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ORIGINAL ARTICLE

The correlation between a chronic inflammatory marker Tartrate-Resistant Acid Phosphatase 5a with cancer cachexia

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Summary

Purpose: *The mechanism of cancer cachexia remains unclear and inflammatory cytokines may play a role in its development. Interleukin-6 (IL-6), C-reactive protein (CRP) and tumor necrosis factor-a (TNFa) are known to be associated with cancer cachexia. Tartrate-resistant acid phosphatase 5a (TRACP5a) is proposed to be related to chronic inflammation. In this study we hypothesize that TRACP5a is a chronic inflammatory marker that is correlated with cancer cachexia.*

Methods*: Fifty-five cancer patients with and without cancer cachexia were enrolled from January 2009 to December 2012. Body mass index (BMI) was measured and serum total cholesterol, triglycerides and albumin were examined to evaluate the nutritional status. IL-6, CRP and TRACP5a protein activity were evaluated.*

Results: *Inflammatory markers including IL-6, and CRP were significantly elevated in patients with cancer cachexia (p=0.0075 and 0.0021, respectively). Patients with cachexia also had higher CRP/albumin ratio (p=0.0265). TRACP5a activity, TRACP5a protein and their combinations with albumin were increased in the cancer cachexia groups but without significant difference. There were good correlations between IL-6, CRP, and BMI. Patients with higher TRACP5a activity had shorter survival (p=0.004).*

Conclusion*:TRACP5a may be a promising chronic inflammatory marker and may play a prognostic role in cancer cachexia. Further large-scale prospective studies are warranted to confirm its role in the cancer cachexia process.*

Key words: *cancer cachexia, chronic inflammation, tartrate-resistant acid phosphatase 5a*

Introduction

Cancer cachexia increases the morbidity and is associated with 20-40% of cancer deaths [1-4]. Negative balance of energy, chronic inflammation and progressive loss of muscle and fat mass are its characteristics. The current consensus of cancer cachexia definition is more than 5% involuntary weight loss in 3-6 months [1,2]. Cachexia is a continuous event, and may be classified into different stages, including precachexia, cachexia, and refractory cachexia [5]. The mechanism remains unclear and is certainly multifactorial. Evidence from animal models has suggested that various pro-inflammatory cytokines may play a role in the pathogenesis of cancer cachexia. Interleukin (IL)-1, IL-6 and TNF-α are associated with anorexia and weight loss [1-3]. These cytokines are inducers of acute phase protein response. The circulating concentration of IL-6 correlates with markers of systemic inflammation such as CRP in cancer patients. CRP may be used as an indirect m`arker of pro-inflammatory cytokine activity (IL-1, IL-6 and TNF-α), and the serum CRP concentration has been correlated positively with weight loss [1,2]. Therefore, acute phase protein reaction is associated with hypermetabolism, anorexia and increased weight loss in patients with cancer. An elevated serum CRP is also associated with reduced survival in many kinds of cancer and can be used as a prognostic biomarker [6-9]. Glasgow Prognostic Score (GPS) is an inflammation-based prognos-

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Table 1. Association between venous invasion, lymphatic invasion, perineural invasion and other morphopathological factors

	Patients without cachexia N (%)	*Patients with cachexia* N (%)	*p value*
Age*,years (mean±SD)	60 ± 14	61 ± 16	0.791
Gender**			0.788
Male	15 (57.7)	18 (62.0)	
Female	11 (42.3)	11 (38.0)	
BMI (mean±SD)	23.50 ± 2.32	18.03 ± 1.78	<0.0001
Cancer diagnosis**			0.121
GI tract	10 (38.5)	13 (44.9)	
Lung	3 (11.5)	6 (20.7)	
Breast	8 (30.8)	1 (3.4)	
Lymphoma	2 (7.7)	6 (20.7)	
GU tract	2 (7.7)	2 (6.9)	
Head and neck	1 (3.8)	1 (3.4)	
Stage			0.146
I	0 (-)	1 (3.4)	
II	3 (11.5)	1 (3.4)	
III	6 (23.0)	2 (6.9)	
IV	17 (65.5)	25 (86.3)	

*Student's t-test, **Chi-square test

tic scoring system that consists of albumin and CRP assessments and it is proposed to predict the treatment efficacy in patients with cancer cachexia [10].

