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Original article

Plasma Pyruvate Kinase M2 as a marker of vascular inflammation in giant cell arteritis

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Abstract

Objectives. GCA is a large vessel vasculitis in which metabolically active immune cells play an important role. GCA diagnosis is based on CRP/ESR and temporal artery biopsies (TABs), in combination with ^{18}F-fluorodeoxyglucose ([^{18}F]FDG)-PET/CT relying on enhanced glucose uptake by glycolytic macrophages. Here, we studied circulating Pyruvate Kinase M2 (PKM2), a glycolytic enzyme, as a possible systemic marker of vessel wall inflammation in GCA.

Methods. Immunohistochemical detection of PKM2 was performed on inflamed ($n = 12$) and non-inflamed ($n = 4$) TABs from GCA patients and non-GCA ($n = 9$) patients. Dimeric PKM2 levels were assessed in plasma of GCA patients ($n = 44$), age-matched healthy controls ($n = 41$), metastatic melanoma patients ($n = 7$) and infection controls ($n = 11$). CRP, ESR and macrophage markers calprotectin and YKL-40 were correlated with plasma PKM2 levels. To detect the cellular source of plasma PKM2 in tissue, double IF staining was performed on inflamed GCA TABs. [^{18}F]FDG-PET scans of 23 GCA patients were analysed and maximum standard uptake values and target to background ratios were calculated.

Results. PKM2 is abundantly expressed in TABs of GCA patients. Dimeric PKM2 plasma levels were elevated in GCA and correlated with CRP, ESR, calprotectin and YKL-40 levels. Elevated plasma PKM2 levels were downmodulated by glucocorticoid treatment. PKM2 was detected in both macrophages and T cells at the site of vascular inflammation. Circulating PKM2 levels correlated with average target to background ratios PET scores.

Conclusion. Elevated plasma PKM2 levels reflect active vessel inflammation in GCA and may assist in disease diagnosis and in disease monitoring.

Key words: GCA, Pyruvate Kinase M2, metabolism, vascular inflammation, [^{18}F]FDG-PET/CT

BASIC SCIENCE

Rheumatology key messages

- Pyruvate Kinase M2 is a candidate biomarker of vessel wall inflammation in GCA.
- Macrophages and T cells are metabolically active in untreated GCA patients.

Introduction

GCA is the most common type of vasculitis, affecting large- and medium-sized blood vessels. Systemic markers of inflammation such as IL-6 and CRP are known to be elevated in the blood of patients with GCA, and the IL-6 receptor blocker tocilizumab is the first targeted prednisolone-sparing treatment in GCA patients [1, 2]. The gold standard for the diagnosis in patients with suspected GCA is the presence of vascular

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inflammatory infiltrates, mainly CD4 T cells, macrophages and multinucleated giant cells, in temporal artery biopsies (TABs). However, TABs have limited sensitivity. The ^{18}F-fluorodeoxyglucose ([^{18}F]FDG)-PET scan is emerging as a more sensitive diagnostic imaging tool in GCA [3]. This technique relies on high glucose uptake by active immune and resident cells in vessel wall tissue. To identify new biomarkers for early detection of vessel wall inflammation in GCA diagnosis, we focussed on the relation between cell metabolism and inflammatory signatures in GCA.

In resting immune cells, glycolysis and the tricarboxylic acid cycle are in balance. However, upon activation, the energy demands of immune cells increase and a metabolic switch to aerobic glycolysis is induced. This is also known as the Warburg effect, which supports immune cell proliferation and immune function. Pyruvate Kinase M2 (PKM2) is a glycolytic enzyme involved in the last step of glycolysis. Emerging evidence indicates non-canonical functions of PKM2 in immune cells, where it can act as an immunomodulatory protein kinase and transcription factor [4–7].

PKM2 is allosterically regulated and can exist in a tetrameric and a dimeric form. The tetrameric form has a high affinity for binding phosphoenolpyruvate. It acts as an enzyme kinase and transfers a phosphate group from phosphoenolpyruvate to adenosine diphosphate, thereby generating pyruvate and adenosine triphosphate. The dimeric form of PKM2 has a lower affinity for phosphoenolpyruvate and is, therefore, less active in the glycolysis pathway than tetrameric PKM2. Various mechanisms such as metabolic intermediates or post-translational modifications favour the dimerization of PKM2 [8]. Dimeric PKM2 was first detected in the circulation of cancer patients. Different non-glycolytic functions of dimeric PKM2 have been characterized, especially in tumour cells and in the promotion of inflammatory responses, due to its protein kinase activity [6, 7, 9]. Dimeric PKM2 contributes to cytokine production and chronic tissue inflammation via phosphorylating STAT3 and STAT1 [10–12]. Dimeric PKM2 can also act as a transcription factor, translocating to the nucleus to regulate gene expression. In activated macrophages, transcriptional activity of dimeric PKM2 promotes pro-inflammatory signalling via HIF-1α, IL-1β and glycolytic enzymes [13–15].

So far, the role of the glycolytic enzyme PKM2 in GCA pathogenesis has not been investigated. In our study, we aimed to investigate systemic and local expression of PKM2 as an immune metabolic marker of vascular inflammation in GCA and to define its cellular source.

