UNIVERSITÀ VITA-SALUTE SAN RAFFAELE

CORSO DI DOTTORATO DI RICERCA INTERNAZIONALE IN MEDICINA MOLECOLARE

Curriculum in Clinical and Experimental Medicine

LONG-TERM PATHOPHYSIOLOGIC AND PROGNOSTIC EVALUATION OF DIFFERENT DISEASE SUBSETS IN GIANT CELL ARTERITIS

DoS: Professor Lorenzo Dagna
Second Supervisor: Professor Christian Dejaco

Tesi di DOTTORATO di RICERCA di Alessandro Tomelleri
matr. 017591
Ciclo di dottorato XXVI
SSD MED09

Anno Accademico 2022/2023

Prof. Lorenzo DAGNA

CONSULTAZIONE TESI DI DOTTORATO DI RICERCA

Il/la sottoscritto/*I*	Alessandro Tomelleri
Matricola / *registration number*	017591
Iscritto al / Enrolled in	International PhD Course in Molecular Medicine
Curriculum in	Clinical and Experimental Medicine
nato a/ *born in*	Verona (Italy)
il/*on*	12th April 1990

autore della tesi di Dottorato di ricerca dal titolo / *author of the PhD Thesis entitled*

LONG-TERM PATHOPHYSIOLOGIC AND PROGNOSTIC EVALUATION OF DIFFERENT DISEASE SUBSETS IN GIANT CELL ARTERITIS

DICHIARA DI aver ricevuto completa informativa, di seguito riportata, ai sensi dell'art. 13 del Regolamento CE n. 679/2016 ed esprime il proprio consenso affinché l'Università Vita-Salute San Raffaele, tratti e comunichi i dati che lo riguardano, nei limiti, per le finalità e con le modalità precisati nell'informativa./ *I, the undersigned, declare that I have received a full disclaimer under art. 13 of Regulation (EU) 2016/679 and hereby authorise the Università Vita-Salute San Raffaele to use and disclose my personal data, within the limits, for the purposes and by the procedures stated in the disclaimer.*

Inoltre DICHIARA DI/*I also* ☐ CONFERIRE/*to give* X NON CONFERIRE/*to not give declare*:

il proprio consenso al trattamento e alla divulgazione dei dati personali nei confronti di Società ed Enti pubblici e privati che ne facciano richiesta, per eventuali collaborazioni professionali./ *my consent to the processing and disclosure of personal data to companies and public and private organizations that so request, for any professional collaborations*.
All'interessato competono i diritti ai sensi del Regolamento CE 679/2016./ *The data person is entitled to the rights in accordance with regulation (EU) 2016/679.*

Firma/*Signature* _____ Data/*Date* _____

DECLARATION

This thesis has been:

- Composed by myself and has not been used in any previous application for a degree. Throughout the text I use both 'I' and 'We' interchangeably.
- Has been written according to the editing guidelines approved by the University.

Permission to use images and other material covered by copyright has been sought and obtained.

All the results presented in this thesis have been obtained by myself, except for the following experiments, which have been carried out in collaboration with one or more persons, as detailed below:

- The ELISA tests for the determination of inflammatory cytokines, including *ex vivo* treatment with 2-Deoxy-D-Glucose (***Results****; Figures 1, 2, 15, 16*), were conducted in collaboration with Dr. Eleonora Cantoni, Dr. Raffaella Molteni, Dr. Maddalena Panigada, Dr. Davide Stefanoni, Dr. Laura Merlo-Pich and Dr. Vito Giordano (Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy);
- The immunometabolomics experiments (i.e., ultra-high-pressure liquid chromatography mass spectrometry metabolomics and tracing experiments) (***Results****; Figures 3, 4, 5, 6, 7*) were conducted in collaboration with Dr. Eleonora Cantoni, Dr. Raffaella Molteni, Dr. Maddalena Panigada, Dr. Davide Stefanoni, Dr. Angelo D'Alessandro, Dr. Laura Merlo-Pich and Dr. Vito Giordano (Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy), and Dr. Marina Ferrarini and Dr. Elisabetta Ferrero (Division of Experimental Oncology, San Raffaele Scientific Institute, Milan, Italy);
- The epigenetic experiments (i.e., ATAC-sequencing, chromatin immunoprecipitation) (***Results***; *Figures 8, 9, 10*) were conducted in collaboration with Dr. Ivan Merelli (San Raffaele-Telethon Institute for Gene Therapy, San Raffaele Scientific Institute, Milan,

Italy), and Dr. Jorge Domìnguez-Andres, Prof. Mihai G Netea and Prof. Leo A B Joosten (Department of Internal Medicine and Radboud Center for Infectious diseases, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands);

- The transcriptomic experiments (i.e., RNA-sequencing) (***Results***; *Figures 11, 12, 13*) were conducted in collaboration with Dr. Ivan Merelli (San Raffaele-Telethon Institute for Gene Therapy, San Raffaele Scientific Institute, Milan, Italy), and Dr. Eleonora Cantoni, Dr. Raffaella Molteni and Dr. Eleonora Panigada (Division of Genetics and Cell Biology, San Raffaele Scientific Institute, Milan, Italy);
- The confocal analysis on histological specimens from temporal arteries (***Results****; Figure 14)* were conducted in collaboration with Dr. Barbara Vergani and Dr. Biagio Eugenio Leone (Department of Pathology, Bicocca University, Milan, Italy), and Dr. Marina Ferrarini and Dr Elisabetta Ferrero (Division of Experimental Oncology, San Raffaele Scientific Institute, Milan, Italy).

In addition, I also declare that some of the results presented in this thesis have already been published in the paper titled "*Myelomonocytic cells in giant cell arteritis activate trained immunity programmes sustaining inflammation and cytokine production*" (Cantoni E, et al. Rheumatology 2023;62(10):3469-3479), which I co-authored. I declare that I actively contributed to the realisation of this paper by participating in the conception of the study design, collecting biological samples, managing patients clinically, discussing results, and drafting and reviewing the manuscript.

All sources of information are acknowledged by means of reference.

ABSTRACT.

Introduction. Giant cell arteritis (GCA), an inflammatory disease primarily affecting medium/large vessels, is characterised by an unprovoked nature and frequent relapses. In addition, GCA has different clinical/imaging phenotypes. Trained immunity (TI) is a pro-inflammatory programme characterised by epigenetic and immunometabolic modifications leading to enhanced production of cytokines.

Aims. i) To assess the contribution of maladaptive activation of TI in GCA development; ii) to evaluate possible correlations between different clinical GCA phenotypes and different levels of TI activation; iii) to assess the prognostic influence of different GCA phenotypes on clinical outcomes.

Materials and Methods. First, cytokine production, intracellular metabolomics and epigenetic modifications were assessed in monocytes from 20 newly diagnosed GCA patients and matched healthy donors (HDs). Then, differences in cytokine production and intracellular metabolomics between different GCA phenotypes were assessed. Finally, the association between different disease phenotypes and disease-related outcomes (i.e., visual loss, aortic aneurysms, relapses) was evaluated in both a small prospective cohort and a large multicentre retrospective cohort.

Results. Monocytes from patients with GCA showed the typical characteristics of TI, including increased IL-6 production after stimulation, enhanced glutaminolysis and enhanced glycolysis, and epigenetic modifications favouring increased transcription of pro-inflammatory genes. GCA patients with aortitis and patients without ischaemic involvement at baseline showed higher stimulated IL-6 production but no significant immunometabolic differences. In the prospective cohort (n=20), these patients also showed a higher tendency to relapse over 24 months of follow-up. In the retrospective cohort (n=1048) we found that cranial ischaemic symptoms were risk factors for visual loss but protective factors for aneurysm development and that aortitis and polymyalgia rheumatica were protective factors for visual loss and risk factors for aneurysm development and relapses.

Discussion. GCA monocytes display activation of TI programs which promote enhanced inflammatory activation. This inflammatory activation differs between cranial and extra-cranial disease phenotypes and correlates with different disease-related outcomes.

TABLE OF CONTENTS.

ACRONYMS AND ABBREVIATIONS.

2-DG, *2-Deoxyglucose*

DAMP, *damage associated molecular pattern*

DC, *dendritic cell*

ELISA, *Enzyme-Linked Immunosorbent Assays*

FDG-PET, *^{18}F-Fluorodeoxyglucose Positron Emission Tomography*

GCA, *giant cell arteritis*

GPSD, *GCA-PMR spectrum disease*

HD, *healthy donors*

IL, *interleukin*

LPS, *lipopolysaccharide*

PAMP, *pathogen associated molecular pattern*

PMR, *polymyalgia rheumatica*

ROS, *reactive oxygen species*

TLR, *toll-like receptor*

TI, *trained immunity*

TNF, tumour-necrosis factor

US, *ultrasound*

LIST OF FIGURES AND TABLES.

FIGURES.

TABLES

INTRODUCTION.

Epidemiology of Giant Cell Arteritis (GCA).

Giant cell arteritis (GCA) is an inflammatory disease predominantly affecting the medium and large arteries (Tomelleri et al, 2023). The incidence of GCA ranges from 6 to 22 per 100,000 people over the age of 50 and the disease is more common in North America and Northern Europe (Watts et al). The women-to-men ratio is approximately 3:1 and the onset typically occurs between 60 and 80 years (Watts et al; Weyand & Goronzy, 2014).

Clinical features of GCA.

The GCA spectrum is broad, ranging from characteristic cranial features to less specific constitutional symptoms. Although the classic cranial pattern often raises the possibility of GCA, maintaining a high level of awareness in the face of atypical presentations is crucial. Minimising diagnostic delay is imperative given the potential for acute and irreversible ischaemic complications in GCA.

Cranial symptoms.

The predominant clinical manifestation in GCA is persistent headache, which is present in about two-thirds of cases (Myklebust et al, 1995). Although traditionally associated with the temporal regions, GCA-related headaches may also be occipital, frontal, or generalised. Cranial arteries involvement can also provoke tenderness over the scalp and jaw and/or tongue claudication (Salvarani et al, 2012). In GCA patients with cranial features, a thorough examination may reveal abnormalities over the temporal arteries, such as pulselessness and tenderness (Gonzalez-Gay et al, 2005). Nevertheless, it should be noted that the diagnosis of GCA cannot be excluded in the presence of a normal appearance of the temporal arteries (Widico & Newman, 2005).

The most dreaded of the clinical manifestations of GCA are visual symptoms, since they may foretell irreversible ischaemic complications (Vodopivec & Rizzo, 2018). Patients may report temporary and partial visual field defects (known as *amaurosis fugax*), blurring vision, or diplopia, which are highly specific for GCA (Liozon et al, 2001). If an

appropriate treatment is not started promptly, these symptoms can progress to irreversible vision loss, occasionally occurring abruptly without any warning symptoms (Gonzalez-Gay et al, 2000). Previous reports suggest that vision loss can be experienced by a variable fraction of patients, from 10 to 20% (Gonzalez-Gay et al, 2000). The primary mechanism of vision loss is optic ischaemic neuropathy, usually anterior, which is a consequence of the inflammatory occlusion of one of branches of the ophthalmic artery, usually the posterior ciliary artery, (González-Gay et al, 2016). However, clinicians must be aware that, in most patients, optic neuropathy is caused by atherosclerotic lesions and not by an inflammatory condition; in these cases, sometimes, there can be spontaneous recovery (Hayreh et al, 2014). Nevertheless, an arteritic aetiology must always be considered when investigating optic neuropathy. Other causes of visual complication in GCA can be central retinal artery occlusion and posterior ischaemic optic neuropathy (Dumont et al, 2020). Models to predict ocular ischaemic complications are currently lacking, with previous *amaurosis fugax* being the only clinical predictor (Liozon et al, 2016). Once visual loss is established, it is rarely reversible (Salvarani et al, 2005). Furthermore, if monocular at onset, the other eye may be involved within one week in up to half of cases if left untreated (Danesh-Meyer et al, 2005). Timely initiation of an appropriate treatment is therefore essential to prevent this complication (Soriano et al, 2017).

Ischaemic stroke is rare in GCA and represents a cause of cerebrovascular events very rarely in the non-GCA population (González-Gay et al, 2009). Notably, strokes in GCA are typically caused by an occlusion of vertebrobasilar branches (Wiszniewska et al, 2007). Therefore, GCA should always be suspected (if patients are older than 50 years) when both vertebral arteries are involved (Rüegg et al, 2003).

Vascular and systemic symptoms.

Notably, in people with more than 50 years, GCA is among the most common causes of fever of unknown origin (Knockaert et al, 1993). Fever can be present in more than half of patients with GCA; however, temperature in GCA is rarely above 39ºC and in such cases other diagnoses (e.g., systemic infections) should be always sought (Knockaert et al, 1993). Weight loss and fatigue are other typical constitutional symptoms (Tomelleri et al, 2023).

Systemic symptoms are usually present in patients where the extracranial arteries (e.g., aorta, carotid arteries, subclavian arteries) are affected (Tomelleri et al, 2021). Therefore, if these manifestations predominate, investigations of the extracranial arteries should be always adequately performed. In addition, it is important to underline that patients with an extracranial phenotype are typically younger than patients where only cranial arteries are affected (Gonzalez-Gay et al, 2009; Salvarani et al 2004).

Inflammation of the aorta and its branches can also lead to more specific symptoms, as it happens in patients affected by the other main large-vessel vasculitis, Takayasu arteritis (Gribbons et al, 2020). These symptoms include *arteriodynia* (i.e., pain in the area corresponding to an inflamed artery) and limb claudication (i.e., pain due to limb ischaemia caused by arterial stenosis) (van der Geest et al, 2020; Brack et al, 1999). Particularly, limb claudication has been suggested as one of the most informative symptoms for diagnosing GCA (van der Geest et al, 2020). In such cases, a careful physical examination can allow to auscultate vascular bruits and feel diminished arterial pulses (van der Geest et al, 2020).

Polymyalgia rheumatica.

Polymyalgia rheumatica (PMR) is a disorder characterised by symmetrical stiffness and pain over the shoulders, hips, and neck (González-Gay et al, 2017). Pain and stiffness typically lead to functional limitation, mostly in the morning, with a significant impact on everyday activities. In PMR, inflammation involves periarticular structures (e.g., tendons and bursae), with the most common findings being biceps tenosynovitis, subdeltoid/subacromial bursitis, trochanteric bursitis, iliopsoas bursitis, and iliopectineal bursitis; these structures are usually involved symmetrically (Camellino et al, 2020).

There is a strong connection between GCA and PMR. Notably, around 50% of GCA patients also display features of PMR, whereas patients with apparently isolated PMR can eventually develop GCA in 20-25% of cases (Tomelleri et al, 2023). Notably, it seems that the concomitant presence of PMR is higher in patients with the extracranial subset of GCA (Dejaco et al, 2017). Hence, to avoid delays in diagnosis, when approaching a patient with PMR who complains of symptoms suggestive of GCA, appropriate investigations should be immediately performed (Koster et al, 2018). Other suspected symptoms include uncommon features of PMR (e.g., low back pain), strong constitutional

symptoms and resistance to conventional treatments (Prieto-Peña et al, 2019). A correct diagnosis (i.e., distinguishing between isolated PMR and PMR in the setting of GCA) is crucial, as the therapeutic approach in these two situations is different (De Miguel et al, 2023).

GCA and PMR should therefore not be considered as separate entities, but rather as integral components of a unified spectrum of disease, aptly termed GCA-PMR spectrum disease (GPSD) (Tomelleri et al, 2023; Dejaco et al, 2017). This conceptual framework is based on shared epidemiological aspects, common clinical features and overlapping pathogenesis (Tomelleri et al, 2023).

Diagnosis of GCA: role of imaging.

In the current landscape, imaging plays a central role in the diagnosis of GCA (Prieto-Peña et al, 2021). Ultrasonography (US) is a valuable instrument for detecting inflammation not only in the cranial but also in some extracranial arteries, obviating the necessity to obtain histological confirmation by temporal artery biopsy. ^{18}F-Fluorodeoxyglucose-Positron Emission Tomography (FDG-PET) can detect inflammation over the aorta and all major extracranial arteries (Dejaco et al, 2023). Although imaging is critical, it must always be interpreted after a careful assessment of the clinical and laboratory background. This is necessary to avoid misdiagnosis and overtreatment (Dejaco et al, 2023).

Vascular ultrasound.

