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IMAGING AND SEROLOGICAL PROFILING OF PATIENTS WITH POLYMYALGIA RHEUMATICA AND GIANT CELL ARTERITIS

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ABSTRACT

Background: Polymyalgia rheumatica (PMR) and giant cell arteritis (GCA) are two inflammatory conditions affecting people aged over 50 years. PMR is characterized by pain and stiffness in the shoulder and hip girdles. GCA, a large vessel vasculitis, is the most common form of primary systemic vasculitis. About 40-60% of patients with GCA present with concomitant PMR, and histologic features consistent with GCA can be detected on temporal artery biopsy of about 16% to 21% of patients with PMR. It is still debated whether PMR and GCA are different conditions or represent different clinical manifestations across the spectrum of a single disease. The aim of this research project was to profile immunological and imaging aspects of these two conditions to better characterize their similarities and differences.

Patients and methods: A cohort of unselected, consecutive patients with PMR, GCA or both was studied. PMR was diagnosed according to Bird et al. criteria, whereas patients with cranial (C)-GCA were diagnosed according to the 1990 ACR classification criteria; a subset of these patients underwent temporal artery biopsy. Five further patients with fever of unknown origin (FUO) and imaging evidence of large vessel vasculitis (LVV) were included. All patients underwent a detailed and standardized clinical examination and, subsequently, a 18F-Fluorodeoxyglucose (FDG) positron emission tomography (PET) scan.. Joint and vascular uptake were evaluated by a qualitative visual score, using the liver uptake as a reference, and with semi-quantitative mean standardized uptake value (SUV). Each value of the qualitative joint and vascular scores of every region were summed up to obtain a total joint score (TJS) and a total vascular score (TVS). In a subgroup of patients, serum samples were collected just before the injection of FDG on the same day of the PET scan. The soluble (s) immune

checkpoints cytotoxic T-lymphocyte antigen-4 (CTLA-4), soluble programmed death-1 (sPD-1) and programmed death-ligand 1 and 2 (PD-L1 and PD-L2) were measured in this subgroup. The serum of fifty healthy controls were studied for comparison.

Results: One hundred and thirty-one patients underwent FDG-PET/CT scanning, including 89 females and 42 males, with a median age of 74 years (range 47-92). Ninety-seven patients were diagnosed as PMR, 13 as C-GCA, 16 with both PMR and C-GCA and five patients presented with FUO.

Soluble CTLA-4, sPD1, sPD-L1, and sPD-L2, evaluated in 40 patients (32 with PMR and 8 with PMR+C-GCA), were increased in comparison with controls ($p<0.001$ for all the comparisons), although no statistically significant difference between patients with PMR+C-GCA and those with isolated PMR was found.

Conclusions: Patients with PMR and GCA share many immunological and imaging abnormalities. Results from this study demonstrate that available and evaluated biomarkers are unable to precisely differentiate these two conditions.

1. INTRODUCTION and BACKGROUND

Polymyalgia rheumatica (PMR) is an inflammatory disease of the elderly, characterized by aching and stiffness in the scapular and pelvic girdles[1,2]. Giant cell arteritis (GCA) is the most frequent systemic vasculitis in the adult over fifty years of age, involving large and medium size vessels. The hallmark of these two diseases is a strong inflammatory response and both occur in people of the same age range as well as ethnical and geographic groups.

About 40-60% of patients with GCA present concomitant PMR, and histologic features consistent with GCA can be detected on temporal artery biopsy in about 16% to 21% of patients with PMR[3]. As a consequence, some authors have hypothesized that PMR and GCA could be different expressions of the same disease[4].

Large vessel vasculitis (LVV) is a generic term used to define the presence of inflammation in the aorta and its major branches. According to the 2012 Revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides[5], LVV "*is vasculitis that affects large arteries more often than do other vasculitides*". The two main diseases in this category are GCA and Takayasu arteritis, which are considered very similar in terms of histology and are classically differentiated by age of onset, although recent studies have underlined more similarities between these two diseases than previously thought[6]. LVV however is an umbrella term, under which other diseases are also included. In this group, one emerging entity is the so-called "idiopathic aortitis", also referred to as "isolated aortitis", defined as the presence of pathologic inflammation in aortic segments (detected by imaging techniques or surgical

pathology), without clear clinical evidence of a systemic rheumatologic disease to account for the vasculitis[7].

Imaging studies, especially with 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET), have shown the presence of LVV in up to 30-40% of patients with apparently isolated PMR[8] and in 70-80% of patients with GCA[9], although these figures are strongly influenced by the techniques used to interpret imaging results[9–11]. The detection of an otherwise occult LVV is a crucial issue, because LVV could be related to the development of aneurysms[12,13] and ischaemic complications. Nevertheless, clinical or laboratory features, predictive of the presence of LVV in patients with PMR or GCA, are not yet available[8,14].

LVV is increasingly clinically recognized, mostly because of the evolution and wider utilization of advanced imaging techniques. However, defining the clinical prevalence of LVV is difficult, because many clinical characteristics of vasculitis, such as fever, weight loss and malaise are nonspecific and difficult to attribute to a rheumatologic disease, especially in non-specialized settings[15]. In routine clinical practice, and in specialty tertiary centres, patients with isolated LVV are generally classified as having either GCA or Takayasu arteritis[16], but there is a real possibility that these patients constitute a different nosologic group. In fact, patients with isolated aortitis seem to be younger than patients with GCA[16,17] and more susceptible to aortic damage requiring surgery[17].

The purpose of the investigations forming the basis of this thesis is to examine the relationship between PMR, GCA and LVV, in order to understand if they are separate entities or are in the same disease spectrum.

2. ROLE OF POSITRON EMISSION TOMOGRAPHY (PET)

2.1. PET and polymyalgia rheumatica

The first paper on the use of PET in PMR patients was published by Blockmans et al, in 1999[18]. In four out of five patients with PMR without clinical signs of GCA, they found increased FDG uptake in at least one vascular district, among arteries of thorax, upper and lower limbs. The only patient with PMR without vascular uptake had a lower C-reactive protein (CRP) than those with uptake. Among the 6 patients with biopsy proven GCA, only 1 did not show vascular uptake. The control group comprised 23 patients with various diseases, including leukaemia, rheumatoid arthritis, monoclonal gammopathy or unspecified diagnosis. In the control group, 8/23 patients had increased vascular uptake, which in two of them was thought possibly related to atherosclerosis. The most inflammation-specific site was the thoracic aorta, with only 1/23 controls showing uptake in that district. Interestingly, the only patient with biopsy-proven GCA but no FDG-uptake suffered from diffuse atherosclerosis, although the diagnostic criteria used to ascertain it were not clear. The four PMR patients underwent a second PET scan after glucocorticoid treatment, when symptoms disappeared and inflammatory parameters returned to normal, showing a clearly decreased vascular uptake[18].

The same authors subsequently increased the number of observations studying 25 patients with GCA or PMR, of whom 13 had biopsy proven temporal arteritis and 12 had negative biopsy[19]. PMR was diagnosed according to Healey and Hunder criteria. Vascular FDG uptake was described in 10 of these patients with GCA (76.9%), in nine patients with PMR (75%) and in 10 out of 44 controls (22.7%) who suffered from other diseases such as infections, rheumatoid arthritis or small vessel vasculitis. Among the patients with PMR or GCA who

did not show FDG vascular uptake, four out of six had marked uptake in the shoulders. Uptake in the large joints was seen in 12 of 25 (48%) patients with PMR or GCA, as well as in 14 of 44 (31.8%) controls, some of whom had diagnosed arthritis. Thoracic vascular FDG uptake had a sensitivity of 56% for the diagnosis of GCA or PMR, a specificity of 98% and a positive predictive value of 93%. A negative scan had a negative predictive value of 80%. Vascular FDG uptake in the legs had a slightly greater sensitivity of 64% but a lower specificity of 77%.

Moosig et al.[20] used FDG-PET evaluated 13 patients with a diagnosis of PMR according to the Chuang et al criteria. A group of six patients with inflammatory diseases other than PMR served as controls. Three of the patients with PMR had presented with concomitant GCA, one had a negative biopsy and the remaining 9 had no clinical symptoms nor duplex-ultrasound alterations suggestive of temporal arteritis. The PET scans were evaluated visually and also by placing a region of interest (ROI) on 9 vascular regions, including the ascending thoracic aorta, descending thoracic aorta, abdominal aorta, right and left subclavian arteries, right and left external carotid arteries, and the right and left common iliac arteries. An index was calculated by dividing the value of every ROI with that of a peripheral region of the lung. All 12 patients showed increased tracer uptake of the aorta or its major branches compared with the controls. However, the control subjects were few and significantly younger than the patients with PMR, a fact that could have biased the results.

The mean ROI index for all nine regions was 1.58±0.37 in the PMR group and 0.93±0.12 in controls (p=0.001). Among the various vascular areas evaluated, the locations best discriminating between active PMR and controls were the

subclavian and external carotid arteries. Eight patients underwent a second PET scan after three months of high dose glucocorticoid treatment, while they were in partial or complete remission. In this group, the uptake decreased from a ROI index of 1.50±0.16 to 1.09±0.08 (p=0.001). However, the uptake in the subclavian arteries remained higher in patients in remission than in controls. In patients with PMR, there was a strong correlation between CRP, erythrocyte sedimentation rate (ESR) and platelet count with the intensity of vascular uptake. In a more recent study by Blockmans et al.[21], which included some of the patients reported in the previous studies, FDG-PET was performed in 35 patients with isolated PMR, diagnosed according to Healey and Chuang criteria and after excluding GCA by temporal biopsy, obtained in 30 of 35 patients. To increase specificity, patients with visual disturbance, headache or jaw claudication were excluded.

Two different nuclear medicine specialists scored the FDG uptake in seven vascular regions (thoracic aorta, abdominal aorta, subclavian arteries, axillary arteries, carotid arteries, iliac arteries and femoral arteries) as negative (0) or positive, and further scored the uptake semi-quantitatively as 1 (minimal but not negligible FDG uptake), 2 (clearly increased FDG uptake) or 3 (very marked FDG uptake). Then they calculated a 'total vascular score' (TVS) ranging from 0 (no vascular FDG uptake in any of the seven vascular regions) to 21 (vascular FDG uptake scored 3 in all seven territories). Both subclavian, axillary, carotid, iliac and femoral arteries were counted as one vascular region; when the score differed from right to left artery, the highest score was taken for that vascular region. FDG uptake in the shoulder and hip regions and in the spinous processes

of the vertebrae was scored as 0 (no uptake), 1 (moderate uptake) or 2 (intense uptake).

At the first PET scan, 11/35 (31.4%) patients had vascular uptake with mean TVS of 0.8±1.7. Increased uptake was noted in the subclavian arteries in 10 patients, in the thoracic aorta in four, in the axillary arteries, the abdominal aorta and the iliac arteries in two each, and in the femoral and carotid arteries in one each. Thirty-three out of 35 (93.4%) patients had FDG uptake in the shoulders with a mean score of 1.7±0.6.

FDG uptake was seen in the spinous processes (mean score 0.6±0.7) of the lumbar vertebrae in 15 patients, at the dorsal level in seven patients (of whom five had also an increased FDG uptake in the spinous processes of the lumbar spine) and in the cervical spine in three patients (of whom two also had uptake at the lumbar level). At follow up, vascular and joint uptake persisted, although diminished, in most patients, especially at six months. Interestingly, the intensity of uptake did not predict the occurrence of PMR relapses, although the power of the study was limited by the fact that only a few patients completed it[22].

Similar findings, although with different frequency and severity, were also observed in a study on patients with GCA[23]. Indeed, twenty-nine of 35 (82.9%) patients with GCA had FDG uptake at least in one district, with a mean TVS of 6.0 ± 6.2. Eleven of 35 (31.4%) patients showed FDG uptake in the shoulders with a score ≥2, and 17 (48.6%) with a score of 1.