Methods

Patient sample collection and characteristics

Newly diagnosed GCA patients were prospectively followed according to a fixed study protocol. All GCA patients started treatment with glucocorticoids (GCs), according to the British Society for Rheumatology guidelines with a starting dose of 40–60 mg/day [16]. GCs were tapered by 10 mg every 2–3 weeks to 20 mg/day as long as there were no clinical signs and symptoms of disease activity and guided by CRP and ESR levels. For the PKM2 ELISA, samples of GCA ($n = 44$) at baseline, and at 6 weeks ($n = 32$) and 1 year ($n = 31$) of follow-up were selected for this study. All baseline samples were collected before treatment. As a reference, we included plasma samples of age-matched healthy controls ($n = 41$). Metastatic melanoma ($n = 7$) patients [17] were included as positive controls. Hospitalized infection control ($n = 11$) patients diagnosed with pneumonia or a urinary tract infection were included as inflammation controls.

For histological examination, inflamed ($n = 12$) and non-inflamed TABs ($n = 4$) of GCA patients and TABs from GCA negative (non-GCA) patients ($n = 9$) were stained for PKM2. The non-inflamed TABs are from patients suspected of having GCA who had aclinical diagnosis of GCA with negative histology. Written informed consent was obtained from all study participants. All procedures were in compliance with the declaration of Helsinki. The study was approved by the institutional review board of the University Medical Center Groningen (UMCG) (METc2012/375 for healthy controls, METc2011.388 for melanoma and METc2010/222 for GCA patients). Baseline characteristics of the study groups and details of included patient samples per analysis/assay can be found in Table 1 and supplementary Tables S1 and S2, available at *Rheumatology* online, respectively.

Immunohistochemistry and IF staining

Formalin-fixed, paraffin-embedded negative and positive TABs from GCA and non-GCA patients were deparaffinized. Next, antigen retrieval was performed and endogenous hydrogen peroxidase was blocked followed by incubation with a PKM2-specific antibody recognizing the monomeric, dimeric and tetrameric form of PKM2 (pH 6, 1:400, Cell Signalling and Technology, Leiden, The Netherlands) or with anti-IL-1β (1:100, pH9, Abcam, Cambridge, UK). Isotype control for PKM2 staining was included (supplementary Fig. S1A, available at *Rheumatology* online). Slides were stained with a secondary antibody for 1 h at room temperature (RT). After detection of peroxidase activity with 3,3′-diaminobenzidine, slides were counterstained with haematoxylin. Tissue stainings for calprotectin (pH9, 1:100, DAKO, Santa Clara, CA, USA) and YKL-40 (pH 9, 1:40, R&D Systems, Minneapolis, MN, USA) were essentially performed as described [18]. CD68 staining (Ventana Medical Systems, Oro Valley, AZ, USA) was performed on the Ventana Benchmark platform by a pathologist.

In order to identify co-localization of PKM2 with cell-specific markers, we performed IF double stainings. After antigen retrieval at pH9, slides were incubated for 1 h at RT with primary antibodies against PKM2 (1:100, Cell Signalling Technology, Danvers, USA), CD3 (1:25, DAKO) and CD68 (1:50, DAKO, Santa Clara, CA, USA). Next,

TABLE 1 Baseline characteristics of healthy controls, GCA, melanoma and infection control patient groups

Characteristics	HCs	GCA	Melanoma	INF
N	41	44	7	11
Age, years, median (range)	73 (58–96)	70 (52–89)	73 (51–82)	77 (55–93)
Female, *n* (%)	25 (59)	31 (70)	5 (71)	3 (27)*
GCA diagnosis (TAB/PET-CT/both), *n*	NA	13/21/8	NA	NA
GCA symptoms (cranial/systemic/combined), *n*	NA	15/12/17	NA	NA
Fulfilled ACR criteria, *n* (%)	NA	31 (70)	NA	NA
Ischaemic ocular involvement, *n* (%)	NA	21 (47)	NA	NA
Claudication, *n* (%)	NA	9 (20)	NA	NA
Six-week follow-up, median, days (range)	NA	42 (25–65)	NA	NA
One-year follow-up, median, days (range)	NA	380 (332–442)	NA	NA

HC: healthy control; INF: infection control; NA: not applicable; TAB: temporal artery biopsy. *$P < 0.01$.

slides were stained for 1 h (RT) with secondary antibodies. Lastly, fluorescence-labelled tertiary antibody was added and incubated for 1 h (RT). To block autofluorescence, the Vector TrueVIEW auto-fluorescence quenching kit (SP-8400, Vector Laboratories, Burlingame, CA, USA) was used according to the manufacturer's protocol. DAPI was used for nuclear conunterstain. Sections stained were scanned using a Nanozoomer Digital Pathology Scanner (NDP Scan U10074–01, Hamamatsu Photonics K.K., Shizuoka, Japan). The single stainings are depicted in supplementary Fig. S1B (available at *Rheumatology* online) demonstrating no bleed-through between channels. Antibody reagents and experimental details are shown in supplementary Table S3, available at *Rheumatology* online.

Percentages of positive cells in scanned tissues were scored with automated imaging software, QPath (University of Edinburgh, Edinburgh, UK) [19]. IF stainings were visualized with the LSM780, AxioObserver (Carl Zeiss BV, Breda, The Netherlands) confocal microscopy, and images were analysed using Zen Lite (Carl Zeiss BV, Breda, The Netherlands) software.

