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Letter to the Editor (Matters arising from published papers)

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Comment on: Plasma Pyruvate Kinase M2 as a marker of vascular inflammation in giant cell arteritis: reply

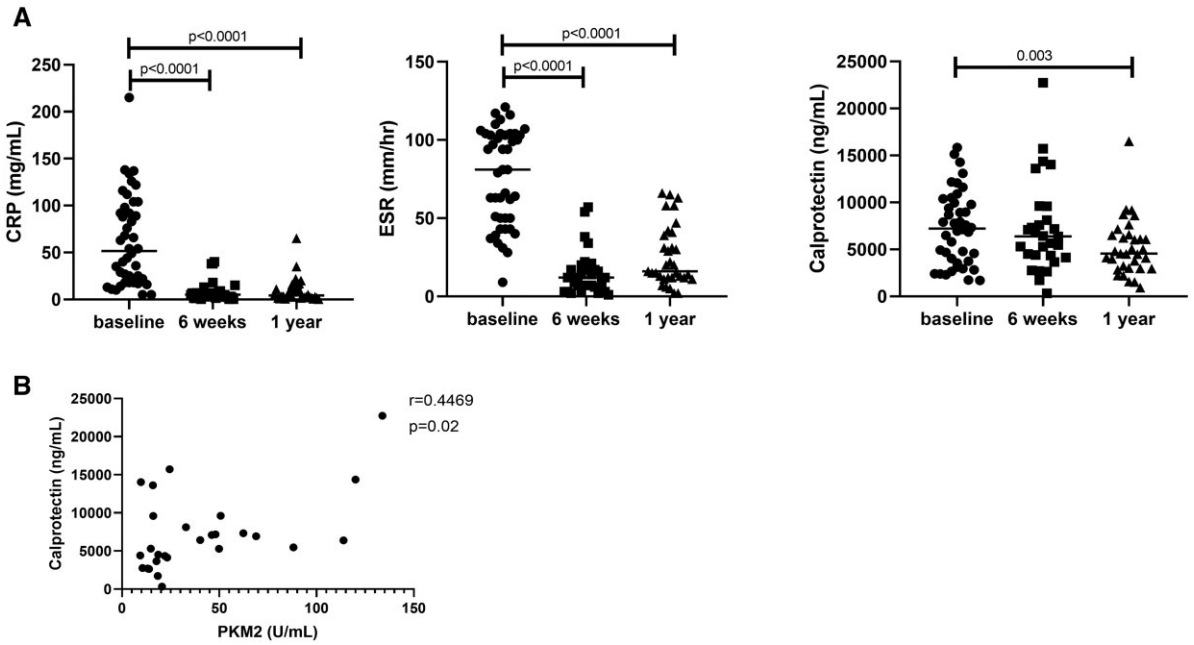
Dear Editor, We thank Dr R. Watanabe and Dr M. Hashimoto for their interest in our recently published article on pyruvate kinase M2 (PKM2) expression in giant cell arteritis (GCA). We are happy to respond to the issues raised in their letter [1] and to share some of our additional observations in a point-by-point reply below.

Firstly, as pointed out by the authors, plasma PKM2 levels are indeed elevated not only in active GCA but also in malignancies and infections [2]. Thus, elevated PKM2 levels are not specific for GCA but rather reflect glycolytic inflammatory processes. Therefore, we fully agree that there is an urgent clinical need for novel diagnostic biomarkers that can differentiate between GCA and other inflammatory diseases/infections. It may well be that a combination of biomarkers is required to achieve this. Of note, 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 biomarkers in GCA. Whereas CRP and ESR levels decrease after glucocorticoid (GC) treatment, both macrophage markers remain elevated despite GC treatment, which may reflect ongoing vascular inflammation [3]. For this reason, we performed an additional analysis of CRP, ESR, calprotectin and plasma PKM2 levels after 6 weeks and 1 year of GC treatment (Fig. 1A). We found a positive correlation between calprotectin and plasma PKM2 levels ($r = 0.447$, $P = 0.02$) after 6 weeks of treatment (Fig. 1B). There was no correlation between PKM2 and calprotectin levels after 1 year of GC treatment. This association between circulating PKM2 and calprotectin at 6 weeks may indicate that plasma PKM2 also reflects ongoing vascular inflammation in GCA patients.