In a short report, three out of eight patients with glucocorticoid-resistant PMR investigated with FDG-PET were found to be affected by LVV[14]. This percentage, which is not different from that found in untreated PMR patients, suggests that the low-dose glucocorticoid therapy, which is usually given to

patients with PMR, might be not sufficient for the treatment of concomitant LVV. Yamashita et al.[24] evaluated 14 patients with PMR with FDG-PET/CT and 17 patients with other rheumatic diseases (mainly rheumatoid arthritis). They found that the intensity of FDG uptake in ischial tuberosities, greater trochanters and spinous processes was higher in patients with PMR, whereas wrists and hips showed higher uptake in controls. Shoulders, elbows and sternoclavicular joint did not differ significantly between the two groups.

2.2. PET and giant cell arteritis

GCA is also known as Horton's or temporal arteritis, for its peculiar involvement of the superficial temporal arteries. Although autopsy studies demonstrated the presence of large vessel involvement in patients with PMR and GCA[25,26], the first descriptions of large vessel uptake detected by FDG-PET in patients with fever of unknown origin (FUO) appeared in the late 1990's[18,27]. The exact prevalence of large vessel involvement in GCA is controversial, and depends on the selection of the patients, disease duration, ongoing treatment, and the technique used to evaluate large vessels. Regarding FDG-PET/CT, one critical issue is the definition of vasculitis, since an accepted definition of pathological large vessel uptake is still lacking[28,29]. Several scores have been proposed for the evaluation of large vessel uptake, both qualitative and semi-quantitative, but a definitive consensus has not yet been reached[9].

In 2003, Meller et al. evaluated 15 patients with FDG-PET and introduced one of the first and most widely used methods for grading large vessel uptake, based on a visual score[30]. They found increased uptake in 59 of the 104 (56%) arterial districts studied. Fourteen of these patients also underwent (magnetic resonance imaging (MRI); pathologic alterations were detected in 13 of these patients in at

least one arterial site. Fourty-seven vascular regions out of the 76 (61.8%) evaluated with both PET and MRI were concordantly positive or negative with both techniques.

Using a visual score ≥2 as positive, the presence of large vessel involvement was reported in 15 out of 26 (58%) patients with GCA or TAK by Walter et al.[31], in 29 out of 35 (83%) patients with GCA by Blockmans et al.[23], and in 20 out of 25 (80%) patients with GCA by Both et al.[32]. In the latter study, all patients additionally underwent thoracic MRI[32]; clinical and serological parameters showed a weak correlation with FDG-PET findings, whereas no significant correlation was found with MRI[32].

In a retrospective study[33], 62 out of 304 (20%) of PET scans performed on the basis of clinical suspicion of LVV were positive for large vessel uptake. In comparison with patients with a negative scan, those with a positive PET were more frequently female, older, received a previous diagnosis of temporal arteritis, suffered less frequently from arthralgia and presented with higher levels of thrombocytes and ESR.

Prieto-González et al.[34], in a well-designed study, showed that maximum standardised uptake values (SUV) of all arteries studied in 71 GCA patients were higher than those of 20 controls without systemic inflammatory diseases, and that SUV correlated with inflammatory markers.

Stellingwerff et al.[11] retrospectively reviewed 18 patients with GCA and evaluated their PET scans. They used two qualitative visual scoring methods and four semi-quantitative methods and reported that the best performance of the score for presence of vasculitis was when vascular uptake was higher than liver uptake using the aorta-to-liver ratio with a cut-off of 1.03. In both cases,

diagnostic accuracy increased when patients taking glucocorticoids were excluded.

It is well known in clinical practice that some patients whose onset is typical of PMR relapse with symptoms of GCA[35], underscoring the strong relationship between these two disease entities.

There is increasing evidence of the existence of two different patterns of GCA. Cranial disease is characterized by typical symptoms such as headache, jaw claudication and visual disturbances. The other pattern is dominated by large vessel involvement, in which signs of vascular insufficiency, aneurysms/stenosis and polymyalgia rheumatica are prevalent[36–38]. In clinical practice, these presentations may be mixed with different nuances. The 1990 American College of Rheumatology criteria, although intended for classification and not for diagnostic purposes, completely neglected the presence of LVV[39]. The Diagnostic and Classification Criteria in Vasculitis Study (DCVAS) will soon provide new classification criteria for LVV, taking into account how the tremendous technological progress in imaging techniques has changed our understanding of LVV[40].

2.3. PET and large vessel vasculitis

In the 2012 Chapel Hill Consensus Conference nomenclature scheme, LVV was defined as vasculitis that involves large arteries more often than other vasculitides, and include GCA and TAK as the two main entities of LVV[5]. However, the involvement of large vessels, especially the aorta, by inflammatory processes is seen in a wide variety of conditions, including rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, relapsing polychondritis,

Cogan syndrome, Behçet's disease and immunoglobulin G4-related disease (IgG4-RD)[7].

IgG4-RD can present with lymphoplasmacytic aortitis and can exclusively involve the adventitia, as a periaortitis. This condition can affect the aorta and other large and medium size vessels. FDG-PET can show the protean manifestations of IgG4-RD, which range from vascular to lymphatic and other glands involvement. Indeed, virtually every organ can constitute a target[41–43].

One major issue of published literature on FDG-PET in LVV is the mixture of patients with GCA and TAK in the same cohort. While there is some evidence to suggest that these two conditions could be different manifestations of the same disease[44], other authors highlight significant differences that justify classifying them as separate disease entities[45].

The major problem in applying imaging techniques currently in use for evaluation of LVV is the absence of a pathologic confirmation of the underlying disease. In fact, every study addressing sensitivity and specificity of MRI or FDG-PET is based on clinical diagnosis, or in some cases on histology of temporal arteries. A systematic histologic description of vessels which show uptake with FDG-PET is lacking, hampering our confidence in published results, especially in patients with a mild grade vasculitis. Few cases with pathologic description of FDG large vessel uptake have been published. In one of them, labelled as GCA, a markedly thickened aortic wall and extensive multifocal perivascular chronic inflammation, composed mainly of lymphocytes and plasma cells was seen at surgical pathology, findings that could also be consistent with IgG4-RD[7].

In 2018, the European League against Rheumatism (EULAR) published evidence-based recommendations for the use of imaging in LVV[46]. An early imaging test is suggested in patients with suspected GCA to confirm the diagnosis. Ultrasound is the first-choice imaging technique to detect cranial arteries involvement, whereas PET and MRI are best suitable for the assessment of aortic and large-vessel involvement.

2.4. PET and fever of unknown origin

The key to identifying the correct diagnosis in patients with fever of unknown origin (FUO) is the clinical history and examination, that, if carefully and repeatedly conducted, can successfully lead to the correct diagnosis in up to one-third of patients when combined with laboratory testing[47]. FDG-PET can identify the most frequent causes of FUO (i.e. neoplasms, infections, inflammatory diseases). Nevertheless, in up to 10-40% of patients with FUO a final diagnosis is lacking[48,49]. An emerging entity is the "inflammation of unknown origin" (IUO), defined as an increase of CRP or of ESR, in patients presenting with nonspecific signs and symptoms including fatigue, malaise, weight loss, anorexia and night sweats in whom a diagnosis is not reached after conventional diagnostic procedures[50]. These features are in many cases the presentation of patients with vasculitis. Whereas substantial literature has been published on FUO, few papers have analysed the characteristics of patients with IUO, although one paper found striking similarities with FUO[50], suggesting adoption of the same diagnostic workup for both conditions.

FDG-PET/CT has been proposed as a fundamental step, although not in the early stage, of diagnostic work-up of patients with inflammation and fever of unknown

origin. Although expensive, it could ultimately be cost-effective, sparing time and other diagnostic resources[51].

In a cohort of 240 patients with FUO and IUO[52], fifteen percent of those with FUO received a final diagnosis of adult-onset Still's disease, whereas LVV and PMR accounted for the final diagnosis in 21% and 18.3% of cases with IUO, respectively. In 136 (57%) patients of the total cohort, FDG-PET/CT was considered helpful in reaching the final diagnosis[52].

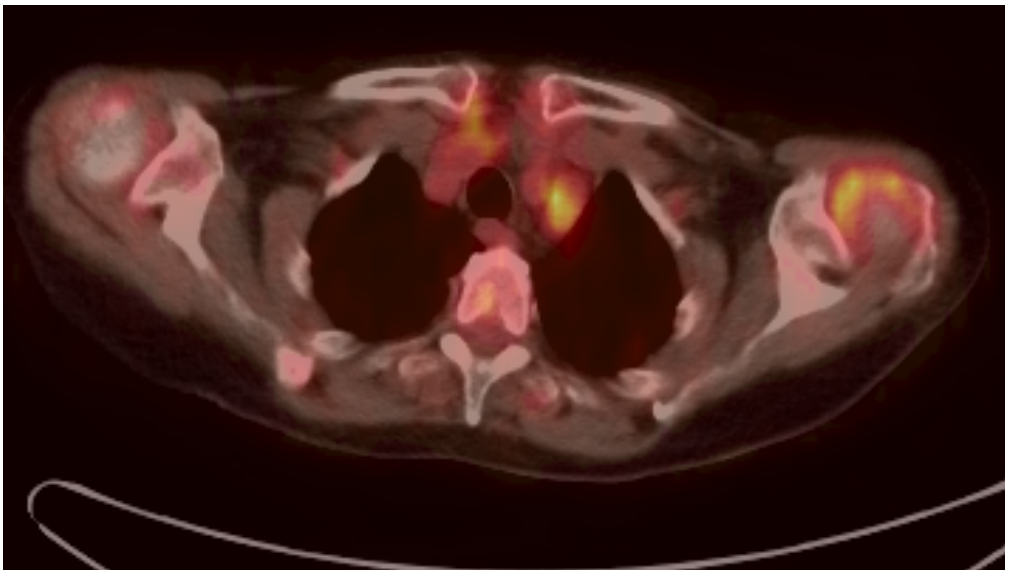

Figure 1. PET/CT: transaxial view at the shoulder girdle level in a patient diagnosed as polymyalgia rheumatica. Note the intense uptake of gleno-humeral and sterno-clavicular joints and of the origin of the left subclavian artery.

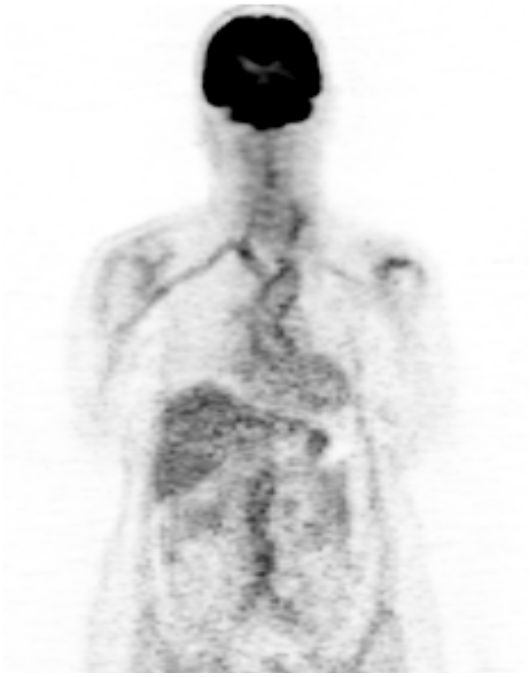

Figure 2. PET, coronal reconstruction. Same patient as figure 1. Note the intense uptake in aortic arch, subclavian and carotid arteries in addition to that of shoulders.

3. IMMUNE CHECKPOINTS

The immune system is fine-tuned to achieve a balance between excessive activation, which may lead to self-damage due to uncontrolled inflammation, and excessive inhibition, which may lead to an unopposed proliferation of pathogens and tumour cells. Immune checkpoints are a group of receptors that inhibit the activation of immune cells. They comprise the anti-cytotoxic T lymphocyte antigen-4 (CTLA-4) and the programmed cell death protein-1 (PD-1)[53,54].

CTLA-4 is a member of the immunoglobulin superfamily that is expressed on the surface of lymphocytes and acts as an immune checkpoint. It is involved in the costimulatory pathways of T cells, competing with CD28 in the binding with CD80/86 and thus exerting inhibitory effects on the immune system[55]. CTLA-4 has been shown to play a role both in neoplastic[56,57] and autoimmune diseases[58]. A soluble form of the CTLA-4 molecule, resulting from alternative splicing, has been identified. Soluble CTLA-4 retains the ability to bind its physiological ligands and may exert an immunomodulatory function via competitive binding. Serum soluble CTLA-4 levels have been reported to be markedly increased in several autoimmune diseases[58].