The potential of US in the diagnosis of GCA was first demonstrated 25 years ago, when the first description of a hypo-echoic thickening (i.e., halo sign) around the temporal arteries was made (Schmidt et al, 1997). This circumferential area, suggestive of mural oedema, may extend to the main branches and other cranial arteries (Schmidt, 2013). The bilateral halo sign, which persists on compression, is highly specific for GCA (Arida et al, 2010). Notably, US surpasses TAB in sensitivity for diagnosing GCA (Chrysidis et al, 2021). Therefore, according to current European guidelines, US should be chosen as the first test to be performed when GCA is suspected, due to its low radiation load and its ability to provide an immediate result (Dejaco et al, 2023). However, it should be

underlined that glucocorticoid therapy significantly impacts the sensitivity of US, highlighting the importance of obtaining US within the first week of therapy initiation (Hauenstein et al, 2012). US also allows the examination of extracranial arteries and is a first-line test for screening for extracranial involvement in patients with GCA (Czihal et al, 2012). In addition, US can provide prognostic insight through the recently developed 'Halo Score', which correlates with systemic inflammation and the likelihood of ischaemic complications (Van Der Geest et al, 2019). This score is also valuable for monitoring patients and assessing treatment response, as evidenced by the fact that it correlates well with markers of GCA activity and cumulative glucocorticoid dose (van der Geest et al, 2020).

^{18}F-Fluorodeoxyglucose Positron Emission Tomography (FDG-PET).

FDG-PET is acquiring a growingly central role in the diagnosis of GCA, helping to rule out alternative diagnoses and to detect vascular (mainly extracranial) involvement (Dejaco et al, 2023). According to current European guidelines, FDG-PET should be performed when signs and/or symptoms evocative for extracranial disease are present (Dejaco et al, 2023): in these patients, particularly in the absence of cranial involvement, whole-body imaging is essential to identify vasculitic lesions (Slart et al, 2018). Of note, FDG-PET sensitivity decreases after the first three to ten days of glucocorticoid therapy, and this emphasises the need for early post-therapy scans (Nielsen et al, 2018). Although useful for monitoring treatment, persistent vascular hypermetabolism does not always indicate active inflammation, but could signal vessel wall fibrosis and remodelling (Martínez-Rodríguez et al, 2017). FDG-PET has limitations due to its high cost, limited availability, and radiation exposure (Dejaco et al, 2023).

Management of GCA.

Glucocorticoids.

The initiation of high-dose systemic glucocorticoids is mandatory when the suspect of GCA is high, in order to prevent and avoid ischaemic manifestations (Hellmich et al, 2019). Current European guidelines advocate starting with high dose glucocorticoids (i.e., 1 mg/kg of prednisolone daily); as no additional benefit has been demonstrated, doses

higher than 60 mg daily should be avoided (Hellmich et al, 2019). Before starting oral glucocorticoids, intravenous pulses of methylprednisolone (250-1000 mg) for 3-5 days are recommended for patients with visual loss (either permanent or transient) to prevent the involvement of the fellow eye or to limit the progression of damage (Hellmich et al, 2019). However, regardless of the intensity of steroid therapy, once visual loss is established, reversibility is rare.

Ideally, the starting glucocorticoids dose should be maintained for 1 month, or at least until remission is established (Hellmich et al, 2019). Tapering should then begin, with European guidelines suggesting that a dose lower than 5 mg daily (prednisolone-equivalent) should be reached within 12 months (Hellmich et al, 2019). Throughout the dose reduction, close clinical monitoring and acute phase reactant measurements are essential to detect disease flares. In the event of a flare, glucocorticoid dose should be increased. If the flare is mild (i.e., without ischaemic manifestations), the dose can be returned to the last effective dose; if there are ischaemic manifestations, the induction dose should be restarted (Hellmich et al, 2019).

Conventional steroid-sparing agents.

Non-steroidal immunosuppressive drugs may be required in some patients with GCA, particularly those with recalcitrant disease or risk factors for glucocorticoid-induced complications (Hellmich et al, 2019). Among these drugs, methotrexate, which has been studied for over 20 years, is the only one which has shown some sort of efficacy. Specifically, it has been demonstrated to be able to reduce the cumulative dose of glucocorticoids and the rate of relapses, particularly when extracranial involvement prevails (Mahr et al, 2007). Therefore, European guidelines recommend starting methotrexate as the first alternative to tocilizumab (*see next Section*) and advocates to use a minimum dose of 15 mg weekly (Hellmich et al, 2019). Although promising results have been reported for azathioprine, cyclophosphamide and leflunomide (Tomelleri et al, 2021; De Silva et al, 1986), these drugs have not been evaluated in randomised trials and are not currently included in the European recommendations for the treatment of GCA (Hellmich et al, 2019).

Biologic steroid-sparing agents.

Tocilizumab, targeting the IL6 receptor, exhibits notable anti-inflammatory effects and can be administered subcutaneously or intravenously. Tocilizumab, when added to glucocorticoids, achieves higher remission rates than glucocorticoid monotherapy, even with accelerated tapering (Stone et al, 2017). While not universally indicated, patients prone to or experiencing steroid-related complications benefit from tocilizumab, associated with lower cumulative glucocorticoid doses (Hellmich et al, 2019). However, it is still unclear how long tocilizumab should be continued; what is known is that discontinuation after only 12 months is linked to a 50% relapse rate (Stone al, 2021; Tomelleri et al, 2023).

Other biologic therapies, like abatacept, mavrilimumab, secukinumab, and baricitinib, show promise in preventing relapses or achieving sustained remission, although larger clinical trials are warranted for definitive recommendations (Sebastian et al, 2021). Tumour-necrosis factor (TNF) inhibitors have demonstrated effectiveness in Takayasu arteritis but lack conclusive evidence for routine GCA use (Mekinian et al, 2021).

Long-term outcomes of GCA.

Despite an often satisfactory response to glucocorticoids, over 50% of patients with GCA have relapses during and after tapering (Alba et al, 2014; Restuccia et al, 2016). These flares range from subclinical inflammation, detectable only by imaging and acute phase markers, to symptomatic manifestations, with ischaemic complications being exceptionally rare during flares (Alba et al, 2014). Notably, marked systemic inflammation at baseline seems to correlate with a higher risk of relapse (Restuccia et al, 2016; Hernández-Rodríguez, et al, 2002; Restuccia et al, 2016). In addition, unfavourable disease outcome appears to be associated with extracranial involvement (Dumont et al, 2019).

The most feared long-term complication of GCA is represented by vascular remodelling, which can lead to stenoses and, mostly, aortic aneurysms (de Boysson et al, 2018). Indeed, in around one-fifth of patients with GCA aortic aneurysms can be found, mostly over the thoracic aorta (de Boysson et al, 2018; González-Gay et al, 2004). Predictors of this structural complication remain unidentified, although it seems that aortic aneurysms are more common after 5 years and in patients with aortitis on FDG-PET at baseline (Moreel

et al, 2023). On the other hand, cranial symptoms/signs may confer protection against aortic dilation (de Boysson et al, 2018). Notably, some patients develop aneurysms also when the disease is clinically inactive; this suggests that inflammation is not the only mechanism beyond this complication and also atherosclerotic risk factors can play a role (González-Gay et al, 2004). In particular, disarray of elastic fibres, rather than inflammatory cells, is a prominent feature of GCA-related aortic aneurysms (Evans et al, 1995).

Despite the undoubted clinical importance of aortic aneurysms, to date, there is a lack of consensus on the frequency and modality for their monitoring (Dejaco et al, 2023).

General aspects of the pathogenesis of GCA.

GCA is probably an antigen-driven immune-mediated disease, as indicated by the increased expression of MHC class-II genes (Carmona et al, 2015); however, the specific antigen(s) associated with GCA onset have not identified yet. In addition, a specific response to particular epitopes has been suggested by the finding of clonally expanded T lymphocytes in arterial lesions (Weyand et al, 2012).

The inflammatory cascade in GCA unfolds primarily within the arterial mural layers of medium and large-sized vessels (i.e., with a diameter > 0.2 cm), which have three mural layers (i.e., the intima, media, and adventitia). While diffusion from the lumen cannot adequately supply all layers with necessary nutrients, a microvascular system, termed *vasa vasorum*, becomes critical for nutrient transfer to all these three arterial layers. In contrast to small arteries, which lack *vasa vasorum*, large arteries harbour resident vascular dendritic cells (DCs) at the media-adventitia interface.

The role of the *innate immune system* in GCA pathogenesis.

Dendritic cells.

DCs play a critical role as a link between the adaptive and innate immune systems (Weyand et al, 2013). Studies have consistently highlighted the dysregulated vascular DC population as central to GCA (Weyand et al, 2013). These vascular DCs, which normally reside at the adventitia-media junction, exhibit tolerogenic behaviour in normal arteries,

implying their inability to stimulate T lymphocytes (Ma-Krupa et al, 2004). One hypothesis is that, in susceptible individuals, damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) from the circulation, possibly derived from bacterial or viral pathogens, can reach the media-adventitia interface via the *vasa vasorum* (Ma-Krupa et al, 2004; Deng et al, 2009). In individuals predisposed to GCA, possibly due to genetic factors such as toll-like receptor (TLR) polymorphisms, vascular DCs can be activated in response to danger signals and acquire the ability to interact with T lymphocytes (Deng et al, 2009; Song et al, 2012). Thus, DC migrate into the media, where they release chemotactic factors (e.g., CCL21) that subsequently induce the activation and migration of monocyte-macrophages and T lymphocytes (Ma-Krupa et al, 2004; Weyand et al, 2013). The ensuing inflammatory activation, mainly involving Th1 and Th17 lymphocytes, contributes to the formation of granulomas typically observed in patients with GCA (Deng et al, 2010).

Macrophages.

In healthy people, T lymphocytes and monocyte-macrophages are normally absent from the arterial wall of medium-/large-sized vessels. However, in GCA, orchestrated recruitment of monocyte-macrophages to the arterial layers appears to occur through the *vasa vasorum*, probably facilitated by activated vascular DCs and T lymphocytes (Koster et al, 2017). IFN-γ-induced chemokine release from vascular smooth muscle cells has pivotal function in this recruitment (Corbera-Bellalta et al, 2015).

Monocyte-macrophages from GCA patients show increased production of matrix metalloproteinase (MMP) 9. This increased level of MMP-9 enables monocyte-macrophages to erode the *vasa vasorum* capillaries, giving them the ability to invade tissues. This also enables the infiltration of other inflammatory cells (Watanabe et al, 2018).

Within macrophages, two dominant phenotypes emerge: M1 and M2. M1 macrophages specialise in pro-inflammatory actions, whereas M2 macrophages focus on tissue repair mechanisms (Weyand et al, 2013; Wagner et al, 1994).

Activated M1 macrophages are found in both the adventitia and media in GCA. Adventitial macrophages help maintain the inflammatory response by producing interleukin (IL)-1 and IL-6, the main inflammatory cytokines (Weyand et al, 2012).

Meanwhile, in the media, activated M1 macrophages release molecules such as MMPs, contributing to the degradation of the arterial layers (Rodriguez-Pla et al, 2005; Rittner et al, 1999).

Activated M2 macrophages position themselves at the interface between the intima and the media layers, where they produce angiogenic factors (e.g., vascular endothelial growth factor). This involvement contributes to the anatomical modification of the vascular lumen, eventually leading to thickening of the vessel wall or, sometimes, even to overt stenoses (Carmona et al, 2017).

The characteristic granulomas in GCA paint a picture made not only by macrophages and T lymphocytes, but also histiocytes and multinucleated cells (i.e., 'giant cells') (Weyand et al, 2013). These giant cells, which give GCA its name, are present in approximately 70% of diagnostic arterial biopsies (Cavazza et al, 2014) and are formed by the fusion of macrophages. This process is driven, at least in part, by colony-stimulating factors such as M-CSF (monocyte colony stimulating factor) and GM-CSF (granulocyte monocyte colony stimulating factor) (Jiemy et al, 2020).

Neutrophils.

Recent evidence has demonstrated that circulating immature neutrophils can extravasate into the perivascular tissues of the temporal arteries of GCA patients. This migration culminates in the generation of increased levels of extracellular reactive oxygen species (ROS), thereby exacerbating vascular damage (Wang et al, 2020; Michailidou et al, 2022).

In addition, histological studies of arteries affected by GCA show neutrophil infiltration in the adventitia and media layers (Nadkarni et al, 2013). At the same time, an increased concentration of neutrophil extracellular traps (NETs) has been clearly identified in the temporal arteries of individuals diagnosed with GCA (Palamidas et al, 2021).

This nuanced understanding of neutrophil involvement highlights their multifaceted contribution to the inflammatory milieu that characterises GCA pathogenesis.

The role of the *adaptive immune system* in GCA pathogenesis.

Like macrophages, T lymphocytes are normally absent from arteries of medium and large size. However, in the context of GCA, activated vascular DCs, strategically positioned in the proximity of *vasa vasorum*, at the interface between the media and the adventitia, secrete factors that can attract T lymphocytes. This interaction leads to the differentiation of T cells into two prominent lines: Th1 lymphocytes and Th17 lymphocytes (Weyand et al, 2013).

Th1 lymphocytes.

Th1 lymphocytes appear to be unaffected by glucocorticoids as demonstrated by the fact that their numbers and associated cytokines (mainly, IFN-γ) remain elevated in both arterial tissue and blood (Deng et al, 2010). The differentiation of naïve T lymphocytes towards the Th1 lineage is orchestrated by different cytokines, with the main being IL-12 (Weyand et al, 2013). Notably, in addition to this role, IL-12 is also involved in stimulating the release of damaging molecules and the activation of macrophages and vascular smooth muscle cells (Weyand et al, 2013).

Th17 lymphocytes.

Th17 lymphocytes play a pivotal role in GCA pathogenesis. This is supported by the fact that the concentration of Th17 lymphocytes in the peripheral blood is invariably higher in patients with GCA compared to healthy controls (Samson et al, 2012; Terrier et al, 2011). The differentiation of naïve T lymphocytes towards the Th17 phenotype is mainly influenced by IL-6, TGF-β and IL-21, and Th17 lymphocytes release a spectrum of cytokines, such as IL-21, IL-17, IL-22, and chemokines, such as CCL20 (Maddur et al, 2012). These cytokines/chemokines play an important role to induce not only local arterial damage, but mostly systemic constitutional manifestations. Notably, in contrast to Th1 lymphocytes, Th17 lymphocytes and Th17-associated cytokines/chemokines are significantly reduced in both arterial tissue and peripheral blood following the initiation of glucocorticoid therapy (Deng et al, 2010).

T-regulatory lymphocytes.

Flow cytometry measurements indicate low levels of T-regulatory lymphocytes in GCA patients (Samson et al, 2012). These low levels are probably caused by the fact that some

cytokines that play a pivotal role in the pathogenesis of GCA, such as IL-21, IL-23, and IL-26, hinder the expression of FOXP3, a key transcription factor involved in the differentiation of T-regulatory lymphocytes (Kimura et al, 2010). Additionally, the same cytokines upregulate RORγt, a transcription factor that stimulates Th17 differentiation (Barbi et al, 2013; Kimura et al, 2010).

Cytokine interplay.

Cytokine signatures within GCA reveal intricate immunological orchestrations.

The *IL-6–IL-17 signature* highlights the centrality of IL-6, a versatile cytokine secreted not only by immune but also by stromal cells such as vascular smooth muscle cells, endothelial cells, and fibroblasts (Weyand et al, 2013; Camporeale et al, 2012). IL-6, in conjunction with TGF-β, triggers the enhanced production of inflammatory markers by hepatocytes and orchestrates the differentiation of naïve T lymphocytes towards the pro-inflammatory Th-17 subset; in addition, along with IL-23 and IL-21, inhibits FOXP3, which is critical for the anti-inflammatory T-regulatory lymphocytes (Weyand et al, 2013; Camporeale et al, 2012; Saravia et al, 2019). As a result, IL-6 exerts pro-inflammatory effects and, at the same time, inhibits the potential countermeasures of the immune system (Weyand et al, 2013). The Th-17 lymphocytes that result from this differentiation release various pro-inflammatory cytokines and chemokines (e.g., IL-17, IL-21, IL-22, CCL20), which stimulate hepatocytes to produce acute phase reactants and activate endothelial cells, macrophages, vascular smooth muscle cells and fibroblasts (Weyand et al, 2013; Rutz et al, 2013; Torchinsky et al, 2010). Notably, the Th-17 axis can be inhibited by treatment with glucocorticoids, which can suppress the production of IL-6, IL-1 and IL-23, all cytokines essential for Th17 cell differentiation. Therefore, glucocorticoids can lead to suppression of IL-17 production both in inflamed arteries and in the peripheral circulation (Deng et al, 2010).

