



## Research Paper

# Tartrate-Resistant Acid Phosphatase 5a Activity as a Novel Biomarker for Flare of Systemic Lupus Erythematosus

Accepted 13<sup>th</sup> February, 2017

## ABSTRACT

Tartrate-resistant acid phosphatase (TRAP) 5a is strongly expressed in inflammatory macrophages. Serum TRAP 5a protein concentration increases in patients with chronic inflammatory diseases such as end-stage chronic kidney disease, rheumatoid arthritis and systemic lupus erythematosus (SLE). SLE can involve the joints, kidneys, mucous membrane and blood vessel walls. In this cross-sectional, proof-of-concept study, patients with SLE were analyzed for substantiating our hypothesis that TRAP 5a activity and protein are potential biomarkers for SLE flare. Forty-one (41) female patients with SLE were enrolled: 18 had SLE flare and 23 had stable SLE. In addition, 20 healthy women without SLE served as controls. Serum TRAP 5a activity and protein concentration and C-reactive protein concentration were evaluated in all participants. Serum TRAP 5a activity was significantly higher in patients with SLE flare than in those with stable SLE ( $P < 0.0001$ ). However, serum TRAP 5a activity was significantly lower in patients with stable SLE than in controls ( $P = 0.0135$ ). Serum TRAP 5a protein concentration was significantly higher in SLE patients with and without flare than in controls ( $P = 0.005$  and  $0.0012$ , respectively). Furthermore, serum TRAP 5a protein concentration was slightly higher in patients with SLE flare than in those with stable SLE ( $P = 0.0634$ ). To compare TRAP 5a activities and protein concentrations between the flare and stable groups, receiver operating characteristic curves were constructed; the areas under these curves were  $0.9662$  ( $P < 0.0001$ ) and  $0.6763$  ( $P = 0.0055$ ) for the activities and protein concentrations, respectively. Serum TRAP 5a protein concentration increases in patients with SLE and tends to increase further when patients develop the flare. TRAP 5a activity was lower than normal in patients with stable SLE, but it increased significantly after the flare developed. These data suggest that TRAP 5a activity and protein concentration are potential biomarkers for SLE flare.

Jia-Hong Chen<sup>1,5</sup> Yi-Ying Wu<sup>1,4</sup>, Tzu-Chuan Huang<sup>1</sup>, Chun-Chi Lu<sup>2</sup>, Feng-Cheng Liu<sup>2</sup>, Anthony Janckila<sup>3</sup> and Tsu-Yi Chao<sup>1,3,4\*</sup>

<sup>1</sup>Division of Hematology and Oncology, Department of Medicine, Taipei Medical University- Shuang Ho Hospital, Taipei, Taiwan, No. 291, ZhongZheng Rd., Zhonghe District, New Taipei City 23561, Taiwan, Republic of China.

<sup>2</sup>Division of Rheumatology, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan, Republic of China.

<sup>3</sup>Special Hematology Laboratory, Veterans Administrative Medical Center, Louisville, KY, U.S.A.

<sup>4</sup>Graduate Institute of Life Sciences, National Defense Medical Center,

<sup>5</sup>Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University.

\*Corresponding author. E-mail:

j0607@ms6.hinet.ne. Tel:

+886287927208. Fax: +886287927209.

**Key words:** Tartrate-resistant acid phosphatase 5a, macrophage, inflammation, SLE.

## INTRODUCTION

Systemic lupus erythematosus (SLE) is possibly the most diverse autoimmune disease serologically and clinically, with widely varying immunopathogenic abnormalities and clinical manifestations being continually uncovered among patients with SLE. Immune dysregulation causes excessive

autoantibodies and immune multiplex production, excessive complement activation and severe tissue inflammation in patients with SLE, which together can cause clinical disorders with multi-organ involvement and difficult-to-predict courses.

**Table 1:** The clinical and laboratory data of SLE in flare status, stable status and control groups.

Variables	SLE with flare up	SLE in stable	Control	p value
Number	18	23	20	-
Age	44.4 + 14.0	38.8 + 11.5	28.1 + 5.8	<0.0001
C <sub>3</sub>	80.9 + 25.6	76.0 + 22.0		0.5172
C <sub>4</sub>	18.1 + 13.1	13.5 + 5.4		0.1347
dsDNA	54.8 + 105.2	62.9 + 99.6		0.8033
TRACP5a protein	12.7 + 10.8	8.2 + 3.2	5.4 + 1.7	0.0027
TRACP5a activity	2.4 + 0.6	1.3 + 0.3	1.6 + 0.4	<0.0001
CRP	1.0 + 0.9	0.6 + 0.2		0.0775

\*Mean  $\pm$  SD, TRACP 5a activity Cut-off point: 1.724, Sensitivity: 94.4%, Specificity: 91.3%, Positive predictive value: 89.5%, Negative predictive value: 95.5% and Youden's index: 0.82.