PD-1 is expressed on the surface of T cells, B cells and monocytes[59]. T cells activation causes an up-regulation of PD-1, which is crucial for the termination of the immune response. PD-L1 and PD-L2, the ligands of PD-1, are expressed on antigen presenting cells and other non-hematopoietic cells, including vascular endothelial cells[59]. Soluble forms of PD-1 and PD-L1 have been described as well[60,61]. The binding of the sPD-1 to the membrane-bound form may have an immunostimulatory effect, preventing the connection with PD-L1 and therefore

blocking the transduction of the inhibitory message[62]. An up-regulation of the PD-1/PD-L1 pathway is present in rheumatoid arthritis[63]. Knock-out mice for the PD-1 gene showed an increased susceptibility to the induction of collagen-induced arthritis, but this was reversed with a treatment with PD-L1Fc[63].

James P. Allison demonstrated in 1996 that anti-CTLA-4 antibodies enhanced the immune response against tumour in mice[64]. Tasuku Honjo isolated the PD-1 gene, demonstrating its role in the programmed cell death[65], and showed that the ligand of PD-1 (PD-L1), a member of the B7 gene family, can inhibit T lymphocyte proliferation via the binding with PD-1[66]. In 2018, the Nobel prize in Medicine was awarded to Allison and Honjo for their pivotal discovery of cancer therapy based on the inhibition of the negative regulator of the immune system.

Ipilimumab is a fully human monoclonal antibody against CTLA-4 which "enhances" T lymphocytes activation through the increased binding of CD 80/86 with CD28. It is licensed for the treatment of metastatic melanoma and metastatic renal cell carcinoma[56]. A number of drug-related or drug-induced immune adverse events have been reported, as a consequence of the growing use of immune checkpoint inhibitors, targeting CTLA-4 and PD-1[67]. Involvement of CTLA-4 and its soluble form has been demonstrated in several autoimmune diseases[58,68].

The rationale for studying the role of immune checkpoints in PMR and GCA is provided by the anecdotal evidence of drug-induced PMR/GCA in patients treated with ipilimumab[69,70] and other checkpoint inhibitors[71,72]. Moreover, there is evidence that temporal artery specimens from patients with active GCA show low levels of PD-L1 transcripts and high levels of PD-1 transcripts[73]. On the

contrary, arteries from healthy donors showed high levels of PD-L1 transcripts but almost complete absence of PD-1 transcripts, indicating lack of T cells in the wall of normal vessel. The use of an anti-PD-1 antibody in a mouse model of GCA increased vascular inflammation and T cell infiltration[73]. These findings suggest a deficiency of the immune checkpoint activity in GCA[74], a hypothesis that is supported by cases of immune checkpoint inhibitor-induced vasculitides[75].

4. THE STUDY

4.1. Objectives

The objectives of the present study were:

1. to evaluate via FDG-PET/CT patients with PMR, GCA, the association of both of them, and FUO; to compare their pattern of uptake; and to correlate their clinical characteristics with imaging findings
2. to analyse the soluble checkpoint inhibitors in patients with PMR and GCA and to test whether these biomarkers can be used to identify patients with GCA and/or LVV
3. to provide insight as to whether these conditions can be considered as different entities or as a continuum within the same disease or syndrome.

4.2. Patients and methods

4.2.1. Inclusion criteria

All patients were recruited from January 2009 to April 2016. Patients with PMR were diagnosed on the basis of Bird et al. criteria[76], patients with cranial (C)-GCA according to the 1990 American College of Rheumatology criteria[77], and patients with FUO according to Durack and Street criteria[48]. More details are provided in the appendix.

4.2.2. Clinical and laboratory assessment

All patients underwent a careful clinical history and subsequent standardized physical examination. Disease duration, morning stiffness, presence of fever, weight loss, headache, jaw claudication, visual disturbance, spontaneous pain in the girdles and in the spine and previous glucocorticoid therapy were recorded. Clinical examination included pain on application of digital pressure at the long

head of the biceps (LHB), the sub-acromial area (SA), the ischiatic (IB) and trochanteric (TB) bursae, the sacro-iliac joints, and on mobilization of the shoulder and hip girdles, recorded in a dichotomous manner as “presence” or “absence” of pain. Also assessed were degree of arm elevation, presence of peripheral arthritis, abnormalities of temporal arteries and presence of vessel murmurs and of limb claudication. All sites of spontaneous or provoked pain (“total sites of pain”, TSP) were summed. CRP, ESR and blood cell count were obtained for all patients. In patients with FUO, a diagnostic work-up was carried out to exclude the main causes of fever, including obtaining of blood cultures, screening for neoplastic diseases, etc. on the basis of the underlying clinical picture.

4.2.3. PET/CT acquisition

FDG-PET/CT was performed in all patients. After a minimum of 6 hour fasting, a dose of 4.8-5.2 MBq of F18-FDG per kilogram body weight was injected through a peripheral vein catheter. Patients were placed in a quiet room and instructed remain still. Data acquisition started ≥60 minutes after intravenous administration of 18F-FDG. Patients underwent simultaneous FDG-PET and computed tomography (CT) imaging from the skull base to the thighs using an integrated PET/CT scanner (Hirez; Siemens Medical Solutions, Knoxville TN, USA). In some patients, the scan included also legs and feet, if they seemed clinically involved. PET raw data were reconstructed by means of ordered subset expectation maximization and attenuation correction was performed using the CT raw data. The entire CT dataset was fused with the 3-dimensional PET images using an integrated software interface (Syngo Image Fusion; Siemens

Erlangen, Germany) to create anatomical images superimposed with FDG uptake.

4.2.4. Image analysis

Joint and vascular uptake was scored both semi-quantitatively with a visual score and quantitatively. Regions of interest (ROIs) were placed on the anatomic CT images to identify four aortic segments (ascending, arch, descending and abdominal), subclavian arteries, common carotid arteries, iliac and femoral arteries; ROIs were drawn on the theoretical vessel wall (figure 3) to exclude the uptake of the blood inside the vessel lumen. A further region was drawn within the left ventricular chamber using the PET image to estimate the tracer concentration in the arterial blood (blood-pool, BP). To assess joint metabolism CT-based ROIs were bilaterally drawn on the gleno-humeral, sterno-clavicular, and coxo-femoral joints, and on the trochanteric and ischiatic bursae (figure 4). Arterial FDG uptake was quantified by calculating the mean standardized uptake value (SUV) within each ROI. To take into account the contribution of FDG activity in the blood, results were expressed as the ratio between mean SUV value of each ROI and blood-pool ROI (SUV/BP), expressing true arterial wall metabolic activity; joint FDG uptake was considered without BP ratio.

To evaluate the presence and extent of atherosclerosis, total arterial calcium load (ACL) was also estimated in the same arterial segments. For this purpose, calcium density was graded according to a semi-quantitative 5 point scale based on percentage of calcification of the arterial ring documented in the trans-axial CT views: 0=no calcific deposits; 1=0-25%; 2=25-50%; 3=50-75% and 4=75-100%, with a possible score ranging between 0 and 48.

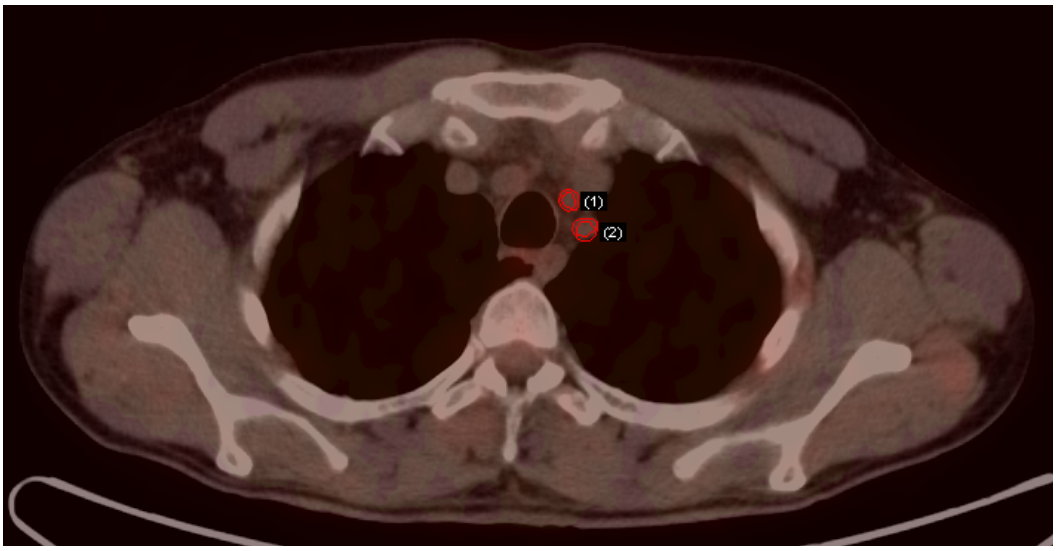

Figure 3. PET/CT, transaxial view. Vascular ROIs drawn on the left carotid and subclavian arteries.

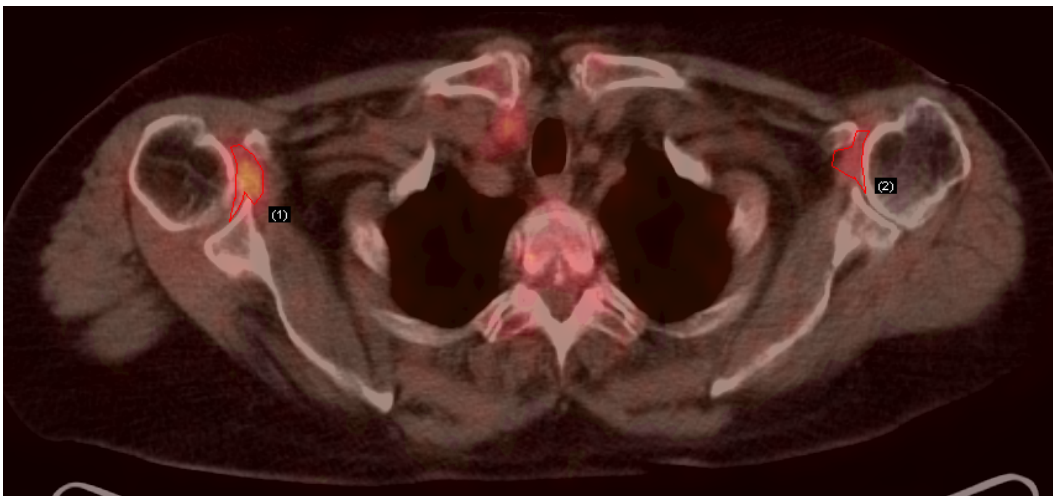

Figure 4. PET/CT, transaxial view. Articular ROIs drawn on both gleno-humeral joint spaces.

Arterial and joint uptake was visually graded using a four-point scale, as proposed by Walter et al.[31]: 0=no uptake present, 1=uptake present but lower than liver uptake, 2=similar to liver uptake, 3=uptake higher than liver uptake. To determine the prevalence of each finding, these scores were further

subdivided as “negative” (0 and 1) and “positive” (2 and 3) (figure 5). For each patient, the sum of the four-point score of vascular and joint uptake was recorded as total visual vascular score (TVS), with a maximum score of 36, and total visual joint score (TJS), also taking into account uptake in cervical and lumbar interspinous bursae, with a maximum score of 36.

