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Image guided thermal ablation of tumors increases the plasma level of IL-6 and IL-10

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Abstract

PURPOSE—To identify changes in plasma cytokine levels following image-guided thermal ablation of human tumors and to identify the factors that independently predict changes in plasma cytokine levels.

MATERIALS AND METHODS—Whole blood samples were collected from 36 patients at 3 time points: pre-ablation, post-ablation (within 48 hours), and in follow-up (1–5 weeks after ablation). Plasma levels of IL-1a, IL-2, IL-6, IL-10 and TNFa were measured using a multiplex immunoassay. Univariate and multivariate analyses were performed using cytokine level as the dependent variable and sample collection, time, age, sex, primary diagnosis, metastatic status, ablation site, and ablation type as the independent variables.

RESULTS—There was a significant increase in the plasma level of IL-6 post-ablation when compared to pre-ablation (9.6+/-31 fold, $p<0.002$). IL-10 also showed a significant increase postablation (1.9 +/-2.8 fold, $p<0.02$). Plasma levels of IL-1a, IL-2, and TNFa were not significantly changed after ablation. Cryoablation resulted in the largest change in IL-6 level (>54 fold), while radiofrequency and microwave ablation showed 3.6 and 3.4-fold changes, respectively. Ablation of melanomas showed the largest change in IL-6 48 hours after ablation (92×), followed by ablation of kidney (26×), liver (8×), and lung (6×) cancers. Multivariate analysis revealed that ablation type ($p<0.0003$), and primary diagnosis ($p<0.03$) were independent

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predictors of changes to IL-6 following ablation. Age was the only independent predictor of IL-10 levels following ablation ($p < 0.019$).

CONCLUSION—Image guided thermal ablation of tumors increases the plasma level of IL-6 and IL-10, without increasing the plasma level of IL-1a, IL-2, or TNF α .

Keywords

thermal ablation; radiofrequency; microwave; cryoablation; cytokine; inflammation; growth factor

INTRODUCTION

Image-guided thermal ablation offers patients a repeatable and low morbidity treatment that has been shown to effectively palliate, salvage, or cure both primary and metastatic malignancies [1–3]. Heat-based thermal ablation (radiofrequency and microwave) kills tumors via direct thermal denaturation and coagulation of tumor proteins [4]. Cold-based thermal ablation (cryoablation) relies on the formation of intracellular ice crystals which arrest tumor cell function by damaging intracellular organelles and disrupting the plasma membranes of tumor cells [5]. All thermal ablation techniques leave devitalized and damaged tumor cells within the body, stimulating a robust inflammatory response demonstrating the cardinal signs of inflammation, including fever, pain and swelling in ablated tissues [6]. This inflammatory response that can be seen both radiologically [7] and pathologically [8].

Thermal ablation creates an internal wound which initiates a stereotypical wound healing response consisting of inflammatory, proliferative, and maturational phases mediated by growth factors and cytokines [9]. Following thermal injury, activated platelets release interleukin (IL)-1, tumor necrosis factor alpha (TNF α), and transforming growth factor beta (TGF β), which increase vascular permeability and promote chemotaxis. Neutrophils are attracted to the region and perform debris scavenging by releasing free radicals via oxidative burst mechanisms aimed at destroying inciting agents (cellular debris and foreign cells). Macrophage migration ensues, transitioning to the proliferative stage, during which macrophages secrete growth factors [10] including TNF α , vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (bFGF). Tissue remodeling factors like hepatocyte growth factor (HGF, also known as scatter factor) and matrix metalloproteinases (MMPs) degrade stroma, facilitating cellular motility and granulation tissue formation. The level of proliferative activity of macrophages and lymphocytes is mediated by IL-6 positively [11] and IL-10 negatively [12].

In the event of an incomplete ablation or multifocal disease, viable tumor cells (in the sublethal ablation margin or at remote site of disease) may be affected positively or negatively by the concomitant inflammatory response including increased levels of cytokines (such as IL-6 [13]) and growth factors (such as hepatocyte growth factor [14]). Inflammation may augment tumor cell death, by increasing neutrophil infiltration and subsequent destruction of the tumor cells by oxidative burst mechanisms, similar to mechanism for the destruction of bacterial pathogens [15]. On the other hand, inflammation following ablation may have

negative effects via the production of growth factors and cytokines by macrophages and lymphocytes which could stimulate tumor cell growth within the sublethal margin [16].