Recently, TRACP5a has been proposed to be a secretory inflammatory protein of macrophages [11-14]. It is consistently elevated in one-fourth to one-third of patients with established rheumatoid arthritis [11]. TRACP5a protein level also reflects the inflammatory burden of cardiovascular disease and sarcoidosis [15,16]. Because cancer cachexia is a chronic systemic inflammation [10], TRACP5a may reflect the disease burden and severity of cancer [17].

In this study, we propose that the serum TRACP5a can reflect the burden of cancer and is related to the prognosis of cancer patients. We investigated the clinical relevance of serum TRACP5a, IL-6, CRP and clinical nutritional parameters and their correlation in patients with and without cancer cachexia.

Methods

Inclusion / Exclusion criteria

Fifty-five cancer patients were enrolled from January 2009 to December 2012. The inclusion criteria were: (1) age≥18 years; (2) body weight loss 5-10% in past 3-6 months; (3) confirmed diagnosis of cancer and completed anti-cancer treatment (chemotherapy, radiotherapy, surgery) for more than one month; (4) predicted life expectancy more than one month. The exclusion criteria were: (1) patients having active or chronic infection; (2) uncontrolled autoimmune diseases; (3) receiving non-steroid anti-inflammatory drugs, corticosteroids, anabolic steroids, progestational hormones within one month; (4) organ failure, such as heart failure (New York Heart Association Function Classification IV), chronic renal insufficiency (creatinine ≥3.0mg/dl), maintenance hemodialysis; (5) uncontrolled hypo- or hyperthyroidism.

The Tri-Service General Hospital Review Board approved the study. Written informed consent was obtained from all of the patients.

Body mass index measurement

Trained staff measured body height and weight. BMI was calculated as body weight (in kilograms) divided by body height squared.

Laboratory measurement

All sera were stored at −80°C and were thawed at room temperature immediately before biochemical parameters measurement. A venous blood sample (20ml) was taken after a 12-h fasting for total cholesterol, triglycerides, CRP, IL-6, and TRACP5a measurements. Serum IL-6 was determined using a commercial immunoassay kit according to the manufacturer's instructions (RayBiotech Inc., Georgia, USA) and expressed as pg/mL. Serum CRP was determined by a high-sensitivity

