Dynamic changes in cytokine profiles and their impact on tumour recurrence following thermal ablation in hepatocellular carcinoma

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Abstract

Background

Thermal ablation is widely accepted as a radical HCC therapy. However, the 5-year recurrence rate is high, and whether this local treatment induces a systemic immune response remains unclear. Herein, we investigated the effects of thermal ablation on HCC patients’ immune cytokine profiles and explored predictive biomarkers of tumour recurrence.

Methods

Twenty-two HCC patients were enrolled. We collected peripheral blood before ablation (baseline) and 5–7 days (week 1) and 28–30 days (week 4) after ablation and measured 27 cytokine/chemokine levels at the three time points. Dynamic changes in cytokine profiles and impacts on tumour recurrence were observed.

Results

We found that most cytokines/chemokines (12/27) in HCC patients at baseline were significantly decreased, while MCP-1 was elevated compared to that in healthy controls. IL-6 was significantly elevated at week 1 and decreased at week 4 after ablation, and there were positive correlations between IL-6 levels and ALT and WBC at week 1. IL-10 was slightly decreased at week 1 and dramatically decreased at week 4. The MCP-1, MIP-1β and TNF-α dynamics were similar (decreasing at week 1, increasing at week 4). IL-17, PDGF-BB and RANTES were significantly elevated at week 4 compared with baseline and week 1. We also found that patients with high levels of IL-10 at baseline and low levels of TNF-α, PDGF-BB and RANTES at week 4 were at risk of tumour recurrence.

Conclusion

Our results suggest that thermal ablation relieves tumour immune suppression and activates systemic immune responses by circulating cytokines linked to tumour recurrence.

Background

Hepatocellular carcinoma (HCC) is the sixth most common type of cancer and ranks third in mortality globally [1]. In China, HCC ranks second in terms of the mortality rate of malignancies, and the majority of HCC cases are caused by chronic hepatitis B [2, 3]. Surgical resection and liver transplantation are curative therapies for HCC; however, due to the complex features (such as cirrhosis, underlying liver function, and organ shortage), a major proportion of patients are not eligible for these treatments. In recent decades, local thermal ablation therapies, such as radiofrequency ablation (RFA) and microwave
Ablation (MWA), have become potentially curative strategies for early-stage HCC in most clinical practice guidelines due to their excellent curative effect and minimal invasiveness [4–10]. However, local and distant recurrence rates are still high. The 5-year recurrence rate of HCC with thermal ablation is as high as 70% [11].

Evidence has indicated that thermal ablation may induce a systemic antitumor immune response by the release of cytokines [12], such as the immunosuppressive cytokines interleukin (IL)-10 and transforming growth factor (TGF)-β, which not only directly suppress cytotoxic T cells and natural killer (NK) cells but also promote tumour progression and a poor prognosis. IL-10 and TGF-β significantly decreased at 1 week after RFA; nevertheless, the serum interferon (IFN)-γ level, which is an important proinflammatory molecule stimulating antitumour immunity, was significantly increased at 1 week after RFA [13]. However, there are still few data about the immune cytokine/chemokine dynamics induced by thermal ablation, with only a limited number of plasma biomarkers available to predict prognosis after thermal ablation.

The purpose of this study was to investigate the effects of thermal ablation on 27 cytokine profiles, namely, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, FGF basic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, PDGF-BB, MIP-1β, RANTES, TNF-α and VEGF, at three different time points (baseline, week 1 and week 4) and to identify new biomarkers to predict HCC recurrence.

Materials And Methods

Patients enrolled

In this prospective study, twenty-two HCC patients were recruited at Beijing You’an Hospital, Capital Medical University from January 2019 to February 2020. The diagnosis of HCC was based on the American Association for the Study of Liver Diseases (AASLD) [5], and the classification of HCC stages was based on the Barcelona Clinic Liver Cancer (BCLC) staging system [14]. The inclusion criteria were as follows: (1) age between 18 and 75 years; (2) transcatheter arterial chemoembolization (TACE) combined with complete thermal ablation (RFA/MWA) therapy; (3) Child–Pugh class A or B; and (4) BCLC 0/A/B.