Isolated human and animal studies of immunohistochemical staining of tissues, ELISA of tissue lysates, and ELISA of plasma have shown 2–150 fold changes in specific inflammatory and immunomodulatory mediators following thermal ablation [17–22]. However, it remains unknown how tumor characteristics (like tumor site, primary diagnosis, metastatic status) and ablation characteristics (like modality, ablation size and time from ablation) affect plasma cytokine levels following ablation. The purpose of this study is to identify changes in plasma cytokine levels following image-guided thermal ablation of human tumors and to identify the factors that independently predict changes in plasma cytokine level using multivariate analysis.

MATERIALS AND METHODS

Patient Characteristics

Institutional Review Board approval was obtained for this prospective biospecimen study. Inclusion criterion was planned thermal ablation in patients being treated for primary or secondary malignancy. Exclusion criteria included prior ablation or embolization procedure within 30 days, combined embolization/ablation procedure, or no require clinical blood testing prior to the procedure. Using these criteria, between Feb 2011 and July 2011, 49 patients were recruited into and were consented to participate in the study. For patients consenting to the study, in accordance with our institutional biospecimen protocol, research blood draws were obtained at the time of a patient's clinical blood draws. Under this biospecimen protocol, if clinical blood draws were not indicated at a particular timepoint, research blood samples were not drawn. Of the 49 patients, 4 patients subsequently withdrew from the study or refused the research blood draws, and 9 patients were discharged on the same day of the procedure and did not undergo post-procedure laboratory testing. The remaining 36 patients were evaluated.

Patient and ablation demographics are listed in Table 1. The mean age of patients was 63.4 years. The most common primary diagnosis was colorectal cancer (39%), followed by sarcoma (22%), liver (11%), head and neck (8%), lung (8%), melanoma (6%), kidney (6%) and gynecologic (1%) malignancy. The majority of ablations were performed in liver (42%) or lung (44%). Ablations were predominantly heat-based (89% microwave, laser, or radiofrequency ablation), while 11% of ablations were cold-based (cryoablation).

Thermal Ablation

All tumors were treated using thermal ablation performed under general anesthesia and using sterile technique. A single dose of prophylactic antibiotic (Ancef, GlaxoSmithKline, Research Triangle Park, NC) was administered intravenously prior to the procedure. The skin and subcutaneous tissue was infiltrated with lidocaine 1%. Type of thermal ablation and protocol was determined by operator preference, tumor size, geometry, and location. Using CT or MR guidance, the applicator was guided to the tumor, and position to create an adequate margin around the tumor. When necessary, multiplanar reconstructions were

performed to confirm that the planned ablation zone would encompass the tumor. Radiofrequency ablation was performed using the Radiotherapeutics (Boston Scientific, Natick, MA), RITA (Angiodynamics, Latham, NY), or Cool-tip (Covidien, Latham, NY) system (12–36 minutes, maximum temp of 60–95 degrees centigrade). Cryoablation was performed with the Endocare (Healthtronics, Austin Texas) or Seednet (Galil, Arden Hills, MN) system (28–32 minutes, minimum temperature of –140 to –120 degrees centigrade). Microwave ablation was performed with a 2.45 Ghz generator: the Certus (Neuwave Medical, Madison Wisconsin) or AMICA (Mermaid Medical, Stenlose, Denmark) system (5–25 minutes, 30–140 watts). Laser ablation was performed with the Visualase (Visualase, Houston, Texas) system (10 mins, 30 watts). Once the ablation device was appropriately positioned, ablation was performed. The endpoint of ablation in all cases was creation of an ablation zone with a margin of at least 5 mm circumferentially around the tumor [23]. When necessary, the ablation probe was repositioned and further ablation was performed in order to achieve the desired endpoint.