Laboratory measurements

Blood samples were drawn at the rheumatology and clinical immunology outpatient clinic of the UMCG. Blood serum and plasma were stored at –20°C until use. Plasma PKM2 levels of patients and controls were assessed using the ScheBo® M2-PK™ EDTA Plasma Test, Giessen, Germany according to the manufacturer's instructions. Notably, the ScheBo kit specifically detects the dimeric form of PKM2 [20, 21]. Serum IL-6 levels ($n = 38$) at baseline were measured by Human IL-6 High Sensitivity Magnetic Luminex Performance Assay (R&D Systems, Minneapolis, MN, USA). To investigate associations between plasma PKM2 levels and macrophage markers, we relied on previously published concentrations of calprotectin ($n = 42$) and YKL-40 ($n = 36$) in the same patients [18]. Details of laboratory measurements are shown in supplementary Tables S2–S4, available at *Rheumatology* online.

High-sensitive CRP and ESR were assessed in the context of standard medical care. CRP levels were determined using the Cobas 8000 modular analyser (Roche, Basel, Switzerland). ESR (Westergren method) was determined by the XN-9000 (Sysmex, Kobe, Japan).

FDG-PET/CT assessment

Twenty-nine GCA patients underwent [^{18}F]FDG-PET/low-dose CT (LDCT) scan examination. Patients who had taken GC for >3 days at the time of the FDG-PET/CT scan were excluded from analysis [22]. Also, patients were excluded when plasma sample collection and PET/CT were >14 days apart. Furthermore, patients with FDG-PET/LDCT images not meeting the EARL criteria were excluded [23, 24]. After exclusion, a total of 23 newly diagnosed GCA patients were included for [^{18}F]FDG-PET uptake analysis.

[^{18}F]FDG-PET/CT scans that were performed before May 2018 were analysed by Biograph mCT camera system (Siemens Healthineers, Erlangen, Germany) while the Biograph Vision camera system (Siemens Healthineers, Erlangen, Germany) was used for scans after May 2018. Prior to i.v. [^{18}F]FDG injection (3 MBq/kg), patients fasted at least 6 h and imaging was performed 1 h after injection.

[^{18}F]FDG PET images were obtained within 2–3 min per bed position in three-dimensional setting. Images were reconstructed using a time-of-flight iterative reconstruction method (three iterations; 21 subsets) with point-spread-function correction [23]. Images were corrected for random coincidences, scatter and attenuation (CT-based), and were smoothed with a Gaussian filter of 6.5 mm in full-width at half-maximum. [^{18}F]FDG-PET data from GCA patients were acquired from head to proximal femur or from head to feet.

FDG uptake was assessed visually in the subclavian arteries, common carotid arteries, ascending aorta, aortic arc, descending aorta and abdominal aorta. A visual score was performed by grading the FDG uptake in the arteries compared with uptake in the liver on a 0–3 scale where 0 = no visible uptake, 1 = uptake was lower than liver uptake, 2 = uptake equals liver uptake and 3 = uptake is higher than liver uptake. A total PET Vascular Activity Score (PETVAS) was calculated for each patient by summation of the visual scores of all artery segments, with a maximum of 27 [25]. [^{18}F]FDG uptake in the temporal arteries, maxillary arteries, occipital arteries, vertebral arteries, subclavian arteries, axillary arteries, carotid arteries, ascending aorta, aortic arch and descending aorta were assessed by maximum standardized uptake value (SUVmax). SUVmax scores for each patient were corrected for patient weight and injected radiotracer dose. Target-to-background ratio (TBR) was calculated using the mean standardized uptake value (SUVmean) of the superior caval vein as background.

For measurements, in-house developed software based on the syngo.via Frontier development kit (Siemens Healthineers, Erlangen, Germany) was used. For segmentation, volume of interest contours were manually drawn around arterial segments without including surrounding tissue. The SUVmax for all arteries and SUVmean of superior caval vein was measured. The maximal and the average value of SUVmax (maximal SUVmax, average SUVmax) and TBR (maximal TBR, average TBR) for all arterial segments were used for analysis.

Statistical analysis

Statistical differences by Mann–Whitney *U* test between two groups are shown only if the Kruskal–Wallis test showed significant differences between the study groups (three or more groups). Fisher's exact test was used for comparing sex differences between study groups. Follow-up patient samples were analysed by Wilcoxon signed rank test. Spearman's rank test was used for correlation analyses. Graphs and statistical analysis were established with Graphpad Prism 8.

Results

PKM2 expression detected at the site of vascular inflammation in GCA

Tissue expression of PKM2 was investigated by immunohistochemistry in TAB of GCA and suspected GCA patients. For this purpose, a PKM2-specific antibody recognizing all forms of PKM2 was used. Sections of inflamed and non-inflamed TABs from GCA patients were stained and compared with TABs from non-GCA patients (Fig. 1A). Histological analysis revealed an abundant PKM2 expression in infiltrates as well as in resident cells such as vascular smooth muscle cells and endothelial cells in inflamed GCA TABs (Fig. 1A, supplementary Fig. S2, available at *Rheumatology* online). Quantitative scoring of the adventitia, media and intima vessel wall layers revealed that both inflamed and non-inflamed TABs of GCA patients showed higher percentages of PKM2 expressing cells than TABs of non-GCA patients (Fig. 1B). PKM2 staining scores were significantly higher in inflamed GCA TAB than in non-GCA and non-inflamed GCA TABs. In the intima, the percentage of PKM2-positive cells in non-inflamed GCA TABs was higher than in non-GCA TABs.