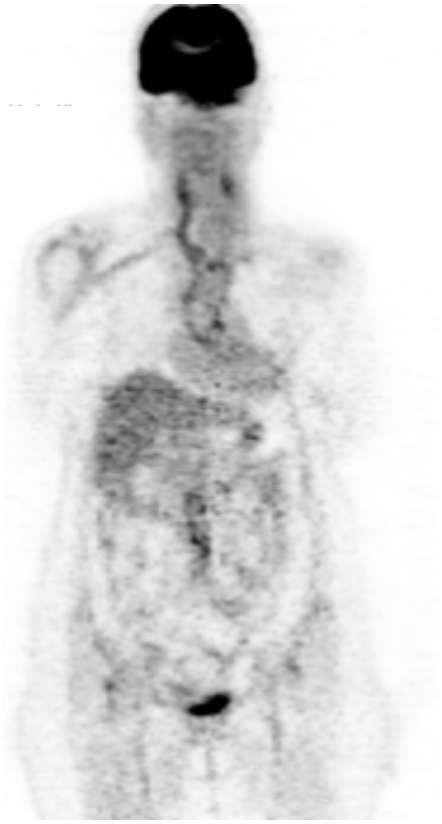

Figure 5. PET, coronal reconstruction. Grade 3 uptake of the right and left carotid arteries, of the ascending aorta and of the aortic arch (uptake is higher than that of the liver). Right subclavian artery shows a grade 2 uptake (left subclavian artery is not displayed in this slice).

4.2.5. Laboratory analysis

In a subgroup of 40 patients, serum samples were collected the same day of the PET scan, before the infusion of FDG. Serum sCTLA-4, sPD-1, sPD-L1 and sPD-L2 were measured by ELISA (EMELCA Bioscience,

www.emelcabio.com) according to the manufacturer's instructions. Each sample was diluted 1:10 and tested in duplicate. Deviation between duplicates was <10% for any reported value and the detection limit of the assay was 0.1 ng/ml. The analytical response was linear between 0.162 and 1.200 of absorbance values (corresponding to 0.1 - 50 ng/ml) as assessed by serial dilution test using a strongly positive serum sample. Intra-assay Precision (Precision within an assay) was <8%, whereas Inter-assay Precision (Precision between assays) was <6%.

Serum sPD-1 was measured by ELISA (EMELCA Bioscience, www.emelcabio.com) according to the manufacturer's instructions. Each sample was diluted 1:10 and tested in duplicate. Deviation between duplicates was <10% for any reported value and the detection limit of the assay was 0.156 ng/ml. The analytical response was linear between 0.162 and 3.000 of absorbance values (corresponding to 0.156 - 10 ng/ml) as assessed by serial dilution test using a strongly positive serum sample. Intra-assay Precision (Precision within an assay) was <10%, whereas Inter-assay Precision (Precision between assays) was 12%.

Serum sPD-L1 was measured by ELISA (EMELCA Bioscience, www.emelcabio.com) according to the manufacturer's instructions. Each sample was diluted 1:10 and tested in duplicate. Deviation between duplicates was <10% for any reported value and the detection limit of the assay was 0.156 ng/ml. The analytical response was linear between 0.162 and 2.800 of absorbance values (corresponding to 0.156 - 10 ng/ml) as assessed by serial dilution test using a strongly positive serum sample. Intra-assay Precision

(Precision within an assay) was <10%, whereas Inter-assay Precision (Precision between assays) was 12%.

Serum sPD-L2 was measured by ELISA (EMELCA Bioscience, www.emelcabio.com) according to the manufacturer's instructions. Each sample was diluted 1:10 and tested in duplicate. Deviation between duplicates was <10% for any reported value and the detection limit of the assay was 0.062 ng/ml. The analytical response was linear between 0.162 and 2.600 of absorbance values (corresponding to 0.062 - 10 ng/ml) as assessed by serial dilution test using a strongly positive serum sample. Intra-assay Precision (Precision within an assay) was <10%, whereas Inter-assay Precision (Precision between assays) was 12%.

Serum interleukin 6 (IL-6) was measured by ELISA (ImmunoTools GmbH, Friesoythe, Germany), according to the manufacturer's instructions. Each sample was diluted 1:10 and tested in duplicate. The deviation between duplicates was <10% for any reported value, and the detection limit of the assay was 6.1 pg/ml. The analytical response was linear between 0.162 and 1.400 of absorbance values (corresponding to 6.1 - 500 pg/ml) as assessed by serial dilution test using a strongly positive serum sample.

White blood cells (WBC), platelets (PLT), haemoglobin (Hb), CRP and ESR values were retrieved from patients' routine laboratory examinations, performed in the week preceding the FDG-PET/CT scan.

A group of 40 age- and sex-matched healthy controls, without any known inflammatory and neoplastic disease, was also included in this study.

4.2.6. Statistical analysis

Analysis was performed using IBM SPSS Statistics software. The comparison of means was evaluated with Student's t-test and ANOVA with Bonferroni correction. Medians were compared with the Mann-Whitney or Kruskall-Wallis tests. The correlations between variables were evaluated with the Pearsons' test if normally distributed and with the Spearman's rho test if non-parametrical. Statistical significance was assumed as $p<0.05$.

4.3. Results

4.3.1. Patient characteristics

One hundred and thirty-one patients were included, 89 women and 42 men, with a median age of 74 years (range 47-92). Ninety-seven patients were diagnosed as PMR, 13 as C-GCA, 16 with both PMR and C-GCA and 5 patients presented with FUO (figure 6). Demographic, clinical and laboratory data are shown in table 1. Sixteen patients underwent temporal artery biopsy, of these, 10 were positive. Five out of thirteen (38.4%) patients with GCA and four out of sixteen (31.2%) patients with both PMR and GCA had a positive temporal artery biopsy. Forty-three out of 131 (32.8%) patients were already taking glucocorticoids at the time of PET/CT (29 with PMR, 4 with PMR+C-GCA, 8 with C-GCA and 2 with FUO).

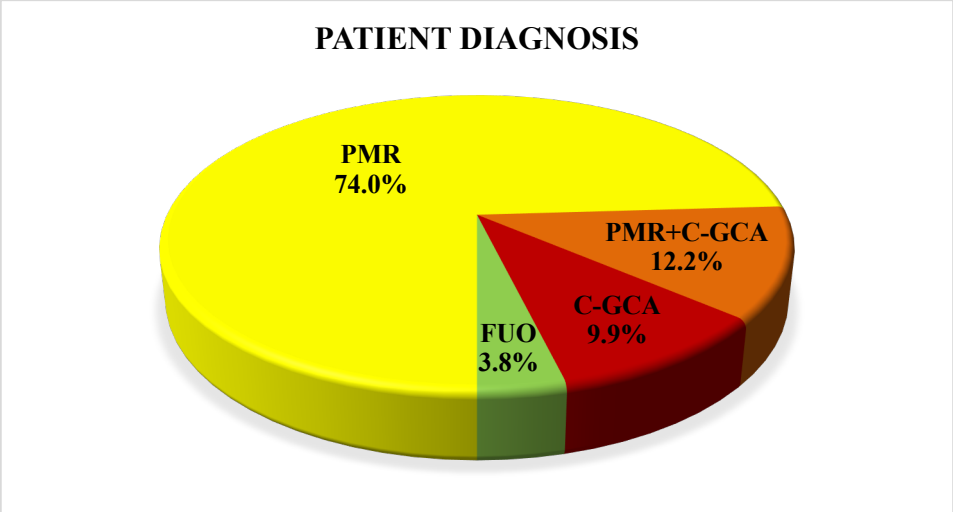

Figure 6. Pie chart showing subdivision of the patients according to clinical diagnosis.

Variable (unit of measure)	Value (range)
Men (n)	42
Women (n)	89
Median age (years)	74 (range 47-92)
Median disease duration (days)	85 (range 4-1957)
Median Hgb (g/L)	126 (range 82-168)
Median WBC ($x10^9$/L)	8.4 (range 4.3-15)
Median PLT ($x10^9$/L)	321 (range 108-643)
Median ESR (mm/h)	59 (range 8-140)
Median CRP (mg/L)	34.7 (range 0.4-162)

Table 1. Demographic, clinical and laboratory characteristics of the patients.

4.3.2. Evaluation of vascular uptake

4.3.2.1. Comparison of vascular uptake between patient groups

Mean SUV of arterial districts are shown in figure 7. Patients with PMR showed a statistically significant lower mean arterial SUV in comparison to patients with FUO (0.77 vs. 1.15, p=0.004); patients with C-GCA showed a tendency towards lower uptake in comparison to patients with FUO (0.81 vs 1.14, p=0.052).

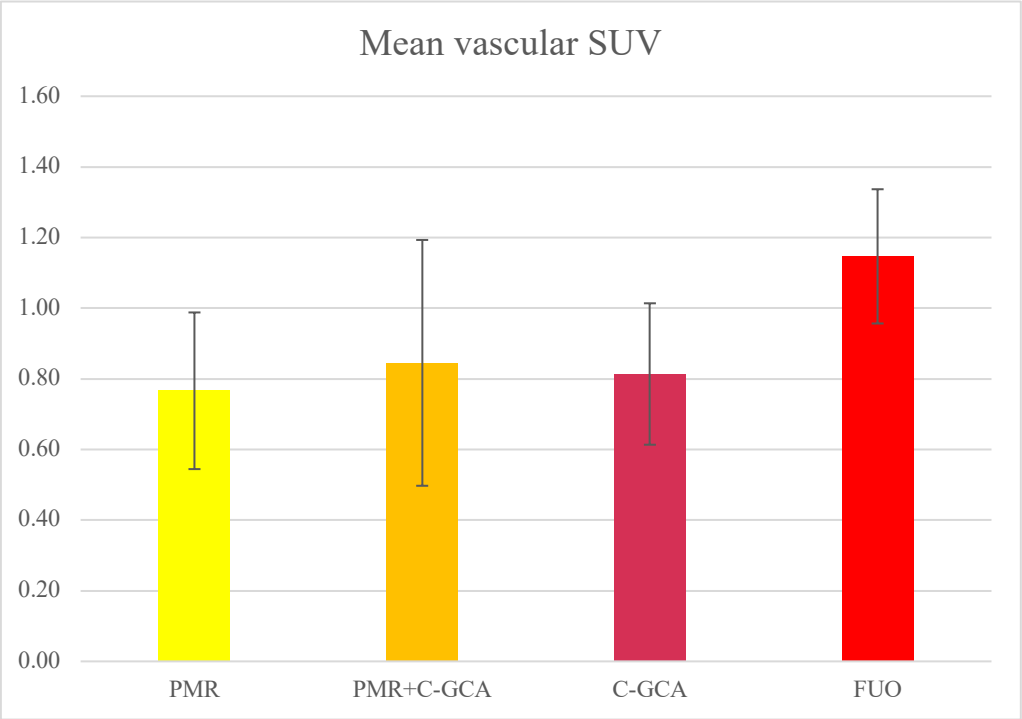

Figure 7. Columns represent the means of vascular SUV, with standard deviation of each group.

Similar results were obtained using visual scoring, although differences between groups seemed more striking. Mean TVS values are shown in figure 8. Patients with a clinical diagnosis of FUO showed increased uptake in large vessel in all cases. PMR patients showed statistically lower TVS than patients with PMR+C-GCA, C-GCA alone, and FUO (table 2).

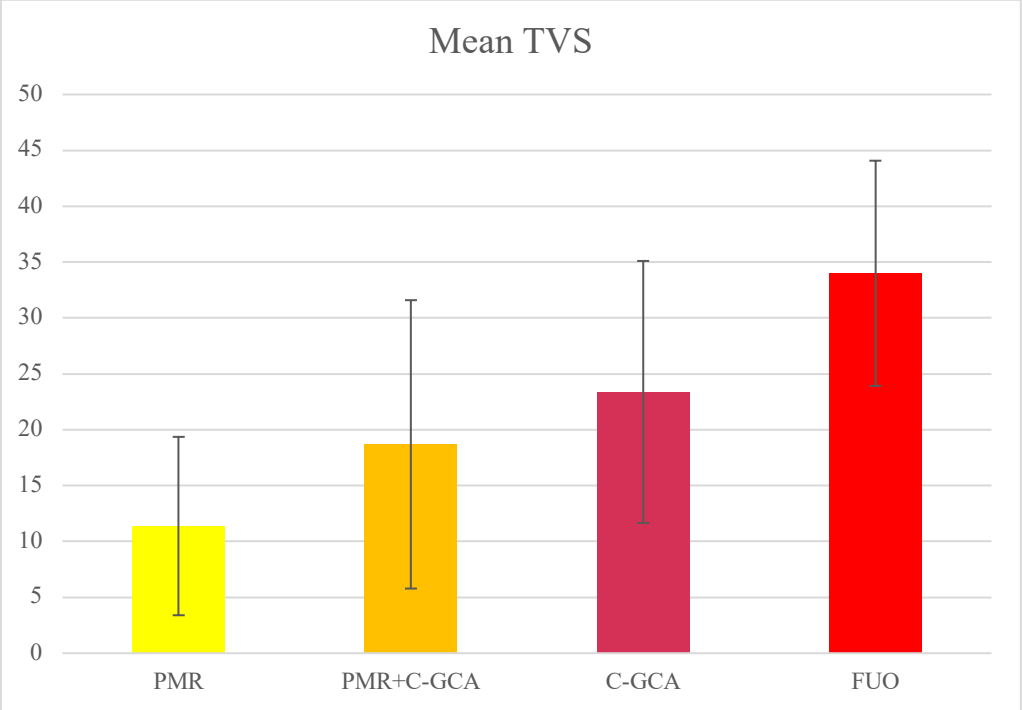

Figure 8. Columns represent the means of total vascular score (TVS) with standard deviation of each group.

