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Original article

^{18}F-Fluorodeoxyglucose positron emission tomography and serum cytokines and matrix metalloproteinases in the assessment of disease activity in Takayasu's arteritis

Anne E. D. Arraes[a], *Alexandre W. S. de Souza*[a], *Henrique A. Mariz*[a], *Neusa P. Silva*[a], *Ivone C. G. Torres*[b], *Paula N. V. Pinto*[b], *Eduardo N. P. Lima*[b], *Emilia I. Sato*[a,*]

[a] *Rheumatology Division, Escola Paulista de Medicina, Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil*
[b] *Radiology Division, Hospital A.C. Camargo, São Paulo, SP, Brazil*

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ABSTRACT

Objective: To evaluate ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake on positron emission tomography–computed tomography (PET–CT) and serum levels of different cytokines and matrix metalloproteinases (MMPs) in patients with Takayasu arteritis (TA) and associations with disease activity.

Methods: Serum levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-2, IL-6, IL-8, IL-12, IL-18, MMP-3 and MMP-9 were measured in 36 TA patients and 36 controls. Maximum standard uptake value (SUV_{max}) of ^{18}F-FDG in arterial walls was determined by PET–CT scans. TA patients were classified as active disease, inactive disease and possible active disease.

Results: Serum IL-6 and MMP-3 levels were higher in TA patients than in controls ($p < 0.001$). Serum IL-6 was higher in patients with active disease and in patients with possible active disease than in inactive disease ($p < 0.0001$). Patients with active disease had higher serum TNFα levels than patients with inactive disease ($p = 0.049$) while patients with possible active disease presented higher IL-18 levels than patients with inactive disease ($p = 0.046$). Patients with active disease had higher SUV_{max} values than those with inactive disease ($p = 0.042$). By receiver operating characteristic (ROC) curve SUV_{max} was predictive of active disease in TA and values ≥1.3 were associated with disease activity ($p = 0.039$). Serum TNF-α levels were higher in patients with $SUV_{max} \geq 1.3$ than <1.3 ($p = 0.045$) and controls ($p = 0.012$). Serum IL-6 levels were higher in patients with $SUV_{max} \geq 1.3$ than in controls ($p < 0.001$). No differences regarding other biomarkers were found between TA patients and controls.

Conclusions: Higher serum IL-6 and TNFα levels as well as higher ^{18}F-FDG uptake in arterial wall are associated with active TA.

* *Corresponding author.*
E-mail: eisato@unifesp.br (E.I. Sato).

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Tomografia por emissão de pósitrons com 18F-fluordesoxiglicose e citocinas séricas e metaloproteinases da matriz na avaliação da atividade da doença na arterite de Takayasu

Palavras-chave:
Citocinas
Metaloproteinases da matriz
Tomografia computadorizada
Arterite de Takayasu

RESUMO

Objetivo: Avaliar a captação de 18F-fluordesoxiglicose (18F-FDG) na tomografia por emissão de pósitrons–tomografia computadorizada (PET–CT) e os níveis séricos de diferentes citocinas e da metaloproteinases da matriz (MMP) em pacientes com arterite de Takayasu (AT) e associações com a atividade da doença.

Métodos: Foram mensurados os níveis séricos do fator de necrose tumoral-α (TNF-α), interleucina (IL)-2, IL-6, IL-8, IL-12, IL-18, MMP-3 e MMP-9 em 36 pacientes com AT e 36 controles. O valor padronizado de captação máximo (SUV_{max}) de 18F-FDG nas paredes arteriais foi determinado por exames de PET–CT. Os pacientes com AT foram classificados como tendo doença ativa, doença inativa e possível doença ativa.

Resultados: Os níveis séricos de IL-6 e MMP-3 foram mais altos em pacientes com AT do que nos controles ($p < 0{,}001$). Os níveis séricos de IL-6 eram mais elevados em pacientes com doença ativa e em pacientes com possível doença ativa do que naqueles com doença inativa ($p < 0{,}0001$). Os pacientes com doença ativa apresentaram níveis séricos mais elevados de TNF-α do que os pacientes com doença inativa ($p = 0{,}049$), enquanto os indivíduos com possível doença ativa apresentaram maiores níveis séricos de IL-18 do que os pacientes com doença inativa ($p = 0{,}046$). Aqueles com doença ativa apresentaram maiores valores de SUV_{max} do que aqueles com doença inativa ($p = 0{,}042$). De acordo com a curva ROC, o SUV_{max} era capaz de predizer a doença ativa na AT e valores ≥1,3 estavam associados à atividade da doença ($p = 0{,}039$). Os níveis séricos de TNF-α foram maiores em pacientes com $SUV_{max} \geq 1{,}3$ do que naqueles com valor <1,3 ($p = 0{,}045$) e controles ($p = 0{,}012$). Os níveis séricos de IL-6 foram mais elevados em pacientes com $SUV_{max} \geq 1{,}3$ do que nos controles ($p < 0{,}001$). Não foram encontradas diferenças em relação a outros biomarcadores entre pacientes com AT e controles.

Conclusões: Níveis séricos elevados de IL-6 e TNF-α, bem como uma maior captação de 18F-FDG na parede arterial, estão associados a AT ativa.