Owing to its complex nature, SLE remains one of the main challenges for physicians (Rahman and Isenberg, 2008; Crispin et al., 2010; Tsokos, 2011). The SLE Disease Activity Index (SLEDAI) computes disease activity by weighting the importance of each organ system involved; it is reliable, reproducible and sensitive to changes in patient status. However, the SLEDAI has a few limitations—it does not consider some life-threatening factors, such as pulmonary hemorrhage and hemolytic anemia.

Moreover, the SLEDAI can be challenging sometimes because its score may remain unchanged even if the patient status improves, stabilizes or worsens.

In addition, the SLEDAI does not consider subjective symptoms, such as fatigue, arthralgia, or myalgia, which may considerably reflect SLE activity and may be crucial (Ward et al., 2000; Fortin et al., 2000; Chang et al., 2002).

Human serum tartrate-resistant acid phosphatase (TRAP) has two distinct isoenzymes, 5a and 5b (Lam et al., 1978; Janckila et al., 2003; Halleen et al., 2002; Janckila et al., 2009, 2007; Chung et al., 2006; Lu et al., 2006; Chao et al., 2005; Chu et al., 2003, 2004) primarily expressed by monocyte lineage cells (Radzun et al., 1983; Yaziji et al., 1995; Hayman et al., 2000; Pradella et al., 2011).

TRAP 5a is mainly secreted by inflammatory macrophages and dendritic cells (DCs). TRAP 5a activity and protein concentration can be evaluated. Serum TRAP 5a protein concentration is higher in rheumatoid arthritis patients with rheumatoid nodules than in those without the nodules; however, TRAP 5a activity remains unchanged (Janckila et al., 2008). TRAP 5a protein concentration is independently associated with acute myocardial infarction; thus, it may also reflect the inflammatory burden in patients at the risk of cardiovascular diseases.

Research on the clinical usefulness of serum TRAP 5a in disease diagnosis and management for a wide variety of chronic inflammatory diseases recently increased (Janckila and Yam, 2009); however, the role and importance of the TRAP 5a activity remains unknown. Based on these observations, we hypothesized that circulating TRAP 5a activity is derived from the function of macrophage in accordance with their systemic number and it possibly reflects the severity of a chronic inflammatory disease (Janckila et al., 2011, 2002; Shih et al., 2010; Chao et al.,

2005). If this hypothesis is accurate, TRAP 5a activity or protein concentration will increase in other systemic chronic inflammatory diseases as well. In this proof-of-concept study, we investigated serum TRAP 5a activity and protein concentration in a cohort of Taiwanese patients with SLE to substantiate our hypothesis.