Secondly, authors argue that PKM2 is a critical determinant for Th17 differentiation [4, 5] which we did not include in our analysis. We agree that a correlation between plasma PKM2 and IL-17 is worth studying. Unfortunately, we did not study this in the PKM2 study cohort. However, we measured absolute counts of Th17 cells (defined as CD4+/IFNγ-/IL-17+) in a limited group of treatment-naïve GCA patients ($n = 7$). No significant association between Th17 cell counts and plasma PKM2 levels in baseline GCA patients was found in this small group ($r = 0.357$, $P = 0.444$).

Lastly, the authors raise an interesting point concerning the effect of PKM2 on the expression of the immunoinhibitory ligand, programmed death protein ligand 1 (PD-L1), on macrophages. In the pathogenesis of GCA, a dysregulation of the PD-1/PD-L1 pathway was previously reported by Zhang *et al.* [5]. To investigate the possible contribution of immune checkpoint pathways in GCA, our group previously explored the expression of several immune checkpoint molecules by peripheral blood monocytes and CD4+ T cells of GCA patients and in age- and sex-matched healthy controls [6]. Our data did not reveal a change in frequencies of PD-L1 expressing cells of GCA patients and HCs in the periphery. Additionally, we examined temporal artery biopsies (TAB) of GCA and non-GCA patients for checkpoint expression and demonstrated increased infiltration of PD-L1+ cells in GCA TAB compared with non-GCA TAB. In various studies, dimeric PKM2 has been reported as a regulator of PD-L1 expression on macrophages due to its nuclear translocation and effect on gene transcription [7–9]. Our observations in GCA-affected tissues on PD-L1 and PKM2 expression may indeed suggest a possible link between them. Interestingly, Watanabe and coworkers previously demonstrated that glucose availability in the microenvironment is a critical determinant for enhancing PD-L1 expression on macrophages from HCs but cannot overcome the PD-L1 deficiency they found for GCA macrophages [10]. Their findings exhibit an important link between cellular metabolism and the immune response in GCA. Thus, targeting the metabolic pathway may grant a new therapeutic direction in the treatment of GCA. Previously, in LPS-induced macrophages targeting PKM2 with TEPP-46, a potent activator of tetrameric PKM2, was found to downregulate PD-L1 expression [9] which may exacerbate the pathology of GCA as remarked by authors. However, it has also been shown that inducing the tetrameric form of PKM2 via TEPP-46 suppresses pro-inflammatory responses by inhibiting IL-1ß production and inducing IL-10 [11]. In our study, we observed PKM2 expression in areas of IL-1ß+ macrophages and reported an association of PKM2 with other inflammatory and macrophage markers in both blood and tissue [2]. Therefore, we believe that TEPP-46 has potential as a therapeutic agent in GCA treatment. However, we fully agree that more detailed investigations into the link between changes in the metabolic machinery of macrophages and GCA vasculopathy are warranted. More insight may help the identification of the highly awaited novel diagnostic and disease monitoring biomarkers in GCA.

FIG. 1 Plasma PKM2 levels in GCA patients are associated with continuing vascular inflammation



(**A**) CRP, ESR and calprotectin levels in 6-week and 1-year follow-up patients. (**B**) Correlation of calprotectin levels and plasma dimeric PKM2 in GCA after 6 weeks of treatment. (**A**) Wilcoxon test (**B**) r, Spearman's rank correlation coefficient were used for statistical analysis. P-values are indicated in the graph.

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Data availability statement

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

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