Introduction

Takayasu arteritis (TA) is a primary systemic vasculitis of unknown etiology that affects large arteries, mainly the aorta and its main branches and less frequently pulmonary and coronary arteries. The chronic granulomatous inflammation occurs in all layers of the vessel wall and may lead to stenosis, occlusion, dilation or aneurysm formation.[1,2]

Cell-mediated autoimmunity plays a key role in the pathogenesis of TA. Immunohistochemical studies of the infiltrating cells in the aortic tissue have shown mainly gamma-delta T-cells, CD4+ and CD8+ T-cells, NK cells and macrophages.[3] The presence of granulomatous inflammation in the internal elastic layer is the most characteristic pathologic finding of TA. The inflammatory process begins in the *vasa vasorum* and it is believed to be triggered by the activation of dendritic cells and dendritic cell/T-cell interaction in the vascular microenvironment of the adventitia of large arteries leading to the induction of a Th1 response.[4]

The detection of vascular inflammation in TA patients is a major challenge in clinical practice, since up to 60% of asymptomatic patients developed new angiographic lesions on sequential arteriographic evaluation, and 44% of patients considered to be in remission by clinical evaluation showed histopathological evidence of active inflammation on surgical specimens.[1] Due to the evidence of progression of vascular damage in asymptomatic TA patients, additional evaluation is necessary to guide therapeutic decisions. Nevertheless, to date there is no reliable surrogate parameter to detect subclinical disease activity in TA patients. Potential biological markers of inflammation and tissue degradation, such as cytokines and metalloproteinases (MMPs) have been evaluated separately in different studies but some results have not been reproduced.[5–7] Different imaging techniques have been used to assess the extent of arterial involvement in TA, among them ^{18}F-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography with computed tomography (PET–CT) scan can detect active arterial wall inflammation. Some studies have been promising[8–10] whereas others have found conflicting results with the use of PET–CT scans to evaluate disease activity in TA.[11,12]

The aims of this study were to evaluate the intensity of ^{18}F-FDG uptake on PET–CT scans on arterial walls and quantify serum levels of interleukin-2 (IL-2), IL-6, IL-8, IL-12, IL-18, tumor necrosis factor-α (TNF-α), MMP-3 and MMP-9 in patients with TA and controls as well as to evaluate possible associations with disease activity in TA.

Materials and methods

Patients and controls

Patients under regular follow-up at the Vasculitis Unit of the university hospital of Universidade Federal de São Paulo (Unifesp) or at private offices of rheumatologists who work at Unifesp were consecutively invited to participate in this cross-sectional study. They underwent clinical, laboratorial and imaging evaluation within a 20-day period. Inclusion criteria were the fulfillment of the American College of Rheumatology (ACR) classification criteria for TA,[13] age above 18 years and the absence of malignancy, active infection and pregnancy. The control groups comprised laboratory controls (lab-controls) and PET–CT scan controls (PET-controls). The former group included 36 age- and gender-matched subjects without chronic inflammatory or infectious disease and the latter group consisted of six healthy individuals who underwent a PET–CT scan, using the same protocol used by TA patients. All subjects signed informed consent form approved by the Institutional Ethics Committees from Unifesp and AC Camargo Hospital.

Clinical and laboratory assessment

The clinical assessment of TA patients was performed by two physicians experienced in TA management (AWSS and HAM) at routine medical visits. Disease activity was defined whether the onset or worsening of at least two of the following features was present: (a) systemic features with no other identified cause; (b) the onset of features of vascular ischemia or inflammation, such as limb claudication, unequal or absent pulse, bruit, vascular pain (carotidynia) or asymmetric blood pressure; (c) elevated acute phase reactants including erythrocyte sedimentation rate (ESR); (d) development of new lesions in previously unaffected vascular territories on serial vascular imaging evaluation.[1] TA patients presenting abnormal levels of acute phase reactants (i.e. ESR $\geq$ 40 mm/h and/or serum CRP levels $\geq$ 10 mg/dL) without signs and symptoms of active TA or new angiographic lesions were considered a distinct subgroup named as possible active disease.

Blood samples were collected from patients and lab-controls for cytokines, MMPs, ESR (Westergren) and CRP measurements. Serum samples were separated and stored at $-70\,^{\circ}C$ until tests were performed. The enzyme-linked immunosorbent assay (ELISA) technique was used to quantify CRP (Diagnostics Systems Laboratories, EUA), IL-18 (Kit Med & Biologic Lab Co, Japan), MMP-3 and MMP-9 (R&D Systems, EUA). All tests were performed in duplicate. The measurement of IL-2, IL-6, IL-8, IL-12 and TNF-α was performed by LUMINEX Millipex kits.

Image assessment

PET–CT scans were acquired using a Gemini PET–CT scanner (Philips) after at least a 6-h fasting period. Capillary glucose levels were checked before scans and no one presented levels above 200 mg/dL prior to ^{18}F-FDG administration. Image acquisition was performed 60 min after the infusion of 0.154 millicurie (mCi)/kg of ^{18}F-FDG and participants were scanned from the skull to proximal thighs. Firstly, CT images were obtained without the infusion of intravenous contrast using the following parameters: 120 kV, 120 mAs, 0.75 s per rotation, pitch 1.5, 5 mm slice thickness and a 3 mm reconstruction interval. Then PET scan was acquired during 2 min and 15 s at each bed position in a total of 11 bed positions. PET-control subjects had undergone the same protocol and their scans were retrieved from the archive. All PET–CT scans were performed at AC Camargo Hospital and images were evaluated by a radiologist (PNVP) and a nuclear medicine physician (ICGT) who were unaware of clinical and laboratory assessments of TA patients.