Systemic levels of PKM2 are elevated in GCA patients at baseline but decrease upon treatment

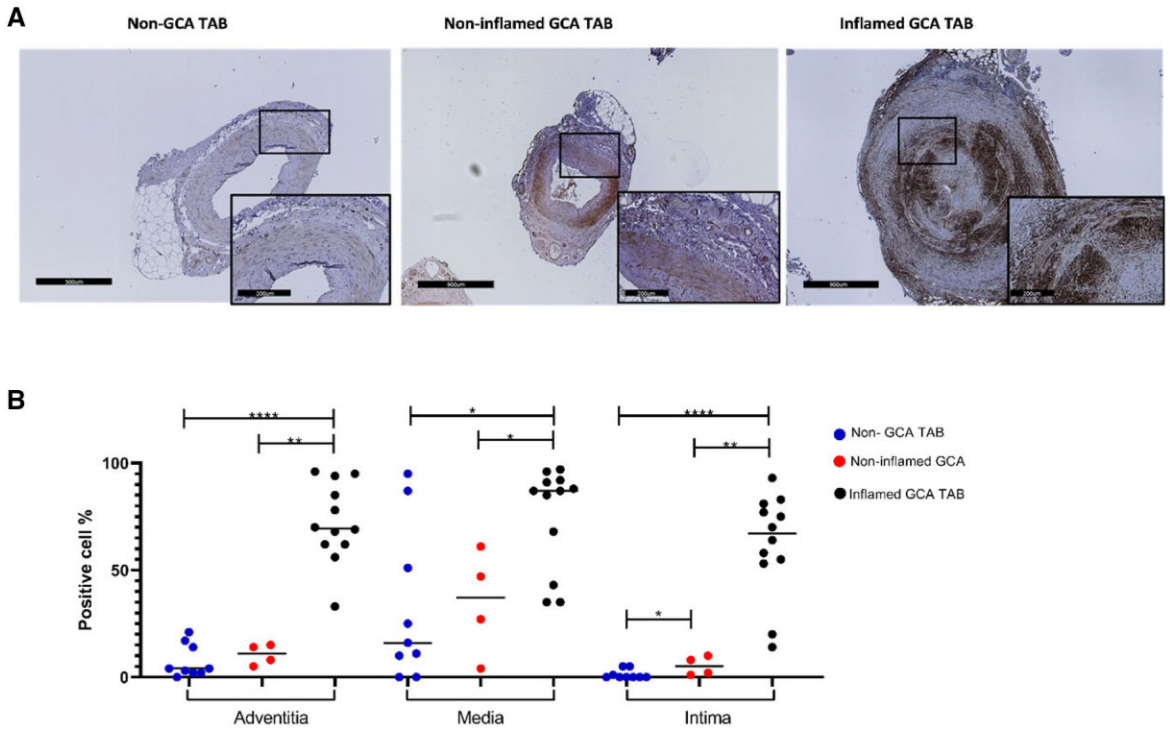
To analyse circulating PKM2 plasma levels in GCA patients, we performed an ELISA detecting dimeric PKM2. Samples from melanoma patients were included as positive control [20, 21, 26–28]. Infection controls were added as inflammation controls. A significant elevation of plasma PKM2 was detected in baseline samples of treatment-naïve GCA patients. Plasma PKM2 levels were also significantly elevated in infection controls, indicating that plasma PKM2 levels indeed associate with inflammation (Fig. 2A). To determine the effect of treatment on plasma PKM2 expression, dimeric PKM2 plasma levels at 6 weeks and 1 year after treatment were measured and compared with their baseline levels. A significant decrease of plasma PKM2 levels was observed upon treatment (Fig. 2B).

PKM2 plasma levels correlate with inflammation and macrophage activation markers in GCA patients at baseline

Plasma PKM2 levels correlated with systemic markers of inflammation such as CRP ($r = 0.42$, $P = 0.005$), ESR ($r = 0.362$, $P = 0.016$) and IL-6 ($r = 0.564$, $P = 0.0003$) (Fig. 3A). Beside inflammation markers, PKM2 levels correlated with YKL-40 and calprotectin, markers of macrophage activation, previously found to be elevated in GCA [18]. Plasma PKM2 levels correlated weakly with serum levels of calprotectin ($r = 0.379$, $P = 0.013$) and YKL-40 ($r = 0.376$, $P = 0.023$), both of which were also readily detected in vascular lesions (Fig. 3B). Additional immunohistochemical staining for calprotectin, and YKL-40 confirmed that tissue PKM2 expression was detected in macrophage-rich areas (Fig. 3C).

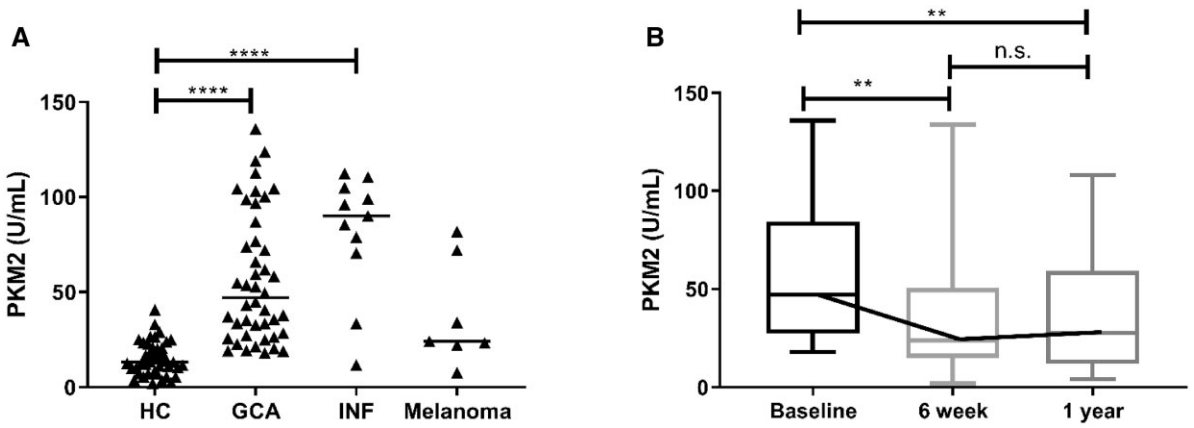
Previously, it was shown that lipopolysaccharide (LPS)-activated macrophages express dimeric PKM2 and that transcription of HIF-1α regulated genes such as IL-1β increases [29]. Thus, we investigated the expression of IL-1β in TABs of GCA patients. Histological