Immediately following ablation, noncontrast computed tomography (for lung) or contrast enhanced computed tomography (for liver and other sites) was performed to confirm the ablation zone. In the case of lung ablation, immediate and 2 hours post procedure chest radiographs were also obtained to evaluate for pneumothorax. All cases of enlarging or symptomatic pneumothoraces were managed with a pigtail thoracostomy catheter. The patients recovered in the post-anesthesia care unit and were observed for a minimum of 24 hours. Follow-up CT or MR imaging was performed within 4–6 weeks. The ablation diameter was defined as the maximum length of the zone of non-enhancement post procedure contrast enhanced CT, or in the case of lung ablation, the size of post treatment ground glass or lung consolidation, as measured on an axial imaging. In the case of multiple site of ablation, the maximum diameter of each ablation site were summed. The size of the ablation zone was defined as the maximum area of the zone of non-enhancement post procedure contrast enhanced CT, or in the case of lung ablation, the size of post treatment ground glass or lung consolidation, as measured on an axial imaging.

Cytokine measurement

36 patients underwent pre-procedure research blood draws in conjunction with a clinical blood draw. 33/36 patients underwent a post-procedure blood draw in conjunction with a clinical blood draw within 48 hours of ablation. 21/36 patients underwent a follow-up research blood drawn in conjunction with standard of care blood work. A total of 90 research blood samples were obtained. Whole blood samples were collected in calcium-EDTA tubes, transported to our CLIA-certified clinical chemistry laboratory, and centrifuged to obtain plasma. Plasma samples were stored at –80 degrees centigrade until analysis.

Plasma levels of inflammatory mediators were measured using the Searchlight multiplex immunoassay system (Aushon Biosystems, Billerica, MA). Plasma levels of interleukin 1 alpha (IL-1a), IL-2, IL-6, IL-10, and tumor necrosis factor alpha (TNFa) were measured. Plasma leukocyte count (WBC) was also measured automatically using the Advia 2120

Hematology System (Siemens, Erlangen, Germany) using both flow cytometry and the peroxidase method..

Statistical Analysis

Fold changes of cytokine levels and WBC count were computed in comparison to pre-ablation levels. Univariate analysis of mediator level as outcome was performed on the 90 samples for patient and ablation variables. Because the majority of patients received multiple blood draws, sampling time (pre, post, and follow-up) was treated as repeated measures variables. The subject variable was treated as a random effect. For mediators that showed a significant change following ablation, multivariate linear regression was performed for patient and ablation variables to identify the factors that were independent predictors of the change. Significance level was taken to be $p < 0.05$. All analyses were performed in SAS (version 9.2, SAS Institute Inc., Cary, NC).

RESULTS

On average, pre-procedure blood draws were obtained -7.1 ± 5.7 day prior to ablation. Following thermal ablation, the mean time to the post-ablation blood draw was 0.8 ± 0.2 days (Table 1). Mean time to follow-up blood draws was 17.4 ± 9.9 days. The average ablation size was 13.3 ± 7.0 cm².

Post-ablation, the plasma levels of IL-6 and IL-10 showed robust changes (Table 2 and Figure 1). IL-6 levels rose significantly from 16.7 to 46.6 pg/ml ($p = 0.002$), and IL-10 rose from 1.9 to 3.3 pg/ml ($p = 0.02$). Leukocyte count also rose significantly immediately post-ablation, from 6.0 to 7.6 ($p = 0.0003$). IL1a, IL-2, and TNF α showed no significant changes in levels 48-hours following ablation. In follow-up 1–5 weeks after ablation, neither cytokines levels nor leukocyte count showed a significant change from pre-ablation levels.

To identify relationships between changes in cytokine levels following ablation and other factors, tabulation of the fold change for cytokine levels post ablation was performed, with stratification by primary diagnosis, metastatic status, ablation site, ablation type, and ablation size (Table 3). The largest changes were seen in plasma levels of IL-6. With regard to diagnosis, ablation of melanomas showed the largest increase in IL-6 (92-fold) <48 hours after ablation, followed by ablation of kidney (26-fold), liver (8-fold), and lung (5-fold) malignancies. With regard to ablation site, ablation of bone and kidney produced the greatest fold change in IL-6 level <48 hours after ablation (91-fold and 26-fold, respectively). Cryoablation showed a significantly greater change in IL-6 levels compared to both radiofrequency and microwave ablation (54-fold vs 3.6-fold and 3.4-fold, $p < 0.0027$ and $p < 0.0034$, respectively). Ablation size showed a nonlinear relationship between fold change and cytokine level, with ablations in the 10–20 cm² range (3–4.5 diameter range) showing the highest fold change in IL-6 following ablation (24-fold).