A semi-quantitative assessment of ^{18}F-FDG uptake was done at using the standard uptake value (SUV). The SUV was calculated dividing the maximum tissue concentration by the total injected dose of ^{18}F-FDG per body weight. The region of interest (ROI) was manually placed on artery walls of the following sites: vertebral, internal carotid, subclavian and common carotid arteries, pulmonary artery, brachiocephalic trunk, aortic arch, ascending, descending and abdominal aorta, celiac trunk, superior and inferior mesenteric arteries, renal and iliac arteries. The highest value of SUV found in each artery was recorded and only the highest value on the whole PET–CT scan (SUV_{max}) was used for statistical analysis.

Statistical analysis

Statistical analysis was performed with the software *Statistical Package for the Social Sciences* (SPSS) version 17.0 (Chicago, IL). Categorical data was presented as total number and percentage, and numerical data as median (range) or mean (standard deviation) as appropriate. The Shapiro–Wilk test was used to test the normality of continuous variables. Comparisons regarding numerical variables were performed with Mann–Whitney *U* test or Student's *t* test for two groups or with Kruskal–Wallis test or one-way ANOVA test for three or more groups. The Mann–Whitney *U* test with Bonferroni's correction was used as a post hoc test. Values of $p < 0.05$ were considered significant while p values between 0.10 and 0.05 were considered as statistical tendency.

SUV_{max} values for the diagnosis of disease activity was evaluated by the ROC (receiver operating characteristic) curve and the Youden index was used to assess the best cutoff values. Results of the ROC curve were expressed in area under the curve, 95% confidence interval (95% CI), positive predictive value, negative predictive value, accuracy, sensitivity and specificity for cutoff points of SUV_{max}. Correlations between numerical variables were performed by Spearman's test. The presence of active disease in TA determined by the cutoff of SUV_{max} was compared to disease activity determined by clinical evaluation using Fisher's exact test. Statistical significance was considered for $p < 0.05$.

Results

Patients and controls

Thirty-six TA patients were evaluated in this study, 14 patients (38.9%) had active disease while 11 (30.6%) patients were

Table 1 – Demographic and disease features of TA patients evaluated in this study.

Variables	Active disease ($n = 14$)	Inactive disease ($n = 11$)	Possible active disease ($n = 11$)	p
Age, years	35.0 (22.0–54.0)	32.0 (24.0–68.0)	32.0 (21.0–56.0)	0.818
Females, n (%)	14 (100.0)	9 (81.8)	10 (90.9)	0.262
Time since first TA symptoms, months	72.0 (24.0–216.0)	85.0 (23.0–372.0)	118.0 (9.0–207.0)	0.800
Time since diagnosis, months	45.5 (2.0–216.0)	66.0 (17.0–292.0)	58.0 (1.0–240.0)	0.628
ESR, mm/h	20.00 (5.00–115.00)	11.00 (1.00–26.00)	26.00 (7.00–70.00)	0.059
Serum CRP levels, mg/dL	18.17 (0.10–99.05)	1.53 (0.17–7.77)	78.13 (8.16–91.89)	<0.0001

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.
Data are presented as mean and standard deviation or as median and range.

considered with inactive disease and 11 (30.6%) had possible active disease. Table 1 describes demographic features, time since the onset of symptoms attributable to TA, time since diagnosis and acute phase reactants in subgroups of TA patients. Patients with active disease had higher ESR and serum CRP levels than patients with inactive disease. TA patients with possible active disease had significantly higher serum CRP levels than patients in remission and a trend to have higher ESR than patients in remission (Table 1). At the time of assessment, 21 patients were on steroids and immunosuppressive agents, three patients only on steroids, six were treated only with an immunosuppressive agent and six other patients were not receiving any therapy for TA. The median prednisone daily dose was 12.5 mg (5.62–27.50). The following immunosuppressive agents were used by patients: methotrexate (50.0%), mycophenolate sodium (11.1%), azathioprine (8.3%), and cyclophosphamide (5.6%). Only one patient used etanercept.

The lab-control group comprised 33 women and 3 men, with a median age of 34.0 (20–68) years. All lab-controls did not present any medical condition and were not under any medical therapy. The PET-control group consisted of six healthy individuals (four women, two men) with median age of 32.5 (28–45) years.

Cytokines and metalloproteinases

Table 2 shows serum levels of cytokines and MMPs in TA patients and lab-controls. TA patients presented higher IL-6 levels ($p < 0.001$) and higher serum MMP-3 levels ($p < 0.001$) and a tendency for higher TNF-α levels ($p = 0.069$) than lab-controls. No significant differences were found regarding serum levels of IL-2, IL-8, IL-12, IL-18 and MMP-9 when comparing TA patients and lab-controls (Table 2).

Serum cytokine and MMPs levels in TA patients with active disease, patients with inactive disease and patients with possible active disease are shown in Table 3. Serum IL-6 levels were higher in patients with active disease and in patients with possible active disease compared to with patients with inactive disease ($p < 0.0001$) (Fig. 1). Although a tendency for differences was found regarding serum TNFα and IL-18 levels among TA subgroups, when those subgroups were analyzed separately, TA patients with active disease had significantly higher serum TNFα levels than patients with inactive disease ($p = 0.049$) while patients with possible active disease presented higher serum IL-18 levels than patients with inactive disease ($p = 0.046$).