The *IFN-γ-IL-12 signature* highlights the role of IL-12 derived from activated vascular DCs within GCA-affected arteries (Weyand et al, 2013; Nizzoli et al, 2013). Indeed, IL-12 can stimulate naïve CD4+ T lymphocytes to polarise into the Th1 lineage by inducing the expression of Th1-related genes while suppressing Th2-specific genes (Saravia et al, 2019). Activated Th1 cells in turn release IFN-γ, a potent pro-inflammatory molecule

typically associated with Th1 lymphocytes, DCs and macrophages. IFN-γ can exacerbate tissue damage by interacting with vascular smooth muscle cells and endothelial cells, but can also amplify the inflammatory cascade (Watanabe et al, 2020). Interestingly, IL-12-IFN-γ cytokines and related Th-1 responses remain largely unaffected by conventional immunosuppressive drugs currently available for the treatment of GCA, highlighting the distinct regulatory landscape (Weyand et al, 2010). Finally, recent evidence of elevated plasma IFN-γ levels prior to clinical disease onset underscores the central role of this pathway in early disease mechanisms (Wadström et al, 2022).

Trained immunity (TI): a pathogenic mechanism beyond autoimmune inflammatory disorders.

The immune response of the vertebrates has been traditionally divided into adaptive and innate components. The former relies on the involvement of T and B lymphocytes that, upon encountering a pathogen, first orchestrate a response and then form a memory. In contrast, the latter have historically been based on systems that operate rapidly, lack specificity and, most importantly, do not form a memory, such as phagocyte activity and complement activation.

However, an increasing amount of evidence substantiates the triggering of a memory-like reaction also within the innate immune system when exposed to pathogens (Barton et al, 2010).

Trained immunity (TI) was introduced to characterise the heightened innate immune reaction upon a subsequent encounter with the same or different pathogens (Netea et al, 2011). This innate form of memory lacks specificity to antigens and, instead, relies on enhancing the innate response during subsequent encounters with microorganisms. This unique response does not fit neatly into the conventional categories of innate or adaptive, defining it as a distinct mechanism that emerges following a subsequent encounter (Netea et al, 2011). TI predominantly involves monocytes-macrophages, excluding the need for T and B lymphocytes, and is linked to metabolic and epigenetic alterations that influence gene expression.

Training mechanisms.

In vertebrates, stimulation of innate cells by TLRs or other pattern recognition receptors (PRRs), induces permanent changes that affect epigenetics and cell metabolism (Domínguez-Andrés et al, 2018; Owen et al, 2021). PRRs engage with PAMPs and DAMPs and possess the capability to elicit sterile inflammation by identifying non-damaging elements (Chen Gabriel et al., 2010).

Metabolic modifications.

At a metabolic level, quiescent cells exhibit a minimal biosynthetic demand, primarily metabolising glucose through glycolysis and oxidative phosphorylation (Pearce et al, 2013). Conversely, activated cells elevate the use of glucose by employing oxidative phosphorylation and aerobic glycolysis and generating biosynthetic precursors (Lachmandas et al, 2016). Notably, β-glucans, prototype agonists inducing TI, redirect cellular metabolism to aerobic glycolysis in monocytes, amplifying metabolic activity and bolstering long-term innate immunity (Arts et al, 2016; Cheng et al, 2014).

Metabolic processes, encompassing fatty acid metabolism and glycolysis, not only yield energy but also shape immune cell functionality (Jung et al, 2019). Metabolic reprogramming, in conjunction with other pivotal immunoregulatory events, assumes a central role in moulding immune activation. Cellular metabolic adaptability proves crucial for responding to environmental shifts and functional requirements, enabling cells to reconfigure their metabolism in reaction to alterations in nutrient availability or signals from PRRs, cytokine receptors, and antigen receptors (O'Neill et al, 2015).

The transition towards aerobic glycolysis and fatty acid synthesis characterises activated macrophages, DCs, and diverse immune cells, influencing the modulation of immune activation or suppression (Tannahill et al, 2013). For instance, M1 macrophages exhibit a preference for aerobic glycolysis, whereas M2 macrophages favours oxidative phosphorylation and the Krebs cycle (Galván-Peña et al, 2014). Similarly, DCs undergo alterations in morphology, cytokine synthesis, antigen presentation, and heightened glycolysis upon stimulation by PRRs (Everts et al, 2014).

Epigenetic modifications.

Importantly, the above metabolic changes do not occur in isolation within cellular networks but are closely linked to epigenetic changes that regulate innate immune memory. This link is partially due to the association of many epigenetic events with metabolic pathways, providing substrates and co-factors essential for enzymatic activities (Donohoe et al, 2012).

Notably, epigenetic modifications are dependent on changes in cellular metabolism, and their prevention is observed when metabolic changes are bypassed (Arts et al, 2015). Cell activation induces shifts in intracellular metabolite levels that impact the activity of enzymes with a role in histones and DNA modification (Schvartzman et al, 2018). For instance, there is a perceived association between acetyl coenzyme A levels and histone acetylation (Netea et al, 2016). Furthermore, the accumulation of fumarate (derived from the Krebs cycle) inhibits demethylases and amplifies epigenetic alterations in histones, thus contributing to TI responses (Arts et al, 2016).

Epigenetic regulation entails alterations in phenotype without changes in genotype, involving both transient and enduring structural modifications in chromatin that impact the expression of genes (Funes et al, 2021). Histones post-transcriptional modifications (e.g., methylation, acetylation), DNA chemical alterations, and non-coding RNAs regulation are integral to these mechanisms (Chen et al, 2019). Exposing monocytes to stimuli such as β-glucans leads to a sustained enhancement of signatures like lysine acetylation (H3K27ac) or methylation (H3K4me) on histone H3 in the promoters of pro-inflammatory genes, thereby increasing their expression (Quintin et al, 2012).

Effects of activated TI on myeloid cells.

The impact on myeloid cells is contingent on the character of the stimulus (and the involved receptor) and the concentration during exposure. Consequently, the same component may elicit a subdued or intensified response when administered at different concentrations (Arts et al, 2016). Prominent microbial ligands for TLR and NLR have been assessed for their capacity to modulate the immune response in monocytes during a subsequent encounter. For instance, muramyl dipeptide (MDP) and flagellin have been identified as triggers of TI, with the significance of flagellin in the development of inflammatory bowel diseases (IBD) being particularly highlighted (Ifrim et al, 2014). Moreover, exposure to *Candida albicans* induces enduring epigenetic alterations,

specifically H3K4me, leading to the activation of inducible genes (Quintin et al, 2012). Human monocytes, when stimulated with β-glucans, exhibit both H3K27ac and H3K4me3 after one week, aligning with the induction of the glycolysis pathway (Cheng et al, 2014).

TI can be stimulated also by vaccines, which can confer non-specific protective effects against unrelated pathogens. Notably, the *Bacillus Calmette-Guérin* (BCG) vaccine can induce in monocytes epigenetic and metabolic modification both *in vivo* and *in vitro* (Soto et al, 2022). Indeed, monocytes exposure to the BCG vaccine results in an augmented cross-response (with heightened cytokine production) upon subsequent exposure to a different pathogen after one week (Kleinnijenhuis et al, 2014).

Trained immunity in autoimmune diseases.

The promotion of TI may prove detrimental to individuals predisposed to autoimmune disease. For example, a TI signature has been suggested in mice and patients affected by systemic lupus erythematosus (SLE). This signature involves a reprogramming of haematopoietic stem cells towards the myeloid lineage and seems to contribute not only to SLE-related flares but also to a tendency to heightened immune responses (Grigoriou et al, 2019). Therefore, the inflammatory milieu in SLE may induce a memory of immune training in bone marrow progenitors, similar to the identified β-glucan signature of haematopoietic stem cells (Mitroulis et al, 2018). Furthermore, administration as an adjuvant of *Candida albicans* β-glucan in a murine model of arthritis (collagen-induced arthritis, CIA) is able to increase the severity of joint inflammation (Hida et al, 2005). Conversely, an *Aureobasidium pullulans* β-glucan administered to the CIA murine model can reduce significantly signs of arthritis (Kim et al, 2012).

Hence, interactions with elements able to induce TI may not necessarily be detrimental to autoimmune processes. For instance, BCG vaccination primes cells and enhances their response to secondary pathogen stimulation without an increased cytokine production upon the second stimulation (Kleinnijenhuis et al, 2013). In line with this, some Authors have reported a beneficial impact of BCG vaccination on autoimmunity. Specifically, the inoculation of non-obese diabetic (NOD) mice with components of *Mycobacterium tuberculosis* was able to prevent type 1 diabetes and to reverse early stages of the disease

(Shehadeh et al, 1994; Ryu et al, 2001). This effect was associated with selective destruction of autoreactive T lymphocytes, TNF production, and facilitation of pancreatic renewal (Ryu et al, 2001).

In a model of systemic sclerosis, BCG-treated macrophages have been shown to adopt a pro-inflammatory phenotype and exacerbate inflammation (Jeljeli et al., 2019). Conversely, macrophages from the same systemic sclerosis models exposed to low doses of lipopolysaccharide (LPS) showed higher expression of iCOS ligand, indicative of a mixed M1/M2 phenotype (Jeljeli et al, 2019).

As said previously, TI depends on epigenetic and metabolic changes (Netea et al, 2020). Certain components derived from pathogens, such as helminths, have demonstrated the ability to cause epigenetic changes in stem cells. These changes tend to support an augmented anti-inflammatory activation over a pro-inflammatory one in subsequent encounters. (Quinn et al, 2019). Notably, compounds from helminths, like *Fasciola hepatica total extract*, have been reported to induce an attenuated form of TI. This form is characterised by an anti-inflammatory profile that provides protection against the development of experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis (Quinn et al., 2019). Furthermore, products secreted *by F. hepatica* prompted an anti-inflammatory profile in hematopoietic stem cells by augmenting the proliferation of Lys6Clow monocytes, resulting in an increased proportion of M2 macrophages. These events mitigated and delayed the onset of murine experimental autoimmune encephalomyelitis for at least 8 months.

Therefore, despite the uncertainties surrounding the mechanisms of training during autoimmunity, comprehending the processes that promote inflammation may open avenues for innovative strategies based on epigenetic and metabolic modifications.

GCA as a candidate disease model for the study of TI.

GCA emerges as a prime candidate for exploring the role of TI based on several clinical observations:

(i) histological studies consistently identify activated macrophages as the predominant players in GCA lesions;

(ii) macrophages within GCA arterial lesions display evident signs which indicate metabolic activation (as evidenced by increased uptake FDG in FDG-PET scans);
(iii) the disease is characterised by heightened production of pro-inflammatory cytokines (e.g., IL-6);
iv) the disease is characterised by a chronic and relapsing-remitting course.

AIMS OF THE WORK.

The apparently unprovoked nature of the onset of GCA and subsequent flares, as well as the frequent recurrences after discontinuation of treatment, suggest that patients with GCA are predisposed to develop excessive and uncontrolled inflammatory responses.

The main research hypothesis of this study is that a maladaptive activation of TI may contribute to this predisposition and the first aim of this study is therefore to determine the role of TI in the development of GCA.

In addition, we would like to explore a possible correlation between different clinical disease phenotypes of GCA and different degrees of TI activation.

Finally, we will evaluate the prognostic impact of different clinical phenotypes of GCA on different clinical outcomes. This evaluation will be performed both in a small prospective monocentric cohort and in a larger retrospective cohort.

RESULTS

Prospective study cohort.

Peripheral monocytes were isolated from a cohort comprising 20 individuals diagnosed with GCA and 20 healthy donors (HD), age- and sex-matched. All GCA patients satisfied the "2022 ACR/EULAR classification criteria for GCA" (Ponte et al, 2023). In all patients with GCA, clinical diagnosis was corroborated by either vascular ultrasound or FDG-PET. Detailed clinical and demographic characteristics of GCA patients and HDs are presented in **Table 1**.

Table 1. Patients with GCA and healthy donors from the OSR prospective cohort: baseline features.

	GCA patients (n = 20)	**Healthy donors (n = 20)**
Male sex ***Number (%)***	6 (30)	6 (30)
Age at sample collection ***Years, mean ± SD***	72 ± 7.6	71 ± 6.8
Smoking ***Number (%)***	2 (10)	1 (5)
Hyperlipidaemia ***Number (%)***	3 (15)	4 (20)
Arterial hypertension ***Number (%)***	12 (60)	10 (50)
Diabetes mellitus ***Number (%)***	1 (5)	2 (10)
Ischaemic heart disease ***Number (%)***	1 (5)	0
Obesity ***Number (%)***	3 (15)	2 (10)
History of stroke ***Number (%)***	0	1 (5)
History of cancer ***Number (%)***	1 (5)	1 (5)
Dementia ***Number (%)***	0	0
Osteoporosis ***Number (%)***	2 (10)	1 (5)

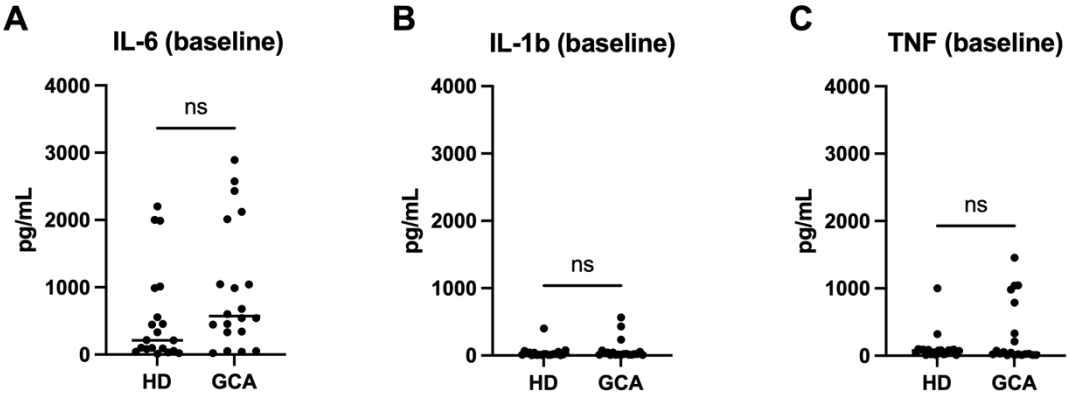
Cytokine production.

First, to probe the activation of TI programs, we performed polyfunctional assessments on monocytes, initiating with cytokine production assays. Cytokine production was evaluated in both HDs and GCA patients.

We assessed baseline and stimulated production of cytokines by monocytes from HDs and GCA patients using Enzyme-Linked Immunosorbent Assays (ELISA), specifically focusing on IL-1β, IL-6, and TNFα.

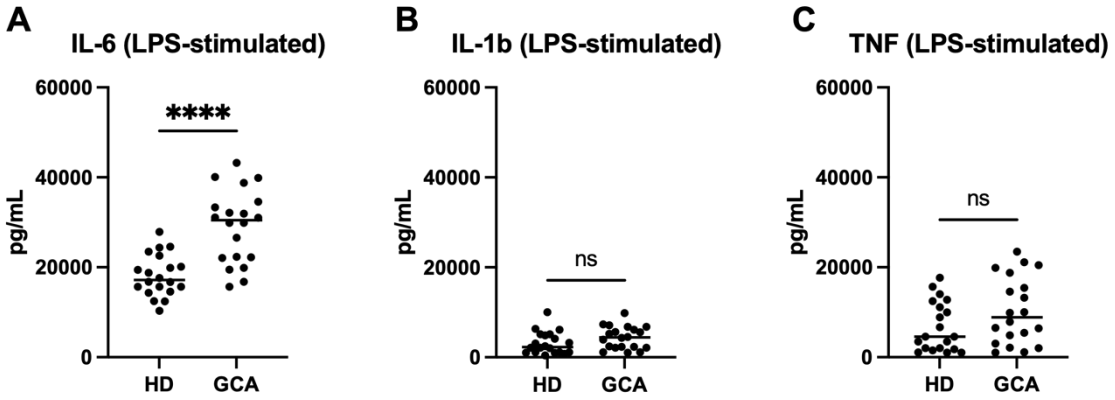
At baseline, we observed that GCA and HD monocytes exhibited comparable cytokine levels (**Figure 1 A-C**).

Figure 1. Production of IL-6 (A), IL-1b (B) and TNF (C) at baseline (Cantoni et al, 2023).
ns = not statistically significant.



However, upon stimulation with LPS, a mimic of pathogen encounter, monocytes from GCA patients demonstrated a significant increase in IL-6 production compared to monocytes from HDs (**Figure 2A**). On the other hand, differences in IL-1b and TNFα production were less pronounced and not statistically significant (**Figures 2B and 2C**). This elevation in IL-6 production after LPS stimulation, absent at baseline, aligns with TI activation in monocytes from GCA patients.

