New blood biomarkers and imaging for disease stratification and monitoring of giant cell arteritis

Alessandro Tomelleri 1 , Christian Dejaco 2,3

ABSTRACT
Relapses and late complications remain a concern in giant cell arteritis (GCA). Monitoring strategies are required to effectively tailor treatment and improve patients’ outcomes. Current monitoring of GCA is based on clinical assessment and evaluation of traditional inflammatory markers such as C reactive protein and erythrocyte sedimentation rate; however, this approach has limited value in patients receiving interleukin (IL)-6 blocking agents. New blood biomarkers that are less dependent on the IL-6 axis such as IL-23, B cell activating factor, osteopontin and calprotectin have been explored, but none of them has yet accumulated sufficient evidence to qualify as a routine follow-up parameter. Imaging techniques, including ultrasound and 18F-fluorodeoxyglucose positron emission tomography/computed tomography, potentially offer additional insights; however, the choice of the imaging method as well as its interpretation must be investigated further. Future studies are required to investigate the outcome of patients with GCA whose treatment decisions are based on traditional plus novel (laboratory and imaging) biomarkers as compared with those undergoing conventional monitoring strategies.

CURRENT MONITORING STRATEGIES IN GIANT CELL ARTERITIS
Giant cell arteritis (GCA) is the most common primary vasculitis in older adults. Glucocorticoids (GCs) are the treatment of primary choice; however, sustained remission is achieved only in a minority of patients (10%–20%) when GCs are used in monotherapy. In patients with refractory or relapsing disease, the addition of GC-sparing agents such as tocolizumab or methotrexate is recommended. These agents should also be considered in cases with an increased risk for GC-related adverse events. The recently published treat-to-target recommendations for GCA and polymyalgia rheumatica suggest regular monitoring of patients with GCA in order to control symptoms, prevent disease-related damage and minimise treatment-related adverse events. In most centres, patients are followed up every 3–6 months, and disease monitoring is based on clinical assessment and traditional inflammatory markers such as C reactive protein (CRP) and erythrocyte sedimentation rate (ESR). The main limitations of this strategy are the facts that GCA-related symptoms may be non-specific during a flare and that CRP and ESR are directly suppressed by interleukin (IL)-6 receptor inhibitors, preventing an increment of these markers even when the disease is active. No reliable alternative laboratory biomarker has been established yet; hence, vascular imaging has become an attractive additional tool for monitoring, particularly in situations of clinical uncertainty. The most commonly used imaging technique in GCA is vascular ultrasound, which has the advantages of prompt availability, absence of radiation and low resource consumption. Additionally, it has the potential to intercept disease flares by demonstrating the reappearance of the halo sign in the most accessible vascular territories (usually the common superficial temporal arteries and their branches as well as the axillary arteries). At cranial arteries, a decrease of arterial wall swelling can be detected already a few days after start of GCs, while at axillary arteries, the improvement is usually more gradual. In many patients, slight intima-media thickening might even persist for a long time, and it is often associated with a change of the ultrasound pattern towards a hyperechogenic appearance with multilineal bands. The main limitations of ultrasonography are the operator dependence, the lack of training with this technique among many rheumatologists and the difficulty in assessing certain vascular territories such as the thoracic aorta.

A complementary role can be assigned to 18F-fluorodeoxyglucose positron emission tomography (18F-FDG PET)/computed tomography (CT), which provides a total-body assessment (including the entire aorta)
and may sometimes yield a positive result even when the ultrasonography is negative. However, given the high costs, time consumption and invasiveness, $^{18}$F-FDG PET/CT cannot be applied for regular monitoring several times a year but should be reserved to situations where the ultrasonography result is inconclusive. In addition, $^{18}$F-FDG PET/CT results may be influenced by high blood glucose levels or concomitant metformin therapy, which is why special care must be taken when patients who have GCA with diabetes mellitus are studied.

Another application of vascular imaging is to detect and monitor vascular damage, particularly with regard to the development of aortic aneurysms and arterial stenoses, which can affect 20%-30% (mostly the descending thoracic and the ascending aorta) and 10% of patients, respectively. Even though aortic aneurysms occur frequently in people with GCA, only a minority of these aneurysms (and usually those located at the level of the ascending aorta) eventually undergo surgical repair. CT angiography (CTA) and magnetic resonance angiography (MRA) are the most appropriate techniques for detecting vascular damage, although it must be considered that not all patients can undergo such procedures due to renal insufficiency, allergy to the contrast medium or (in the case of MRA) the presence of metal implants. Furthermore, there is still much uncertainty about when, how often and in which patients they should be applied, especially considering that vascular complications can occur a long time (>5 years) after the disease outset and despite the disease having been quiescent for years.