Correlations among inflammatory markers in TA patients

IL-6 levels correlated with ESR (rho = 0.475; $p = 0.005$) and with CRP levels (rho = 0.673; $p < 0.001$). CRP levels and ESR values (rho = 0.585; $p < 0.001$) were significantly correlated as well. TNF-α levels correlated with IL-6 (rho = 0.365; $p = 0.029$), IL-8 (rho = 0.346; $p = 0.039$), as well as with CRP levels (rho = 0.370; $p = 0.026$). Furthermore, serum IL-2 and IL-12 levels were correlated (rho = 0.529, $p = 0.001$). No correlations were found among other cytokines and MMPs.

Medical therapy, cytokines, metalloproteinases and acute phase reactants

MMP-3 levels were higher in TA patients using steroids than in those without steroids (45.05 ± 26.96 ng/mL vs.

Table 2 – Serum levels of cytokines and metalloproteases in TA patients and controls.

Markers	TA patients ($n = 36$)	Lab-controls ($n = 36$)	p
IL-2, pg/mL	0.36 (0.00–23.38)	0.25 (0.00–21.87)	0.710
IL-6, pg/mL	7.55 (0.17–76.22)	1.74 (0.17–16.21)	<0.0001[a]
IL-8, pg/mL	4.83 (1.88–17.92)	4.49 (2.02–22.02)	0.327
IL-12, pg/mL	0.00 (0.00–46.39)	0.00 (0.00–11.97)	0.908
IL-18, pg/mL	151.71 (0.00–463.26)	121.63 (0.00–745.22)	0.130
TNF-α, pg/mL	5.64 (1.91–15.28)	4.26 (1.41–12.89)	0.056
MMP-3, ng/mL	27.00 (6.00–102.70)	12.05 (4.30–75.50)	<0.0001[a]
MMP-9, ng/mL	495.55 (183.70–2338.80)	452.15 (221.20–2044.00)	0.457

IL, interleukin; MMP, matrix metalloproteinase; TNF, tumor necrosis factor.
Continuous data are presented as median and range or as mean and standard deviation.
[a] Statistically significant values.

Table 3 – Serum levels of cytokines and metalloproteases in TA patients with active disease, inactive disease and with possible active disease.

Variables	Active disease ($n = 14$)	Inactive disease ($n = 11$)	Possible active disease ($n = 11$)	p
TNFα, pg/mL	5.93 (3.84–10.71)	4.16 (1.91–15.28)	6.76 (3.69–9.23)	0.079
IL-2, pg/mL	0.19 (0.00–1.57)	1.12(0.00–12.04)	0.44 (0.00–23.38)	0.354
IL-6, pg/mL	9.15 (0.65–76.22)	2.48 (0.17–10.80)	8.48 (1.25–37.56)	0.003[a]
IL-8, pg/mL	5.47 (2.83–17.92)	4.53 (1.88–10.56)	4.61 (2.74–8.82)	0.368
IL-12, pg/mL	0.00 (0.00–2.22)	0.00 (0.00–12.56)	0.00 (0.00–46.39)	0.587
IL-18, pg/mL	183.58 ± 129.05	119.96 ± 57.98	246.20 ± 147.29	0.059
MMP3, ng/mL	22.40 (6.00–94.90)	26.30 (9.30–102.70)	31.10 (7.10–84.90)	0.682
MMP9, ng/mL	515.10 (208.40–2338.80)	424.20 (183.70–1422.50)	491.30 (274.40–1160.40)	0.547

IL, interleukin; MMP, matrix metalloproteinase; TNF, tumor necrosis factor.
Continuous data are presented as median and range or as mean and standard deviation.
[a] Statistically significant values.

13.11 ± 6.36 ng/mL; $p < 0.001$) and a positive correlation was observed between MMP-3 levels and daily prednisone dose (rho = 0.463; $p = 0.023$) (Fig. 2). No associations were found among steroids use, daily prednisone dose, immunosuppressive therapy and levels of MMP-9, cytokines or acute phase reactants (data not shown).

PET–CT scan and biomarkers

Median SUV_{max} values were higher in TA patients than in PET-controls [1.57 (0.87–3.04) vs. 0.99 (0.67–1.23); $p = 0.001$] and in patients with active disease compared with those in remission [1.97 (1.19–3.04) vs. 1.19 (0.87–2.59); $p = 0.015$]. However, no significant differences regarding SUV_{max} could be found between TA patients with active disease and those with possible active disease [1.97 (1.19–3.04) vs. 1.58 (1.00–2.72); $p = 0.324$] or between patients with possible active disease and those with inactive disease [1.58 (1.00–2.72) vs. 1.19 (0.87–2.59); $p = 0.212$]. Furthermore, patients with inactive disease presented higher SUV_{max} values than PET-controls [1.19 (0.87–2.59) vs. 0.99 (0.67–1.23); $p = 0.049$]. Fig. 3 illustrates the PET–CT findings in a TA patient with active disease.

TA patients on immunosuppressive therapy presented lower median SUV_{max} values than patients without those agents [1.41 (0.87–3.04) vs. 2.57 (0.96–3.00); $p = 0.044$]. However, no differences in median SUV_{max} values were found in patients with and without steroids [1.48 (0.87–3.04) vs. 2.14 (0.96–3.00); $p = 0.127$] as well as no correlation was found between SUV_{max} and daily prednisone dose (rho = 0.233; $p = 0.274$). Moreover, no correlations were found between SUV_{max} values and ESR (rho = 0.081; $p = 0.647$) or CRP levels (rho = 0.139; $p = 0.419$) and between SUV_{max} values and levels of any cytokine or MMPs evaluated in this study (data not shown).