FIG. 1 Elevated PKM2 expression in temporal artery biopsies of GCA patients.



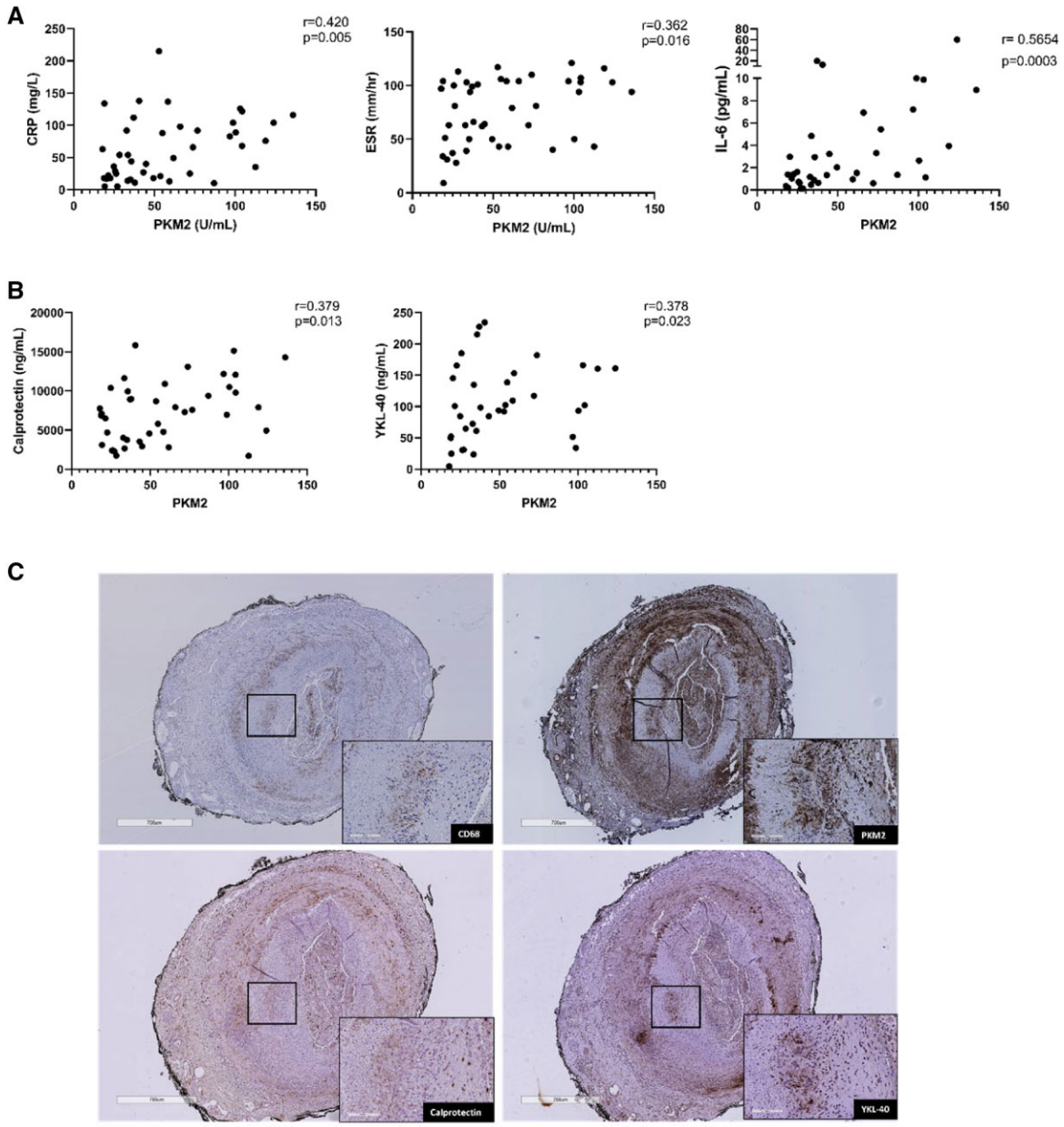
(**A**) Representative images of immunohistochemical staining of PKM2 in non-GCA, non-inflamed GCA and inflamed GCA TABs (left to right). (**B**) Quantification of PKM2 expression in non-GCA, non-inflamed GCA and inflamed GCA TABs by Qpath imaging software. Statistical differences by Mann–Whitney *U* test (A) between groups are displayed only if Kruskal–Wallis testing was significant. Non-GCA TABs, $n = 9$; non-inflamed GCA TABs, $n = 4$; inflamed GCA TAB, $n = 12$. ****$P < 0.0001$, **$P < 0.001$, *$P < 0.05$. PKM2: Pyruvate Kinase M2; TAB: temporal artery biopsy.