The exclusion criteria were as follows: (1) Child–Pugh class C, (2) incomplete ablation, (3) presence of infection, (4) secondary liver cancer, (5) the presence of immune-related diseases, and (6) combined immunotherapy. The healthy control subjects included 10 self-reported healthy volunteers who were HCV-, HBV-, and HIV antibody-negative without a history of alcohol consumption. The study protocol conformed to the Helsinki Declaration and was approved by the ethics committee of Beijing You’An Hospital affiliated with Capital Medical University. Written informed consent was obtained from all candidates.

Ten millilitres of blood was collected from each patient at three time points: baseline and week 1 and week 4 after ablation. Plasma was frozen in a -80°C freezer.

Therapeutic procedure
All patients enrolled underwent TACE (microguide wire and microcatheter; Asahi Intecc, Co., Ltd.) combined with locoregional ablation (RFA electrode needle; AngioDynamics, Inc.) or MWA (microwave ablation needle; Nanjing Eco Microwave System, Co., Ltd.) within 2 weeks after TACE. A safety margin of 0.5–1.0 cm of the adjacent nonneoplastic tissue was ablated to ensure complete coverage. Abdominal dynamic contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) was used to evaluate the ablation effect.

**Measurements of cytokines/chemokines**

We determined the serum cytokine and chemokine levels using a Human Cytokine 27-plex assay kit (Bio-Rad, Hercules, CA, USA) with Bio-Plex Manager software version 6.0 in a Bio-PlexTM 200 system (Bio-Rad). This system allows quantitative measurement of 27 different chemokines and cytokines, namely, IL-1β, IL-1ra, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, FGF basic, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1α, PDGF-BB, MIP-1β, RANTES, TNF-α and VEGF.

**Follow-up**

The patients were followed up 1 month after ablation and then every 3 months, including AFP/PIVKA-II and enhanced CT or MRI, to monitor tumour recurrence until follow-up ended in January 2022. Tumour recurrence was defined as abnormal arterial phase contrast enhancement and a washout in the portal or delay phase in CT or delay phase in CT or MRI as either local or distant.

**Statistical analysis**

Continuous variables are expressed as the mean ± standard deviation (SD) for those with a normal distribution and, if not, as the median (interquartile range, IQR). Comparisons between HCC patients and healthy controls were performed using the independent samples T test, and the Mann–Whitney U test was used when the data were not normally distributed. Repeated measurement data were analysed by repeated-measures ANOVA or generalized estimating equations with a Bonferroni post hoc test comparing all pairs of time points (baseline, week 1, and week 4). Differences were considered significant at P < 0.05. Spearman's rank correlation coefficient was used for linear correlation analysis between plasma cytokine levels and ALT and WBC. A receiver operating characteristic (ROC) curve was used to determine the cut-off value. Analyses were performed with SPSS software v 25 (IBM, New York, USA).