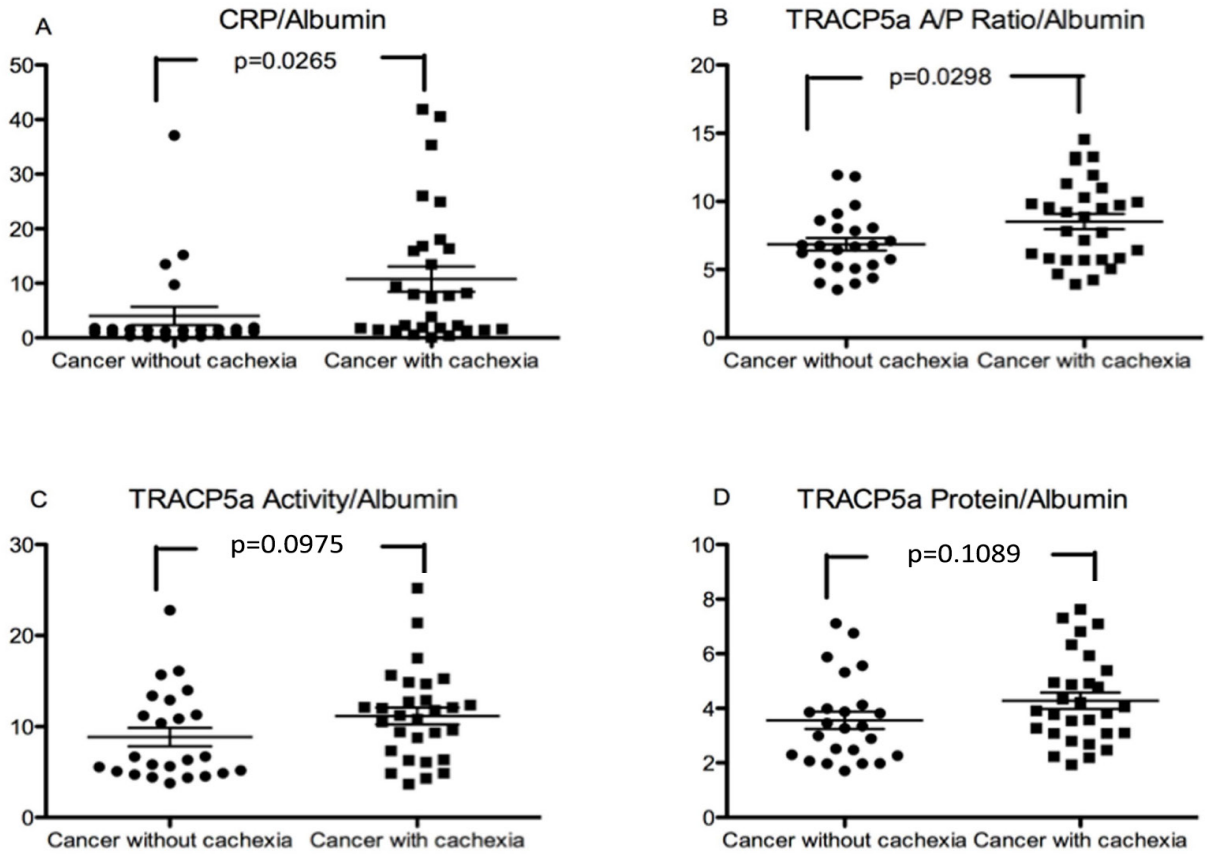

Figure 1. The distribution plots of inflammatory marker-albumin combination in cancer patients with and without cachexia categorized according to CRP/albumin **(A)**, TRACP5a A/P ratio/albumin **(B)**, TRACP5a activity/albumin **(C)** and TRACP5a protein/albumin **(D)**

in-house immunoassay constructed from commercial antibodies and purified human CRP as standard (Dako Corp.) and expressed as mg/L. Serum TRACP5a activity and protein were determined by two-site immunoassay as previously described [1,2] using the in-house immunoassay TRACP5a-specific antibody mab220 to assess serum TRACP5a. TRACP5a activity/protein (A/P) ratio was calculated to evaluate the active percentage of TRACP5a. We also combined inflammatory markers with albumin and expressed them as TRACP5a (protein or activity or A/P ratio)/albumin, IL6/albumin, CRP/albumin to evaluate the inflammation-malnutrition status.

Statistics

Differences of age and BMI between cancer patients with or without cachexia were analyzed with the Student's t-test. Differences in gender, different cancer subtypes and stages were assessed with chi-square test. The correlation between TRACP5a protein, TRACP5a activity, IL-6 and CRP were analyzed with the Spearman's correlation test. Kaplan–Meier method with log rank test were used to compare cachexia and non-cachexia groups. By using the median value of each biomarker as a cutoff value, we dichotomized the cancer patients and evaluated the significance of each biomarker in the survival analysis. A p value <0.05 was considered statistically significant. SPSS version 19.0 statistical software (SPSS Inc., Chicago, Ill) was used to perform all statistical analyses.

Results

Serum inflammatory markers and nutritional parameters in cancer patients

There were 29 (52.7%) patients with cancer cachexia and 26 (47.3%) patients without cancer cachexia.