FIG. 2 Plasma PKM2 levels at baseline in GCA patients and modulation of plasma PKM2 by treatment.



The Mann–Whitney *U* test (**A**) and Wilcoxon tests (**B**) were used for statistical analysis. Median with minimum and maximum values are shown (A, B). Statistical differences by Mann–Whitney *U* (A) between groups are displayed only if Kruskal–Wallis testing was significant. ****$P < 0.0001$, **$P < 0.01$, ns = not significant. HC: healthy control; INF: infection controls; PKM2: Pyruvate Kinase M2.

FIG. 3 Dimeric PKM2 levels correlate with inflammation and macrophage markers



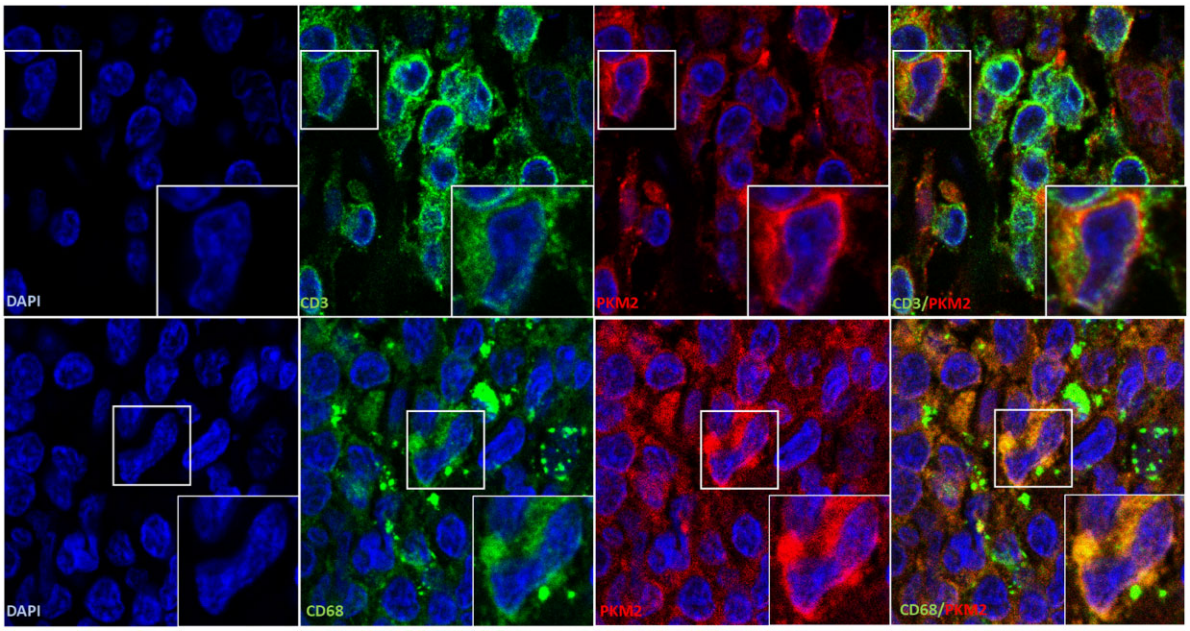
Association of plasma PKM2 with (**A**) CRP ($n = 44$), ESR ($n = 44$), IL-6 ($n = 38$) and (**B**) calprotectin ($n = 44$) and YKL-40 ($n = 36$) at baseline in GCA patients. r, Spearman's rank correlation coefficient and *P*-values are indicated in the graphs. (**C**) Representative images showing immunohistochemistry detection of CD68 (upper left), PKM2 (upper right) and macrophage products calprotectin (lower left) and YKL-40 (lower right) in GCA temporal artery biopsy. PKM2: Pyruvate Kinase M2.

examination of the tissues showed that PKM2 and IL-1β are indeed expressed in macrophage-rich areas (supplementary Fig. S3, available at *Rheumatology* online).

Macrophages and T cells express PKM2 in GCA

As tumour cells can release dimeric PKM2 into the circulation [26, 30], we hypothesized that abundant PKM2 expression by glycolytic cells in the inflamed vessel may underlie the elevated plasma PKM2 levels in GCA. As T cells and macrophages are regarded as key contributors to vessel wall inflammation in GCA, we focussed on PKM2 expression by these cell types in the GCA vascular lesions. The IF data show PKM2 expression in areas with infiltrated T cells and macrophages (Fig. 4). Taken together, the data suggest

Fig. 4 IF reveals PKM2 expression in macrophages and T cells in temporal artery biopsy from a GCA patient



From left to right stainings for: DAPI: blue; PKM2: red; CD3/CD68: green. Regions of interest are shown in white boxed areas and magnified in the lower right corner. T cell (upper panel) and macrophage (lower panel) areas. PKM2: Pyruvate Kinase M2.

that metabolically active (glycolytic) macrophages and T cells in vasculitis tissue express PKM2 and may thus contribute to the elevated PKM2 plasma levels in GCA.