To determine the factors that were predictors of cytokine level changes following thermal ablation, univariate analysis was performed on the cytokines which showed a significant overall effect (IL6 and IL-10), as well as leukocyte count (Table 4). Univariate analysis showed that age, primary diagnosis, ablation site and ablation type were significant

predictors of change in IL-6 and IL-10 level following ablation. In addition, primary tumor type (primary vs metastasis) also predicted changes in IL-10 level. In contrast, ablation size was the only predictor of leukocyte count increase following thermal ablation.

To determine which patient and ablation factors were independent predictors of changes in cytokine level following thermal ablation, multivariate analysis was performed (Table 5). After controlling for the other variables, the only independent predictors of the IL-6 level increase following ablation were primary diagnosis ($p<0.039$) and ablation type ($p<0.0001$). For IL-10, age remained the only independent factor, and was a positive predictor of IL-10 level increase following ablation. Ablation size was the only independent positive predictor of leukocyte count increase following ablation ($p<0.015$).

DISCUSSION

These results show that percutaneous thermal ablation of tumors invokes an inflammatory response which is characterized by elevation in the plasma levels of specific cytokines. IL-6 and IL-10 show the most robust increases in plasma levels within 48 hours of ablation, whereas TNF α and IL-1 were unchanged. Both the type of ablation (particularly cryoablation) and primary diagnosis (particularly melanoma, kidney, and liver cancers) profoundly impact changes in IL-6 levels following percutaneous ablation.

Our results are consistent with investigations of cytokine changes following open surgical ablation. Schell et al [18] showed that following open surgical radiofrequency ablation of hepatic malignancy, IL-6 levels increased 4-fold and after open hepatic cryoablation, IL-6 levels increased by over 100-fold [20]. Similarly, Li et al. demonstrated lower elevations in IL-6 following high intensity focused ultrasound (a heat based ablation technique) compared to prostate cryoablation, [24]. In this patient image guided ablation population, heat based ablation techniques showed 3.5-fold increases in IL-6 compared to a 54-fold increase with cryoablation. The higher levels of cytokines after open surgical ablation may be due to the increased inflammatory response from the larger incision required for open surgical ablation. In our patient population, difference in cytokine levels following heat and cold based ablation may be due to the fact that cryoablation results in the shattering of cellular membrane and less denaturation of proteins than the hydrolysis of proteins seen in heat based ablation. This could potentially lead to increased deposition of tumor antigens into the extracellular space, serving as a stimulus for the inflammatory response. It is interesting to speculate to what degree non thermal ablation techniques like irreversible electroporation will elicit serum cytokine changes. It is intriguing why the changes in WBC showed a linear increase with ablation size, but IL-6 levels did not. It is possible that the size of ablation determine the degree of generalized inflammatory response, as indicated by the serum WBC count, but differences in tumor and ablation characteristics more profoundly affect actual level of IL-6 than ablation size.

IL-6 and IL-10 are categorized as T Helper 2 (Th2) cytokines as they are both produced by Th2 lymphocytes [25], where as IL-2 and TNF are categorized as T Helper 1 (Th1) cytokines as they are both produced by Th1 lymphocytes [26]. Our data showing that plasma levels of IL-6 and IL-10 (but not IL-2 and TNF α) are elevated after thermal ablation support

our hypothesis that the lymphocytes which infiltrate the site of thermal ablation [27] are predominantly a Th2 population. Our hypothesis is also supported by studies which show that Th2 cytokines promote fibrocyte differentiation [28] and fibrotic scar formation after hepatic injury [29]. Recovery after liver injury is mediated by the NF- κ B (Nuclear Factor Kappa-light-chain-enhancer of activated B cells) and STAT3 (Signal Transducer and Activator of Transcription 3) signaling pathways, both of which are activated by IL-6 [30]. In fact, IL-6 deficient mice show impaired liver regeneration following partial hepatectomy, and treatment of mice with IL-6 before injury resulted in improved liver regeneration [31]. Recovery of soft tissues after injury is also dependent on IL-6, as IL-6 knockout mice show markedly increased wound healing time following punch biopsy compared to controls [32].

