines in the peripheral blood demonstrated that patients with different stages of disease responded differently to MWA. IL-2 levels were higher in patients with advanced stage disease, indicating that these patients were more susceptible to

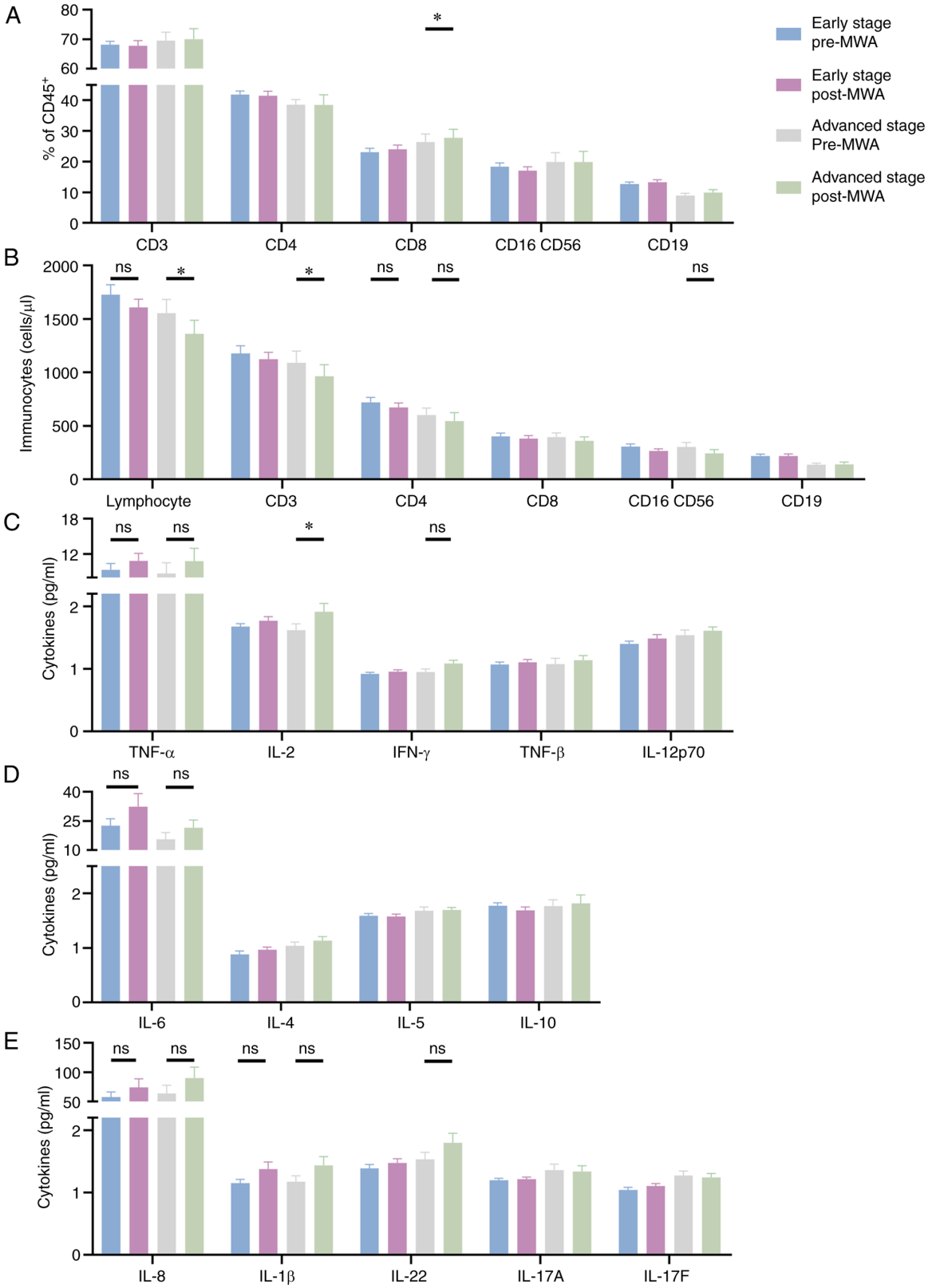

Figure 6. Phenotypical characterization of peripheral lymphocytes and cytokine levels before and after ablation in patients with early or advanced stage disease. Changes in (A) proportion and (B) levels of immunocytes after ablation in patients with early or advanced stage disease. Changes in (C) TNF-α, IL-2, IFN-γ, TNF-β and IL-12p70, (D) IL-6, IL-4, IL-5 and IL-10, and (E) IL-8, IL-1β, IL-22, IL-17A and IL-17F peripheral cytokine levels following ablation in patients with early or advanced stages of disease. *P<0.05. MWA, microwave ablation.