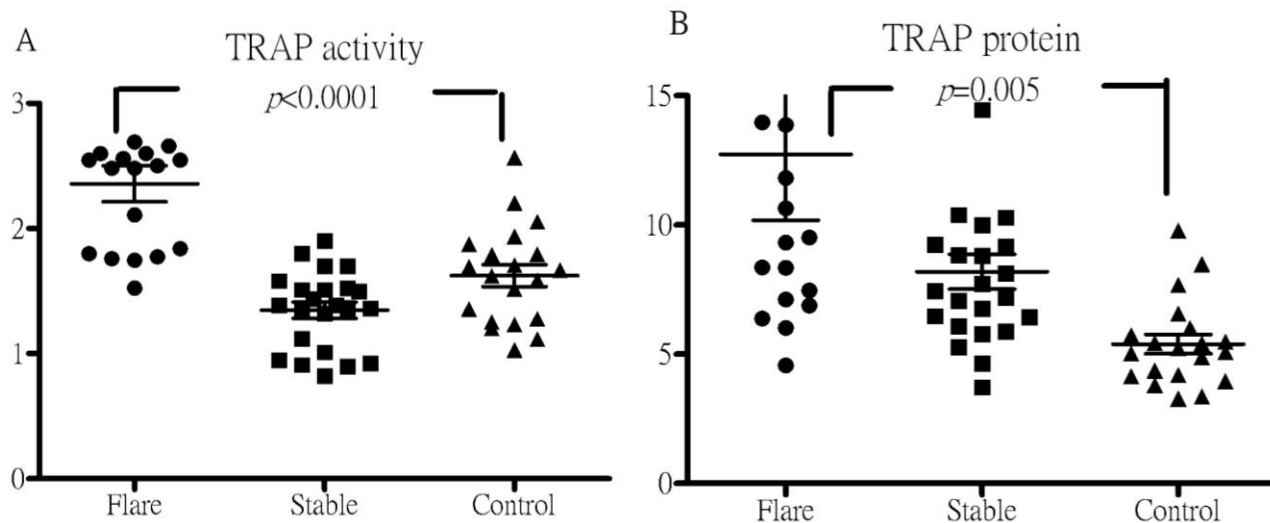
## MATERIALS AND METHODS

### Study patients

Forty-one (41) female patients with SLE defined according to the American College of Rheumatology criteria were enrolled from Tri-Service General Hospital (TSGH) during March 2011 to December, 2012; of these patients, 23 with low disease activity (SLEDAI score of <8 and/or disease not requiring any increase in therapy for at least 3 week) were stratified into the stable group, whereas 18 (SLEDAI score of  $\geq$ 8) were included in the flare group. The disease activity was determined through C<sub>3</sub>, C<sub>4</sub> and dsDNA concentrations and clinical symptoms. To establish a reference range for biomarkers, serum was drawn from 20 women in a healthy control group present at the health-check clinic of TSGH (Table 1). The study was conducted in accordance with the Helsinki Declaration and approval obtained from the Human Studies Committee of TSGH. Informed consent was obtained from all participants.

### Serum TRAP 5a immunoassay

Three immunoassays were conducted for TRAP isoforms. TRAP 5a activity and protein concentration was determined separately through in-house published assays by using monoclonal antibody 220 (mab220) with minor modification. TRAP 5a bound to immobilized mab220 was detected using anti-TRAP mab162 conjugated to horseradish peroxidase. Mab162 was generated against the same recombinant TRAP antigen preparation as that for mab220 (Chao et al., 2005; Janckila et al., 2005). Mab162 reacted with both TRAP 5a and 5b and recognized the same epitope as mabJ1B used to develop the original



**Figure 1:** a) Scatter plot of TRAP-5a activity level compare SLE flare, SLE stable groups and normal groups; b) scatter plot of TRAP-5a protein level compare SLE flare, SLE stable groups and normal groups; 1c, scatter plot of CRP level compare SLE flare and SLE stable groups.

TRAP 5a immunoassay. The activity of the bound TRAP 5a was measured using 10mM 4-NPP (pH 5.8). Serial dilutions of 4-nitrophenolate in 1M NaOH equivalent to 0 to 10 IU of TRAP activity were used as standards for the TRAP activity assays. Serial dilutions of partially purified serum containing 0 to 5 g/L TRAP 5a were used to construct a standard curve for the TRAP 5a assay.

### Other biomarker assays

Serum CRP concentration (mg/dL) was determined using a high-sensitivity in-house immunoassay constructed from commercial antibodies with purified human CRP as the standard (Dako Corp.).

### Statistical analyses

Using SPSS version 19, one-way analysis of variance was used to compare mean biomarker concentrations among the flare and stable groups. To identify significant differences in pairwise comparisons, follow-up t tests were performed. Pearson correlation was used to estimate the strength of the associations between each biomarker pair.

## RESULTS

### Serum Biomarkers in Patients with SLE

The SLEDAI scores of the 23 stable group patients were low (mean 4.1), whereas those of the 18 flare patients were high (mean 12.6). Table 1 lists the median value of all biomarkers, including TRAP 5a activity and protein

concentration, CRP concentration and the demographic characteristics of both patient groups. Age,  $C_3$ ,  $C_4$ , dsDNA and CRP concentrations did not differ significantly between the groups.