Reference group	Comparison group	p
PMR vs.	PMR+C-GCA	0.022
	C-GCA	<0.001
	FUO	<0.001
PMR+C-GCA vs.	C-GCA	n.s.
	FUO	0.009
C-GCA vs.	FUO	n.s.

Table 2. Multiple comparison of mean TVS between patient groups (n.s.: not significant).

4.3.2.2. Comparison of quantitative and visual vascular uptake

As expected, vascular SUV and TVS showed correlation ($p<0.001$), with a coefficient of 0.299.

4.3.2.3. Comparison of vascular uptake between patients with and without glucocorticoid therapy

There were no significant differences in vascular uptake between the 88 glucocorticoid-naïve patients at the time of PET/CT and the 43 who were already taking glucocorticoids, considering both mean arterial SUV (0.78 vs 0.82, respectively) and visual score (mean TVS of 14.15 vs 14.7).

4.3.2.4. Arterial calcium load

Patients with GCA showed a tendency to higher mean ACL in comparison with the other patient groups (figure 9), although the difference was not statistically significant ($p=0.058$). ACL did not differ between patients who were taking GC and those who did not.

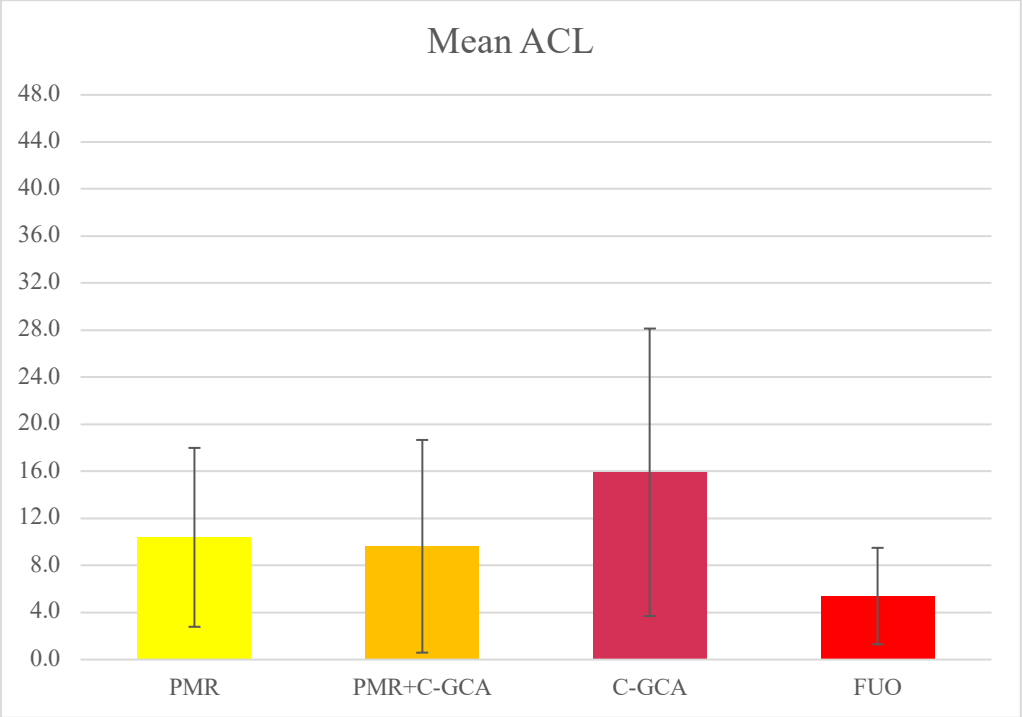

Figure 9. Columns represent the means of total arterial calcium load (ACL) with standard deviation of each group.

4.3.2.5. Correlation between vascular uptake and temporal artery biopsy

Mean vascular SUV was not significantly higher in patients in whom the temporal artery biopsy was positive compared to those with negative biopsy, although those with a positive showed a tendency toward higher values. In contrast, patients with a positive biopsy showed a statistically significant higher TVS compared to those with a negative biopsy (p=0.015) or those not undergoing biopsy (p=0.003).

4.3.2.6. Correlation between vascular uptake and clinical and laboratory variables

Mean arterial SUV did not correlate significantly with the presence of fever (n=41), weight loss (n=48), headache (n=27), jaw claudication (n=10), visual

alterations (n=8), and palpable (n=15), pulseless (n=9), or tender temporal artery (n=16). Mean arterial SUV correlated inversely with haemoglobin concentration (p=0.05), and positively with PLT, CRP, ESR (p=0.026, p=<0.001, p=0.002, respectively); no correlation was noted with WBC.

Conversely, patients who presented with fever showed a higher mean TVS than those without (p=0.007). The same was true for patients with headache (p=0.04), patients with visual alterations (p=0.036), and patients with a thickened temporal artery (p=0.027). Pain at pressure of the temporal artery showed no significant correlation with mean TVS, as well as weight loss and jaw claudication. Mean Hgb concentration was inversely correlated with mean TVS (p=0.002), whereas PLT, CRP and ESR showed a positive correlation with mean TVS (p=0.005, p=0.001, p=004 respectively); WBC did not correlate with TVS.

4.3.2.7. Dichotomic evaluation of vascular uptake

Using a visual uptake of ≥2 in at least one large vessel to define a positive scan, forty-nine out of 97 (50.5%) patients with PMR had a positive PET scan, as well as 12 out of 16 (75%) of patients with PMR+C-GCA, eleven out of 13 (84.6%) patients with C-GCA and 5 out of 5 (100%) with FUO. Using the same standard of a visual uptake >2 as a positive scan, fifteen out of 97 (15.4%) patients with PMR had a positive scan for vasculitis, seven out of 16 (43.7%) of patients with PMR+C-GCA, seven out of 13 (53.8%) patients with C-GCA and 4 out of 5 (80%) of patients with FUO.

4.3.3. Evaluation of joint uptake

4.3.3.1. Comparison of joint uptake between patient groups

Mean SUV of articular and extra-articular areas considered is shown in figure 10. Patients with PMR showed a statistically significant higher uptake than C-GCA patients (p=0.01).

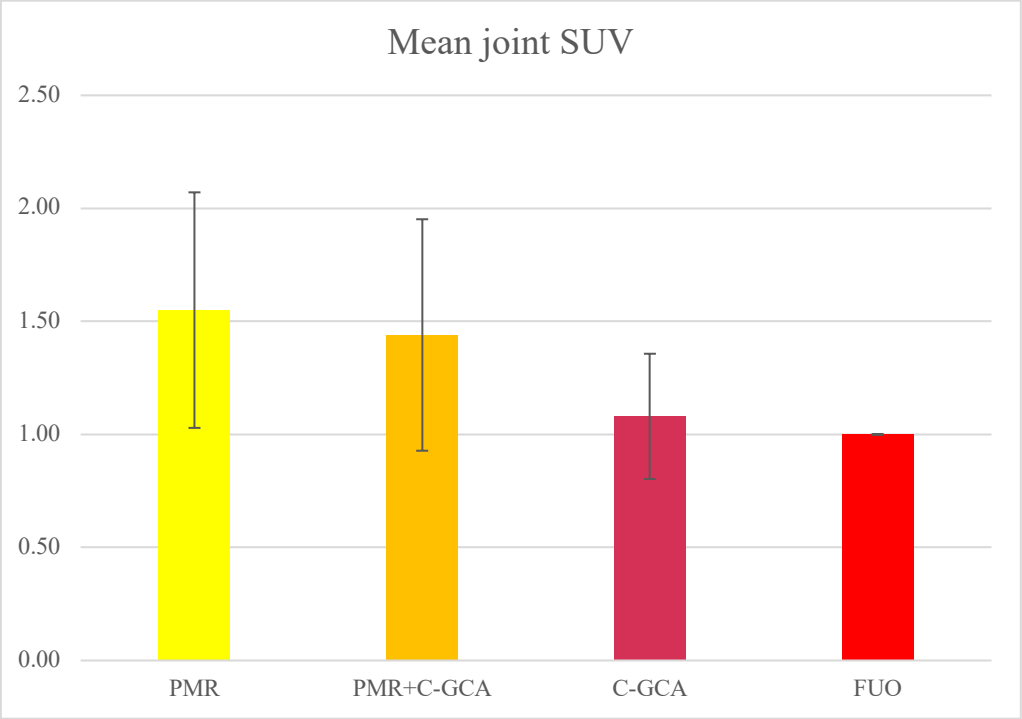

Figure 10. Columns represent the mean SUV of articular and extra-articular areas with standard deviation of each group.

Mean TJS values are shown in figure 11. Patients with a clinical diagnosis of PMR showed the highest mean articular uptake, whereas patients with FUO showed the lowest mean articular uptake. Mean TJS of patients with PMR was higher than mean TJS of patients with either C-GCA or FUO ($p<0.001$ for both comparisons).

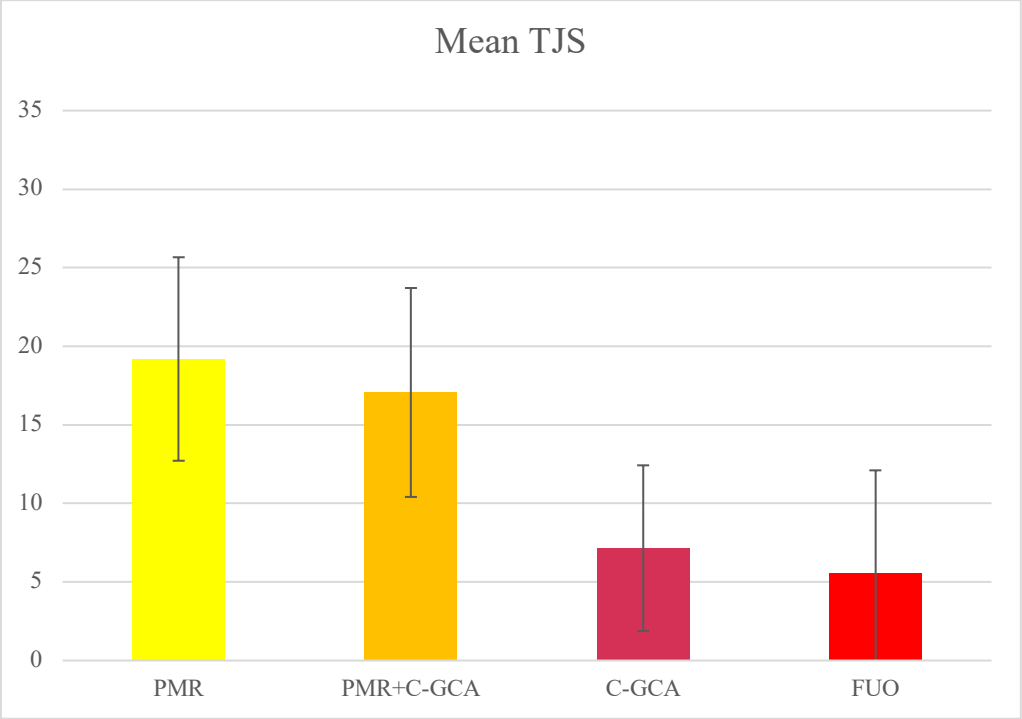

Figure 11. Columns represent the mean TJS of articular and extra-articular areas with standard deviation of each group.