Table 1 lists the patient demographic characteristics and shows no significant difference between the two groups in terms of gender, age, tumor sites and stage distribution. The cancer cachexia group had significantly lower albumin and BMI (p=0.0248 and <0.001, respectively). Significantly higher IL-6 and CRP (Table 2, p=0.0075 and 0.0021, respectively) were noticed in the cancer cachexia group. The ratio of CRP/albumin, and TRACP5a A/P ratio/albumin were significantly higher in the cancer cachexia group (Figure 1A-1B,

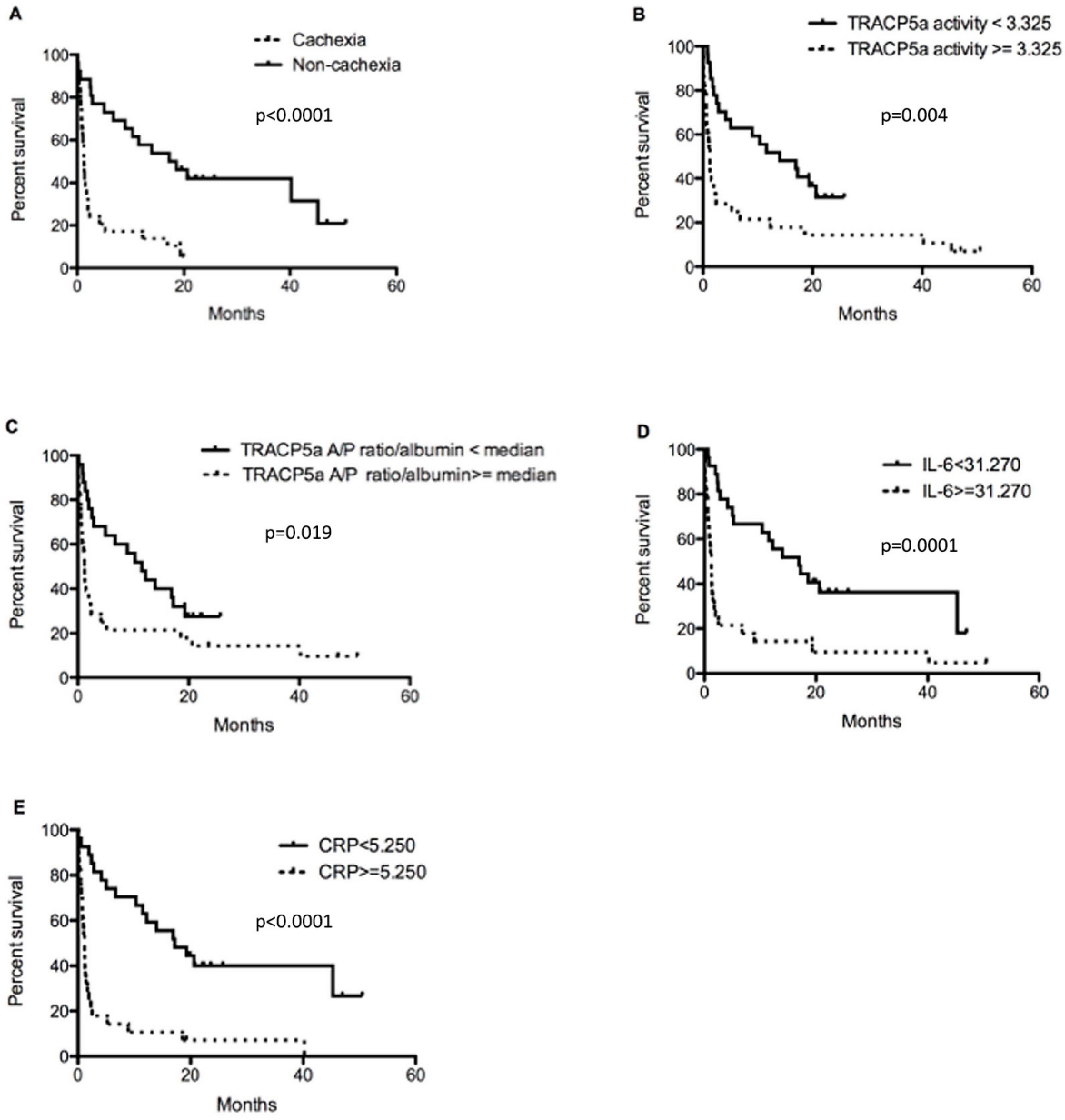

Figure 2. Kaplan-Meier plots of the probability of overall survival in cancer patients categorized according to cachexia **(A)**, TRACP5a activity **(B)**, TRACP5a A/P ratio/albumin **(C)**, IL-6 **(D)**, and CRP **(E)**.