**Results**

**Clinical and laboratory characteristics**

In this study, we prospectively recruited 22 HCC patients and 10 healthy controls. The demographic characteristics of the HCC patients are shown in Table 1. The present study included 18 males (81.8%) and 4 females (18.2%), the average age was 54.86 ± 9.37 years, and the average tumour size was 3.02 ± 1.56 cm. In addition, 77.3% of patients were BCLC stage 0/A, and the rest were BCLC stage B; 86.4% of patients were classified as Child–Pugh class A (Table 1).
Table 1
Baseline characteristics of HCC patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>HCC patients (n = 22)</th>
<th>Healthy controls (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.86 ± 9.37</td>
<td>52.2 ± 4.42</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>18/4</td>
<td>8/2</td>
</tr>
<tr>
<td>Child–Pugh class (A/B)</td>
<td>19/3</td>
<td>/</td>
</tr>
<tr>
<td>BCLC stage (0/A/B/)</td>
<td>2/15/5</td>
<td>/</td>
</tr>
<tr>
<td>Tumour size (cm)</td>
<td>3.02 ± 1.56</td>
<td>/</td>
</tr>
<tr>
<td>Ablation method (RFA/MWA)</td>
<td>12/10</td>
<td>/</td>
</tr>
<tr>
<td>White Blood Cell (10^9/L)</td>
<td>5.15 ± 1.70</td>
<td>6.33 ± 1.42</td>
</tr>
<tr>
<td>Neutrophil (10^9/L)</td>
<td>3.27 ± 1.65</td>
<td>3.64 ± 1.13</td>
</tr>
<tr>
<td>Lymphocyte (10^9/L)</td>
<td>1.41 ± 0.59</td>
<td>2.09 ± 0.38</td>
</tr>
<tr>
<td>Platelet count (10^9/L)</td>
<td>130.82 ± 70.78</td>
<td>227.01 ± 38.87</td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)</td>
<td>34.77 ± 25.69</td>
<td>/</td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)</td>
<td>31.14 ± 14.05</td>
<td>/</td>
</tr>
<tr>
<td>Total bilirubin (µmol/L)</td>
<td>17.58 ± 10.13</td>
<td>/</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>41.62 ± 5.55</td>
<td>/</td>
</tr>
<tr>
<td>International normalized ratio</td>
<td>1.12 ± 0.13</td>
<td>/</td>
</tr>
<tr>
<td>α-Fetoprotein (IQR), ng/ml</td>
<td>27.83(3.21–196.6)</td>
<td>/</td>
</tr>
<tr>
<td>PIVKA-II (IQR), mAU/ml</td>
<td>55(32–208)</td>
<td>/</td>
</tr>
</tbody>
</table>

**Different profiling of circulating cytokines/chemokines between HCC patients and healthy controls**

As shown in Table 2, HCC patients showed a systemic decrease in 12 cytokines/chemokines compared with healthy controls, specifically IL-1β, IL-4, IL-9, IL-12p70, IL-17A, eotaxin, FGF basic, IFN-γ, MIP-1β, PDFF-BB, RANTES and TNF-α (P < 0.05). The MCP-1 level in HCC patients was higher than that in healthy controls (P < 0.05). Our data suggest an immune suppression tumour microenvironment in early HCC (BCLC stage 0-B).
Table 2
Summary of 27 cytokines/chemokines in HCC patients and healthy controls