ROC curve analysis showed the value of SUV_{max} as a predictor of active disease in TA (AUC = 0.703; 95% CI = 0.534–0.832; $p = 0.043$). The best cutoff points obtained for SUV_{max} values (with their respective sensitivity and specificity) were:

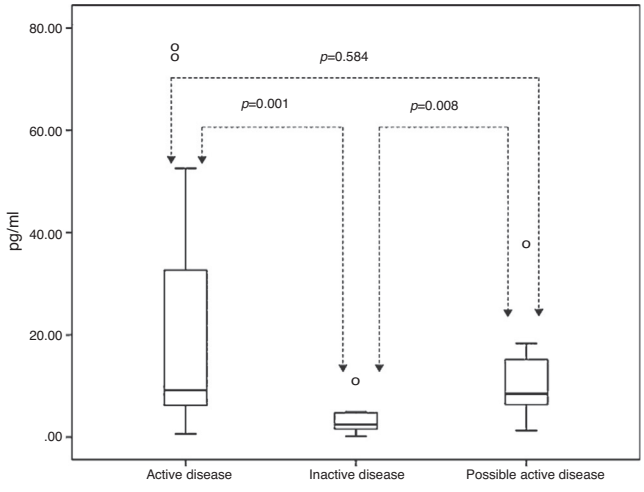

Fig. 1 – IL-6 levels in patients with active disease, inactive disease and possible active disease. Serum IL-6 levels were significantly higher in patients with active disease and in patients with possible disease activity than in patients with inactive disease. No significant differences could be found between TA patients with active disease and patients with possible active disease.

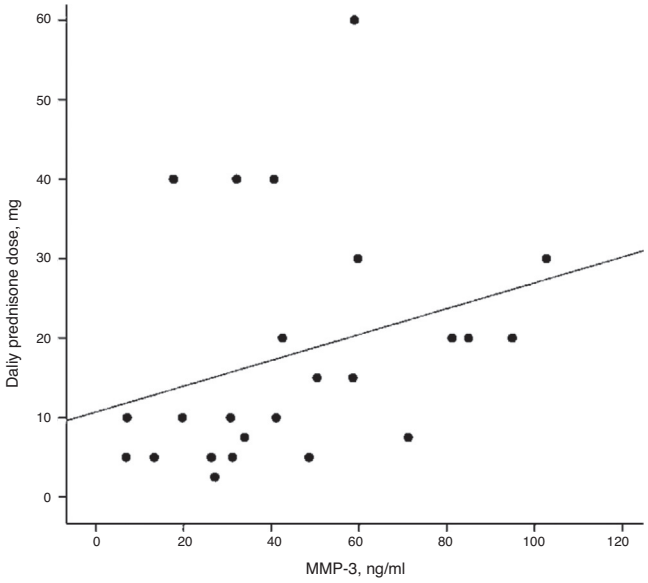

Fig. 2 – Correlation between serum levels of MMP-3 and prednisone daily dose. A significant correlation was found between serum MMP-3 levels and daily prednisone dose (rho = 0.463; $p = 0.023$).