Cytokines like IL-6 not only support the regeneration of normal liver parenchyma after injury, but also directly support survival of cancer cells [33]. IL-6 has been shown to promote the growth of lung [34], colon [35], liver [36], as well as other cancers [37–39]. The relationship between IL-6 and the development of cancer is particularly strong in the liver, and increased levels of IL-6 may predispose hepatitis patients to develop HCC. Patients with hepatitis B and high a plasma level of IL-6 (>7 pg/ml) have a 3 $\times$ shorter HCC-free survival than those with low IL-6 levels, even after controlling for age, gender, cirrhosis, use of antiviral treatments, HBV DNA, and peak ALT levels [40]. It is not surprising that in cultured HCC cells challenged with doxorubicin, IL-6 inhibition using an anti-IL-6 antibody resulted more cell death than controls without IL-6 inhibition [41].

Our finding that IL-6 levels are increased after thermal ablation of tumors has several potential ramifications for both locoregional cancer therapy and systemic targeted therapy. Since focal ablation can induce high systemic levels of IL-6 post-ablation, viable tumor cells that persist either in the margin of the ablation zone or are remote from the ablation site may be inadvertently stimulated to proliferate by circulating IL-6. The development of adjuvant therapies that decrease IL-6 levels, increase IL-10 levels, or affect the downstream targets of these molecules (e.g. STAT3) may prove useful in decreasing the rate of local recurrence and/or distant metastasis after thermal ablation. Thus, IL-6 and IL-10 are attractive potential targets for the development of adjuvant therapies which could enhance the treatment efficacy of image guidance thermal ablation of tumors [42]. In addition, since thermal ablation leads to increase in both blood and ablation zone lymphocytes, immunotherapeutic strategies designed to shift the balance of lymphocytes from the Th2 phenotype (which produce IL-6) to the Th1 phenotype (which support cell-mediated tumor killing) may prove useful for future research [43].

This study has several limitations. Because of the observational nature of this study, certain subgroups have small samples, which skew the analyses and does not allow for rigorous hypothesis testing. Future studies larger studies should be performed to validate these results, and should focus on undersampled subpopulations in this study (e.g. melanoma ablations) to confirm the observed cytokine changes. Cytokine blood specimens were obtained at the time of clinical blood draws rather than at fixed time points, which could lead to lack of precision in the average cytokine levels in the post-procedure and follow-up groups. Since assay of a limited panel of cytokines was performed, it is possible that the other relevant cytokines which play a vital role in shaping the post ablation

microenvironment were omitted from the analysis. Future studies of our existing plasmas samples will hopefully allow for a more complete understanding of cytokine changes following thermal ablation. It is important to note that other confounding variables were not measured (like ablation time, ablation power, number of ablations, or use of anesthesia), which may affect the lack of significance of factors (like ablation size). Finally, plasma levels of cytokines are only a surrogate marker for changes in the ablation zone microenvironments and local cytokine levels could vary greatly from those in plasma. We hope to validate the presumed correlation between cytokine levels in plasma and biochemical changes in the local ablation zone with animal studies.

In conclusion, percutaneous thermal ablation of tumors results in increased plasma level of IL-6 and IL-10 in the first 48 hours after ablation in our patient population. Cryoablation of tumors produces greater changes in IL-6 levels than heat-based ablation techniques.

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Figure 1.

Plasma cytokine level and leukocyte count following thermal ablation measured post procedure (<48 hours, N=33) and at follow-up (1–5 weeks, N=21). Data presented is log of fold change from baseline level. * p<0.002. † p<0.02. ‡ p<0.0003

Table 1**Patient And Ablation Demographics**

Variable	Value
Sex	
Female	13 (36)
Male	23 (64)
Age (years)	63.4 +/- 13.5
Primary Diagnosis	
Colorectal	14 (39)
Sarcoma	8 (22)
Liver	4 (11)
Head and Neck	3 (8)
Lung	3 (8)
Melanoma	2 (6)
Kidney	1 (3)
Gynecologic	1 (3)
Target Tumor: Primary or Metastasis	
Metastasis	26 (72)
Primary	10 (28)
Ablation Site	
Lung	16 (44)
Liver	15 (42)
Soft tissue	2 (6)
Bone	2 (6)
Kidney	1 (3)
Ablation Type	
Radiofrequency Ablation	16 (44)
Microwave	15 (42)
Cryoablation	4 (11)
Laser	1 (3)
Time of Blood Sampling from Ablation (days)	
Pre-Ablation (n=36)	-7.1 ± 5.7
Post-Ablation (n=33)	0.8 ± 0.2
Follow-up (n=21)	16.6 ± 9.2
Ablation Max Diameter (cm)	4.6 ± 1.5
Ablation Max Diameter	
<3cm	4 (11)
3–4.5 cm	14 (39)
4.5–6 cm	12 (33)
>6 cm	6 (17)
Ablation Area (cm ²)	13.8 ± 7.6
Ablation Area	