Figure 2. Production of IL-6 (A), IL-1b (B) and TNF (C) after LPS stimulation (Cantoni et al, 2023).
*ns = not statistically significant; **** = $p < 0.0001$*

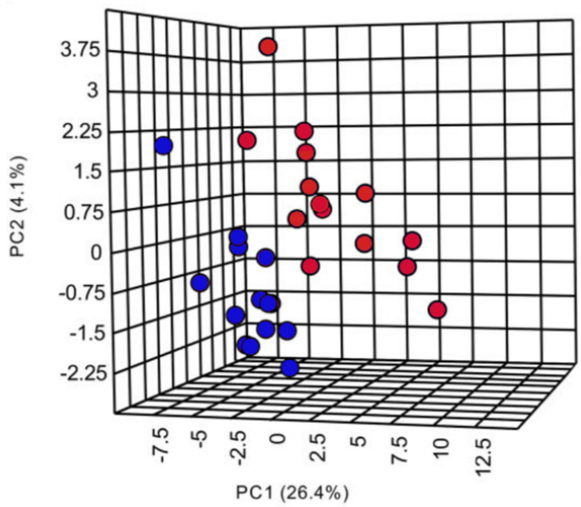


Intracellular metabolomics.

We further explored whether the immunometabolic changes classically associated with TI (i.e., increased glycolysis and increased glutaminolysis) sustained the heightened inflammatory activation of monocytes from patients with GCA.

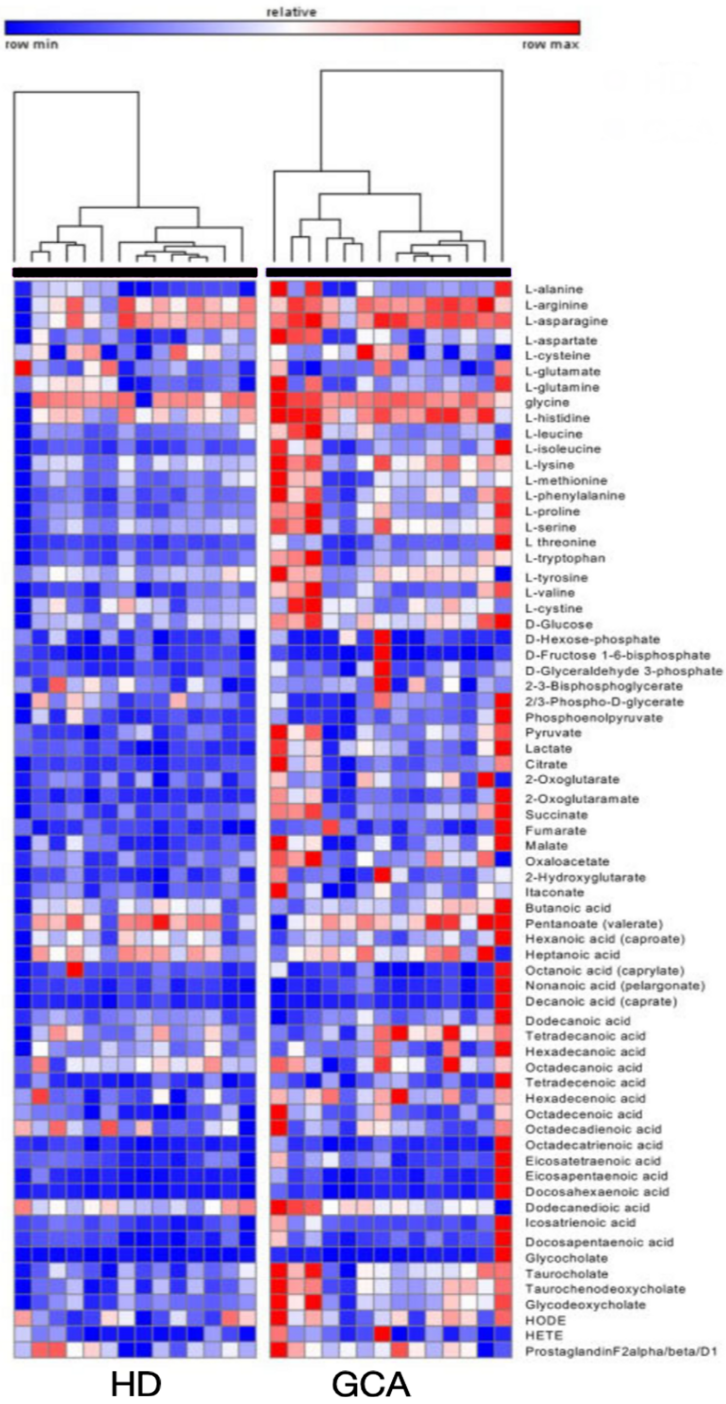
First, principal component analysis showed that monocytes from patients with GCA and from HDs exhibited a distinct metabolic profile (**Figure 3**). This result is indicative of deep metabolic rewiring

Figure 3. Principal component analysis of metabolomics (Cantoni et al, 2023).
HDs are represented as blue dots; GCA patients are represented as red dots.



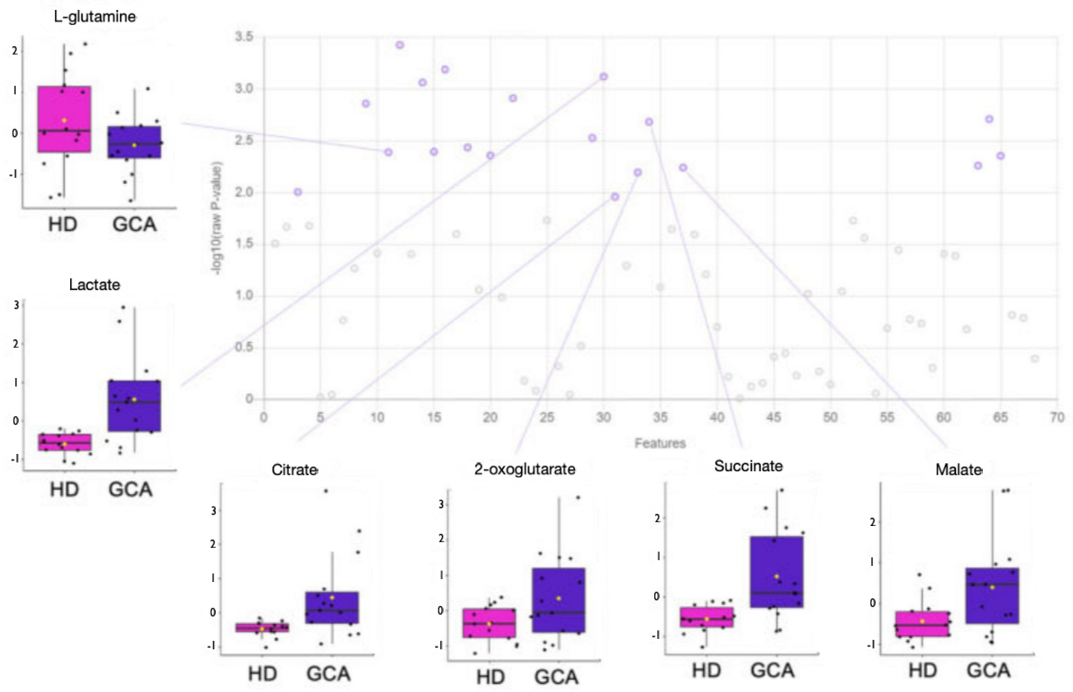
Specifically, global changes in individual metabolites reflected the activation of TI pathways, characterised by an increase in intermediates and end-products associated with glycolysis and tricarboxylic acid cycle (**Figure 4**).

Figure 4. Metabolites involved in the activation of TI pathways: hierarchical clustering analysis (Cantoni et al, 2023).



Notably, we observed many differentially regulated metabolites, all in line with the activation of TI in monocytes obtained from GCA patients. These metabolites included tricarboxylic acid cycle intermediate, such as malate, succinate, and citrate, and glycolysis end-products, such as lactate (**Figure 5**).

Figure 5. Scatter plots showing differential metabolite expression (Cantoni et al, 2023).
The relative abundance of each metabolite is reported in the y-axis.



Tracing metabolomics.

To corroborate the activation TI, we performed tracing experiments in monocytes from patients with GCA using either $^{13}C_6$-glucose or $^{13}C_5$-glutamine. These experiments enabled the assessment of the integration of these metabolites into particular intermediates.

We specifically found reduced intracellular levels of glucose paralleled by increased levels of lactate, both indicators of heightened glycolysis (**Figure 6**). On the other hand, we also observed increased incorporation of $^{13}C_5$-glutamine-derived carbons into the tricarboxylic acid cycle, an indirect sign of increased tricarboxylic acid cycle activity (**Figure 7**). Both these findings provide robust evidence that GCA monocytes exhibit the TI classic metabolic features.

Figure 6. Tracing experiments (with $^{13}C_6$-glucose) (Cantoni et al, 2023).

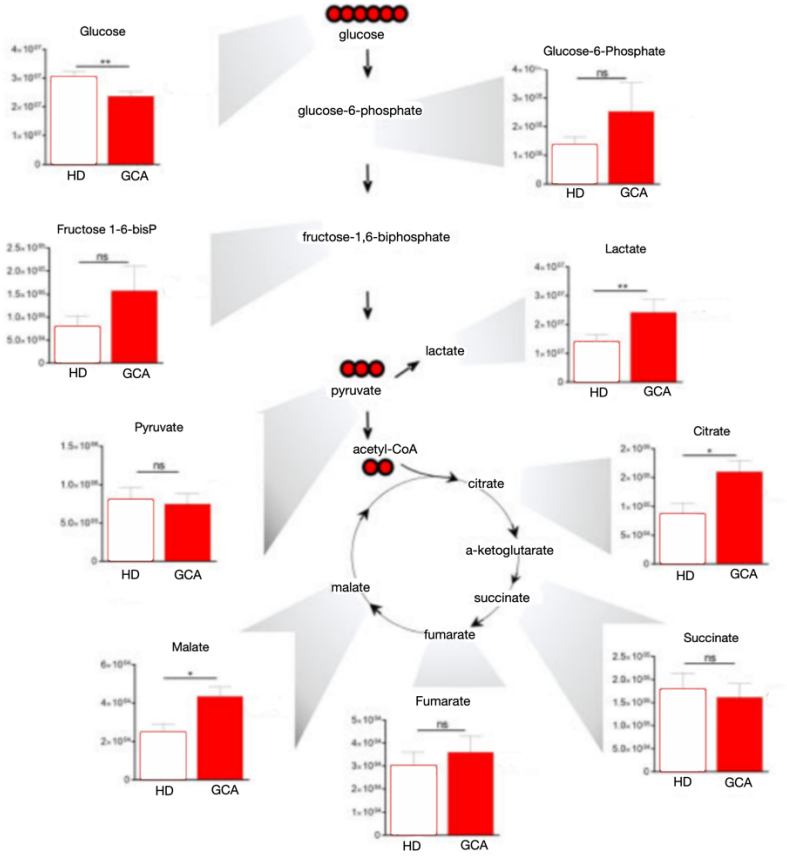
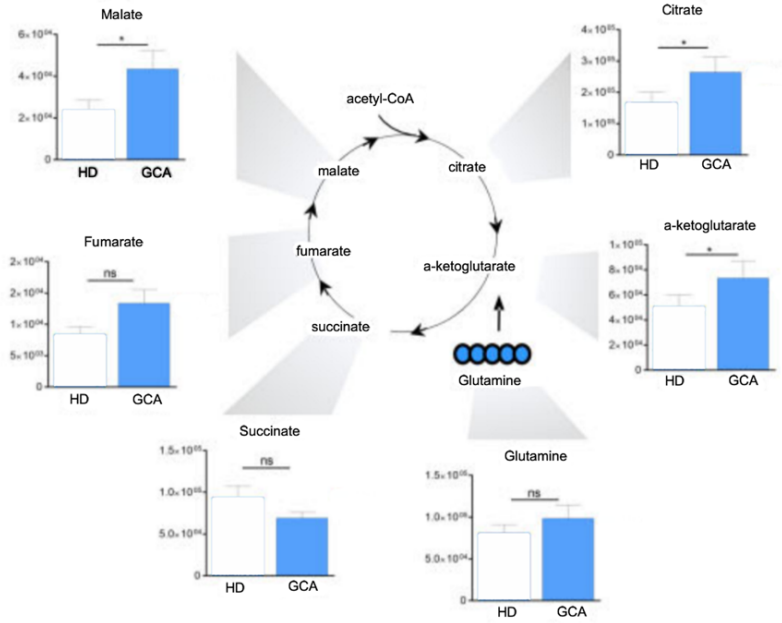


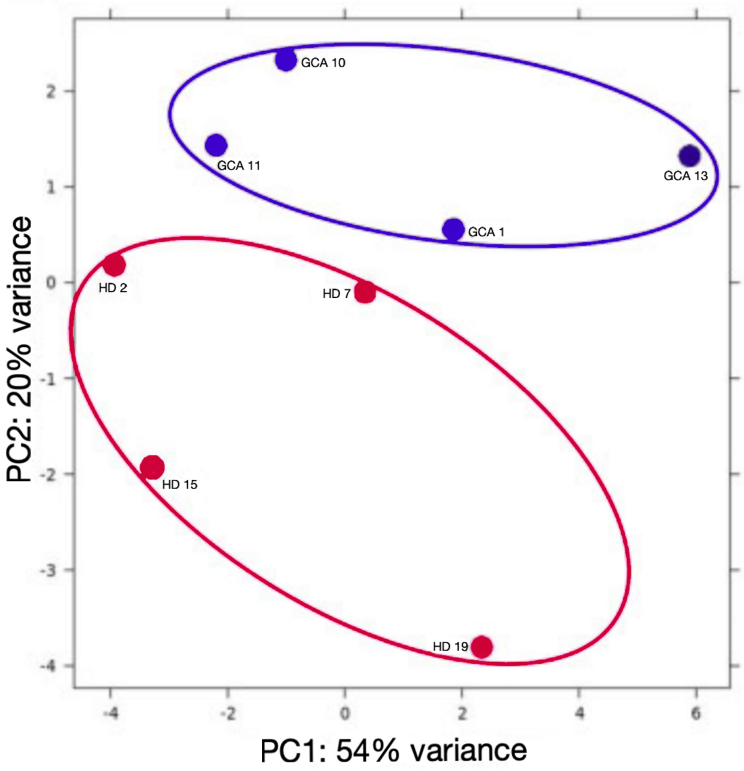
Figure 7. Tracing experiments (with $^{13}C_5$-glutamine) (Cantoni et al, 2023).



Epigenetic and global gene expression.

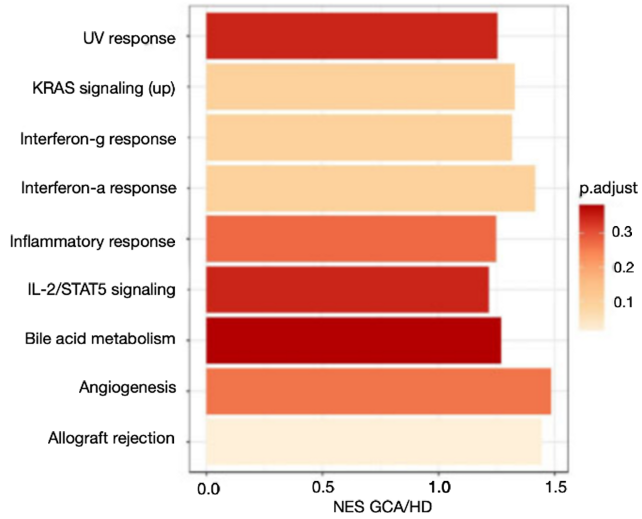
TI is characterised by the triggering of chromatin-modifying enzymes induced by immunometabolic changes, which results in epigenetic changes (i.e., chromatin modifications) and consequent increased transcription of genes associated with cytokine production. When we performed epigenetic studies, including Assay for Transposase Accessible Chromatin Sequencing (ATAC) sequencing, we observed substantial differences in chromatin accessibility between GCA monocytes and HD (**Figure 8**).

Figure 8. Chromatin accessibility: principal component analysis (Cantoni et al, 2023).
HDs are represented as red dots; GCA patients are represented as blue dots.