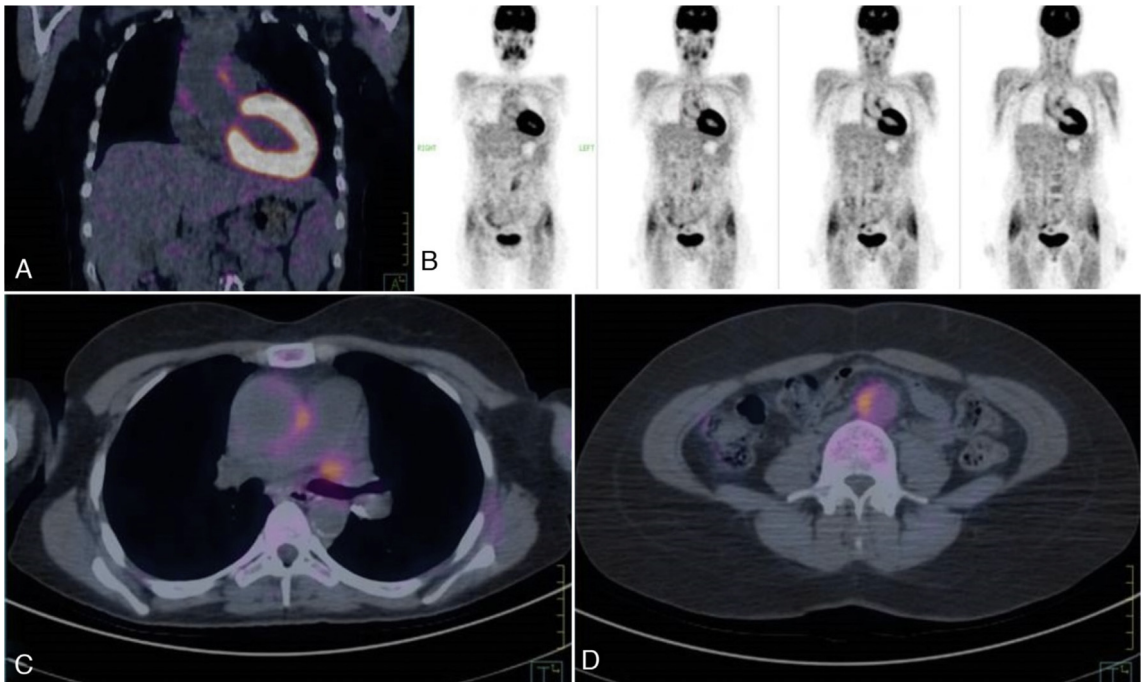

Fig. 3 – Representative PET–CT scan images from a patient with Takayasu arteritis and active disease.
PET–CT scan images of a 32-years-old female patient with clinically active disease: (A) fusion image in coronal reconstruction with ^{18}F-FDG uptake in ascending aorta, (B) images of PET scan in coronal plane with ^{18}F-FDG uptake in the abdominal and ascending aorta; (C) fusion image in axial section showing ^{18}F-FDG uptake in ascending aorta and right pulmonary artery, (D) fusion image in axial section with ^{18}F-FDG uptake in the abdominal aorta.

1.23, (93% and 45%), 1.29 (86% and 50%) and 1.83 (57% and 73%). More TA patients presenting active disease based on NIH criteria had $SUV_{max} \geq 1.3$ on arterial walls when compared to those with $SUV_{max} < 1.3$ ($p = 0.039$) (Table 4). This cutoff value yielded a positive predictive value of 52.0%, a negative predictive value of 78% and an accuracy of 63.8% for active disease in TA. Patients with SUV_{max} values ≥1.3 presented higher TNF-α ($p = 0.015$) and IL-6 levels ($p = 0.036$) in comparison to those with SUV_{max} value <1.3 and lab-controls (Fig. 4A and B). No significant differences were found regarding levels of IL-2, IL-8, IL-12, IL-18, MMP-3, MMP-9, ESR and CRP regarding the SUV_{max} cutoff value of 1.3 (Table 4).

Discussion

This is the first study evaluating the majority of biomarkers of inflammatory activity already reported in the literature, including several cytokines and MMPs as well as ^{18}F-FDG uptake in large arteries on PET–CT scan in TA patients. TA

Table 4 – Comparison of disease activity parameters, cytokines and matrix metalloproteinases in TA patients based on SUV_{max} cutoff value of 1.3.

Variables	$SUV_{max} \geq 1.3$ ($n = 23$)	$SUV_{max} < 1.3$ ($n = 13$)	p
Active disease, n (%)	12 (52.2)	2 (15.4)	0.039[a]
ESR, mm/h	19.00 (5.00–115.00)	14.00 (1.00–70.00)	0.139
CRP, mg/dL	24.56 (0.10–99.05)	5.23 (0.17–82.34)	0.096
TNFα, pg/mL	6.76 (3.56–15.28)	4.37 (1.91–9.23)	0.015[a]
IL-2, pg/mL	0.44 (0.00–23.38)	0.05 (0.00–8.50)	0.484
IL-6, pg/mL	8.48 (0.43–76.22)	4.46 (0.17–37.56)	0.036[a]
IL-8, pg/mL	5.15 (1.88–17.92)	4.53 (2.16–10.56)	0.553
IL-12, pg/mL	0.00 (0.00–46.39)	0.00 (0.00–5.06)	0.130
IL18, pg/mL	164.60 (0.00–424.01)	132.05 (60.95–463.26)	0.987
MMP-3, ng/mL	26.90 (6.00–94.90)	27.10 (7.10–102-70)	0.542
MMP-9, ng/mL	499.80 (183.70–2338.80)	461.60 (283.20–1442.40)	0.564

CRP, C-reactive protein, ESR, erythrocyte sedimentation rate; IL, interleukin; MMP, matrix metalloproteinase; SUV, standard uptake value; TNF, tumor necrosis factor.

[a] Statistically significant values. Continuous data are presented as median and range.

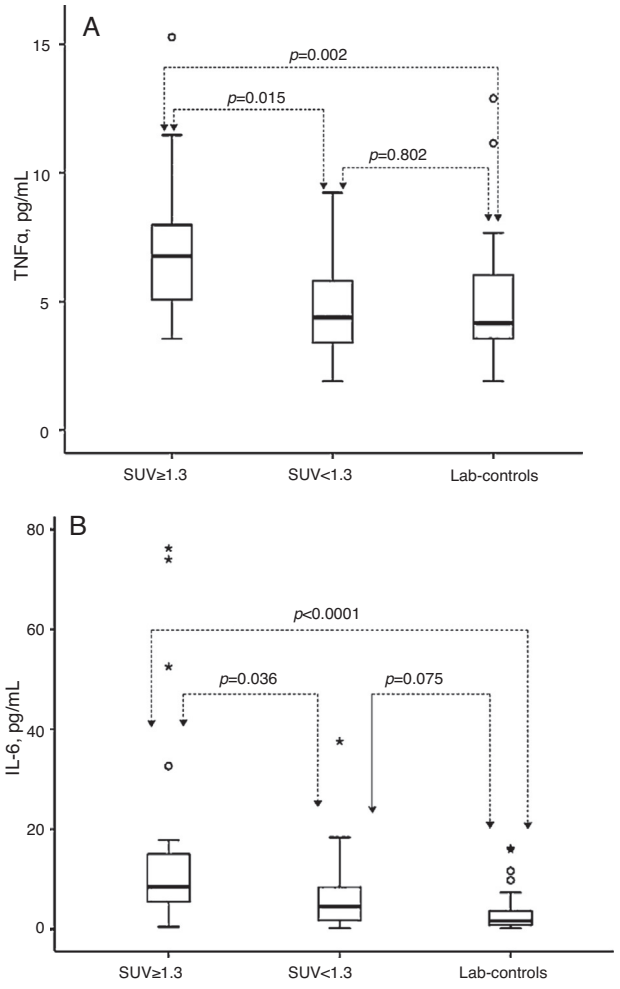

Fig. 4 – Serum TNFα and IL-6 levels in patients with $SUV_{max} \geq 1.3$, patients with $SUV_{max} < 1.3$ and lab-controls. Serum TNFα (A) and IL-6 (B) levels were significantly higher in TA patients presenting $SUV_{max} \geq 1.3$ compared to patients with $SUV_{max} < 1.3$ and lab-controls. No difference could be found regarding serum TNFα and IL-6 levels between patients with $SUV_{max} < 1.3$ and lab-controls.