4.3.3.2. Comparison of quantitative and visual joint uptake

Mean joint SUV and mean TJS were positively correlated ($p<0.001$).

4.3.3.3. Comparison of joint uptake between patients with and without glucocorticoid therapy

Patients who were already taking glucocorticoids at the time of PET/CT showed lower uptake than glucocorticoid-naïve patients ($p=0.002$) when evaluated with SUV.

Also, when evaluated with TJS, patients who were already taking glucocorticoids showed lower uptake.

4.3.3.4. Correlation between joint uptake and clinical and laboratory variables

Mean SUV of the assessed articular and extra-articular areas correlated positively with morning stiffness (MS) ($p<0.001$) and with total number of clinically involved sites. No correlation was found between mean SUV and Hgb concentration, PLT, WBC, ESR or CRP.

Mean TJS correlated positively with MS ($p<0.001$) and with the total number of clinically involved sites ($p<0.001$). No correlation was found between mean TJS and Hb concentration, PLT, WBC, ESR or CRP.

4.3.4. Correlation between vascular and joint uptake

Because joint involvement is considered more characteristic of PMR but can occur in a substantial percentage of patients with GCA, it was of interest to assess possible correlation between vascular and joint uptake. TVS and TJS correlated negatively ($p=0.01$), as also did mean vascular SUV and mean joint SUV ($p=0.001$).

4.3.5. Correlation between clinical diagnoses and laboratory/imaging findings

The ratio between TVS and TJS of patients with PMR was significantly different to that of patients with PMR+C-GCA, with C-GCA and with FUO ($p<0.01$ for all comparisons). The ratio between TVS and TJS of patients with PMR+C-GCA was significantly different to that of patients with PMR, with C-GCA and with FUO. The ratio between TVS and TJS of patients of patients with C-GCA and FUO was significantly different to that of patients with PMR and PMR+C-GCA, but they did not differ significantly between each other. Patients with a positive PET for vasculitis (i.e. a visual score of 3 in at least one vascular region) showed a higher value of TVS/TJS in comparison to those without PET-defined vasculitis [2 (range 0.6-41) vs 0.5 (range 0-8.5), $p>0.01$]. Patients with a PET positive for vasculitis showed a higher ratio of PLT/WBC (45.8 ± 13.4 vs 36.2 ± 11.1, $p<0.01$). These and other comparisons are provided in supplementary table 1.

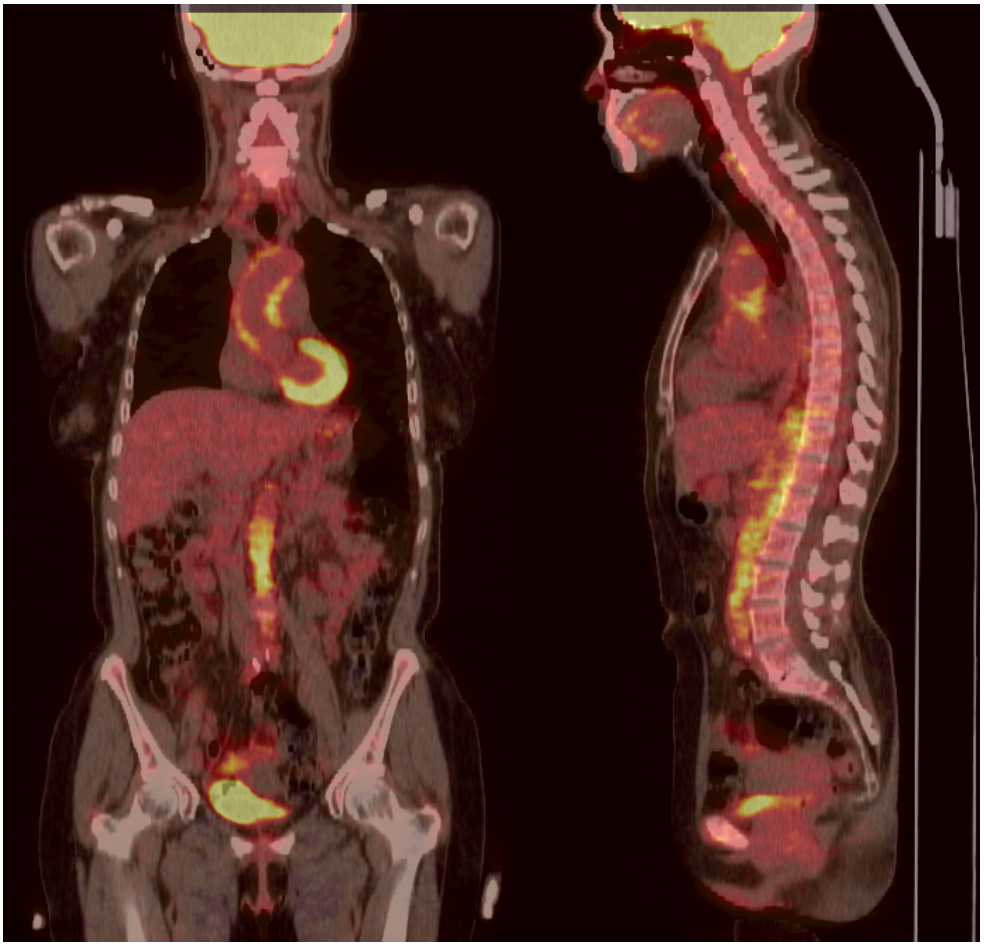

Figure 12. Coronal (on the left) and sagittal (on the right) PET/CT reconstructions. Intense uptake of both thoracic and abdominal aorta in a patient with FUO.

4.3.6. Soluble immune checkpoints

Forty consecutive patients with PMR/GCA underwent both FDG-PET/CT and serological analysis of soluble immunological checkpoints. Of these, 32 had clinically isolated PMR, and 8 patients had PMR with associated C-GCA. The blood for these serologic tests was drawn at the time of venepuncture for FDG injection. Patient characteristics are given in Table 3.

The median serum levels of sCTLA-4, sPD-1, sPD-L1 and sPD-L2 in these patients were 1.23 ng/ml (range 0.1-122.4), 2.93 ng/ml (range 0.1-7.3), 3.38

ng/ml (range 0.1-112.7), and 18.49 ng/ml (range 0.1-411), respectively. In control subjects, the median serum levels of sCTLA-4, sPD-1, sPD-L1 and sPD-L2 were 0.1 ng/ml (range 0.1-1.7), 0,1 ng/ml (range 0.1-0.96), 0,1 ng/ml (range 0.1-16-1) and 0.3 ng/ml (range 0.1-4.5). Patients had higher concentrations of all the analysed soluble immune checkpoints ($p<0.001$ for all the comparisons). Median IL-6 concentration was higher in patients (65.37 pg/ml; range 1-755) than in controls (3.4 pg/ml; range 1-6).

Male patients had a higher median concentration of sCTLA-4 (12.7; range 0.1-40.7) than female patients (0.1 ng/ml; range 0.1-122.4), $p=0.03$, whereas levels of PD-1, PD-L1, and PD-L2 were comparable. The correlations of sCTLA-4, sPD-1, sPD-L1 and PD-L2 with several clinical, laboratory and imaging characteristics were explored, including age, morning stiffness, disease duration, WBC, PLT, Hgb, CRP, ESR, TVS and TJS, but none of those gave significant results. Levels of IL-6 did not correlate with the levels of soluble immune checkpoints, nor with the aforementioned clinical and imaging parameters. The differences in the concentration of soluble immune checkpoints between patients with PMR+C-GCA and those with isolated PMR were not statistically significant. This was the also case when comparing patients with and without systemic manifestations (i.e. fever and/or loss of weight), those with and without potential ischaemic manifestations (i.e. headache, visual disturbances and jaw claudication) and those with and without peripheral arthritis and/or tenosynovitis. The six patients already taking glucocorticoid treatment had values of soluble immune checkpoints comparable to the 34 glucocorticoid-naïve patients.

Table 3. Characteristics of patients undergoing soluble immune checkpoints analysis at the time of the assay.

Total number	40
Male, n (%)	8 (20)
Median age, years	76 (range 50-85)
Patients with isolated PMR, n (%)	30 (75)
Patients with PMR+C-GCA, n (%)	10 (25)
Already taking GC, n (%)	6 (15)
Systemic manifestations, n (%)	17 (42.5)
Peripheral arthritis, n (%)	10 (25%)
Median disease duration, days	87.5 (range 4-1086)
Median morning stiffness, minutes	60 (range 0-360)
Mean WBC, 10^9/L	8.4±1.6
Mean PLT, 10^9/L	318.8±92.4
Mean Hgb, g/L	124±14
Mean ESR, mm/h	67.7±33.7
Median CRP, mg/L	31.6 (range 2-124.3)

5. DISCUSSION

The relationship between PMR and GCA has been always elusive. The high prevalence of PMR features among patients with GCA suggests that isolated PMR could be a sort of "incomplete form" of GCA, an hypothesis supported by imaging[19,78] and autopsy studies[25]. Indeed, the term "polymyalgia arteritica" has been suggested because of the presence of vasculitis in apparently isolated forms of PMR[25]. However, some differences seem to exist between isolated ("pure") PMR and PMR associated with GCA[79] in terms of age, presence of systemic symptoms and laboratory abnormalities. Moreover, patients with PMR who have "incidental" findings of a positive temporal artery biopsy, but who do not have clinical GCA features, have a lower risk of ischemic complications[4], suggesting that PMR and GCA could be overlapping, but not necessarily the same disease.

The widespread use of imaging technique has revealed the presence of LVV in a high proportion of patients with PMR[80], even though the prognostic implications of this finding are still unclear. In this study, the occurrence of LVV in patients with apparently "pure" (isolated) PMR was 15.4%, using the strictest definition of vasculitis on FDG-PET. On the other hand, PMR is the most frequent extra-cranial manifestation of GCA[38]. There are conflicting results about the prognostic implications of concomitant PMR in patients with GCA, with some reports suggesting a lower risk of ischaemic events in these patients when compared to patients with isolated GCA, even though this finding has not been confirmed by others[81].

In the 2012 revised Chapel Hill consensus nomenclature, GCA is defined as an arteritis "*often granulomatous, usually affecting the aorta and/or its major branches, with a predilection for the branches of the carotid and vertebral arteries*"[5]. The prototypical clinical picture of temporal arteritis with the

presence of headache, temporal artery abnormalities, jaw claudication and visual disturbance, is now referred to as “cranial-GCA”[38]. Patients presenting with prevailing involvement of the aorta and its branches, with possible peripheral stenosis and limbs claudication, are grouped as “large-vessel-GCA”[38]. The design of this study and the type of referral pattern in our unit facilitated the examination of “pure” (isolated) PMR patients, who composed the large majority of the present cohort. Although there is a numerical imbalance with the other two classes of patients, those with isolated LVV seem to differ in terms of PET uptake.