Variable	Value
<10 cm ²	14 (39)
10–20 cm ²	12 (33)
20–30 cm ²	9 (25)
>30 cm ²	1 (3)

Note.- Values presented as mean $\pm$ standard deviation. Values in parentheses are percentages.

Table 2

Cytokine Levels and Leukocyte Count following Thermal Ablation

	IL-1a	IL-2	IL-6	IL-10	TNFa	WBC
Pre-Ablation						
Level (n=36)	4.7 ± 11	3.8 ± 5.4	16.7 ± 23	1.9 ± 0.6	15.5 ± 33	6.0 ± 1.8
Range	1.6–65	1.6–25	1.1–136	1.0–3.8	9.4–212	1.7–9.6
Post-Ablation						
Level (n=33)	5.9 ± 17	3.9 ± 6.5	46.6 ± 51	3.3 ± 4.2	14.7 ± 28.5	7.6 ± 3.6*
Range	1.6–98	1.6–32	5–200	1.6–25	9.4–173	2.5–17
Fold (n=33)	1.1 ± 0.4	0.98 ± 0.27	9.6 ± 31	1.9 ± 2.8	0.98 ± 0.1	1.3 ± 0.4*
P-value	0.18	0.62	0.002	0.02	0.15	0.0003
Follow-up						
Level (n=21)	1.9 ± 1.4	2.2 ± 1.3	27 ± 40	1.6 ± 0.0	9.8 ± 1.8	6.9 ± 2.2†
Range	1.6–8.2	1.6–7.2	3.1–192	1.6–1.6	9.4–17	3.7–11
Fold (n=21)	0.98 ± 0.7	1.01 ± 0.2	9.9 ± 37	0.96 ± 0.1	0.98 ± 0.8	1.2 ± 0.5†
P-value	0.72	0.86	0.15	0.7	0.41	0.08

Note. - Values presented at mean ± standard deviation. Levels were measured post procedure (<48 hours) and at follow-up (1–5 weeks). Values in parentheses are sample size. Cytokine level are in pg/ml. White blood cell count in 10³cells/microliter. P-value are based on comparison to pre-procedure levels.

* N=30.