### TRAP 5a activity and concentration and CRP concentration

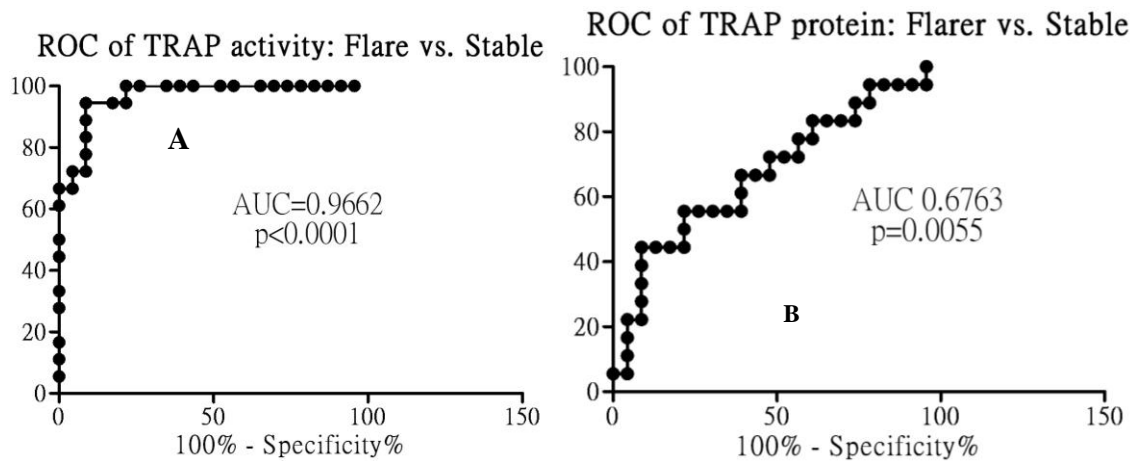
Serum TRAP 5a activity was significantly higher in the flare group patients than in the stable group patients and controls ( $P < 0.0001$ ). However, serum TRAP 5a activity was significantly lower in the stable group patients than in controls ( $P = 0.0135$ ; Figure 1a).

Serum TRAP 5a protein concentration was significantly higher in the flare group patients than in controls ( $P = 0.005$ ); however, the difference was non-significant when the slightly higher TRAP 5a protein concentration in the flare group patients was compared with that in the stable group patients ( $P = 0.0634$ ). Serum TRAP 5a protein concentration was significantly higher in the stable group patients than in controls ( $P = 0.0012$ ; Figure 1b). Serum CRP concentration was measured only in the patients with SLE (mean  $\pm$  standard deviation: flare group,  $1.0 \pm 0.9$  mg/dL; stable group,  $0.6 \pm 0.2$  mg/dL); the concentrations did not differ significantly between the 2 groups ( $P = 0.07$ ; Figure 1c).

### Receiver operating characteristic curves of TRAP 5a activity and protein

#### Concentration in patients with SLE

Receiver operating characteristic (ROC) curves were



**Figure 2:** ROC curves for comparisons between TRAP 5a activity; a) TRAP 5a and b) Protein.

constructed to compare TRAP 5a activity between the flare and stable groups. The area under the ROC curve for TRAP 5a activity in the flare versus stable group was 0.9662 ( $P < 0.0001$ ; **Figure 2a**). TRAP 5a activity cut-off point was 1.724 (sensitivity 94.4%; specificity 91.3%; **Table 1**). ROC curves were also constructed to compare TRAP 5a concentrations between the flare and stable groups. The area under the ROC curve for TRAP 5a protein concentration in the flare versus stable group was 0.6763 ( $P = 0.0055$ ; **Figure 2b**).

## DISCUSSION

Over the past decades, numerous SLE-related challenges—such as understanding the etiopathogenesis of SLE by developing tests and biomarkers and improving the quality of care for patients with SLE—have been addressed. Although considerable progress has been made, many requirements in SLE research and patient care remain unfulfilled; this is primarily due to lack of reliable SLE biomarkers for monitoring and predicting SLE flare (1 to 3). In this study, we determined whether TRAP 5a is an SLE flare biomarker. Serum TRAP 5a protein concentration increased in patients with SLE as compared with those without SLE (controls), regardless of the presence of flare.

Notably, TRAP 5a activity was lower in patients with stable SLE than in controls; however, the activity was significantly higher in patients with SLE flare. The 2 isoenzymes of TRAP, 5a and 5b are derived from the product of a common gene, ACP5, located at chromosome 19p13, through differential post-translational modification. TRAP 5a protein and activity can be detected in macrophages, monocytes and DCs, whereas TRAP 5b is majorly present in osteoclasts. TRAP 5a is the major circulating isoform of TRAP, whereas TRAP 5b accounts for the rest (Zenger et al., 2011). ACP5 mutations can cause SLE in case of an immuno-osseous disease, spondyloenchondrodysplasia (SPENCD).