A pioneering study from Mayo Clinic published in 1975[82], showed that among 248 patients with GCA, 34 (14%) had involvement of the aorta or its major branches. In 1999, a case-control study showed that patients with angiographic signs of vasculitis in the upper limbs rarely presented with cranial manifestations. The same also showed a lower frequency of positive temporal artery biopsy in patients with vasculitis of the arms, in comparison with a control group of "classical" temporal arteritis[36]. In a Swedish cohort of 164 patients with biopsy proven GCA, 24 (15%) had ectasia, aneurysm or stenosis of the aorta or its branches[83]. In a cohort study, patients with C-GCA were compared with patients with LV-GCA, defined as those having radiological signs of vasculitis in the subclavian arteries[37]. Patients with LV-GCA were younger than those with C-GCA and presented less frequently cranial symptoms (41% vs 83%) and vision loss (4% vs 11%). As expected, patients with LV-GCA fulfilled less frequently the ACR classification criteria for GCA than those with C-GCA (39% vs 95%). Patients with LV-GCA presented a more severe disease course, having more relapses and requiring prolonged treatment. Other studies support a lower

risk for cranial ischaemic events in patients with LV-GCA compared to those with C-GCA[81], as well as a more refractory disease course[84]. . In keeping with this study, a currently ongoing follow-up evaluation of a part of the patients described in this thesis demonstrated that PMR patients with concomitant LVV at PET-CT do not show a worse prognosis than the patients with "pure" (isolated) PMR. There is a growing body of evidence for the existence of at least two patterns of GCA (i.e. C-GCA vs LV-GCA)[38]. However, more than half of patients with biopsy proven GCA can present with large vessel involvement[85], suggesting an overlap between these two phenotypes. There is a discrepancy between diagnoses based only on clinical and laboratory data and those obtained with the aid of imaging. This observation suggests that a new nomenclature is necessary to define the spectrum of these conditions. The present results may help in this regard. In addition, standardization of PET-CT and other imaging techniques is crucial in trying to classify these disorders.

GCA and Takayasu arteritis represent the two major variants of LVV, according to the 2012 revised Chapel Hill consensus nomenclature[5]. Although they share some common clinical[44], imaging[86] and histopathological[7] features, they appear to be two different diseases with different manifestations[87,88], even when patients with Takayasu arteritis are compared with those with extra-cranial GCA[89].Isolated aortitis is another enigmatic entity: is it a separate condition, characterized by exclusive inflammation of the aortic arch, or is it simply another presentation of GCA? Although comparison between published papers are difficult because of different study designs (imaging vs. surgical vs. autopsy studies) and different settings (rheumatology vs. cardiac surgery vs. pathology), most of the cases of giant cell aortitis were discovered accidentally, in absence

of any specific symptom or sign related to GCA[90,91]. Among a cohort of 7551 patients undergoing surgery of the thoracic aorta[92], 156 (12%) showed the histologic presence of aortitis. Only a minority of them had clinical or serological evidence of systemic inflammation, and in 82% of cases, the diagnosis was made after the histologic examination, suggesting that a significant proportion of patients with aortitis could remain unnoticed. Another important drawback of the studies of this topic is that only very few patients did have a biopsy of a large artery; as a result, a classical gold standard is lacking.

It is still unclear whether isolated aortitis represents a sort of aborted, incomplete form of GCA[93]. In a French cohort of patients with radiologically defined aortitis[17], seventy-three patients with GCA were compared with 44 with isolated aortitis. The latter group appeared more at risk of developing aortic aneurysm, consistently with other reports[94]. In the French study, however, when patients with isolated aortitis and an age ≥60 were compared with those with GCA, many characteristics appeared comparable between the two groups.

The clinical manifestations of GCA can be protean. In some cases, fever can be the only presenting symptoms of LVV[95]; in fact, PMR and GCA account for up to one-third of cases of FUO[96–98]. In a cohort of 100 patients with biopsy proven GCA, fifteen presented with FUO as the initial manifestations[99]. In these patients, haemoglobin levels were lower, and platelet count and ESR higher compared to the other patients. In a retrospective study of 210 patients with biopsy proven GCA[100], patients presenting with fever before the starting of GC therapy showed lower levels of haemoglobin and higher ESR, but a lower of risk of severe ischaemic manifestations. In a cohort of 693 patients with GCA[101], diagnosed according to biopsy or imaging, sixty-one (9%) initially presented

with fever or inflammation of unknown origin as the sole manifestation. These patients were younger and presented with higher CRP levels. Nevertheless, no differences were seen during follow-up in comparison with patients with different initial manifestations. Interestingly, five of the 61 (9%) patients with fever or inflammation of unknown origin relapsed with cranial symptoms, not present at disease onset.

In our cohort, FDG-PET revealed evidence of inflammation in large vessels in 5 patients with FUO. One of them underwent a temporal artery biopsy, which was negative. Similar to previous reports, patients presenting with FUO as the sole manifestation appeared to have a greater inflammatory response, as shown by the higher vascular uptake. All except one of these patients showed a visual score of FDG uptake of 3 in the thoracic and abdominal aorta, as well in the subclavian, carotid and axillary arteries; the remaining patient, who was already taking prednisone, showed a visual score of 2 in the thoracic aorta and the subclavian arteries. This distribution of the FDG uptake, extending beyond the aorta, makes the diagnosis of isolated aortitis unlikely in these patients. Given the lack of PMR and cranial symptoms, as well the presence of inflammation in aortic branches, these patients with FUO may be considered as having isolated LVV or LV-GCA.

Our results suggest that, although on a “disease continuum”, PMR, C-GCA and LVV present some distinct features. In particular, we noted a sort of gradient from a disease limited to articular and peri-articular areas (“pure” PMR) to a vasculitis without any clinical or imaging findings of joint involvement (“pure” LVV), as demonstrated by the inverse correlation between both qualitative and semi-quantitative scores of joint and vascular uptake. C-GCA (with and without

associated PMR) seems to be situated along the road which connects these two apparently distant conditions. Although examination of only PET/CT images of an individual patient who has of concomitant joint and vascular inflammation doesn't allow the identification of the diagnosis (does the patient have PMR with large vessel involvement, or GCA with joint involvement?), it seems that clinical diagnosis reflects a "cluster" of inflammation, which could be articular, vascular or a combination of the two.

The findings of this study appear to be only partially affected by glucocorticoid treatment. Joint uptake was lower in patients already treated, but vascular uptake, although decreased, was not significantly reduced by glucocorticoid use. This finding indicates that PET/CT maintains, at least in part, its diagnostic value also during established glucocorticoid therapy. In this way, PET/CT is different from temporal artery biopsy, the major inflammatory features of which are markedly reduced or negative in most patients who have been treated for more than one week. More data are needed to understand which doses and duration of glucocorticoid treatment are still associated with a positive vascular uptake. In a prospective study on patients with LV-GCA, FDG vascular uptake decreased after three days of prednisone 60 mg compared to a baseline PET, but the PET scans were still positive for vasculitis[102]. In contrast, after 10 days of treatment, only 5 out of 14 patients (35.7%) still showed a positive PET scan.

In the age group of patients with PMR and GCA, atherosclerosis could be an important confounding factor. Atherosclerotic lesions are reported to show both increased and reduced FDG uptake, probably depending on the grade of inflammation within the plaque[103,104]. Our patients showed a similar degree of ACL, except C-GCA patients who showed higher degree of vascular

calcification. The finding that patients on the opposite ends of the spectrum in terms of vascular uptake (i.e. patients with PMR and FUO) did not show differences in ACL, is reassuring that the study results were not influenced by atherosclerosis.

One major issue in the use of FDG-PET/CT for the evaluation of LVV is the absence of standardization about the definition of pathologic vessel uptake. Several scores have been proposed to identify the presence and to grade the intensity of LVV[9,11], but consensus is still lacking.

One of the most commonly used scores is the visual one chosen for this study. This score is readily understandable and easily applied but may be affected by inter-reader variability. A quantitative method using SUV could have the theoretical advantage of being operator-independent, but it is time consuming. Although the results obtained with these two methods were generally concordant and showed reciprocal correlation, they yielded slightly different results in this study and in a previous one[105].

In the present study, TVS was higher in patients with positive biopsy of the temporal artery, a trend that did not reach statistical significance considering arterial SUV. Similarly, patients with fever, headache, visual disturbances and palpable temporal arteries had higher TVS, a correlation not present with arterial SUV, although a tendency towards higher values in this subgroup was present. Mean Hb, PLT, CRP and ESR correlated positively with both TVS and arterial SUV, suggesting that vascular inflammation is tightly linked with systemic inflammation and that marked alterations in these laboratory parameters, in absence of an alternative explanation, should rise the suspicion of LVV. Conversely, joint inflammation did not appear tightly related to laboratory

parameters of inflammation, since neither joint SUV nor TJS correlated with Hgb, PLT, CRP or ESR. The total number of clinically involved articular/peri-articular sites and MS correlated with joint SUV and TJS, confirming that joint inflammation is clinically more easily detectable than vascular inflammation.

The identification of the most reliable and feasible scoring method for the detection of abnormal vascular and joint uptake may have practical consequences. However, evidence of the need for more active treatment of patients with PMR and concomitant LVV is presently lacking. The GiACTA trial, the most important trial on the use of the humanised monoclonal anti-interleukin (IL)-6 receptor antibody tocilizumab in patients with GCA, considered as an inclusion criterion the presence of a clinical diagnosis of PMR in association with imaging findings of LVV[106]. This could have been a questionable strategy, because, as previously mentioned, it is not actually known whether patients who have PMR and concomitant LVV are the same as patients, or are actually, patients with GCA[4].

In the current study, an inverse correlation was observed between vascular and joint inflammation (expressed both with SUV or visual score). A possible explanation for this finding is that different clinical presentations are likely to be associated with different referral pathways (e.g. a patient with FUO, without joint involvement, may be more likely to be evaluated in an internal medicine ward than in a rheumatology outpatient clinic, and the same could happen to a patient with only cranial GCA symptoms). Therefore, there may be selection bias in the cohort of patients with PMR, and patients with PMR+GCA, who are recruited in a rheumatology unit. This is unlikely to have affected the current study, as most of the other patients were also evaluated by the same

rheumatologists, in some cases as consultants in other wards. Another explanation is that the global "inflammatory burden" could be limited and its expression could be either restricted only to articular or vascular structures, or "equally" divided between these two locations.

In the second part of the study, we tried to understand whether these different disease phenotypes could be attributed to the different expression and functions of the soluble immune checkpoints sCTLA-4, sPD-1 and its ligands.

An analysis of vascular lesions from temporal artery biopsies of patients with GCA showed a reduction in the transcription of the immunoinhibitory ligand PD-L1 together with an increased expression of PD-1^{+} T cells[73], suggesting the presence of a pro-inflammatory environment. The expression of PD-L1 on dendritic cells was particularly low in those patients with higher ESR and CRP[73], in line with the concept that defective immunoregulatory mechanisms in GCA lead to vascular inflammation. This is not the only evidence of the involvement of immune checkpoints in the pathogenesis of GCA. In a cross-sectional study of 30 patients with GCA, of whom 15 were already receiving glucocorticoid treatment, circulating PD-1+ Th cells were reduced in comparison to healthy controls[107]. Circulating T-helper cells expressing the negative checkpoint V-domain Immunoglobulin-containing suppressor of T cell activation (VISTA) were also reduced in number[107]. On the other hand, arteritic lesions from diagnostic temporal artery biopsies showed an increase in VISTA-expressing cells and in PD-L1-expressing cells[107].

The relationship between immune checkpoints and inflammatory rheumatic disease has gained increased interest in recent years. The introduction of immune checkpoint inhibitors (ICIs) has highlighted the close relationship between

autoimmunity and immunity against cancer. On the one hand, ICIs have proved to be efficacious in several types of cancer[56,108–110]. On the other hand, ICIs are associated with a multitude of immune-mediated adverse effects, including endocrine, gastroenterological, renal, cutaneous and articular manifestations[111]. Checkpoints represent a sort of "brake" on the immune system, whose activity would be otherwise uncontrolled and would lead to self-damage. Murine models with CTLA-4 deficiency die after a few days due to a massive tissue infiltration and destruction by lymphocytes[112,113]. ICIs such as ipilimumab, pembrolizumab, atezolizumab and nivolumab block these inhibitory pathways and therefore enhance the immune response against tumour cells. This anti-tumour beneficial effect, however, comes at a price, represented by immune-related adverse events. New-onset inflammatory arthritis appears in about 5-7% of patients with malignancy treated with ICIs[114]. Other possible manifestations include myositis, sicca syndrome and sarcoidosis[114]. A retrospective pharmacovigilance study showed an increased risk of myocarditis, pericarditis and vasculitis in patients receiving ICIs[115]. In that series, sixteen cases of PMR were described, as well as 18 cases of temporal arteritis, of whom five presented with visual impairment[115]. Patients with temporal arteritis were more likely to be treated with anti-CTLA-4 therapy than with anti-PD-1 or anti-PD-L1 therapy.