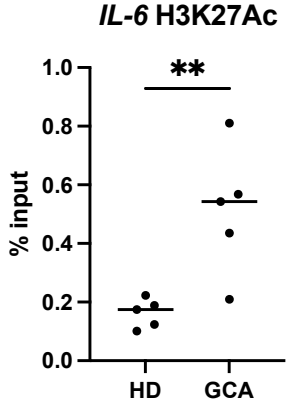
Notably, we observed that increased chromatin accessibility in GCA monocytes affected the regions of the DNA linked to the induction of pro-inflammatory responses, a modification consistent with activated TI (**Figure 9**).

Figure 9. Gene signatures affected by epigenetic changes. (Cantoni et al, 2023).
The bars length represents the normalised enrichment score (NES); colours represent the (adjusted) P value.



Due to the known pathogenetic role of the cytokine IL-6, we then specifically focused on the IL6 gene, and we observed chromatin remodelling. Specifically, chromatin immunoprecipitation-quantitative polymerase chain reaction (qPCR) highlighted a typical epigenetic modification of TI, named histone 3 lysine 27 acetylation (H3K27Ac), at the distal enhancer of the IL6 gene (**Figure 10**). This alteration enhances chromatin accessibility, facilitates transcription, and leads to heightened cytokine production upon stimulation (*as previously described*).

Figure 10. H3K27ac modifications within the IL6 gene (Cantoni et al, 2023).
*** = P < 0.01*



To comprehensively explore the pragmatic effects of epigenetic modification on immune functions, we also performed RNA-sequencing on monocytes from GCA patients and controls. These experiments were conducted both at baseline and after stimulation with LPS. Principal component analysis **(Figure 11)** and hierarchical clustering (**Figures 12 A-B**) revealed distinct clustering of the transcriptome of monocytes from patients with GCA, particularly pronounced after stimulation with LPS.

Figure 11. Principal component analysis of the transcriptional profile (Cantoni et al, 2023). *Analyses were performed at baseline and after LPS-induced stimulation.*

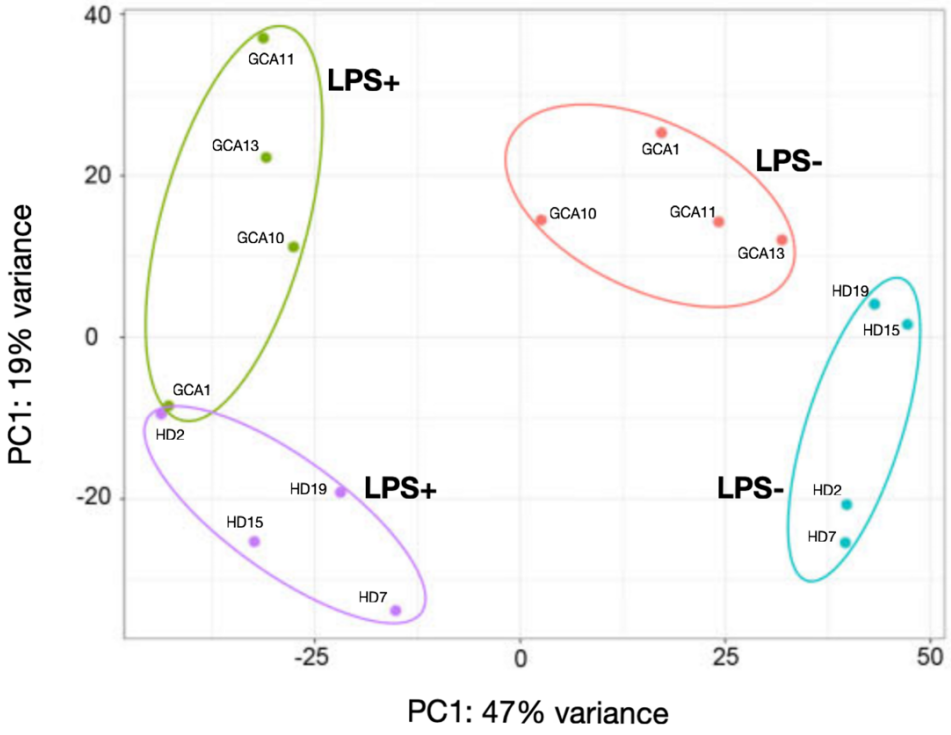
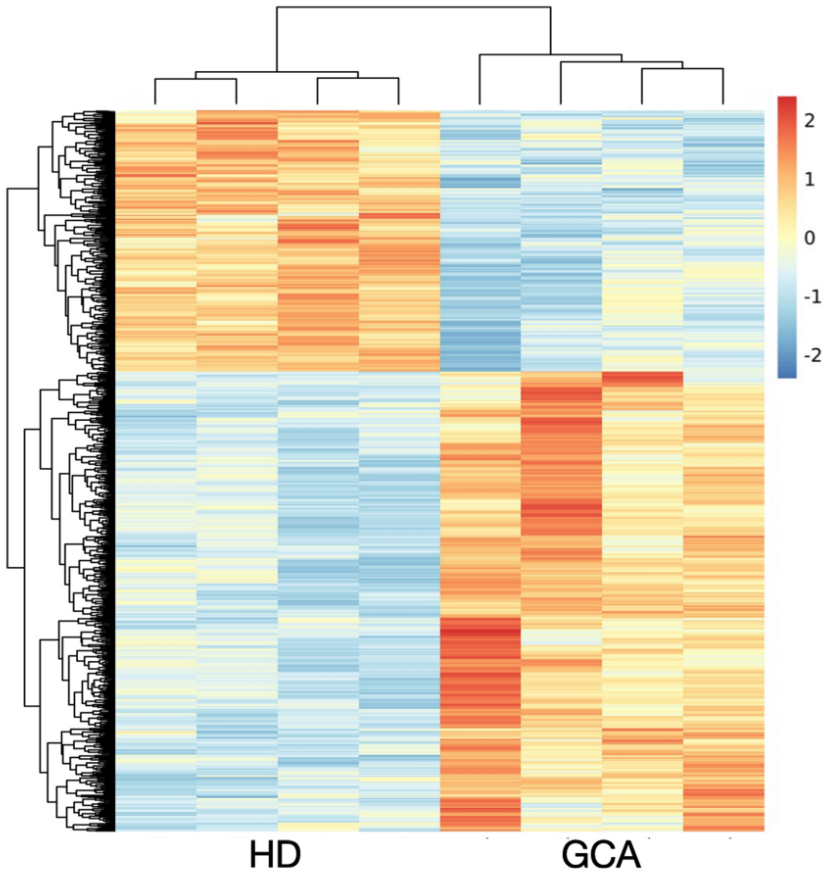
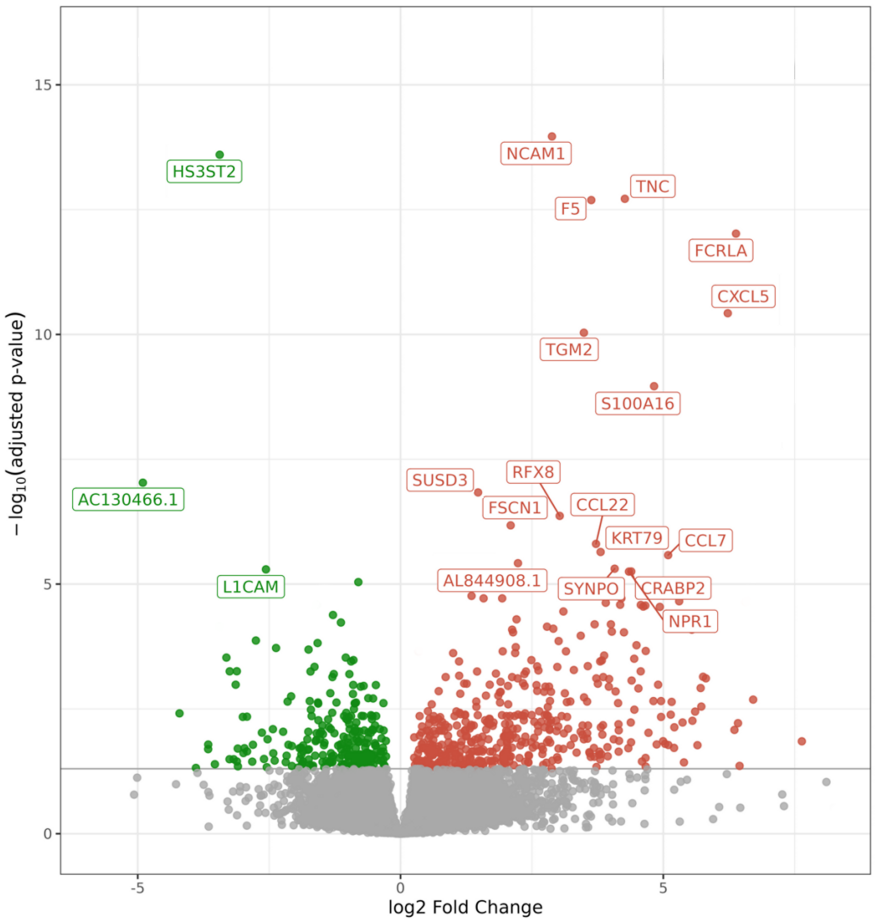


Figure 12. Heatmap of differentially expressed genes (Cantoni et al, 2023).
Unsupervised clustering was performed to samples (columns) and genes (rows).



Notably, monocytes from patients with GCA exhibited 1290 differentially expressed genes (DEGs), with significant enrichment in categories associated with inflammatory responses (**Figure 13**).

Figure 13. Volcano plots showing significant upregulated (red) and downregulated (green) genes in monocytes from GCA patients and HD (Cantoni et al, 2023).



The upregulation of genes involved in immune activation, angiogenesis, chemotaxis, lymphocyte activation, and cytokine production is in line with the established immunopathogenesis of GCA (Weyand et al, 2013).

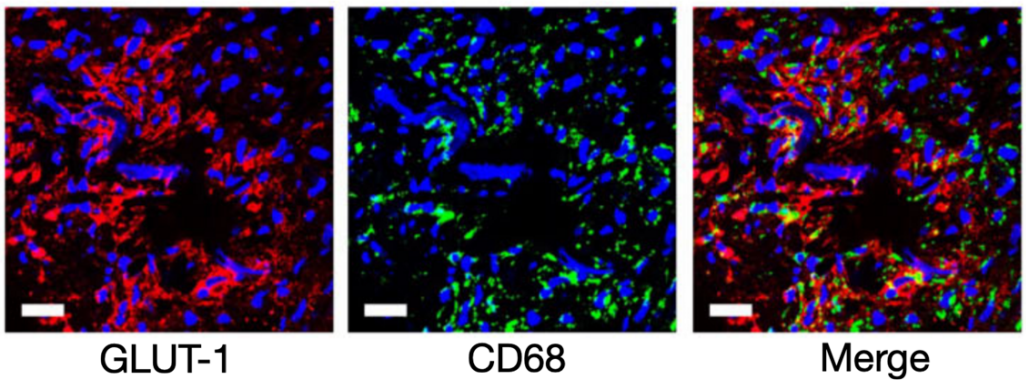
These findings collectively affirm the activation of TI in GCA monocytes, providing insights into the intricate interplay between immunometabolism, epigenetics, and gene expression underlying the hyper-inflammatory phenotype observed in GCA

Histological and confocal microscopy analyses.

Observations from FDG-PET imaging suggest an increased glycolysis in GCA lesions. To corroborate these observations, we performed histological and confocal microscopy

analyses of biopsy samples from temporal arteries of a patient with GCA (a patient not included in the original prospective cohort). These analyses confirmed enhanced glycolysis in $CD68^+$ macrophages, the predominant inflammatory cell type in arterial samples from GCA patients, as demonstrated by a heightened expression of the glucose transporter GLUT-1 (**Figure 14**). Indeed, this increased expression of Glut-1 is indicative of a metabolic shift towards glycolysis, supporting the propensity of these cells for enhanced glucose uptake and utilisation in the inflammatory milieu associated with GCA.

Figure 14. Confocal microscopy which demonstrates a co-localization of GLUT-1 (red) and the macrophage marker CD68 (light green) (Cantoni et al, 2023).
Bars = 15 mm

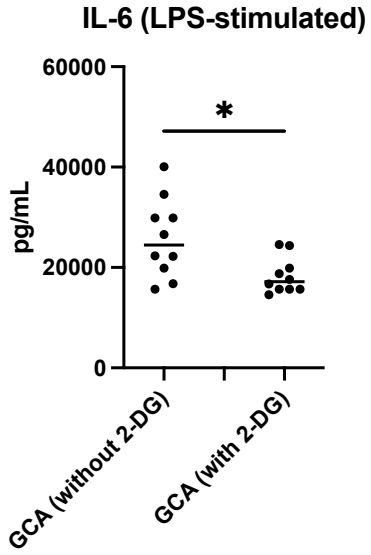


Interfering with glycolysis reduces inflammatory activation of GCA monocytes.

Finally, to assess the relevance of glycolysis to inflammation in GCA, we pharmacologically inhibited GCA monocytes with 2-deoxyglucose (2-DG), an analogue of glucose. 2-DG has the ability to enter cells via Glut-1, but upon entry is phosphorylated by 2-DG hexokinase, resulting in the formation of 2-deoxyglucose-6-phosphate (2DG-P). 2DG-P is non-metabolizable and therefore acts as a potent inhibitor, effectively preventing glycolytic processes within monocytes from patients with GCA. We therefore stimulated monocytes from patients with GCA with LPS, but this time we exposed them to LPS both in presence and in absence of 2DG. Notably, after 24h, IL-6 production was

significantly reduced in monocytes treated with 2-DG, to levels comparable to monocytes from HDs (**Figure 15**).

Figure 15. 2-DG attenuates IL-6 production (Cantoni et al, 2023).
** = $p < 0.05$*



Correlation between TI and baseline features of GCA patients.

Clinical, laboratory and imaging features at disease onset of the 20 patients with GCA, according to the items included in the "2022 ACR/EULAR classification criteria for GCA" (Ponte et al, 2022), are reported in **Table 2**.
Notably, 9 patients had signs of aortitis disclosed on FDG-PET at disease onset, and 8 patients had cranial ischaemic manifestations (i.e., at least one between visual loss and jaw/tongue claudication).

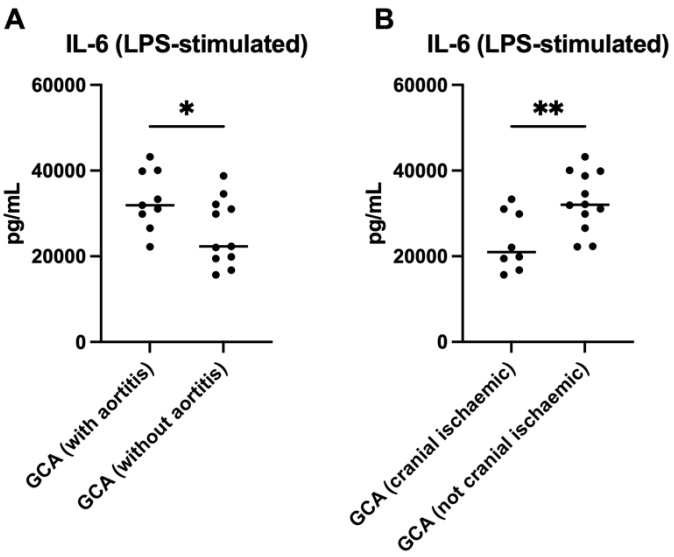
Table 2. Patients with GCA from the OSR prospective cohort: baseline features according to the "2022 ACR/EULAR classification criteria".

Visual loss	2 (10%)
Morning stiffness in shoulders or neck	11 (55%)
New temporal headache	17 (85%)
Jaw and/or tongue claudication	6 (30%)
Scalp tenderness	14 (70%)
Abnormality of temporal arteries	14 (70%)
ESR ≥ 50 mm/h *or* CRP ≥ 10 mg/L	18 (90%)
Aortitis on FDG-PET	9 (45%)
Axillary involvement	7 (35%)
Halo sign on temporal artery ultrasound	16 (80%)

When comparing functional aspects of TI between patients with and patients without aortitis at baseline, we found that the patients with aortitis produced significantly higher concentrations of IL-6 upon stimulation with LPS (**Figure 16A**). Accordingly, when comparing immunometabolic features, patients tended to show higher activation of glycolysis and tricarboxylic acid cycle, but without reaching statistical significance.

Conversely, when segregating patients according to the presence of cranial ischaemic manifestations, patients with such manifestations produced lower concentrations of IL-6 upon stimulation with LPS (**Figure 16B**). Also in this case, no significant immunometabolic differences were observed between patients from the two groups. There was no significant correlation with age at disease onset or sex.