SPENCD is presently considered a monogenic form of SLE associated with TRAP deficiency, which further causes an inflammatory T-cell response and promotes interferon (INF)- $\alpha$  production. Clinically, healthy parents of a child with SPENCD caused by a compound heterozygous ACP5 mutation had a decreased TRAP activity in their sera with the INF- $\alpha$  signature of SLE in their peripheral blood (Briggs, 2014; An et al., 2013; Briggs et al., 2011).

Osteopontin (OPN), a bone matrix protein, is a substrate for TRAP, which dephosphorylates it (Janckila and Yam, 2009). OPN is involved in osteoclast adhesion and migration during bone resorption, 11 executed by TRAP 5b. However, the actual biological function of TRAP 5a remains unclear. An et al. (2013) recently demonstrated that intracellular OPN and TRAP colocalize and interact within the plasmacytoid DCs (pDCs). TRAP deficiency in pDC causes increased INF- $\alpha$  production associated with SLE (An et al., 2013; Briggs et al., 2011). The authors also showed that in SLE patients, heterozygous ACP5 missense variants are in excess with reduced TRAP activity observed in some missense variants.

Defects in phagocytosis and apoptotic cell clearance may have a role in SLE pathogenesis (Udo et al., 2007; Apostolidis et al., 2011; Davis et al., 2011). Since TRAP 5a is present in monocytes, macrophages and DCs and its deficiency is associated with aberrant T-cell response and INF- $\alpha$  production, TRAP 5a may be associated with numerous immunological functions, including antigen presentation, phagocytosis and cytokine production. It was observed that the least TRAP 5a activity was in patients with stable SLE. This could be explained by the defective phagocytic activity of monocytes and macrophages in these patients. Nonetheless, TRAP 5a protein concentration in their serum was paradoxically higher; although the reason for this remains unclear, it may be as a result of patients with SLE who regularly received corticosteroids, non-steroidal anti-inflammatory drugs, anti-malarial or other immunomodulating drugs affecting their TRAP 5a activities.

In this study, we also revealed that both TRAP 5a activity and protein concentration increased significantly when patients with SLE developed flare. Serum TRAP 5a protein concentration was higher in patients with stable SLE than in controls and it increased further during SLE flare. However, TRAP 5a activity was significantly increased only after patients with SLE developed flare. The changes in TRAP 5a activities and protein concentration between patients with stable SLE and those with SLE flare are significant (Figure 2), particularly for the TRAP 5a activity. Therefore, further investigation of whether TRAP 5a protein concentration and activity are useful biomarkers of SLE flare is warranted.

This study has several limitations. First, because its design was cross-sectional, it lacked longitudinal data of individual patients; thus, intra-individual differences could not be demonstrated. Secondly, many patients had received or were receiving treatment and therefore, the study population was non-uniform. Thirdly, the sample size was relatively small. Finally, the severity of flare could not be analyzed in detail; therefore, it remains unclear whether the changes in TRAP5a protein concentration and activity could reflect flare severity.

In summary, TRAP 5a is a potential biomarker demonstrated for the diagnosing and monitoring SLE. Due to the aforementioned limitations, studies involving a larger sample size and a longitudinal design, with a clearly defined stable and flare status are required in a clinically homogeneous population for further development of this biomarker.

## ACKNOWLEDGEMENTS

The authors would like to thank Su-Hui Lee and Hsin-Yi Liu from the Division of Hematology/Oncology, Department of Medicine, Tri-Service General Hospital, for assistance in the biomarker immunoassays and for quality assurance and also Dr. Der-Ming Chang, for referring patients to this study.