One might wonder whether ICI-induced rheumatic diseases represent a valid model to study the "idiopathic" counterparts. In a cohort of patients with ICI-induced PMR and cases from a literature review[72], a considerable portion presented with atypical features: peripheral synovitis (including unusual sites such as the elbow), positivity of autoantibodies and sicca syndrome. Interestingly, two of these patients showed a refractory course and were treated

successfully with tocilizumab[72]. There are other anecdotal reports on the positive effect of tocilizumab in cases of ICI-induced arthritis[116]. This might indicate that either IL-6 is involved in the pathogenesis of immune-related adverse events or that, once the autoimmunity is triggered, each disease follows its own pathway and IL-6 has a pivotal role in rheumatoid arthritis, PMR, and GCA[117]. In a cohort of 14 patients with ICI-induced PMR[118], peripheral arthritis was present in 57%, compared to 28% of the control group composed of 43 "classical" PMR patients. In our study, 25% of patients presented with peripheral arthritis, but its presence was not correlated with the concentrations of soluble immune checkpoints.

The study of ICI-induced rheumatic disease is in its infancy. The available evidence might suggest that ICIs can open the Pandora's box of autoimmunity, but they are not responsible for what comes out from that box. Several mechanisms have been postulated to explain the pathophysiology of immune-related adverse events, including a pre-existing and latent autoimmunity[119]. Another point to emphasize considering the ICIs story is that PMR, considered by some authors an autoinflammatory disease rather than an autoimmune one[120], may ultimately be triggered by autoimmune mechanisms.

The relationship between the soluble and membrane-bound form of immune checkpoints is still not fully understood[121]. Soluble CTLA-4 is almost undetectable in healthy subjects[68]. Levels of sCTLA-4 are increased in patients with systemic lupus erythematosus[122] and systemic sclerosis[123]. In patients with rheumatoid arthritis and spondyloarthropathies, sCTLA-4 was also increased in comparison to controls and, in addition, correlated with disease activity[124,125]. In a recently published report on a cohort of 104 patients with systemic lupus erythematosus, levels of sCTLA-4 correlated closely with those of interferon-

α[126]. There are several lines of evidence in support of an immunoregulatory effect of sCTLA-4, starting from its first description in 2000[127]. In human PBMC cultures, an experimental antibody blocking only sCTLA-4 (but not the membrane-bound form) increased cytokines production[128]. As a confirmation, mice infused with melanoma cells and treated with either an antibody specific to sCTLA-4 or a pan-specific anti-CTLA-4 antibody showed a reduction in the number of the metastatic sites of 44% and 50%, respectively[128]. Silencing of sCTLA-4 mRNA with RNA interference impaired the function of regulatory T cells; the same authors also demonstrated in a murine model that a reduction in sCTLA-4 expression was associated with increased susceptibility of developing type 1 diabetes[129].

There is still debate on the function of sPD-1: it may block the interactions between PD-L1 and CD80, PD-L1 and PD-1, as well as PDL2 and PD-1, thus exerting immunostimulatory and anti-tumour effects[121]. Patients with rheumatoid arthritis patients showed high levels of sPD-1 in both sera and synovial fluid[130], which correlated with disease activity and were reduced by methotrexate treatment. In mice with collagen-induced arthritis, the administration of sPD-1 enhanced Th1/Th17 response and increased the severity of the arthritis[130]. Patients with systemic sclerosis showed increased levels of sPD-1 and sPD-L2, which correlated with different parameters of disease severity[131]. Nevertheless, an inhibitory effect of s-PD1 on T cell activation has also been proposed, possibly through reverse signalling involving dendritic cells[132].

Zoledronate is a nitrogen-containing bisphosphonate which is indicated for the treatment of osteoporosis and bone metastasis. In oncology, zoledronate use is

based on the inhibition of bone resorption by osteoclasts, but it may exert an anti-tumour effect through other mechanisms [133]. In vitro, zoledronate inhibited regulatory T cells with a consequent downregulation of immunoinhibitory molecules, including CTLA-4[134]. This sort of immunostimulatory effect of zoledronate in cancer might be conceptually linked to immunostimulatory action of ICIs. A synergistic action of zoledronate and PD-1 blockade has been suggested in a murine model of breast cancer[135]. We have previously shown that zoledronate infusion significantly reduces circulating levels of sCTLA-4 in patients with inflammatory rheumatic diseases (*Giusti et al, under revision*), suggesting a sort of "pro-inflammatory" transient state, which might be mediated by the reduction of sCTLA-4.

Our patients with PMR and GCA showed significantly higher levels of sCTLA-4, sPD-1, sPD-L1 and sPD-L2 in comparison with controls. This finding is in line with the existing literature in several other autoimmune diseases[68,122,123,125,131]. Given the several reports of onset of PMR and GCA after treatment with ICIs[72,136], we sought to analyse soluble immune checkpoints and their relationship with different clinical, laboratory and imaging characteristics. We did not find significant correlations between the levels of sCTLA-4, sPD-1, sPD-L1 and sPD-L2 and disease duration, morning stiffness, CRP, ESR and the total burden of vascular and articular inflammation as assessed by FDG-PET/CT. We also tested whether patients with GCA were characterized by differences in circulating immune checkpoints. However, no differences were detected in comparison with patients with isolated PMR, either in terms of clinical presentation (e.g. presence or absence of headache, jaw claudication, systemic inflammation) or imaging-detected vasculitis.

There are data suggesting a preferential involvement of one pathway in the development of ICI-induced GCA since patients with temporal arteritis were more likely to have received anti-CTLA-4 treatment in the aforementioned pharmacovigilance study[115]. On the contrary, the study from Zhang et al.[73] pointed to a significant role of the PD-1 pathway. A trial of abatacept, a CTLA-4-Ig used for the treatment of rheumatoid arthritis, in patients with GCA showed a barely significant difference in relapse-free survival at 12 months in patients receiving GC+abatacept compared to those receiving GC only[137]. Although the overall results of this trial were positive, the magnitude of the effect of abatacept may suggest that this pathway is not the pivotal one to be targeted for the treatment of GCA. An industry-sponsored trial on the use of subcutaneous abatacept in patients with GCA (NCT03192969) has been withdrawn. A trial on the use of abatacept monotherapy in patients with early-onset PMR, without associated glucocorticoids for the first 12 weeks of treatment, is currently recruiting (NCT03632187).

Our results confirm that immune checkpoints are involved in PMR and GCA, as highlighted by the increase of their soluble forms in this cohort. However, the CTLA-4 and PD-1 pathways do not seem to be the key players in the PMR/GCA complex, as suggested by the lack of correlations with the principle articular and vascular manifestations in our patients, as well as the limited evidence for the efficacy of abatacept in GCA. The increase in soluble immune checkpoint may represent a generic marker of hyperactivation of the immune system, as observed in many other rheumatic inflammatory diseases[122,124,125]. Another possible interpretation is that the increase of sCTLA-4 in rheumatic diseases may be an attempt to dampening the uncontrolled inflammation.

The strengths of the study presented in this thesis include the prospective inclusion of consecutive, unselected patients; the precise clinical, laboratory and imaging assessment of all the patients, and the relatively low number of patients already taking glucocorticoids at the time of the tests and evaluations done for the study. To the best of our knowledge, this is the largest study analysing immune checkpoint molecular expression in patients with PMR and GCA.

Limitations of this study include the presence of PMR features in almost all patients with C-GCA. The ability to focusing on patients with "pure" cranial-GCA might have allowed the identification of some unique or differentiating characteristics of "articular" vs "vascular" disease in these patients. Only a small number of patients presented with FUO as the unique manifestation and we had no patients with clinical presentations related exclusively to LVV (such as limb claudication). Another limitation is represented by the analysis of the soluble forms of the immune checkpoints exclusively, without studying the membrane-bound counterparts. However, our previous study on the effects of zoledronate on sCTLA-4 showed that the soluble form may be a good "proxy" for the activity of membrane-bound form, as showed by flow-cytometry analysis (*Giusti et al., under revision*).

6. CONCLUSIONS

PMR and GCA, in both its phenotypes related to cranial and large vessel involvement, are diseases with overlapping features of as yet unclear aetiology. Results from this study provide additional evidence that these phenotypes probably represent multiple facets of a syndrome rather than different conditions. FDG-PET/CT is a valuable technique, which offers panoramic view of different sites, allowing the detection of both joint and vascular inflammation, but also of possible neoplastic and infectious diseases. For this reason, it is a valuable tool in the evaluation of patients with FUO. Immune checkpoints are dysregulated in both patients with PMR and GCA, but they cannot be used to differentiate patients with or without LVV.

Prospective studies on the outcomes of patients with PMR and GCA with concomitant LVV are needed to establish the clinical and prognostic impact of inflammation of large vessels, in order to ensure the best therapy for these patients, assuring best possible outcomes while avoiding at the same time overtreatment.

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8. APPENDIX

8.1. Classification criteria used in the study.

Since no formal classification criteria exist for patients with LVV, patients fulfilling the 1990 ACR criteria for GCA were defined as having C-GCA in absence of obvious clinical signs of peripheral vasculitis (such as arm claudication).

Bird criteria for polymyalgia rheumatica[76].

1. Bilateral shoulder pain and/or stiffness
2. Onset of illness within 2 weeks
3. Initial ESR ≥40 mm/hour
4. Morning stiffness >1 hour
5. Age >65 years
6. Depression and/or loss of weight
7. Bilateral upper arm tenderness

Three or more criteria should be fulfilled.

1990 criteria for the classification of giant cell arteritis[77]

1. Age at disease onset ≥50 years: development of symptoms or findings beginning at age 50 or older
2. New headache: new onset of or new type of localized pain in the head
3. Temporal artery abnormality: temporal artery tenderness to palpation or decreased pulsation, unrelated to arteriosclerosis of cervical arteries
4. Elevated erythrocyte sedimentation rate: erythrocyte sedimentation rate ≥50 mm/hour by the Westergren method
5. Abnormal artery biopsy: biopsy specimen with artery showing vasculitis characterized by a predominance of mononuclear cell infiltration or granulomatous inflammation, usually with multinucleated giant cells.

Three or more criteria should be fulfilled.

Durack and Street classification of fever of unknown origin[48].

Classic

1. Temperature >38.3°C
2. Duration of >3 weeks
3. Evaluation of at least 3 outpatient visits or 3 days in hospital

Nosocomial

1. Temperature >38.3°C
2. Patient hospitalized ≥24 hours but no fever or incubating on admission
3. Evaluation of at least 3 days

Immune deficient (neutropenic)

1. Temperature >38.3°C
2. Neutrophil count ≤ 500 per mm3
3. Evaluation of at least 3 days

HIV-associated

1. Temperature >38.3°C
2. Duration of >4 weeks for outpatients, >3 days for inpatients
3. HIV infection confirmed

8.2. Supplementary table 1

	All pts	PMR	PMR+GCA	C-GCA	FUO	p	PET vasc2+	PET vasc2-	p	PET vasc3+	PET vasc3-	p
TVS/TJS Median (range)	0.6 (0-41)	0.5 (0-5)	1 (0.1-4.3)	2.2 (1-5)	9 (2-41)	<0.01	1 (0.2-41)	0.3 (0-2)	<0.01	0.5 (0-8.5)	2 (0.6-41)	<0.01
PLT/WBC Median (range)	38.6±12.4	38.6±11.8	41.2±15	36.3±13.3	37.1±14.9	0.75	39.6±12.9	37.3±11.6	0.3	45.8±13.4	36.2±11.1	<0.01
PLT/CRP Median (range)	9.4 (2.2-707.5)	9.9 (2.2-707.5)	6.8 (3-69.5)	16.3 (3.5-47.2)	4 (2.2-15)	0.21	9.1 (2.2-707.5)	10 (2.6-134)	0.23	7 (2.2-707.5)	10 (2.5-180.7)	0.21

PET vasc2+: PET scan showing at least one vascular region with a visual uptake ≥2. PET vasc3+: PET scan showing at least one vascular region with a visual uptake of 3. TVS: total vascular score. TJS: total joint score. PLT: platelets. WBC: white blood cells. CRP: C-reactive protein.

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