NEW BLOOD BIOMARKERS IN GCA

Currently investigated blood biomarkers in GCA can be divided into two main categories: first, markers to measure actual disease activity that are not directly influenced by the IL-6 pathway (ie, biomarkers for monitoring disease activity) and, second, parameters investigated at baseline or in the early phase of treatment to predict short-term and long-term outcomes (ie, prognostic biomarkers).

Concerning monitoring biomarkers, serum calprotectin is one of the most promising parameters. In states of inflammation, neoplasia or infection, monocytes and neutrophils secrete calprotectin, which acts as a damage-associated molecular pattern and prompts macrophages to release proinflammatory cytokines (eg, IL-1, IL-6 and tumour necrosis factor) via the activation of nuclear factor kappa B and p38 mitogen-activated protein kinase pathways. Increased levels of calprotectin have consistently been associated with active disease in several inflammatory rheumatic diseases, including rheumatoid arthritis and spondyloarthritis, and there seems no apparent direct effect of tocilizumab on this molecule. At least four studies evaluated the performance of calprotectin for monitoring GCA yielding conflicting results, partly due to different study designs and heterogeneous outcome assessments. Specifically, three studies found a positive correlation between calprotectin levels and disease activity of GCA, whereas one study did not confirm this result. The latter study even reported that calprotectin levels remained persistently elevated throughout the disease course, even in phases of treatment-free remission. Disease activity was defined differently in all studies: by the presence of signs/symptoms alone in one study, by a combination of signs/symptoms and acute phase markers, by a combination of acute phase markers and the GC dose required to control disease symptoms, or by the combination of the Birmingham Vasculitis Activity Score and a physician’s global assessment in the other studies. Other sources of heterogeneity were the small sample size (ranging from 10 to 59 patients) and the absence of adequate clinical and imaging phenotyping of patients. In terms of practicability, calprotectin is easy to measure by commercially available ELISA kits and is relatively stable in the preanalytic phase. Calprotectin can be determined from ethylene diamine tetraacetic acid (EDTA) plasma and serum; however, the latter is usually preferred given that calprotectin levels tend to be lower in EDTA plasma due to the presence of calcium altering the protein structure of this molecule.

IL-23 is another key regulator of vascular inflammation in GCA. It is secreted by dendritic cells, macrophages and T cells and is highly expressed in temporal arteries of patients with GCA. IL-23 stimulates the production of both IL-6 and IL-17 and, as part of the IL-6–IL-17 cluster, is highly active in early and untreated patients and rapidly suppressed by GC. IL-23 could be measured in clinical practice using Luminex assays; however, evidence for the value of this cytokine as a biomarker in GCA is limited, given that it has been tested only in a small study of patients treated with GC with or without leflunomide. In that study, relapses were the main outcome, defined by the presence of new or increasing clinical symptoms requiring treatment intensification.

Other potential monitoring biomarkers include the B cell activating factor (BAFF) and serum osteopontin. BAFF is a key regulator of B cell responses, produced mainly by stromal cells but also by monocytes following exposure to proinflammatory cytokines such as interferon-gamma. BAFF levels are higher in patients with new-onset GCA than in healthy controls, and serum concentrations of this molecule correlate strongly with disease activity, as defined by clinical symptoms and ESR levels. A correlation with disease activity (defined by clinical symptoms and acute phase reactants) has also been reported for osteopontin. Osteopontin is expressed by a wide range of cells involved in both innate and adaptive immune responses and is highly upregulated during macrophage differentiation. Its major functions include stimulation of Th1 and Th17 differentiation, inhibition of Th2-mediated responses and contribution to angiogenesis and vascular remodelling. Notably, both BAFF and osteopontin correlate with CRP and ESR, their levels are quickly reduced after GC therapy and they are not affected by IL-6 blocking agents. BAFF and osteopontin can easily be determined by ELISA using...
commercially available kits; however, additional research in larger cohorts is necessary to confirm that these molecules are reliable surrogates of disease activity in GCA.