## REFERENCES

An Jie, Briggs T, Dumax-Vorzet A, Audrey, et al. (2013). Molecular pathogenesis and genetics of tartrate-resistant acid phosphatase deficiency in systemic  
 Apostolidis S, Lieberman L, Kis-Toth K, Crispin J, Tsokos G (2011). The dysregulation of cytokine networks in systemic lupus erythematosus. *J Interferon Cytokine Res.* 31: 769–779.  
 Briggs TA, Rice GI, Daly S, Urquhart J, Gornall H, Bader-Meunier B, Baskar K, Baskar S, Baudouin V, Beresford MW, Black GC, Dearman RJ, de Zegher F, Foster ES, Francès C, Hayman AR, Hilton E, Job-Deslandre C, Kulkarni ML, Le Merrer M, Linglart A, Lovell SC, Maurer K, Musset L, Navarro V, Picard C, Puel A, Rieux-Laucat F, Roifman CM, Scholl-Bürgi S, Smith N, Szykiewicz M, Wiedeman A, Wouters C, Zeef LA, Casanova JL, Elkon KB, Janckila A, Lebon P, Crow YJ. (2011). Tartrate-resistant acid phosphatase deficiency causes a bone dysplasia with autoimmunity and a type I interferon expression signature. *Nat. Genet.* 43(2):127-31.  
 Chang E, Abrahamowicz M, Ferland D, Fortin PR (2002). *CaNIOS Investigators.* Comparison of the responsiveness of lupus disease

activity measures to changes in systemic lupus erythematosus activity relevant to patients and physicians. *J.Clin. Epidemiol.* 55:488-497.  
 Chao TY, Ho CL, Lee SH, Chen MM, Janckila AJ, Yam LT (2004). Tartrate-resistant acid phosphatase 5b as a serum marker of bone metastasis in breast cancer patients. *J Biomed. Sci.*11:511-6.  
 Chao TY, Lee SH, Chen MM, Neustadt DH, Chaudhry UA, Janckila AJ (2005). Development of immunoassays for serum tartrate-resistant acid phosphatase isoform 5a. *Clin. Chim. Acta.* 359:132-140.  
 Chao TY, Yu JC, Ku CH, Chen MM, Lee SH, Janckila AJ, Yam LT (2005). Tartrate-resistant acid phosphatase 5b is a useful serum marker for extensive bone metastasis in breast cancer patients. *Clin. Cancer Res.*11:544-50.  
 Chu P, Chao TY, Lin YF, Janckila AJ, Yam LT (2003). Correlation between histomorphometric parameters of bone resorption and serum type 5b tartrate-resistant acid phosphatase in uremic patients on maintenance hemodialysis. *Am. J. Kidney Dis.* 41:1052-9.  
 Chung YC, Ku CH, Chao TY, Yu JC, Chen MM, Lee SH (2006). Lee SH. Tartrate-resistant acid phosphatase 5b activity is a useful bone marker for monitoring bone metastases in breast cancer patients after treatment. *Cancer Epidemiol. Biomarkers Prev.*15:424-8.  
 Crispin JC, Liossis S, Kis-Toth K, Lieberman LA, Kyttaris VC, Juang YT, Tsokos GC (2010). Pathogenesis of human systemic lupus erythematosus: recent advances. *Trends Mol. Med.* 16: 47–57.  
 Davis L, Hutcheson J, Mohan C (2011). The role of cytokines in the pathogenesis and treatment of systemic lupus erythematosus. *J. Interferon Cytokine Res.* 31: 781–789.  
 Fortin PR, Abrahamowicz M, Clarke AE, Neville C, Du Berger R, Fraenkel L, Liang MH (2000). Do lupus disease activity measures detect clinically important change? *J Rheumatol.* 27:1421-1428.  
 Halleen JM, Ylipahkala H, Alatalo SL, Janckila AJ, Heikkinen JE, Suominen H, Cheng S, Vaananen HK (2002). Serum tartrate-resistant acid phosphatase 5b, but not 5a, correlates with other markers of bone turnover and bone mineral density. *Calcif Tissue Int.* 71: 20-25.  
 Hayman AR, Bune AJ, Bradley JR, Rashbass J, Cox TM (2000). Osteoclastic tartrate-resistant acid phosphatase (acp 5): Its localization to dendritic cells and diverse murine tissues. *J. Histochem. Cytochem.* 48: 219-228.  
 Janckila AJ, Lederer ED, Price BA, Yam LT (2009). Tartrate-resistant acid phosphatase isoform 5a as an inflammation marker in end-stage renal disease. *Clin. Nephrol.*71:387-96.  
 Janckila AJ, Neustadt DH, Yam LT (2008). Significance of serum trapc in rheumatoid arthritis. *J. Bone Miner Res.* 23: 1287-1295.  
 Janckila AJ, Lin HF, Wu YY, Ku CH, Yang SP, Lin WS, Lee SH, Yam LT, Chao TY (2011). Serum tartrate-resistant acid phosphatase isoform 5a (TRACP5a) as a potential risk marker in cardiovascular disease. *Clin. Chim. Acta.* 412:963-969.  
 Janckila AJ, Nakasato YR, Neustadt DH, Yam LT (2003). Disease-specific expression of tartrate-resistant acid phosphatase isoforms. *J. Bone Miner Res.* 18:1916-1919.  
 Janckila AJ, Neustadt DH, Nakasato YR, Halleen JM, Hentunen T, Yam LT (2002). Serum tartrate-resistant acid phosphatase isoforms in rheumatoid arthritis. *Clin. Chim. Acta.* 320: 49-58.  
 Janckila AJ, Parthasarathy RN, Parthasarathy LK, Seelan RS, Hsueh YC, Rissanen J, Alatalo SL, Halleen JM, Yam LT (2005). Properties and expression of human tartrate-resistant acid phosphatase isoform 5a by monocyte-derived cells. *J. Leukoc. Biol.* 77:209–218.  
 Janckila AJ, Slone SP, Lear SC, Martin A, Yam LT (2007). Tartrate-resistant acid phosphatase as an immunohistochemical marker for inflammatory macrophages. *Am. J. Clin. Pathol.* 127:556-66.  
 Janckila AJ, Yam LT (2009). Biology and clinical significance of tartrate-resistant acid phosphatases: new perspectives on an old enzyme. *Calcif Tissue Int.* 85(6):465-83.  
 Lam WK, Eastlund DT, Li CY, Yam LT (1978). Biochemical properties of tartrate-resistant acid phosphatase in serum of adults and children. *Clin. Chem.* 24: 1105-1108.  
 Lu KC, Tseng CF, Wu CC, Yeung LK, Chen JS, Chao TY, Janckila AJ, Yam LT, Chu P (2006). Effects of calcitriol on type 5b tartrate-resistant acid phosphatase and interleukin-6 in secondary hyperparathyroidism. *Blood Purif.* 24:423-30.  
 lupus erythematosus [abstract]. *Arthritis Rheum* 65 Suppl. 10 :2702 - 6510  
 Pradella SD, Slone SP, Wu YY, Chao TY, Parthasarathy RN, Yam LT, Janckila