<table>
<thead>
<tr>
<th>Cytokine /chemokine</th>
<th>HCC (pg/ml)</th>
<th>Healthy controls (pg/ml)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>1.44(0.92–1.76)</td>
<td>2.08(1.54–2.55)</td>
<td>0.005</td>
</tr>
<tr>
<td>IL-1ra</td>
<td>37.35(25.29–55.27)</td>
<td>42.82(29.02–52.23)</td>
<td>0.325</td>
</tr>
<tr>
<td>IL-2</td>
<td>1.83(1.47–2.21)</td>
<td>2.02(1.74–4.73)</td>
<td>0.269</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.97(0.73–1.28)</td>
<td>1.55(1.34–1.93)</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-5</td>
<td>6.41(4.08–10.66)</td>
<td>11.32(6.41–27.31)</td>
<td>0.070</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.71(1.34–2.46)</td>
<td>1.65(1.34–2.98)</td>
<td>0.704</td>
</tr>
<tr>
<td>IL-7</td>
<td>6.80(4.72–12.33)</td>
<td>9.51(8.58–14.30)</td>
<td>0.070</td>
</tr>
<tr>
<td>IL-8</td>
<td>2.18(1.61–3.21)</td>
<td>1.91(1.45–2.69)</td>
<td>0.434</td>
</tr>
<tr>
<td>IL-9</td>
<td>140.60(123.43–159.19)</td>
<td>184.16(166.98–189.77)</td>
<td>0.000</td>
</tr>
<tr>
<td>IL-10</td>
<td>2.62(2.20–4.49)</td>
<td>2.29(1.84–2.56)</td>
<td>0.084</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>3.05(1.80–3.22)</td>
<td>3.91(3.05–7.41)</td>
<td>0.039</td>
</tr>
<tr>
<td>IL-13</td>
<td>0.86(0.53–1.36)</td>
<td>0.94(0.72–1.82)</td>
<td>0.325</td>
</tr>
<tr>
<td>IL-15</td>
<td>5.99(5.34–14.22)</td>
<td>10.61(5.22–67.31)</td>
<td>0.345</td>
</tr>
<tr>
<td>IL-17A</td>
<td>3.94(3.10–5.10)</td>
<td>6.40(5.50–9.29)</td>
<td>0.002</td>
</tr>
<tr>
<td>Eotaxin</td>
<td>9.03(6.43–11.17)</td>
<td>13.41(10.75–19.92)</td>
<td>0.004</td>
</tr>
<tr>
<td>FGF basic</td>
<td>17.70(15.40–21.95)</td>
<td>24.12(21.94–24.64)</td>
<td>0.016</td>
</tr>
<tr>
<td>G-CSF</td>
<td>27.05(20.03–33.03)</td>
<td>33.03(25.05–42.83)</td>
<td>0.092</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>0.82(0.69–1.35)</td>
<td>1.15(0.84–1.87)</td>
<td>0.109</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>3.10(2.23–4.89)</td>
<td>4.67(3.77–7.05)</td>
<td>0.028</td>
</tr>
<tr>
<td>IP-10</td>
<td>89.85(55.67–118.21)</td>
<td>93.62(77.46–115.86)</td>
<td>0.675</td>
</tr>
<tr>
<td>MCP-1</td>
<td>7.86(4.77–9.89)</td>
<td>5.09(3.00–6.13)</td>
<td>0.018</td>
</tr>
<tr>
<td>MIP-1α</td>
<td>0.67(0.48–0.90)</td>
<td>0.73(0.67–0.90)</td>
<td>0.562</td>
</tr>
<tr>
<td>MIP-1β</td>
<td>74.49(70.88–80.29)</td>
<td>88.68(79.32–92.31)</td>
<td>0.002</td>
</tr>
<tr>
<td>PDGF-BB</td>
<td>66.99(33.80–169.52)</td>
<td>226.34(140.99–335.97)</td>
<td>0.005</td>
</tr>
<tr>
<td>RANTES</td>
<td>1022.88(615.78–1984.25)</td>
<td>3615(2073–5353.75)</td>
<td>0.000</td>
</tr>
<tr>
<td>TNF-α</td>
<td>20.10(18.33–23.97)</td>
<td>25.54(22.69–28.07)</td>
<td>0.006</td>
</tr>
</tbody>
</table>
Cytokine/chemokine | HCC (pg/ml) | Healthy controls (pg/ml) | P value
--- | --- | --- | ---
VEGF | 2.15(1.81–6.92) | 7.69(1.81–57.96) | 0.345

Dynamic changes in cytokines/chemokines after thermal ablation

We compared the variations in cytokines/chemokines in 22 HCC patients at baseline and at week 1 and week 4 after thermal ablation. IL-6 was significantly elevated at week 1 with a median level of 3.12 (IQR 2.16–6.97) pg/ml and declined at week 4 ($P = 0.022$ and 0.031, Fig. 1a). IL-10 was slightly decreased at week 1 and then dramatically decreased at week 4, with a median level of 2.10 (IQR 1.81–2.64) pg/ml ($P = 0.012$ and 0.025, Fig. 1b). The dynamics of MCP-1, MIP-1β and TNF-α were similar, decreasing at week 1 and increasing at week 4 (Fig. 1c-e); in particular, TNF-α had a median level of 18.10 (IQR 16.27–19.33) pg/ml at week 1 vs. 20.09 (IQR 18.33–23.97) pg/ml at baseline ($P = 0.018$) and 20.40 (IQR 17.95–22.53) pg/ml at week 4 ($P = 0.026$). The same dynamic changing trends were observed for IL-17, PDGF-BB and RANTES (Fig. 1f-h), which were significantly elevated at week 4 compared with baseline and week 1; in particular, RANTES had a median level of 2082.50 (IQR 1014.89-2720.25) pg/ml at week 4 vs. 1022.88 (IQR 615.78-1984.25) pg/ml at baseline ($P = 0.028$) and 1096.50 (IQR 763.23-1715.25) pg/ml at week 1 ($P = 0.024$). In addition, we did not find statistically significant changes in other cytokines/chemokines between the 3 time points (e.g., IFN-γ, IL-4, and IP-10).