Figure 16. IL-6 production according to the clinical phenotype at baseline
(A) presence vs absence of aortitis on FDG-PET; (B) presence vs absence of cranial ischaemic symptoms.



Prospective study cohort: outcome

During the 24 months of follow-up, 11 patients (55%) had a disease flare while still on glucocorticoid therapy, after a mean of 6.7 ± 2.8 months. All flares were mild and were managed with an increase in glucocorticoid therapy and the addition of a steroid-sparing agent (tocilizumab, n=10; methotrexate, n=1). In the remaining 9 patients, glucocorticoids were stopped after a mean of 12.8 ± 1.1 months. However, 3 of these patients had a flare at a mean of 5.7 ± 0.7 weeks after stopping therapy. In all 3 cases, the flares were mild and managed with tocilizumab start.

Of the 14 patients who had a flare (11 on glucocorticoids and 3 after stopping therapy), 8 had aortitis on FDG-PET at baseline. However, the risk of flare was not significantly increased in this group (p=0.1203). Conversely, only 1 of the 8 patients with cranial ischaemic manifestations had a flare.

Only 1 patient developed ascending aortic aneurysm; notably, this patient showed increased uptake over the ascending aorta on FDG-PET at baseline.

No patient died during the 24-month follow up.

Retrospective study cohort: baseline features.

In order to corroborate the previous clinical findings, data from a total of 1048 patients with a clinical diagnosis of GCA who were followed up at 21 centres in 18 different Italian cities (from 1 January 1989 to 19 May 2023) were reviewed. Patients with complete clinical and laboratory data (*Group 1*) were 1027 at baseline, with a mean age at diagnosis of 72.3 ± 8.5 years. Female patients were 725 (71%). Detailed clinical features of these patients according to the "2022 ACR/EULAR classification criteria" are reported in **Table 3**.

Table 3. GCA patients from Group 1 of the multicentre retrospective cohort: baseline features according to the "2022 ACR/EULAR classification criteria"

Visual loss	197 (19%)
Morning stiffness in shoulders or neck	444 (43%)
Temporal headache	720 (70%)
Jaw and/or tongue claudication	381 (37%)
Scalp tenderness	339 (33%)
Abnormality of temporal arteries	484 (47%)
ESR ≥ 50 mm/h *or* CRP ≥ 10 mg/L	986 (96%)

Patients with not only complete clinical and laboratory data but also imaging data (*Group 2*) were 290 at baseline, with a mean age at diagnosis of 70.2 ± 8.9 years. Female patients were 204 (70%). Detailed clinical features of these patients according to the "2022 ACR/EULAR criteria" are reported in **Table 4**.

Table 4. GCA patients from Group 2 of the multicentre retrospective cohort: baseline features according to the "2022 ACR/EULAR classification criteria".

Sudden visual loss	44 (15%)
Morning stiffness in shoulders or neck	106 (37%)
Temporal headache	185 (64%)
Jaw and/or tongue claudication	95 (33%)
Scalp tenderness	102 (35%)
Abnormality of temporal arteries	125 (43%)
ESR ≥ 50 mm/h *or* CRP ≥ 10 mg/L	276 (90%)
Halo sign on temporal ultrasound	188 (65%)
Aortitis on FDG-PET	153 (53%)
Axillary involvement	78 (27%)

Disease-related outcomes at disease onset.

In *Group 1*, 197 patients suffered from acute visual loss at disease onset (19%). At multivariable analysis, morning stiffness in shoulders or neck was protective for this outcome, while jaw and/or tongue claudication and abnormalities in the examination of the temporal artery were both risk factors (**Table 5**).

Table 5. Baseline features associated with acute visual loss at disease onset in Group 1.
Results of the multivariable logistic regression analysis.

Baseline features	**OR**	**CI 95%**	**p-value**
Morning stiffness in shoulders or neck	0.618	0.445-0.857	0.0040
Jaw and/or tongue claudication	1.727	1.225-2.434	0.0018
Abnormality of temporal arteries	1.650	1.163-2.342	0.0050

In *Group 2*, 44 patients suffered from acute visual loss at disease onset (15%). At multivariable analysis, jaw and/or tongue claudication and morning stiffness in shoulders

or neck were confirmed to be, respectively, risk and protective factors for this outcome. In addition, aortitis on FDG-PET emerged to be a protective factor (**Table 6**).

Table 6. Baseline features associated with acute visual loss at disease onset in Group 2.
Results of the multivariable logistic regression analysis.

Baseline features	**OR**	**CI 95%**	**p-value**
Morning stiffness in shoulders or neck	0.723	0.517-0.981	0.0345
Jaw and/or tongue claudication	1.727	1.225-2.434	0.0158
Aortitis on FDG-PET	0.109	0.024-0.493	0.0040

Disease-related outcomes at 12 months.

In *Group 1*, data on clinical outcome were available for 950 patients (93%) at 12 months. At this time-point, 46 patients (5%) developed aortic aneurysms. At multivariable analysis, both jaw and/or tongue claudication and visual loss were protective factors for this outcome (**Table 7**).

Table 7. Baseline features associated with aortic aneurysm at 12 months in Group 1.
Results of the multivariable logistic regression analysis.

Baseline features	**OR**	**CI 95%**	**p-value**
Visual loss	0.780	0.627-0.897	0.0276
Jaw and/or tongue claudication	0.313	0.127-0.772	0.0117

In addition, 243 patients (26%) had a clinical relapse over 12 months of follow up. At multivariable analysis, morning stiffness in shoulders or neck was a risk factor for this outcome (**Table 8**).

Table 8. Baseline features associated with clinical relapse over 12 months in Group 1
Results of the multivariable logistic regression analysis.

Baseline feature	OR	CI 95%	p-value
Morning stiffness in shoulders or neck	1.431	1.066-1.919	0.0169

In *Group 2*, data on clinical outcome were available for 262 patients (90%) at 12 months. At this time-point, 18 patients (7%) developed aortic aneurysms at 12 months. At multivariable analysis, jaw and/or tongue claudication was a protective factor, whereas aortitis on FDG-PET was a risk factor for this outcome (**Table 9**).

Table 9. Baseline features associated with aortic aneurysm at 12 months in Group 2
Results of the multivariable logistic regression analysis.

Baseline features	OR	CI 95%	p-value
Jaw and/or tongue claudication	0.116	0.015-0.889	0.0381
Aortitis on FDG-PET	1.129	1.035-1.239	0.0377

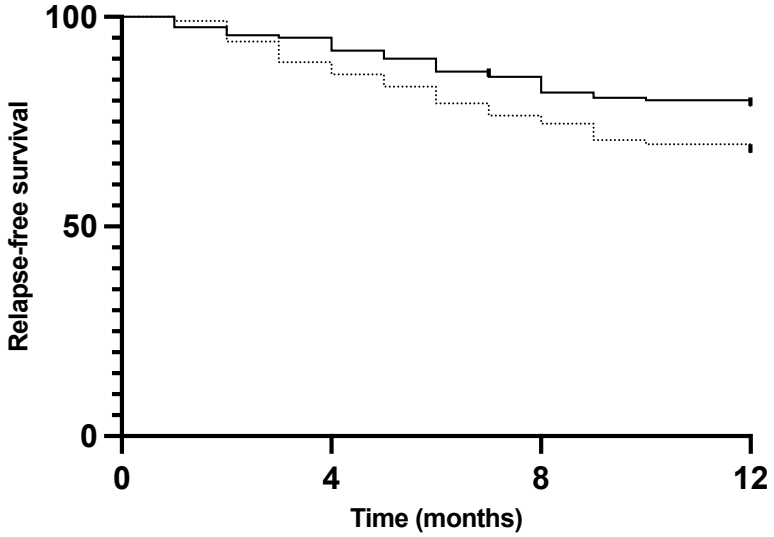
In addition, 66 patients (25%) had a relapse over 12 months of follow up. At multivariable analysis, morning stiffness in shoulders or neck and aortitis on FDG-PET were associated with this outcome (**Table 10**).

Table 10. Baseline features associated with clinical relapse over 12 months in Group 2
Results of the multivariable logistic regression analysis.

Baseline feature	OR	CI 95%	p-value
Morning stiffness in shoulders or neck	1.234	1.019-1.345	0.0034
Aortitis on FDG-PET	1.176	1.045-1.218	0.0272

Finally, we divided the patients in *Group 2* into two subgroups according to the clinical phenotype at onset. Specifically, we distinguished patients with (n=137, 52%) and without (n=125, 48%) the presence of aortitis on FDG-PET at baseline. Notably, patients in the former group had a significantly lower relapse-free survival (p=0.0408) (**Figure 17**).

Figure 17. Relapse-free survival at 12 months in patients with GCA with and without aortitis.
Dotted line: patients with aortitis (n = 465, 49%); solid line: patients without aortitis (n= 485, 51%). Logistic regression analysis; p-value = 0.0408.



Disease-related outcomes at 60 months.

In *Group 1*, data on clinical outcome were available for 494 patients (48%) at 60 months. At this time-point, 30 patients (6%) developed aortic aneurysms at 60 months. At multivariable analysis, visual loss was a protective factor for this outcome (**Table 11**).

Table 11. Baseline features associated with aortic aneurysm at 60 months in Group 1
Results of the multivariable logistic regression analysis.

Baseline feature	**OR**	**CI 95%**	**p-value**
Visual loss	0.456	0.210-0.991	0.0474

In addition, 245 patients (50%) had a clinical relapse over 60 months of follow up. At multivariable analysis, morning stiffness in shoulders or neck was a risk factor for this outcome (**Table 12**).

Table 12. Baseline features associated with clinical relapse over 60 months in Group 1.
Results of the multivariable logistic regression analysis.

Baseline features	**OR**	**CI 95%**	**p-value**
Morning stiffness in shoulders or neck	1.859	1.300-2.657	<0.001

In *Group 2*, data on clinical outcome were available for 113 patients (39%) at 60 months. At this time-point, 12 patients (11%) developed aortic aneurysms at 60 months. At multivariable analysis, no variable was significantly associated with this outcome.
In addition, 60 patients (53%) had a relapse over 60 months of follow up. At multivariable analysis, morning stiffness in shoulders or neck and aortitis on FDG-PET were both risk factors for this outcome (**Table 13**).

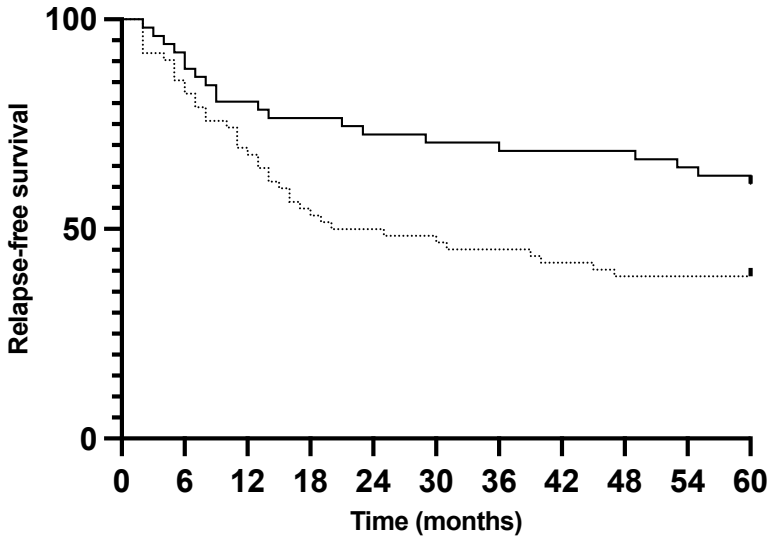
Table 13. Baseline features associated with clinical relapse over 60 months in Group 2.
Results of the multivariable logistic regression analysis).

Baseline features	**OR**	**CI 95%**	**p-value**
Morning stiffness in shoulders or neck	1.239	1.116-1.265	0.0388
Aortitis on FDG-PET	1.301	1.235-1.339	0.0221

Finally, we divided the patients in *Group 2* into two subgroups according to the clinical phenotype at baseline: presence (n=57, 50%) or absence (n=56, 50%) of aortitis on FDG-PET. Also at this timepoint, patients with aortitis had a significantly lower relapse-free survival (**Figure 18**) (p=0.0154).

Figure 18. Figure 17. Relapse-free survival at 12 months in patients with GCA with and without aortitis.

Dotted line: patients with aortitis (n = 57, 50%); solid line: patients without aortitis (n= 56, 50%). Logistic regression analysis; p-value = 0.0154.



DISCUSSION.

In the first part of our study, we have demonstrated the activation of TI programs in myelomonocytic cells from patients with new-onset GCA (Cantoni et al, 2023). This unveils a novel mechanism underpinning the rampant inflammatory activation and the excessive cytokine (mainly, IL-6) production characteristic of this disease.

In physiological conditions, TI functions as a memory programme intrinsic to cells of the innate immune system (i.e., neutrophils and especially monocyte-macrophages) and has evolved to provide defence against recurrent encounters with external pathogens. The induction of TI is therefore contingent upon the recognition of diverse micro-organisms (bacteria, fungi, parasites) and is grounded on 1) alterations in cellular metabolism, in particular with enhanced activation of glycolysis and glutaminolysis through the tricarboxylic acid cycle (Arts et al, 2016; Cheng et al, 2014); 2) epigenetic changes typically localised near to regions that promote genes encoding pro-inflammatory cytokines (e.g., IL-6), such as H3K27ac (Fanucchi et al, 2020). Notably, these modifications do not impact baseline production of these pro-inflammatory cytokines, a characteristic distinguishing TI from conventional chronic or prolonged immune responses. Instead, such modifications facilitate an augmented production of cytokines only after appropriate stimulation. Specifically, epigenetic modifications allow an increased transcription of genes involved in the inflammatory response, whereas metabolic alterations supply the essential energy and substrates to trigger and sustain this response (Arts et al, 2016; Cheng et al, 2014).

In our study, the observation of all these mechanisms, including a baseline physiological production of pro-inflammatory cytokines counterbalanced by a heightened responsiveness after appropriate stimuli (in this case, with LPS), was evident in monocytes obtained from patients with a new diagnosis of GCA. Notably, these monocytes also showed enhanced glycolysis and glutaminolysis through the tricarboxylic acid cycle and epigenetic modifications involving pro-inflammatory genes. All these features are consistent with a maladaptive activation of TI (Netea et al, 2020).

In physiological conditions, TI manifests through immunometabolic and epigenetic reprogramming of myeloid precursors within the bone marrow, providing sustained protection against infections lasting longer than the lifespan of circulating immune cells.

In physiological conditions, it is primarily the myeloid precursors located in the bone marrow that undergo the immunometabolic and epigenetic reprogramming typical of TI to provide long-lasting protection against infection (Bekkering et al, 2021; Cirovic et al, 2020). Consequently, in our study, monocytes served essentially as a surrogate for alterations involving primarily myeloid precursors; however, this approach is line with the standard of human studies on TI (Netea et al, 2020). Monocytes, recognised as pivotal entities in GCA pathogenesis, were deliberately chosen.

Maladaptive TI activation does not invariably characterise all autoimmune and inflammatory diseases. For instance, peripheral monocytes collected from naïve patients affected by rheumatoid arthritis lack the characteristic functional and metabolic features of TI (Messemaker et al, 2017). In the context of GCA, TI activation elucidates two noteworthy clinical features. First, the augmented glycolysis, as evidenced in FDG-PET imaging studies (Tomelleri et al, 2023); second, the increased IL-6 production, which correlates with disease activity (Roche et al, 1993), instigates a robust acute phase response (Weyand et al, 2013), and is clinically targeted through the IL-6 receptor inhibitor tocilizumab (Stone et al, 2017). Moreover, the identification of TI in GCA presents the potential for innovative therapeutic avenues. Current pharmacological strategies for GCA management remain suboptimal (Stone et al 2021; Tomelleri et al 2023), particularly given the significant toxicity compromising the clinical efficacy of glucocorticoids (Matteson et al, 2016), and the limited accessibility and potential disease relapse associated with IL-6 inhibition using tocilizumab (Stone et al 2021; Hellmich et al 2019).

Drugs with the potential of inhibiting excessive activation of TI are now being tested in clinical trials (Mulder et al, 2019), with GCA emerging as a robust candidate for such investigations. Notably, blocking TI offers a theoretical advantage compared to existing therapeutic strategies in GCA. Indeed, such an approach would target an inherent cellular pathway that is causative and has the potential to resolve the enhanced inflammatory response of myeloid cells, thereby addressing the altered inflammation observed in patients with GCA.