T cell activation by MWA (39), which is beneficial in advanced tumor therapy (62). Cancer immune evasion is a major stumbling block to effective anticancer therapeutic strategies as tumors progress, and T cell activation contributes to antitumor effects in these patients (63). IL-1β and IL-6 levels were increased in both groups; however, these

results were not significant. The levels of IL-22 were increased following ablation in patients with advanced stage compared with patients with early stage disease; however, these results were not significant. Thus, MWA may primarily impact IL-22 levels in patients with advanced stage disease and may impact the TME (64). IL-10 levels remained stable after ablation in both groups of patients; however, Leuchte *et al* (22) demonstrated that IL-10 is notably modulated after ablation. The role of IL-10 in tumors is bidirectional, demonstrating protumor effects as a Th2-type cytokine (65). Qiao *et al* (66) reported that IL-10 notably exhibits antitumor effects by preventing dendritic cell-mediated apoptosis of CD8+ T cells; however, further clarification of the antitumor role of IL-10 is required. By contrast, levels of cytokines such as IL-4, IL-5, IL-8, IL-12p70, IL-17A, IL-17F, TNF-α, TNF-β and IFN-γ were not significantly different between groups after ablation and these results are consistent with those of a previous study (41). In summary, the immune response did not differ between patients with early or advanced stage disease and MWA may induce more T cell activation in patients with advanced stage disease, which may be beneficial in treatment of advanced stage disease.

Hyperthermia generated by thermal ablation releases TAAs, which are absorbed by antigen-presenting cells for presentation to T cells to stimulate specific antitumor immune responses (67). Faraoni *et al* (68) reported that RFA notably increases the number of dendritic cells in pancreatic ductal adenocarcinoma and induces $CD4^+$ and $CD8^+$ T cell-mediated abscopal effects. Kanegasaki and Tsuchiya (69) reported that RFA modulates an antitumor immune response in a CC-chemokine receptor 1-dependent manner. However, the immune response induced by thermal ablation alone remains low. Immune checkpoint inhibitors (ICIs) may enhance ablation (70) and combination of thermal ablation and ICIs may amplify the antitumor immune response. Yu *et al* (71) reported that MWA combined with immunotherapy markedly improves the long-term prognosis in patients with NSCLC. Huang *et al* (72) reported that MWA combined with cisplatin notably prolongs local progression-free survival in patients with large NSCLC. In addition, Feng and Lu (73) reported that systemic administration combined with MWA under CT and fiberoptic bronchoscopy is markedly more effective than systemic administration alone in treatment of lung cancer. Notably, incomplete thermal ablation may promote tumor progression and impede immunotherapy (74). The present study provides a novel theoretical basis for the study of abscopal effects and combination of MWA with immunotherapy in lung cancer; however, further investigations are required to improve the feasibility of combination therapy for long-term local efficacy and the systemic immune response.

Several limitations exist in the present study. The study used peripheral blood collected before and 24 h after MWA and changes in immunity in the peripheral blood may only indicate the short-term effects of MWA on the human immune response. Investigations at additional time points, for example at days 1 and 7 and months 1 and 3 following MWA, are required to study the effects of MWA on immunity. Moreover, the sample size was small and further investigations using larger sample sizes are required. Thus, a potential association between the immune response and ablation of healthy lung tissue cannot be excluded.

In conclusion, MWA induced significant systemic immune responses in patients with lung cancer, particularly Th1-type immune responses. However, MWA may be more likely to induce an immune response in patients with advanced stage disease than those with early stage disease. A combination strategy using additional therapeutic approaches that promotes MWA-induced immune responses may exhibit potential in treatment of lung cancer.

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Availability of data and materials

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

Authors' contributions

FM and YL performed the literature review, analyzed the data and wrote the manuscript. XM and XL designed the study and revised the manuscript. ZN designed the study. SW, MZ, XW and ZZ collected blood samples. FM, YL and XM confirm the authenticity of all raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was performed according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Putuo Hospital, Shanghai University of Traditional Chinese Medicine [Shanghai, China; approval. no. PTEC-A-2022-2(S)-1]. Informed written consent was obtained from all subjects involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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