Correlation between ALT and WBC and IL-6 levels at week 1 after ablation

We measured ALT, AST, WBC and neutrophil as indicators of hepatic necrosis and systemic inflammation. As shown in Fig. 2, there was a significant positive correlation between serum ALT and IL-6 levels ($r_s = 0.478$, $P = 0.024$; Fig. 2a). Likewise, the WBC count also correlated with the IL-6 level at week 1 ($r_s = 0.555$, $P = 0.007$; Fig. 2b). The changing characteristic of IL-6 release following thermal ablation suggests that IL-6 is involved in the acute-phase response against tissue damage and inflammation caused by ablation.

The impact of cytokines/chemokines on tumour recurrence

After a follow-up to January 2022, 10 patients experienced tumour recurrence. We studied the potential cytokines/chemokines associated with tumour recurrence. Our data suggested that HCC patients with tumour recurrence had significantly higher levels of IL-10 at baseline ($P = 0.005$; Fig. 3a), while lower levels of TNF-α, PDGF-BB and RANTES at week 4 were significantly associated with tumour recurrence ($P = 0.019$, $P = 0.025$ and $P = 0.013$; Fig. 3b-3d).
Predictive values of IL-10 (baseline), TNF-α (week 4), PDGF-BB (week 4) and RANTES (week 4) levels for tumour recurrence

Based on the analysis of the relationship between the cytokines/chemokines and tumour recurrence, we further analysed the predictive values of IL-10 (baseline), TNF-α (week 4), PDGF-BB (week 4) and RANTES (week 4) for tumour recurrence (Fig. 4a, b). The ROC curves for predicting recurrence were calculated. We found that the area under the curve (AUC) of the IL-10 level at baseline was 0.783 (95% CI: 0.570–0.997, \( P = 0.025 \)), and the cut-off value was 2.99 pg/ml. Low levels of TNF-α, PDGF-BB and RANTES at week 4 were associated with tumour recurrence; the AUC values were 0.792 (95% CI: 0.575-1, \( P = 0.021 \)), 0.750 (95% CI: 0.533–0.967, \( P = 0.048 \)), and 0.783 (95% CI: 0.577–0.989, \( P = 0.025 \)), respectively. Notably, tumour recurrence predicted by the three combinations had a higher AUC (0.9, 95% CI: 0.750-1, \( P = 0.002 \)), with a sensitivity of 83.3% and a specificity of 100% (Table 3). Therefore, these cytokines/chemokines can be used as predictors of tumour recurrence.

<table>
<thead>
<tr>
<th>Variable (s)</th>
<th>AUC</th>
<th>P value</th>
<th>Cut-off (pg/ml)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10 (baseline)</td>
<td>0.783 (0.570–0.997)</td>
<td>0.025</td>
<td>2.99</td>
<td>70%</td>
<td>91.7%</td>
</tr>
<tr>
<td>TNF-α (week 4)</td>
<td>0.792 (0.575-1.000)</td>
<td>0.021</td>
<td>20.4</td>
<td>83.3%</td>
<td>90%</td>
</tr>
<tr>
<td>PDGF-BB (week 4)</td>
<td>0.750 (0.533–0.967)</td>
<td>0.048</td>
<td>107.78</td>
<td>83.3%</td>
<td>70%</td>
</tr>
<tr>
<td>RANTES (week 4)</td>
<td>0.783 (0.577–0.989)</td>
<td>0.025</td>
<td>2303.94</td>
<td>66.7%</td>
<td>90%</td>
</tr>
<tr>
<td>Combined (week 4)</td>
<td>0.900 (0.750-1)</td>
<td>0.002</td>
<td>0.702</td>
<td>83.3%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Discussion

In this prospective cohort, we analysed the key immune cytokines/chemokines in HCC patients (n = 22) before and after thermal ablation. Our results showed that there were many cytokines/chemokines (12 of 27) that were lower in HCC patients before ablation than in healthy controls, whereas only MCP-1 was higher. This result suggested that early HCC (BCLC stage 0-B) developed a complex immunosuppressive environment, especially Th1/Th2 cytokine network imbalance.