The second group of biomarkers comprises parameters predicting the disease course when evaluated at baseline (or at some other point of the disease). The identification of such biomarkers could theoretically help clinicians tailor therapeutic and monitoring strategies according to the individual risk. Relapses might be predicted by baseline levels of osteopontin and matrix metalloproteinase-2; higher levels of the former appear to be linked to an increased relapse risk, whereas higher levels of the latter seem to be protective. Angiopoietin-2, a marker of angiogenesis, was higher in patients with GCA who flared up in the subsequent 4 months, and a direct correlation was found between levels of angiopoietin-2 or YKL-40 (a glycoprotein mainly produced by macrophages during inflammatory states) and the duration of GC therapy.

IL-6 plays a central role in the pathogenesis of GCA, and its levels reflect disease activity correlating almost perfectly with CRP. In contrast to CRP and ESR, however, IL-6 levels tend to increase after the start of tocilizumab with subsequent gradual decrease. In patients treated with tocilizumab, IL-6 does not correlate with disease activity; however, a lack of IL-6 decline is associated with a higher risk of relapse once tocilizumab has been stopped. This might inform the clinician about whether treatment could (or could not) be discontinued in a patient in remission. Whether persistently elevated IL-6 levels indicate ongoing subclinical inflammation or are linked with a positive feedback loop because of IL-6 receptor blockade needs to be investigated further.

Many of the biomarkers described above revealed promising results in small observational studies; however, none of it is yet supported by enough evidence to qualify as a routine parameter. Most studies showed only weak correlations between the biomarker and disease activity and used different criteria to define disease activity and other outcomes. Future research is necessary not only to validate the performance of these parameters in larger cohorts but also to demonstrate that management decisions based on these biomarkers result in better outcomes for patients.

**ADVANCED IMAGING TECHNIQUES FOR PATIENT STRATIFICATION AND DISEASE MONITORING IN GCA**

Similar to blood biomarkers, imaging might be applied for disease stratification, monitoring and prognostic evaluation. Vascular ultrasonography is probably the best candidate for this purpose, given that it is non-invasive, cheap and quickly available. Ultrasonography scores such as the OMERACT GCA Ultrasonography Score (OGUS) or the Halo Score are reliable and sensitive to change and correlate with other markers of disease activity. Scoring systems have also been developed for ¹⁸F-FDG PET/CT (eg, PET Vascular Activity Score and total vascular score); however, these still require prospective validation.

An open question is how positive imaging (either ultrasonography, ¹⁸F-FDG PET/CT or other imaging) should be interpreted in patients in clinical and laboratory remission. Specifically, it must be understood whether such positivity corresponds to subclinical inflammation (and therefore deserves treatment escalation), vascular remodelling (which follows inflammation and precedes vascular damage and is probably less influenced by anti-inflammatory agents) or something else. A related question is the possible significance of a positive ¹⁸F-FDG uptake in a vascular territory where no changes appear on ultrasonography (or any other imaging technique) and vice versa. Until these questions have been answered, caution must be exerted when requesting and interpreting imaging for monitoring of GCA.

As for laboratory biomarkers, there is a major interest in the role of imaging for disease stratification and prediction of long-term outcomes, particularly vascular damage. For instance, a higher ultrasonography score at temporal arteries is associated with a higher risk of blindness, while a positive ¹⁸F-FDG PET/CT of the thoracic aorta predicts the development of an aneurysm at a later time point. In addition, a higher total vascular ¹⁸F-FDG PET/CT score correlates with increased yearly growth of the aortic diameter. However, this seems to apply to the thoracic aorta only, since most vascular territories with increased tracer uptake subsequently neither develop stenosis nor dilatation.

How to monitor vascular damage such as aortic aneurysms is another unresolved issue. The technique with the highest sensitivity and precision is CTA. However, due to the high radiation load and the use of contrast agents, it must be limited to selected time points, which still have to be defined. MRA is an alternative, providing information on structure and inflammation. Ultrasonography has a window over the ascending aorta, but it is impossible to adequately evaluate the descending aorta, which is the territory where GCA-associated aneurysms typically occur.

All imaging techniques require a high level of expertise and are operator dependent. These are presumably the most important limitations of imaging as compared with laboratory biomarkers, where testing can be standardised more easily. This shortcoming will probably decrease in future with increasing training offers to physicians (improving expertise) as well as with the potential integration of artificial intelligence and particularly with deep learning, assisting in the analysis of imaging findings.