In the second part of the study, we evaluated a possible role of different grades of TI activation in defining different disease phenotypes (Dejaco et al, 2017; Hellmich et al,

2019). Specifically, we focused on two main phenotypes: one characterised by evidence of inflammation over the aorta as detected by FDG-PET (extracranial phenotype); the other characterised by signs/symptoms of ischaemic involvement of cranial arterial districts (cranial/ischaemic phenotype) (Tomelleri et al, 2023). These two phenotypes were chosen based on the data available in the previous literature, which recognise in the former a tendency towards increased systemic inflammation at presentation, associated with a more chronic disease course and a greater risk of relapse over time, and in the latter a more favourable long-term prognosis, accompanied by a lower increase in inflammatory indices at presentation (Tomelleri et al, 2023). Indeed, in the clinical part of our study, which prospectively included 20 patients diagnosed with GCA, these observations were confirmed. Specifically, we assisted to a high incidence of disease flares in patients with aortitis on FDG-PET (8/9, 89%) over an observation period of 24 months. Although the difference from patients without aortitis (who had a relapse in 55% of cases) did not reach statistical significance, probably due to the small size of the cohort, this observation still retains clinical value as it shows that obtaining complete clinical remission in GCA patients with aortitis with glucocorticoid monotherapy is an almost impossible task. On the other hand, we observed a better long-term prognosis in patients with an ischaemic/cranial phenotype, as demonstrated by the fact that only 1 of the 7 patients with such a feature had a flare.

We therefore tried to assess whether these clinical observations were also accompanied by a different degree of activation of TI, focusing on functional and metabolomic studies. From a functional point of view, when we segregated patients according to the absence or presence of aortitis, we did indeed observe a higher production of IL-6 in the first group after stimulation with LPS. This could suggest, also in the light of the data presented in the first part of the study, that in the monocytes of patients with GCA, IL-6 activation plays a more pronounced role as a pathogenetic mechanism and contributes more largely to their clinical profile and prognosis. Similarly, LPS-induced IL-6 production was significantly reduced in patients with a cranial/ischaemic phenotype. On the other hand, it should also be pointed out that the metabolomic studies that accompanied these functional studies did not confirm these observations, showing no significant differences between the different groups (aortitis *vs*. non-aortitis; cranial/ischaemic *vs*. non-cranial/ischaemic). This observation therefore does not allow us to draw definitive

conclusions on the possible quantitative role of TI in defining different clinical phenotypes, but on the other hand it could be invalidated by the small sample size. Certainly, future studies including a larger number of patients will be necessary to better define these preliminary observations.

Finally, in the third part of the study, we further evaluated previous clinical observations in a larger population of patients with GCA. To do this, we drew from an Italian national database containing detailed clinical data on more than 1000 patients, one of the largest such case series published to date (Monti et al, 2023). This analysis allowed us to confirm some of the observations made in the second part of the study and to add new ones.

First, we confirmed a strong association between the presence of jaw and/or tongue claudication and the risk of developing visual loss. This association is important because it reinforces the idea that patients with these symptoms should be considered *de facto* as patients with ischaemic complications and should therefore probably be treated aggressively even before ischaemic damage occurs. In fact, the ischaemic complications of GCA, once present, are in most cases irreversible, unless therapy is started very quickly (i.e., within 24-48 hours) (González-Gay et al, 1998); therefore, intervening on the sentinel symptoms, such as jaw and/or tongue claudication, could facilitate their prevention. We also observed that the presence of claudication was a protective factor for the development of aneurysms of the aorta at 12 months (but not at 60 months, probably due to the smaller sample size). This finding is interesting because it supports the idea that this subgroup of patients has a disease phenotype that is more cranially localised (thus sparing extracranial arterial structures such as the aorta and the large arteries that originate directly from it) and that balances greater immediate aggressiveness with a lower tendency to cause long-term damage. Patients with these characteristics therefore might deserve more attention at the onset of the disease but can be probably followed less frequently during disease course.

Also the preliminary observations regarding the role of aortitis on FDG-PET as an element capable of strongly influencing the short- and long-term prognosis of patients with GCA were confirmed. Specifically, the presence of aortitis was confirmed as a risk factor for experiencing disease flares (both at 12 and 60 months), but also for the development of aneurysms of the aorta (only at 12 months). This latter finding is

consistent with data available in the literature, but most importantly confirms the results of a recently published large prospective study (Moreel et al, 2023). Furthermore, while confirming that patients with aortic involvement have a different disease phenotype from those with cranial/ischaemic disease, the presence of aortitis on FDG-PET proved to be a protective factor for visual ischaemic complications. This finding is also in line with previous publications (Muratore et al, 2015; Tomelleri et al, 2021).

Importantly, it should be noted that it is not possible to obtain an FDG-PET at baseline in all patients, given the high cost of this method and the fact that it should be performed no later than 10 days after the start of glucocorticoids (Nielsen et al, 2018). In this context, the identification of an appropriate clinical surrogate for such an imaging finding is very useful. In our study, we identified how such a clinical surrogate may be represented, at least partially, by the presence of symptoms of PMR at the onset of the disease. Indeed, patients with GCA and PMR symptoms were more likely to have aortic involvement on FDG-PET (when this examination was performed) than patients without this clinical manifestation. Consistently, when only the clinical finding (presence of PMR) was considered, it was associated with a reduced risk of vision loss and an increased risk of disease flare, similar to what was observed in patients with positive FDG-PET. However, this association between PMR symptoms and aortitis on FDG-PET is only valid for patients with an established GCA diagnosis.

It is necessary to emphasise that the different disease phenotypes in GCA are not mutually exclusive and that there is a spectrum of disease, with the extremes being a purely extracranial disease, with little or no risk of cranial ischaemic complications, but with a more chronic course, and a purely intracranial/ischaemic disease, with a greater risk of acute complications (i.e., vision loss), but which tends to go into remission more easily in a long-term period (Tomelleri et al, 2023; Dejaco et al, 2017). It is therefore plausible to hypothesise, also in the light of the preliminary results shown in the second part of the study, that in patients in the first group, the pathogenetic role of TI is more dominant and, due to its "immunogenic memory" nature, represents one of the factors involved in the chronicisation of the disease. In the near future, diagnostic algorithms could be developed to profile each patient and modify the therapeutic strategy based on the predicted risk of developing predefined outcomes.

While this study provides valuable insights, it has certain limitations. Regarding the first two parts of the study, we were not able to perform epigenetic studies and RNA sequencing on all GCA patients and controls. Furthermore, it is likely that the study lacked sufficient statistical power to explore variations in TI activation based on the GCA phenotype. Finally, it should be noted that TI is primarily a mechanism within immune cells of the innate response, and while this study focused on myelomonocytic cells, a comprehensive evaluation of the interplay between TI and different cell subtypes that make up GCA lesions, including B and T lymphocytes and stromal and endothelial cells, was not performed. The third part has all the traditional limitations of a multicentre retrospective study. First, the management of patients was not homogeneous, as patients came from different centres and were followed up at different time periods. In particular, the variable access to steroid-sparing drugs such as tocilizumab should be taken into account. In addition, a patient selection bias cannot be excluded with regard to the monitoring of aortic aneurysm development, as patients with aortitis at baseline were probably monitored more closely.

Among the strengths of this study, there is its innovative hypothesis and the use of different experimental methodologies that together encapsulate the complexity of the mechanisms beyond TI activation. In spite of the methodological diversity, the results converged to confirm the central role of monocyte-macrophages in the activation of TI programmes in the context of GCA. In addition, patients on whom the experiments were carried out were followed prospectively and homogeneously by the same physician. Finally, the retrospective cohort included is one of the largest cohorts with the most comprehensive short- and long-term follow-up data available in the literature to date.

In conclusion, this study marks a pioneering demonstration of the involvement of TI activation in the GCA pathogenesis. In addition, it confirms on several levels that GCA is a multifaceted disease with different clinical phenotypes associated with various possible outcomes. This is a fundamental starting point for re-evaluating our clinical approach to patients with GCA, both in terms of monitoring and treatment. In the future, adequate baseline phenotyping of patients with GCA, both clinically and by imaging, will be required prior to initiating treatment, and clinical trials investigating new drugs will likely need to differentiate between the different disease phenotypes.

MATERIALS AND METHODS.

Prospective study cohort: patient recruitment and sample acquisition.

The prospective part of the study enrolled individuals with a new diagnosis of GCA from the Unit of Immunology and Rheumatology at San Raffaele Hospital, Milan (Italy). The diagnosis of GCA was first made by a rheumatologist, starting from clinical signs and symptoms, and further verified by vascular ultrasound and FDG-PET. Vascular ultrasound of the superficial temporal, parietal, frontal and axillary arteries was performed in all patients at the first visit. FDG-PET was performed in all patients no more than 10 days after glucocorticoid therapy was started. Age- and sex-matched HDs served as controls. After informed consent, peripheral blood samples were obtained by peripheral venipuncture using ethylenediaminetetraacetic acid (EDTA)-containing tubes.

Vascular ultrasound.

Ultrasound was performed at baseline on the superficial temporal arteries, parietal arteries, frontal arteries, and axillary arteries, bilaterally. The presence or absence of a halo sign (van der Geest et al, 2019) was used to identify GCA. Ultrasound equipment with high-frequency linear probes was used. Greyscale frequency was ≥ 22 MHz for superficial temporal, parietal and frontal arteries and 4-18 MHz for axillary arteries. Colour Doppler pulse repetition frequency was 2-3.5 kHz for superficial temporal, parietal and frontal arteries and 3-4 kHz for axillary arteries. Colour box had an angle correction < 60°.

FDG-PET assessment.

FDG-PET was used to evaluate *in vivo* glycolysis in affected vessels of patients with GCA. All patients had blood glucose levels below 10 mmol/L when FDG-PET was performed. Injection of FDG in the peripheral circulation preceded a whole-body scan (from the top of the head to the knees) using a Siemens CTI flow scanner with a scan

duration of 5 minutes. Simultaneously, patients underwent a non-contrast low dose computed tomography in order to obtain anatomical representation.

Patient follow-up.

Patients with GCA were clinically followed prospectively for 24 months. In all patients, glucocorticoid monotherapy (i.e., prednisone 1 mg/kg daily; no more than 60 mg daily) was initially started and then gradually tapered according to European recommendations (Hellmich et al, 2019). Patients underwent regular (i.e., every 2-4 months) follow-up visits where they were evaluated for i) disease status (remission *vs*. relapse), ii) development of therapy-related complications. Relapse was defined as a recurrence of symptoms (or signs) interpreted by the clinician as being associated with GCA which led to an increase (or re-start) of glucocorticoids or to the addition of a non-steroidal immunosuppressive drug (i.e., methotrexate or tocilizumab).

Isolation of mononuclear cells and monocytes.

Isolation of blood mononuclear cells involved dilution of blood in RPMI 1640 medium (GibcoTM) followed by differential centrifugation through LymphoprepTM solution (STEM-CELL Technologies). Monocytes were isolated from mononuclear cells through negative selection using magnetic beads (Miltenyi Biotech; Monocyte Isolation Kit). The isolated cells were washed thoroughly and resuspended in culture medium (RMPI 1620) supplemented with GlutaMAXM, 10% fetal bovine serum inactivated by heat (EuroClone; FBS) and 1% streptomycin/penicillin (GicboTM).

Cytokine analysis

For cytokine assays in cultured monocyte supernatants, monocytes (200’000/well) were plated in 96-well U-bottom plates and incubated with i) 250 µL pure culture medium; ii) 250 µL culture medium containing 15 ng/ml of LPS derived from *E. coli* (strain O55:B). The supernatants were collected after 24 hours, placed at -75°C and stored there until

cytokine levels were measured. Concentrations of the cytokines IL-1β, IL-6 and TNFα were measured using ELISA (D&R Systems, Minneapolis, USA).

Treatment with 2-Deoxyglucose.

IL-6 production by monocytes from patients with GCA was evaluated also in the presence of 2-DG, a glycolysis inhibitor. To do this, monocytes were cultured at 37°C with 12 mM of 2-DG for 24 hours before supernatant collection.

Metabolomics evaluation and tracing experiments.

Pellets of monocyte cells (approximately $2x10^6$) were extracted in 700 µL of ice-cold solution (water:acetonitrile:methanol 2:3:5). After a vortex for 45 min at 3-5°C, insoluble material was removed by centrifugation at 20'000 g for 12 min at 3-5°C. The resulting supernatants were used for metabolomic analysis by UPHLC-MS. Analyses were performed using a Q-Exactive mass spectrometer (Thermo-Fisher, Bremen, Germany) coupled to a Vanquish UPHLC. Ten microliters of sample extracts were loaded onto a Kinetex XC-B18 column (2 x 150 mm i.d., 1.8 µm; Phenomenex). Samples were analysed using a 3-minute isocratic condition. MAVEN (Princeton, NJ, USA) was used for metabolite assignments, $^{13}C_5$Glutamine and $^{13}C_6$Glucose tracing experiments, correction for expected natural isotopes abundances, and iso-topologue distributions. Metabolite levels were then normalised to protein quantification. Statistical analyses (principal component analysis and hierarchical clustering analysis) were performed using MetaboAnalyst 4.0, GraphPad Prism 8.5 (GraphPad Inc, La Jolla, California, USA) and GENEE (Broad Inst., Cambridge, Massachusetts, USA).

Confocal analysis.

Confocal analysis of GCA biopsies obtained from temporal arteries of patients with GCA was conducted using antibodies anti-Glut-1 (clone ab15409; Abcam, Cambridge, UK) and anti-CD68 (Dakko, Glostrup, Denmark)

Assay for Transposase Accessible Chromatin Sequencing.

Chromatin accessibility was assessed by ATAC-seq on permeabilised nuclei obtained from monocytes after their isolation. For on-plate ATAC-seq, $2x10^5$ monocytes per sample were seeded onto a 96-well U-bottom plate. After 48 hours of incubation, the cells were washed with phosphate-buffered saline and permeabilized with buffer lysis (0.05% NP-40, 4 mM MgCl, 12 mM Tris-Cl pH 7.4, 12 mM NaCl) for 15 minutes. The subsequent reaction was incubated at 37°C for 40 minutes and followed by purification using a PCR Purification Kit (Qiagen MinElute). The transposed DNA was eluted and amplified using barcoded PCR primers. Quality assessment, alignment and peak calling were performed using FastQC, trimmomatic, BWA, Picard and MACS2 (FDR < 0.05). Peak normalisation and differential analysis were performed using Diff-Bind (false discovery rate < 0.05). Profiles of chromatin accessibility around transcription start sites of genes and heatmaps were made using DeepTools.

Chromatin immunoprecipitation.

Monocytes ($2x10^5$ per sample) were fixed with 0.5% methanol-free formaldehyde. Cell preparations were then sonicated for 10 cycles using a Diagenode Bioruptor UCD-200. 35 µL of chromatin were incubated with 10 µL of protease inhibitor cocktail, 260 µL of dilution buffer, and 0.5 µL of an antibody recognizing H3K27ac (Diagenode). They were finally incubated with rotation overnight at 4°C. Protein A/G magnetic beads were washed in dilution buffer. After that, 0.1% SDS and 0.2% BSA were added to the chromatin/antibody mix and rotated for 1 hour at 4°C. Beads were then washed for 5 min at 4°C with five rounds with 600 µL buffer. After washing, chromatin was eluted using elution buffer for 30 min. Supernatant was recovered and collected, 8 µL NaCl and 2 µL K proteinase were added and samples were incubated at 60°C for 4 h. Finally, samples were purified using QAGEN Qaquick PCR purification Kit and eluted in 20 mL elution buffer.

RNA sequencing.

ReliaPrep RNA Miniprep Systems (Promega Corporation) was used for RNA extraction from 1 x 10^5 cells. Quality assessment was made with the 4000 TapeStation (Agilent). RNA quantification was performed using the Qubit 2.5 Fluorometer (Thermo Fisher). The preparation of the library was performed using the SMART-Seq v5 Ultra Low Input RNA Kit (Takara Bio USA) and sequencing was performed on a NextSeq 500 (Illumina). Quality assessment and alignment of reads to the human reference genome (GCRh38/gh38) was performed using trimmomatic, FastQC, Subread featureCounts and STAR. Transcript counts were processed using edgeR and normalised for library size. P-values were corrected using false discovery rate. Gene Set Enrichment Analysis was performed using clusterProfiler, considering different datasets (from KEGG Pathway Database, Molecular Signatures Database, Reactome Pathway Database and Gene Ontology).