In addition to local tumour tissue necrosis, our study provides evidence that thermal ablation could induce a systemic antitumor immune response. We found that IL-6 was markedly elevated and that there was a significant positive correlation between ALT and WBC and IL-6 levels at week 1 after thermal ablation. IL-6 is a pleiotropic cytokine involved not only in the immune response but also in inflammation by stimulating signal transducer and activator of transcription 3 (STAT3) [15, 16]. Some studies have reported that the IL-6 level is significantly elevated at 3–15 days post-ablation [17–19]. Therefore, we hypothesized that IL-6 is a biomarker reflecting the degree of hepatic trauma and inflammation caused by
thermal ablation and can be used as a biomarker to diagnose complications early. We also found that IL-10, an anti-inflammatory Th2 cytokine that promotes immunosuppression, was decreased dramatically from baseline to week 4 after ablation, while the Th1 cytokine TNF-α was first decreased at week 1 and then elevated at week 4, but there was no significant difference between baseline and week 4. Our findings indicated that thermal ablation may offset the Th1/Th2 balance, which was reshaped and polarized to the Th1 status, thus alleviating HCC tumour-induced immune suppression. In addition, MCP-1 and MIP-1β showed similar dynamics to TNF-α. IL-17, PDGF-BB and RANTES showed the same dynamic changing trends and were significantly elevated at week 4 compared with baseline and week 1. Taken together, our data showed the different dynamic changing trends of cytokines/chemokines after ablation, which could be caused by different mechanisms. First, thermal ablation may cause trauma to the liver, and the wound healing process may result in alterations in some cytokines/chemokines. Second, heat-induced injury could promote acute thermal coagulative necrosis and apoptosis in liver and tumour tissues. The ablated tissue or tumour cells release cytokines/chemokines. Third, importantly, tumour antigens that are released after necrosis drain to antigen-presenting cells, which further stimulate nonspecific and specific immune responses. In conclusion, cytokine expression affected by wound healing, ablated tissue and nonspecific immune responses lasts for a shorter duration than that affected by specific immune responses [20], which may be the reason why the cytokine/chemokine changes lasted for different durations. Therefore, we concluded that thermal ablation induced specific immune cytokine/chemokine changes at week 4.

Interestingly, we further observed that IL-10, TNF-α, PDGF-BB and RANTES were associated with tumour recurrence. IL-10 may contribute to a tumour microenvironment promoting HCC carcinogenesis and progression. Our study showed that a high level of IL-10 at baseline but not after thermal ablation was easily associated with tumour recurrence. This is consistent with the finding of a previous study describing IL-10 as a major biomarker of poor prognosis in HCC [21]. We also observed that the levels of TNF-α, PDGF-BB and RANTES were significantly lower at week 4 and were closely linked with an unfavourable prognosis. The results further indicated that thermal ablation induced antitumor immune function at week 4. TNF-α is one of the most important proinflammatory cytokines and has been demonstrated to be an antitumor cytokine that activates NF-κB formation. Some studies have shown that HCC patients with high expression of TNF-α and NF-κB have longer survival times. The level of TNF-α was higher after ablation and was associated with a good prognosis in patients with cancer [22–24].

PDGF-BB is a pluripotent angiogenic ligand that is present in platelets and released upon degranulation and plays a biological function by activating PDGFR-α and PDGFR-β [25]. PDGF-BB has roles in tumour growth, invasion and metastasis [26–27]. A study revealed that in HCC patients after curative resection, PDGF-BB was lower in the recurrence group than in the nonrecurrence group both before surgery and at 4 weeks post-operation [28]. In a previous study [29], after sorafenib treatment, PDGF-BB was significantly lower in non-long survivors than in long survivors (overall survival ≥ 2 years). We showed for the first time that depleted serum concentrations of PDGF-BB may be associated with tumour recurrence after ablation. In patients with recurrence, the molecular mechanism involved in the exhaustion of serum PDGF-BB concentrations is unknown. The reason could be that platelet dysfunction is already known to
contribute to cancer progression [30–31], and therefore, our hypothesis was that the depletion of serum PDGF-BB might be attributed to platelet exhaustion.