- AJ (2011). Applications and performance of monoclonal antibodies to human tartrate resistant acid phosphatase. *J. Immunol. Methods.* 372:162-70.
- Radzun HJ, Kreipe H, Parwaresch MR (1983). Tartrate-resistant acid phosphatase as a differentiation marker for the human mononuclear phagocyte system. *Hematol. Oncol.* 1: 321-327.
- Rahman A, Isenberg D (2008). Systemic lupus erythematosus. *N. Engl. J. Med.* 358: 929-939.
- Shih KC, Janckila AJ, Kwok CF, Ho LT, Chou YC, Chao TY (2010). Effects of exercise on insulin sensitivity, inflammatory cytokines, and serum tartrate-resistant acid phosphatase 5a in obese Chinese male adolescents. *Metabolism.* 59:144-51.
- Tracey Briggs (2014) TRAP mutations and immunology. *Bone Abstracts.* 3(6):33-6.3
- Tsokos G (2011). Systemic lupus erythematosus. *N. Engl. J. Med.* 365: 2110-2121.
- Udo S, Gaip L, Munoz E, Gerhard G, Kirsten L, Sandra F, Sarter K, Voll RE, Winkler T, Kuhn A, Kalden J, Kern P, Herrmann M (2007) Clearance deficiency and systemic lupus erythematosus (SLE). *J.Autoimmunity.* 28:114-121.
- Ward MM, Marx AS, Barry NN (2000). Comparison of the validity and sensitivity to change of 5 activity indices in systemic lupus erythematosus. *J. Rheumatol.* 27:664-670.
- Yaziji H, Janckila AJ, Lear SC, Martin AW, Yam LT (1995). Immunohistochemical detection of tartrate-resistant acid phosphatase in non-hematopoietic human tissues. *Am. J. Clin. Pathol.* 104: 397-402.
- Zenger S, He W, Ek-Rylander B, Vassiliou D, Wedin R, Bauer H, Andersson G (2011). Differential expression of tartrate-resistant acid phosphatase isoforms 5a and 5b by tumor and stromal cells in human metastatic bone disease. *Clin. Exp. Metastasis.* 28:65-73.