p=0.0265 and p=0.0298, respectively). TRACP5a activity, TRACP5a protein and their combination with albumin were increased in the cancer cachexia group but did not reach significant difference (Table 2, Figure 1C-1D). Spearman's correlation test of biomarkers showed that TRACP5a protein, activity and A/P ratio correlated with each other (Table 3). Triglycerides, cholesterol, and albumin correlated well with each other (Table 3).

Serum inflammatory markers and survival analysis

The cancer cachexia group had significantly shorter overall survival (OS) than the group without cachexia (Figure 2A, p<0.001). While we dichotomized the cancer patients by using the median value of biomarkers, OS was inversely related to TRACP5a activity, TRACP5a A/P ratio/albumin, IL-6 and CRP (Figure 2B-2E, p=0.004, 0.019, <0.001 and <0.001, respectively). There was no statistical difference in OS while we used the median value of TRACP5a protein, TRACP5a A/P ratio alone or albumin to dichotomize the cancer patients (data not shown).

Discussion

Cancer cachexia is commonly found in ad-

Table 2. Inflammatory markers and nutrition parameters in cancer patients with and without cachexia

Parameters	Patients with cachexia N=29	Patients without cachexia N=26	*p value*
TRACP5a activity (U/L)	3.53 ± 1.60	3.28 ± 1.77	0.5218
TRACP5a protein (ng/ml)	13.45 ± 5.06	12.62 ± 4.28	0.82
Activity/Protein (A/P ratio)	0.27 ± 0.09	0.26 ± 0.09	0.5954
IL-6 (pg/mL)	117.65 ± 189.77	64.34 ± 135.02	0.0075
CRP (mg/L)	32.44 ± 37.20	13.09 ± 25.07	0.0021
Cholesterol	165.21 ± 61.10	168.46 ± 47.84	0.6941
Triglyceride	139.28 ± 96.65	124.21 ± 61.75	0.943
Albumin	3.21 ± 0.57	3.51 ± 0.90	0.0248
IL6/Albumin	39.96 ± 63.41	20.31 ± 42.99	0.2024
CRP/Albumin	10.76 ± 12.37	4.026 ± 8.136	0.0265
TRACP5a activity/Albumin	1.116 ± 0.501	0.8838 ± 0.497	0.0975
TRACP5a protein/Albumin	4.279 ± 1.62	3.562 ± 1.557	0.1089
TRACP5a A/P ratio/Albumin	0.085 ± 0.032	0.068 ±0.023	0.0298

Results are given as mean±standard deviation

Table 3. Spearman's correlation between inflammatory markers and nutritional parameters in all patients

		TRACP5a protein (ng/ml)	*TRACP5a A/P ratio*	*IL-6 (mg/L)*	*CRP (mg/L)*	*Cholesterol (mg/dL)*	*Triglycerides (mg/dL)*	*Albumin (g/dL)*	*BMI*
TRACP5a activity	r	0.723	0.695	0.108	0.234	0.187	0.074	0.073	-0.068
	p	<0.0001	<0.0001	0.431	0.085	0.179	0.6	0.601	0.62
TRACP5a protein	r		0.035	0.265	0.223	0.184	0.236	-0.038	-0.03
	p		0.798	0.051	0.101	0.188	0.09	0.784	0.827
TRACP5a A/P ratio	r			-0.141	0.089	0.065	-0.132	0.113	-0.079
	p			0.303	0.517	0.645	0.345	0.414	0.565
IL6	r				0.655	-0.035	0.012	-0.235	-0.375
	p				<0.0001	0.804	0.929	0.087	0.005
CRP	r					-0.12	-0.173	-0.242	0.11
	p					0.393	0.214	0.078	0.433
Cholesterol	r						0.456	0.458	0.038
	p						0.001	0.001	0.787
Triglycerides	r							0.167	0.038
	p							0.232	0,787
Albumin	r								0.144
	p								0.30