patients presented higher SUV_{max} values of ^{18}F-FDG uptake on large arteries by PET–CT scans and higher serum levels of IL-6 and MMP-3 than controls. Moreover, SUV_{max} values of ^{18}F-FDG uptake on large arteries, serum IL-6 and TNFα levels were higher in TA patients presenting active disease when compared to those considered with inactive disease. Patients with possible active disease (i.e. only high acute phase reactants without signs and symptoms of active TA) present higher serum IL-6 and IL-18 levels than patients with inactive disease. However, no significant differences were observed regarding SUV_{max} values in patients with possible active disease compared to those with active disease and those with inactive disease.

Although being a rare disorder, TA often causes high morbidity due to the progression of vascular lesions, and to the lack of reliable parameters to detect disease activity to allow an efficacious intervention. Studies evaluating surrogate markers and imaging methods to assess disease activity in TA patients have provided inconsistent results. The reasons for this include the lack of a gold standard to detect disease activity and the inclusion of small number of TA patients in various studies.

Corroborating previous studies[5,6] we found association of serum IL-6 levels with both the disease status and disease activity in TA. Differently from the results of Park et al.,[6] serum TNFα levels were higher in patients with active disease than those in remission but the difference showed only a tendency to be higher among TA patients than in lab-controls ($p = 0.056$). Serum levels of other cytokines were not different between TA patients and controls and they were not useful to differentiate TA patients with active and inactive disease. Although, a previous study had found an association between disease activity and serum levels of IL-18 in TA patients,[6] we could not find significant differences in IL-18 levels between patients and controls, or between TA patients with active and inactive disease. Indeed, we found higher serum IL-18 levels in TA patients with possible active disease compared to controls and this may indicate that those patients may present subclinical inflammation. Similarly to Trypathy et al., we found no differences in serum IL-2 levels between TA patients and controls. Nevertheless, they observed a lower percentage of IL-2 producing CD3+ T-cells in patients with active disease in comparison to patients with inactive disease and controls.[14] The role of IL-12 as a surrogate marker of disease activity in TA is still controversial. In one study, similar levels of serum IL-12 were found between patients and controls while in another study, plasma IL-12 levels were higher in patients with active disease in comparison to those with inactive disease and controls.[6,15] In the present study, IL-12 levels were similar in TA patients and controls and no association with disease activity could be found. Although IL-8 levels were higher in TA patients than in controls and associated with disease activity in one study,[16] we could not find any differences regarding IL-8 levels between patients and controls.

In a single study, higher MMP-3 and MMP-9 levels were associated with active disease in TA patients, with a subsequent decrease following disease control.[7] In our study, only MMP-3 levels were higher in patients than in controls but no difference related to disease activity was found. MMP-3 levels were higher among patients using steroids with a positive correlation between MMP-3 levels and daily prednisone dose. The association between steroid daily dose and MMP-3 levels was also reported in patients with rheumatic diseases,[17] including a recent study in patients with TA.[18] Sharif et al.[19] found a 53% increase in pro-MMP-3 levels in patients with rheumatoid arthritis using prednisone, even amongst patients presenting a decrease in other inflammatory parameters. However, the mechanism involved in MMP-3 elevation associated with steroids use is still unclear.

Initial studies with PET–CT scans evaluating disease activity in TA showed promising results.[10,20] More recently, Karapolat et al. evaluated the usefulness of PET–CT scans to detect disease activity in TA using the NIH criteria, the disease extent index in TA (DEI.TAK) and physician's global assessment as clinical parameters. They found that PET–CT scans had 100% sensitivity and 88.9% specificity with 66% of positive predictive value and 100% negative predictive value

to detect active disease in TA.[21] However, another study has raised questions about the usefulness of this method, since there is no SUV value defined as normal for the vessel wall.[11] A meta-analysis including six studies found that PET–CT scan had a moderate value to evaluate disease activity in TA. The pooled sensitivity and specificity were 70.1% (95% CI: 58.6–80.0) and 77.2% (95% CI: 64.2–87.3), respectively. Authors considered that PET–CT scan was not suitable to be used as the sole method to evaluate disease activity in TA but it may be additional value to current clinical assessment. Heterogeneity among studies is a limitation of this meta-analysis.[22]

Several studies have considered the SUV values useful in the diagnosis and prognosis for cancers.[23–25] Nevertheless, this imaging technique is still a matter of debate as long as a large variability of SUV values has been observed among several institutes, thus raising the need to calibrate equipments and standardize parameters for data acquisition and processing in order to obtain comparable results.[26] SUV_{max} values become more reliable when used as parameters within the same institute, under the same technical conditions, especially in the comparison of serial imaging studies.