Plasma PKM2 levels correlate with average TBR on PET

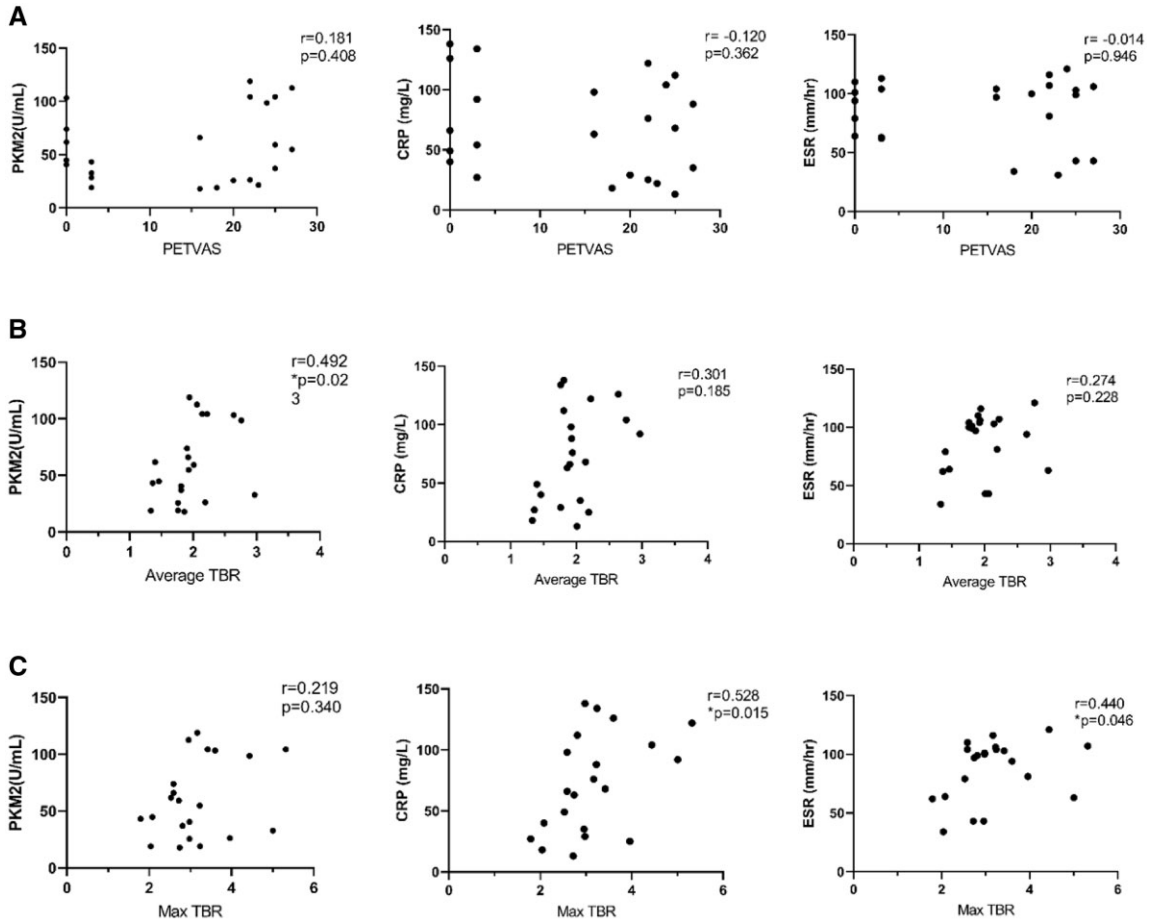
Considering the function of PKM2 in glycolysis, we investigated the utility of systemic PKM2 as a marker for blood vessel inflammation. Inflammation burden was measured by PETVAS, TBR and maximum SUVmax in treatment-naïve GCA patients at baseline having been diagnosed with FDG/PET [31]. PETVAS scores were not associated with PKM2 or any of the systemic inflammation markers (CRP and ESR) (Fig. 5A). However, quantified FDG vessel uptake in GCA patients was correlated with plasma PKM2 and serum CRP levels and ESR. Average TBR correlated with PKM2 ($r = 0.492$, $P = 0.023$) but not with CRP ($r = 0.301$, $P = 0.185$) and ESR ($r = 0.274$, $P = 0.228$), while maximum TBR correlated with CRP ($r = 0.528$, $P = 0.015$) and ESR ($r = 0.440$, $P = 0.046$) but not with PKM2 (Fig. 5B and C). After exclusion of three patients with C-GCA only, PKM2 remained associated with average TBR and CRP with maximum TBR in LV/Combi-GCA patients ($n = 20$) respectively. The association between average TBR and plasma PKM2 levels suggests that PKM2 is a possible systemic marker of vessel inflammation in GCA.

Discussion

Our main finding is that the glycolytic enzyme PKM2 is expressed at the site of vascular inflammation in GCA and that dimeric PKM2 may have utility as a marker of vessel inflammation. One of the hallmarks of tumour cells facilitating their proliferation is their capacity to shift their metabolism to aerobic glycolysis, the so-called Warburg effect. During inflammation, activated immune cells display a similar metabolic profile to tumour cells. Immunometabolism in autoimmune inflammatory conditions has gained much interest and is emerging as a field of intense study as it may provide novel targets for therapy [32–35].