r: Spearman's rank correlation coefficient, p: p value. For abbreviations see text

vanced cancer stages and is associated with anorexia, muscle and fat wasting, psychological stress, low life quality and shorter survival. In addition, cancer cachexia patients respond poorly to chemotherapy and experience increased toxicity during treatment. The nutritional supplements and pharmacological manipulation of appetite cannot reverse the condition [1,2]. Body weight, skin folds thickness, mid-arm circumference and other anthropometric and laboratory tests are commonly used for evaluation of nutritional status. However, they cannot detect cachexia earlier until it develops. Predictive or early biomarkers of cachexia may be helpful to select the patients for early therapeutic intervention.

Inflammatory markers including IL-6, CRP and TRACP5a were evaluated in our study. IL-6 and CRP are previously known be related to cancer cachexia and elevated levels are associated with poor prognosis [1-3,5-8]. Correlation analysis

revealed strong correlation between IL-6 and CRP, but TRACP5a protein and activity had no correlation with IL-6 and CRP. This may be attributed to their different biologic function. Both IL-6 and CRP were markedly increased in the cancer cachexia group. TRACP5a protein and activity were increased in the cancer cachexia group but did not reach significant difference. Therefore, we tried to combine the inflammatory markers with albumin according to the concept of GPS scoring system to evaluate the inflammation-malnutrition status [18,19]. We found that CRP/albumin was significantly higher in the cancer cachexia group. TRACP5a activity/albumin and TRAP5a protein/albumin were more increased in the cancer cachexia group but both of them still did not reach statistical significance. Also TRACP5a A/P ratio/albumin did show significant difference (Figure 1A-B). In previous studies tumor associated macrophages (TAM) might reflect the disease activity of cancer patients [15-17]. We proposed that TRACP5a might be a surrogate of TAM which could reflect the disease burden. Therefore, the total amount of TRACP5a protein in cancer patients might reflect the disease burden. TRACP5a activity might implicate the severity of the disease, and was related to poor survival in our study. Cancer itself is a chronic inflammation and previous data demonstrates that chronic inflammation can predispose an individual to cancer [20]. Our results revealed that TRACP5a protein displayed higher activity in the cachexia patients, which we believe it might be attributable to the burden of TAM.

There are several limitations of this study. Firstly, the patient group had a diversity of cancer types and different performance statuses. The correlation of clinical stage, cancer type with inflammatory markers IL-6, CRP and TRACP5a could not be evaluated because of the small number of patients and disease diversity. Secondly, patients did not undergo uniform palliative treatment, and they were treated according to their physician's decisions. Thirdly, the survival of each patient might be confounded by a variety of factors, including age, performance status, cancer stage, and treatment. The prognostic role of TRACP5a activity in cancer patients might need a larger cohort and clinical risk factors adjustment to make the final conclusion. TRACP5a, a macrophage secreted protein, may be involved in the pathogenesis of cancer cachexia. Though the mechanism of cancer cachexia is not clear, inflammatory cytokines do play important roles. However, TRACP5a activity seems to be prognostic in these cancer patients, which might reflect the inflammatory severity or activity of TAM. More detailed research is worthwhile to elucidate the mechanism between inflammation and TRACP5a activity.

In conclusion, IL-6 and CRP were confirmed inflammatory markers for cancer cachexia in our study. TRACP5a may be a promising chronic inflammatory marker and may play a prognostic role in cancer cachexia. Further large-scale prospective studies are warranted to confirm its role in the cancer cachexia process.

Authors' contributions

Dr. Ping-Ying Chang and Dr. Tsu-Yi Chao conducted the study. Ms. Hsin-Yi Liu and Anthony Janckila, PhD, assisted in biomarker testing. Dr. Yi-Ying Wu collected the clinical data, calculated the statistics, and drafted the paper. Dr. Tzu-Chuan Huang, Jia-Hong Chen, Ming-Shen Dai, and Shiue-Wei Lai assisted patients' enrollment and gave critiques over the manuscript.

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