In this study, PET–CT scans were performed under fasting conditions and capillary blood glucose was checked before ^{18}F-FDG administration to exclude the possibility of false-negative results due to hyperglycemia. Although, the ^{18}F-FDG in TA might be taken up mostly by inflammatory cells in vessel walls, the influence of blood glucose levels has not been still established in this context.[27] Rabkin et al.[28] have described false-negative results in patients with different types of cancer when blood glucose exceeded 180 mg/dL, but not in patients with infectious or inflammatory conditions.

Henes et al. studied the use of PET–CT in patients with large vessel vasculitis, including three patients with TA and found SUV_{max} values between 1.6 and 6.8 within arterial walls. In this study, SUV_{max} values ranged from 2.5 to 5.8 in TA patients with active disease. Among eight patients with surgically treated solid tumors used as controls, SUV_{max} values ranged from 1.4 to 2.3.[8]

Another study evaluated PET–CT scans in 20 patients with large vessel vasculitis including 3 patients with TA. The SUV_{max} cutoff value of 1.78 had the best performance for the diagnosis of a large vessel vasculitis with sensitivity and specificity of, respectively, 65% and 80% by visual scale, and 90% and 45% by the SUV_{max}. The control group was constituted by patients treated for thyroid cancer.[12]

SUV_{max} values in the above-mentioned studies were slightly higher than what we found for TA patients. Technical differences in PET–CT scan protocols may be a hurdle to compare our results with different studies. Furthermore, our study included only TA patients with a mean age of 36 years whereas in the above-mentioned studies,[8,12] most patients had giant cell arteritis with a higher mean age (62 years). Our control group consisted of healthy subjects, instead of cancer patients. The influence of age in the increase of ^{18}F-FDG has been demonstrated in one retrospective study[29] and it may occur in part due to atherosclerosis. Indeed, this fact might reduce PET–CT scans specificity in TA patients, since the prevalence of atherosclerosis is increased in those patients.[30]

Arnaud et al. found low sensitivity and specificity of PET–CT scan to detect disease activity in TA patients. They did not find any association with either inflammatory markers or new lesions on serial MRI angiographies. They used visual scale and quantitative evaluation to assess ^{18}F-FDG uptake and found a strong correlation between both methods.[11] In contrast, Lee et al. have also evaluated the PET–CT scans in TA patients using the visual scale and SUV. They found an association between ^{18}F-FDG uptake in vessel wall and disease activity in both assessments.[31]

Using the SUV_{max} to measure ^{18}F-FDG uptake, we found higher values of SUV_{max} in TA patients with active disease compared to those with inactive disease. Hence in our study, SUV_{max} seemed to be predictive of disease activity in TA. Patients using immunosuppressive agents had lower arterial SUV_{max} values comparing with those without these medications. This finding indicates a suppressive effect on vascular inflammation brought by the use of these agents and adds another argument in favor of the usefulness of PET–CT scans for the detection of the vascular inflammation in TA. In line with this finding, Lee et al.[31] have described a decrease in ^{18}F-FDG uptake during the follow-up of eight patients after controlling disease activity with immunosuppressive treatment.

We found SUV_{max} values similar to those found by Kobayashi et al., despite technical differences in study protocols. The cutoff value of 1.3 was the same in both studies and was based on the highest SUV_{max} value found in controls. However, the sensitivity and specificity for detection of disease activity were 86% and 50%, respectively, in our study, which are lower than those found by these authors. This remarkable difference may be due to different criteria used for clinical evaluation in both studies. They considered clinical remission only when patients were at least 2 years without steroid.[10]

Our study also analyzed the association between a cutoff value of SUV_{max} and serum levels of cytokines and MMPs. Patients with $SUV_{max} \geq 1.3$ had higher levels of TNF-α and IL-6. We also found an association between $SUV_{max} \geq 1.3$ and disease activity. The analysis of biomarkers using this SUV_{max} cutoff value highlights the consistent results achieved from clinical evaluation of disease activity in TA. The significant correlation between serum IL-6 levels and other inflammatory markers indicates the consistency of these findings.

Main limitations of this study include the sample size, the cross-sectional design and the lack of a gold standard to evaluate inflammatory activity in TA, since even the widely used NIH criteria have not been validated yet. Moreover, the weakness of the NIH criteria is highlighted by the fact that TA patients with high acute phase reactants who do not fulfill the NIH criteria for active disease actually present higher serum levels of IL-6 and IL-18 than TA patients considered with inactive disease. Such patients should be followed up more carefully in order to detect clinically significant active disease early. However, the low number of patients in the subgroups of inactive patients and patients with possible active disease may have impaired comparisons among groups and assumptions, especially regarding IL-18 levels should be taken with caution.

Our results suggest that the intensity of ^{18}F-FDG uptake in arterial wall may reflect the intensity of the inflammatory

process and that PET–CT scan seems to be a useful method to detect disease activity in TA, especially to exclude active disease in doubtful cases. A prospective study with a long term follow-up would be necessary to observe changes in the SUV_{max} values after treatment or even to detect the development of new vascular lesions (e.g. vascular stenosis or aneurysms) in sites presenting high ^{18}F-FDG uptake. IL-6 also appears to be a promising parameter of disease activity in TA that should be assessed in a longitudinal study as well. The relevance of IL-6 resides in the fact that it can also be a potential therapeutic target in TA.[32]

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Conflicts of interest

The authors declare no conflicts of interest.

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