The CC-chemokine RANTES (CCL5) is a T-cell chemoattractant and an immunoregulatory molecule. RANTES can be expressed by a number of cell types, including T lymphocyte cells, macrophages, platelets and tubular epithelium [32]. Nevertheless, the role of RANTES in tumour development remains controversial. Some studies have shown that RANTES overexpression facilitates tumour progression and metastasis via the RANTES/CCR5 axis [33–34]. In addition, other studies have reported that increased RANTES expression, which chemotactically attracts a substantial number of immunocytes to tumour tissues to exert antitumor efficacy, may be associated with favourable outcomes in some diseases [35]. In our study, elevated serum levels of RANTES were observed in nonrecurrence patients compared with recurrence patients at week 4 after ablation. This could imply a protective role of RANTES for antitumor immune responses to prevent tumour recurrence.

Our study found immune mediators associated with tumour recurrence and used large panels of 27 cytokines/chemokines, immune mediators and growth factors at three time points before and after ablation, which has rarely been reported in the existing literature. We also recognize that this study has several limitations. First, the sample size was relatively small, and larger cohorts are needed to validate these findings. Second, we merely evaluated circulating cytokines/chemokines, and further studies on their association with the immune response in the ablation zone microenvironment should be conducted.

**Conclusions**

Our results suggest that thermal ablation may be an effective modality to overcome the immunosuppressive tumour microenvironment and induce a systemic immune response. Our data also indicate that the immune cytokine/chemokine levels after thermal ablation for 4 weeks were directly related to tumour recurrence and could be used as noninvasive and useful biomarkers to predict the postablation prognosis. However, a single treatment may lack the therapeutic power to effectively control aggressive tumours. In the future, thermal ablation combined with immune checkpoint inhibitors may enhance the antitumor immune response and improve the prognosis of patients.

**Declarations**

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**Authors’ contributions**

YZ, DG and YZ designed the study and wrote the paper. DG, LQ, JS and KL performed the data analysis. CZ and QW processed the samples. WQ and BL helped provide patient metadata. All authors reviewed the
paper and approved its content.

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**Availability of data and materials**

The data used to support the findings are available from the corresponding author upon request.

**Ethics approval and consent to participate**

The study has been approved by the ethics committee of the Beijing You’an Hospital affiliated with Capital Medical University. This study was in accordance with the Helsinki protocol, the requirement for patients’ informed consent was waived by the same ethics committee that approved the study (Beijing You’an Hospital affiliated with Capital Medical University), and all methods were carried out in accordance with relevant guidelines and regulations.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**References**


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Figures
Figure 1

Dynamic changes in cytokines/chemokines after ablation treatment (a-h). The levels of IL-6 (a), IL-10 (b), MCP-1 (c), MIP-1β (d), TNF-α (e), IL-17 (f), PDGF-BB (g) and RANTES (h) were compared at baseline and at week 1 and week 4 after ablation. Repeated measures data were subjected to repeated measures ANOVA or generalized estimating equations with Bonferroni post hoc test.
There was a positive correlation between ALT and WBC and the IL-6 level at week 1 (Figure 2a, 2b). Spearman’s rank correlation analysis was used for linear correlation analysis.
Figure 3

Cytokine/chemokine levels associated with tumour recurrence. IL-10 at baseline (a), TNF-α at week 4 (b), PDGF-BB at week 4 (c) and RANTES at week 4 (d) were associated with tumour recurrence. Statistical analysis of the data was performed using repeated measures ANOVA or generalized estimating equations with Bonferroni post hoc test.

Figure 4

ROC curves of IL-10, TNF-α, PDGF-BB and RANTES levels to predict tumour recurrence. The ROC curve of IL-10 at baseline to predict tumour recurrence (a). The ROC curves of TNF-α, PDGF-BB and RANTES and their combination at week 4 (b). The AUC values were all more than 0.75 and indicated that these cytokines could predict tumour recurrence well, especially when combined.