Pyruvate Kinase (PK) is a glycolytic enzyme that catalyses the rate-limiting step in glycolysis. PK has four isoforms L, R, M1 and M2. The PKM2 isoform is mainly expressed in rapidly proliferating, differentiating cells [36, 37]. In line with this, elevated PKM2 levels were detected in the circulation of various cancer patients and proposed as a possible diagnostic and prognostic biomarker [20, 21, 26–28, 38]. In LPS-stimulated macrophages, PKM2 was revealed as a critical determinant of the Warburg effect [29]. In RA, increased PKM2 expression was reported [39]. In arthritic rats, upregulated PKM2 expression was shown to be involved in macrophage activation via STAT1 signalling [11]. Moreover, in experimental autoimmune encephalomyelitis increased expression of PKM2 was found to be a regulator of Th17 cells differentiation and inflammation by interaction with STAT3 enhancing its activity [40]. Previous studies

FIG. 5 Correlation of plasma PKM2, CRP and ESR with PETVAS, average and maximal target to background ratio scores



Correlation of plasma PKM2, CRP and ESR with (**A**) PETVAS, and (**B**) average and (**C**) maximal target to background ratio scores. r, Spearman's rank correlation coefficient and *P*-values are indicated in the graphs. PETVAS: PET Vascular Activity Score; PKM2: Pyruvate Kinase M2; TBR: target to background ratio.

demonstrated that the JAK-STAT pathway in GCA contributes to the disease's pathogenesis, and blockade of this pathway was shown to be beneficial in GCA treatment [41–43]. Here, we first showed a clear increase in expression of PKM2 in inflamed TABs of GCA patients compared with non-GCA patients. The abundant PKM2 expression in inflamed TAB can be explained by infiltration and differentiation of metabolically active immune cells. PKM2 can exist as either a monomer, dimer or tetramer in the cell. The monomeric PKM2 is inactive but upon tetramerization, PKM2 gains high enzymatic activity while dimeric PKM2 is largely responsible for the non-canonical, pro-inflammatory functions of PKM2 [36, 37]. In plasma of GCA patients, we found dimeric PKM2 levels were elevated when compared with age-matched healthy individuals. Elevated PKM2 levels in infection controls indicate that PKM2 is not specific for GCA (or melanoma) but rather associates with glycolytic inflammatory processes. The modulation of plasma PKM2 levels upon GC treatment suggests that circulating PKM2 reflects disease activity.

At baseline, plasma PKM2 expression correlated with CRP and ESR and also with levels of macrophage products calprotectin and YKL-40 which were previously identified by us as markers of vessel inflammation in GCA [18]. Furthermore, others demonstrated that dimeric PKM2 regulates HIF-1α genes and induces IL-1β as a determinant of the Warburg effect in macrophages during inflammation [29]. Our histological examination showed PKM2 expression in macrophage-rich areas where calprotectin, YKL-40 and IL-1β seem to be co-expressed. This may suggest a link between PKM2 and metabolically active, pro-inflammatory macrophages in GCA pathogenesis.

As GCA is a granulomatous disease with important roles for both T cells and macrophages at the site of vascular inflammation, we hypothesized that these cells would be the source of local and systemic PKM2

expression. Although the assay we used for plasma PKM2 specifically detects the dimeric form, PKM2 expression in the tissue may reflect the tetrameric, dimeric and even the monomeric form of PKM2 as the antibody used does not discriminate between the different forms. Our data revealed that pro-inflammatory T cells and macrophages express PKM2 at the site of inflammation, which is in line with previous studies [11, 29, 40, 44, 45]. Given that the dimeric form of PKM2 translocates to the nucleus to exert its non-canonical effects [36, 37], nuclear expression of PKM2 in T cells and macrophages is a notion to be further studied in GCA.

The [^{18}F]FDG-PET/CT technique is based on glucose uptake by inflammatory glycolytic cells and is now used as a sensitive diagnostic tool in GCA [31, 46, 47]. Interestingly, plasma PKM2 levels correlated with the calculated average TBR from FDG-PET/CT scans of GCA patients. Other inflammation markers such as CRP and ESR did not correlate with average TBR but correlated with maximum TBR. Average TBR, however, is more representative of the overall systemic vessel inflammation than maximum TBR, which represents the maximum FDG uptake in one vessel of all vessel segments analysed. These findings thus support the role of plasma PKM2 as a marker reflecting the extent of vessel wall inflammation in GCA.

Our study showed tissue expression of the dimeric form of PKM2 in the nucleus of T cells and macrophages. Previous studies revealed that pharmacological targeting of PKM2 by compounds such as TEPP-46 induces PKM2 tetramerization and thereby suppresses subsequent pro-inflammatory gene transcription. This leads to decreased proliferation and cytokine production in immune cells. Indeed, PKM2 inhibition inhibited the development of Th1 and Th17 *in vitro* and suppressed the development of experimental autoimmune encephalomyelitis *in vivo*. This provides evidence that pharmacological targeting of PKM2 affects the T-cell-mediated inflammatory response in this model [44]. Furthermore, Palsson-McDermott *et al.* showed that in LPS-stimulated macrophages PKM2 activation (tetramerization) by TEPP-46, increases IL-10 production, thereby activating an anti-inflammatory circuit [29]. PKM2 activation may thus qualify as an interesting target for treatment in GCA, warranting further investigation.

In conclusion, the identification of biomarkers that predict and monitor vessel wall inflammation in GCA is still awaited. Our findings suggest that elevated PKM2 expression in affected arteries of GCA patients, reflecting TBR [^{18}F]FDG-PET uptake, might aid GCA diagnosis and monitoring of disease activity. Additionally, as non-canonical functions of PKM2 are associated with vessel wall inflammation, the pharmacological induction of tetrameric PKM2 may hold promise for the treatment of autoimmune inflammatory disorders like GCA.

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Disclosure statement: E.B. as employee of the University Medical Center Groningen (UMCG) received speaker/consulting fees from Roche that were paid to the UMCG. The other authors have declared no conflicts of interest.

Data availability statement

The raw data supporting the conclusions of this manuscript will be shared on reasonable request to the corresponding author.

Supplementary data

Supplementary data are available at *Rheumatology* online.

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