† n= 19

Table 3

Cytokine Levels and Leukocyte Count Fold Change Following Thermal Ablation

	IL-1a	IL-2	IL-6	IL-10	TNFa	WBC
Primary Diagnosis						
Melanoma (n=2)	1.0 ± 0.0	1.0 ± 0.0	92 ± 126	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.2
Kidney (n=1)	1.0	1.0	26.0	15.3	1.0	1.9
Liver (n=4)	1.6 ± 0.9	1.4 ± 0.6	8.1 ± 7.0	1.7 ± 0.5	1.0 ± 0.9	1.5 ± 0.5
Lung (n=3)	0.8 ± 0.3	0.8 ± 0.3	5.6 ± 6.0	1.0 ± 0.1	0.9 ± 0.2	1.1 ± 0.3
Sarcoma (n=8)	1.1 ± 0.2	0.9 ± 0.2	3.1 ± 2.9	0.9 ± 0.1	1.0 ± 0.0	1.2 ± 0.4 [†]
Colorectal (n=12)	1.0 ± 0.7	1.0 ± 0.8	2.5 ± 2.0	1.9 ± 2.3	1.0 ± 0.0	1.3 ± 0.4 [*]
Head and Neck (n=2)	0.8 ± 0.08	0.8 ± 0.07	1.2 ± 0.4	1.7 ± 1.0	1.0 ± 0.0	1.4 ± 0.5
Gynecologic (n=1)	1.0	0.8	0.8	1.0	1.0	0.89
Target Tumor: Primary or Met						
Metastasis (n=23)	1.0 ± 0.1	0.9 ± 0.1	10 ± 37	1.5 ± 1.7	1.0 ± 0.6	1.2 ± 0.3 ^{**}
Primary (n=10)	1.2 ± 0.6	1.1 ± 0.4	8.6 ± 8.2	1.0 ± 0.1	1.0 ± 0.6	1.4 ± 0.5
Ablation Site						
Bone (n=2)	1.0 ± 0.0	1.0 ± 0.0	91 ± 128	1.0 ± 0.0	1.0 ± 0.0	1.2 ± 0.05
Kidney (n=1)	1.0	1.0	26.1	15.3	1.0	1.9
Soft Tissue (n=2)	1.0 ± 0.0	1.0 ± 0.0	6.0 ± 5.8	0.9 ± 0.1	1.0 ± 0.0	1.2 ± 0.7
Liver (n=15)	1.2 ± 0.5	1.1 ± 0.3	4.3 ± 4.3	1.9 ± 2.1	1.0 ± 0.9	1.3 ± 0.4 [§]
Lung	0.9 ± 0.1	0.9 ± 0.2	2.6 ± 3.1	1.1 ± 0.3	1.0 ± 0.0	1.2 ± 0.4 ^{//}
Ablation Type						
Cryoablation (n=4)	1.0 ± 0.0	1.0 ± 0.0	54 ± 85	4.6 ± 7.2	1.0 ± 0.0	1.5 ± 0.4
Radiofrequency (n=16)	1.1 ± 0.5	1.0 ± 0.4	3.6 ± 4.2	1.7 ± 2.0	1.0 ± 0.8	1.4 ± 0.4 [‡]
Microwave (n=12)	1.0 ± 0.2	1.0 ± 0.1	3.4 ± 3.4	1.2 ± 0.4	1.0 ± 0.0	1.1 ± 0.3 [*]
Laser (n=1)	0.8	0.8	1.5	2.4	1.0	1.0
Ablation Size						
<10 cm ² (n=13)	1.1 ± 0.5	0.9 ± 0.4	2.5 ± 2.6	1.1 ± 0.4	1.0 ± 0.1	1.2 ± 1.2 ^{††}
10–20 cm ² (n=10)	1.0 ± 0.0	1.0 ± 0.03	24.0 ± 56	2.7 ± 4.5	1.0 ± 0.0	1.2 ± 0.4 ^{‡‡}
20–30 cm ² (n=9)	1.1 ± 0.3	1.0 ± 0.1	4.9 ± 5.3	2.1 ± 2.6	1.0 ± 0.1	1.4 ± 0.4

	IL-1a	IL-2	IL-6	IL-10	TNFa	WBC
>30 cm ² (n=1)	1.0	1.0	0.6	1.0	1.0	1.8

Note.- Values presented at mean ± standard deviation of fold change. Levels were measured post procedure (<48 hours, N=33).

* n=10.
† n=7.
‡ n=15.
§ n=14.
|| n=11.
** n=20.
†† n=12.
‡‡ n=8.

Table 4

Univariate Analysis of Factors Relating to Cytokine Levels and Leukocyte Count Following Ablation

	IL-6	IL-10	WBC
Age	0.2	0.31	0.57
Sex	0.36	0.43	0.29
Primary Diagnosis	0.019	0.0003	0.66
Target Tumor: Primary or Metastasis	0.84	0.01	0.89
Ablation Site	0.003	0.0001	0.58
Ablation Type	0.0001	0.08	0.18
Ablation Size	0.87	0.94	0.007

Note.- Values are p-values. Levels were measured post procedure (<48 hours).

Table 5

Multivariate Analysis of Factors Relating to Cytokine Levels and Leukocyte Count Following Ablation

	IL-6	IL-10	WBC
Age	0.78	0.015	0.79
Sex	0.41	0.19	0.60
Primary Diagnosis	0.039	0.92	0.92
Target Tumor: Primary or Metastasis	0.82	0.20	0.45
Ablation Site	0.69	0.41	0.54
Ablation Type	0.0001	0.10	0.45
Ablation Size	0.44	0.57	0.037

Note.- Values are p-values. Levels were measured post procedure (<48 hours).