**FUTURE DIRECTIONS OF RESEARCH ON LABORATORY AND IMAGING BIOMARKERS IN GCA**

We are entering a new era in the management of GCA. Whereas in the past, the main goals were to prevent vision loss and control disease flares with variable doses of GC, today our ability to individualise management has...
advanced significantly. This advancement undoubtedly stems from novel therapies (already available and on the horizon) but also from an increased availability of laboratory biomarkers and imaging techniques that have the potential to improve monitoring and prediction of disease activity and progression as outlined above. The application of these tools, however, is still grounded on an ‘eminence-based’ approach or relegated to small, experimental studies using heterogeneous comparators and outcomes (Box 1).

In order to homogenise future studies in the field, the next step in research should be the definition of internationally accepted criteria for active disease, remission and relapse, as well as GCA-associated vascular damage, that can be used as comparators or ‘gold standards’. These criteria cannot include any of the experimental biomarkers (risk of circularity) and should be designed for application also in patients receiving drugs that directly interfere with levels of ESR and CRP. Subsequently, all new biomarkers should be tested in large observational studies, which would then be comparable and meta-analyzable. It is important to emphasise that the mere demonstration of a statistically significant association between a candidate biomarker and a specific outcome is not sufficient to qualify such a biomarker for clinical practice. There are several factors that have to be taken into account, including (but not limited to) the sample size of the study, the homogeneity of the population, the duration of the observation and the reproducibility of results.

The most important questions of the research agenda (Table 1), however, are whether and how the new (blood and imaging) biomarker(s) would inform the management decisions of physicians in clinical practice. A trial is needed to answer these questions, but this can only be done after all the previous steps (ie, definition of gold standard and extensive testing in observational studies) have been completed. Such a study would be designed as a randomised controlled trial involving two groups: a control group, where patients are followed up clinically and treatment decisions are based on traditional clinical and laboratory measures, and an experimental group, where novel biomarkers would complement the traditional assessments. The primary endpoint would be the achievement of remission (following the treat-to-target recommendations5) and the prevention of damage according to the novel ‘gold standard’ definitions. Ideally,

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**Box 1 Key learning points**

- Giant cell arteritis (GCA) is a chronic disease with a tendency to recur and cause vascular damage.
- GCA requires disease stratification and individualised management.
- The main goals in the management of GCA are the achievement and maintenance of remission, the prevention of ischaemic complications and vascular damage as well as minimisation of glucocorticoid-induced adverse events.
- Novel laboratory and imaging biomarkers may be useful for disease stratification, for monitoring disease activity and damage as well as for prediction of future clinical outcomes.

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**Table 1 Unmet needs and research agenda for blood and imaging biomarkers in GCA**

<table>
<thead>
<tr>
<th>Unmet needs in GCA</th>
<th>Research questions</th>
<th>Study design</th>
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<tbody>
<tr>
<td>To define internationally accepted criteria for disease activity, remission/relapse and vascular damage that are also applicable in patients treated with drugs directly interfering with levels of acute phase reactants</td>
<td>Do blood biomarkers, such as interleukin-23, B cell activating factor, osteopontin or serum calprotectin, have a role in monitoring disease activity?</td>
<td>Observational study</td>
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<tr>
<td>To identify blood biomarkers that are not influenced by therapy and are useful to monitor disease activity (including subclinical)</td>
<td>Can blood biomarkers, such as osteopontin, matrix metallopeptidase-2, angiopoietin-2 or YKL-40, help in predicting disease outcomes?</td>
<td>Observational study</td>
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<tr>
<td>To identify blood biomarkers able to predict long-term clinical and structural outcomes</td>
<td>How should two discordant results obtained by different imaging methods be interpreted (eg, positive ultrasound with negative 18F-FDG PET/CT, and vice versa)?</td>
<td>Observational study</td>
</tr>
<tr>
<td>To investigate the value of positive imaging findings in patients who are in clinical and laboratory remission</td>
<td>How frequently should an imaging assessment be performed to monitor activity and damage?</td>
<td>Observational study</td>
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<tr>
<td>To determine the role of imaging to predict disease relapses and (occurrence or progression of) vascular damage</td>
<td>Can (blood and imaging) biomarkers inform the management decisions of physicians in clinical practice?</td>
<td>Randomised controlled trial</td>
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18F-FDG PET/CT, 18F-fluorodeoxyglucose positron emission tomography/CT; GCA, giant cell arteritis.
**REFERENCES**