Retrospective study cohort.

The second part of the study included patients with a clinical diagnosis of GCA who were followed for at least 6 months to confirm the diagnosis. The study period was from 1 January 1988 to 19 April 2023 and included different centres affiliated to the Italian Society of Rheumatology Vasculitis Study Group. Patients' medical records were reviewed from the date of GCA diagnosis until the study endpoint, last visit, migration or death.

Variables of interest.

A comprehensive collection of baseline features at the time of diagnosis was categorised into clinical, laboratory and imaging features. Clinical features included morning shoulder and/or neck stiffness, sudden visual loss, jaw and/or tongue claudication, new-onset temporal headache, scalp tenderness, and abnormalities on temporal artery examination. Laboratory features included erythrocyte sedimentation rate (ESR) $\geq$ 50 mm/h and C-reactive protein (CRP) $\geq$ 10 mg/L. Imaging features included temporal artery vasculitis on ultrasound, axillary artery vasculitis on ultrasound and aortitis on FDG-PET. Data were collected using a standardised electronic data collection form. These clinical,

laboratory and imaging variables were chosen because they are included in the "2022 ACR/EULAR classification criteria for GCA" (Ponte et al, 2022) (**Table 14**) and are therefore considered to be most representative of the disease.

Table 14. 2022 ACR/EULAR classification criteria for giant cell arteritis (GCA).
These criteria can only be used to classify a patient as having GCA. Before applying these criteria, a diagnosis of vasculitis must have been made and alternative diagnoses should have been excluded (adapted from Ponte et al, 2023)

ABSOLUTE REQUIREMENT	
Age ≥ 50 years at diagnosis	
CLINICAL CRITERIA	
Jaw and/or tongue claudication	**+2**
Morning stiffness in shoulders or neck	**+2**
Visual loss	**+3**
Abnormal examination of temporal artery	**+2**
Scalp tenderness	**+2**
Temporal headache	**+2**
LABORATORY CRITERIA	
CRP ≥ 10 mg/L or ESR ≥ 50 mm/h	**+3**
BIOPSY/IMAGING CRITERIA	
Halo sign on temporal ultrasound *or* positive temporal artery biopsy	**+5**
Aortitis on FDG-PET	**+2**
Involvement of both axillary arteries	**+2**
A score 6 is required for giant cell arteritis classification	

Outcomes.

Clinical outcomes of GCA patients were assessed at disease onset (for vision loss only), and at 12 and 60 months. Disease-related outcomes included sudden vision loss, development of aortic aneurysm or dissection, and disease flare. A flare was defined as a

recurrence of signs/symptoms of GCA leading to an increase in glucocorticoids or a change in immunosuppressive treatment.

Statistical analysis.

SAS Studio 9.4 and GraphPad Prism 9.1 were used for data analysis. A two-sided p-value < 0.05 was considered statistically significant. Descriptive statistics were used for continuous variables (median and quartiles; mean and standard deviation) and comparisons were performed with Kruskall-Wallis test. Categorical variables were described using counts and percentages (%) and comparisons were performed with Fisher's exact test. Univariate logistic regression analysis with odds ratios (OR) and 95% confidence intervals (CI) were used to assess the correlation between each clinical, laboratory, and imaging features and disease-related and therapy-related clinical outcomes. Survival analysis included relapse-free survival.

REFERENCES.

- Alba M, García-Martínez A, Prieto-González S, Tavera-Bahillo I, Corbera-Bellalta M, Planas-Rigol E, Espígol-Frigolé G, Butjosa M, Hernández-Rodríguez J, Cid MC (2014) Relapses in Patients with Giant Cell Arteritis: Prevalence, Characteristics, and Associated Clinical Findings in a Longitudinally Followed Cohort of 106 Patients. *Medicine (Baltimore)* 93:194-201.
- Arida A, Kyprianou M, Kanakis M, Sfikakis P (2010) The diagnostic value of ultrasonography-derived edema of the temporal artery wall in giant cell arteritis: a second meta-analysis. *BMC musculoskeletal disorders* 8:11:44.
- Arts RJ, Carvalho A, La Rocca C, Palma C, Rodrigues F, Silvestre R, Kleinnijenhuis, J, Lachmandas E, Gonçalves LG, Belinha A, *et al* (2016) Immunometabolic pathways in BCG-induced trained immunity. *Cell reports* 17:2562-2571.
- Arts RJ, Blok BA, Aaby P, Joosten LAB, de Jong D, van der Meer JWM, Benn C, van Crevel R, Netea MG (2015) Long-term in vitro and in vivo effects of γ-irradiated BCG on innate and adaptive immunity. *Journal of leukocyte biology* 98: 995–1001.
- Arts RJ, Novakovic B, Horst RT, Carvalho A, Bekkering S, Lachmandas E, Rodrigues F, Silvestre R, Cheng S, Wang S, *et al* (2016) Glutaminolysis and Fumarate Accumulation Integrate Immunometabolic and Epigenetic Programs in Trained Immunity. *Cell metabolism* 24: 807–819.
- Arts RJ, Joosten LAB, Netea MG (2016) Immunometabolic circuits in trained immunity. *Seminars in immunology* 28: 425–430.
- Barbi J, Drew P, Pan F (2013) Metabolic control of the Treg/Th17 axis. *Immunological reviews* 252: 52–77.
- Barton ES, White DW, Cathelyn JS, Brett-McClellan KA, Engle M, Diamond MS, Miller VL, Virgin HW (2007) Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 447: 326–329.
- Bekkering S, Domínguez-Andrés J, Joosten LAB, Riksen NP, Netea MG (2021) Trained Immunity: Reprogramming Innate Immunity in Health and Disease. *Annual review of immunology* 39: 667–693.
- de Boysson H, Damas A, Vautier M, Parienti JJ, Liozon E, Lambert M, Samson M, Ebbo M, Dumont A, Sultan A, *et al* (2018) Large-vessel involvement and aortic dilation in giant-cell arteritis. A multicenter study of 549 patients. *Autoimmunity* reviews 17: 391–398.
- Brack A, Martinez-Taboada V, Stanson A, Goronzy JJ, Weyand C (1999) Disease pattern in

cranial and large-vessel giant cell arteritis. *Arthritis and rheumatism* 42: 311–317.

- Calamia KT, Hunder GG (1981) Giant Cell Arteritis (Temporal Arteritis) Presenting as Fever of Undetermined Origin. *Arthritis and rheumatism* 24: 1414–1418.
- Camellino D, Duftner C, Dejaco C (2020) New insights into the role of imaging in polymyalgia rheumatica. *Rheumatology (Oxford)* 60: 1016–1033.
- Camporeale A, Poli V (2012) IL-6, IL-17 and STAT3: a holy trinity in auto-immunity? *Frontiers in bioscience* 17: 2306–2326.
- Cantoni E, Merelli I, Stefanoni D, Tomelleri A, Campochiaro C, Giordano V, Panigada M, Baldissera EM, Merlo Pich L, Natoli V *et al* (2023). Myelomonocytic cells in giant cell arteritis activate trained immunity programs sustaining inflammation and cytokine production. *Rheumatology (Oxford)* 62: 3469-3479.
- Carmona FD, Coit P, Saruhan-Direskeneli G, Hernández-Rodríguez J, Cid MC, Solans R, Castañeda S, Vaglio A, Direskeneli H, Merkel PA *et al* (2017) Analysis of the common genetic component of large-vessel vasculitides through a meta-Immunochip strategy. *Scientific reports* 9: 7 – 43953.
- Carmona, FD, Mackie SL, Martín JE, Taylor JC, Vaglio A, Eyre S, Bossini-Castillo L, Castañeda S, Cid MC, Hernández-Rodríguez J, *et al* (2015) A Large-Scale Genetic Analysis Reveals a Strong Contribution of the HLA Class II Region to Giant Cell Arteritis Susceptibility. *The American Journal of Human Genetics* 96: 565–580.
- Cavazza F, Boiardi L, Restuccia G, Pipitone N, Pazzola G, Tagliavini E, Ragazzi M, Rossi G, Salvarani C (2014) Inflamed temporal artery: histologic findings in 354 biopsies, with clinical correlations. *The American Kournal of Surgical Pathology* 38: 1360–1370.
- Chen G, Nunez G (2010) Sterile inflammation: sensing and reacting to damage. *Nature Reviews* I*mmunology* 10: 826–837.
- Chen S, Yang J, Yuquan W, Xiawei W (2019) Epigenetic regulation of macrophages: from homeostasis maintenance to host defense. *Cellular and molecular immunology* 17: 36–49.
- Cheng S, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, Giamarellos-Bourboulis EJ, Martens JH, Appukudige N, Aghajanirefah A, *et al* (2014) mTOR- and HIF-1α–mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* 345: 125–134.
- Chrysidis S, Døhn UM, Terslev L, Fredberg U, Lorenzen T, Christensen R, Larsen K Diamantopoulos A (2021) Diagnostic accuracy of vascular ultrasound in patients with suspected giant cell arteritis (EUREKA): a prospective, multicentre, non-interventional, cohort study. *The Lancet Rheumatology* 3: e865–e873.
- Cirovic B, de Bree C, Groh L, Blok B, Chan J, van der Velden W, Bremmers M, van Crevel R, Händler K, Picelli S, *et al* (2020) BCG Vaccination in Humans Elicits Trained Immunity

via the Hematopoietic Progenitor Compartment. *Cell Host & Microbe* 28: 322-334.e5.

- Corbera-Bellalta M, Planas-Rigol E, Lozano E, Terrades-García N, Alba MA, Prieto-González S, García-Martínez A, Albero R, Enjuane A, Espígol-Frigolé G, *et al* (2015) Blocking interferon γ reduces expression of chemokines CXCL9, CXCL10 and CXCL11 and decreases macrophage infiltration in ex vivo cultured arteries from patients with giant cell arteritis. *Annals of the Rheumatic Diseases* 75: 1177–1186.
- Czihal M, Zanker S, Rademacher A, Tatò F, Kuhlencordt PJ, Schulze-Koops H, Hoffmann U (2012) Sonographic and clinical pattern of extracranial and cranial giant cell arteritis. *Scandinavian Journal of Rheumatology* 41: 231–236.
- Danesh-Meyer H, Savino PJ, Gamble GG (2005) Poor prognosis of visual outcome after visual loss from giant cell arteritis. *Ophthalmology* 112: 1098–1103.
- De Miguel E, Karalilova R, Macchioni P, Ponte C, Conticini E, Cowley S, Tomelleri A, Monti S, Monjo I, Batalov Z *et al* (2023) Subclinical giant cell arteritis increases the risk of relapse in polymyalgia rheumatica. *Annals of the Rheumatic Diseases* ard: 2023-224768
- Dejaco C, Duftner C, Buttgereit F, Matteson EL, Dasgupta B (2017) The spectrum of giant cell arteritis and polymyalgia rheumatica: revisiting the concept of the disease. *Rheumatology (Oxford)* 56: 506 – 515.
- Dejaco C, Ramiro S, Bond M, Bosch P, Ponte C, Mackie SL, Bley TA, Blockmans D, Brolin S, Bolek EC, *et al* (2023) EULAR recommendations for the use of imaging in large vessel vasculitis in clinical practice: 2023 update. *Annals of the Rheumatic Diseases* ard-2023-224543.
- Deng J, Younge BR, Olshen RA, Goronzy JJ, Weyand C (2010) Th17 and Th1 T-Cell Responses in Giant Cell Arteritis. *Circulation* 121: 906–915.
- Deng J, Ma-Krupa W, Gewirtz AT, Younge BR, Goronzy JJ, Weyand C (2009) Toll-Like Receptors 4 and 5 Induce Distinct Types of Vasculitis. *Circulation research* 104: 488–495.
- Domínguez-Andrés J, Netea MG (2018) Long-term reprogramming of the innate immune system. *Journal of Leukocyte Biology* 105: 329–338.
- Donohoe DR, Bultman SJ (2012) Metaboloepigenetics: Interrelationships between energy metabolism and epigenetic control of gene expression. *Journal of Cellular Physiology* 227: 3169–3177.
- Dumont A, Lecannuet A, Boutemy J, Maigné G, Martin-Silva, N, Deshayes S, Audemard-Verger A, Sultan A, Planchard G, Aouba A *et al* (2020) Characteristics and outcomes of patients with ophthalmologic involvement in giant-cell arteritis: A case-control study. *Seminars in Arthritis and Rheumatism* 50: 335–341.
- Dumont JJ, Parienti JJ, Delmas C, Boutemy J, Maigné G, Silva NM, Sultan A, Planchard G,

Aouba A, de Boysson H (2019) Factors Associated with Relapse and Dependence on Glucocorticoids in Giant Cell Arteritis. *The Journal of Rheumatology* 47: 108–116.

- Evans WM, O'Fallon WM, Hunder GG (1995) Increased incidence of aortic aneurysm and dissection in giant cell (temporal) arteritis. A population-based study. *Annals of Internal Medicine* 122: 502–507.
- Everts B, Amiel E, Huang S, Smith AM, Chang C, Lam Y, Redmann V, Freitas TC, Blagih J, van der Windt G, *et al* (2014) TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nature Immunology* 15: 323–332.
- Fanucchi S, Domínguez-Andrés J, Joosten L, Netea MG, Mhlanga MM (2020) The Intersection of Epigenetics and Metabolism in Trained Immunity. *Immunity* 54: 32–43.
- Funes SC, Fernàndez-Fierro A, Rebolledo-Zelada D, Mackern-Oberti JP, Kalergis AM (2021) Contribution of Dysregulated DNA Methylation to Autoimmunity. *International Journal of Molecular Sciences* 22: 11892-NA.
- Galván-Peña S, O'Neill L (2014) Metabolic Reprograming in Macrophage Polarization. *Frontiers in Immunology* 2: 5–420.
- van der Geest KSM, Sandovici M, Brouwer E, Mackie SL (2020) Diagnostic Accuracy of Symptoms, Physical Signs, and Laboratory Tests for Giant Cell Arteritis: A Systematic Review and Meta-analysis. *JAMA internal medicine* 180: 1295–1304.
- van der Geest KSM, Wolfe K, Borg F, Sebastian A, Kayani A, Tomelleri A, Gondo P, Schmidt WA, Luqmani R, Dasgupta B (2020) Ultrasonographic Halo Score in giant cell arteritis: association with intimal hyperplasia and ischaemic sight loss. *Rheumatology (Oxford)* 60: 4361-4366.
- Van Der Geest KSM, Borg F, Kayani A, Paap D, Gondo P, Schmidt WA, Luqmani R, Dasgupta B (2019) Novel ultrasonographic Halo Score for giant cell arteritis: Assessment of diagnostic accuracy and association with ocular ischaemia. *Annals of the Rheumatic Diseases* 79: 393–399
- Gonzalez-Gay MA, Garcia-Porrua C, Llorca J, Hajeer AH, Brañas F, Dababneh A, Gonzalez-Louzao C, Rodriguez-Gil E, Rodriguez-Ledo P, Ollier W (2000) Visual manifestations of giant cell arteritis. Trends and clinical spectrum in 161 patients. *Medicine* 79: 283–292.
- González-Gay MA, Garcia-Porrua C, Piñeiro A, Pego-Reigosa R, Llorca J, Hunder GG (2004) Aortic aneurysm and dissection in patients with biopsy-proven giant cell arteritis from northwestern Spain: a population-based study. *Medicine* 83: 335–341.
- González-Gay MA, Matteson EL, Castañeda S (2017) Polymyalgia rheumatica. *Lancet* 390: 1700–1712.

- González-Gay MA, Castañeda S, Llorca J (2016) Giant Cell Arteritis: Visual Loss Is Our Major Concern. *The Journal of Rheumatology* 43: 1458–1461.
- Gonzalez-Gay MA, Barros S, Lopez-Diaz MJ, Garcia-Porrua C, Sanchez-Andrade A, Llorca J (2005) Giant cell arteritis: disease patterns of clinical presentation in a series of 240 patients. *Medicine* 84: 269–276.
- González-Gay MA, Vazquez-Rodriguez TR, Gómez-Acebo I, Pego-Reigosa R, Lopez-Diaz MJ, Vazquez-Triñanes MC, Miranda-Filloy JA, Blanco R, Dierssen T, González-Juanatey C, *et al* (2009) Strokes at Time of Disease Diagnosis in a Series of 287 Patients with Biopsy-Proven Giant Cell Arteritis. *Medicine* 88, 227–235.
- Gonzalez-Gay MA, Vazquez-Rodriguez TR, Lopez-Diaz MJ, Miranda-Filloy JA, González-Juanatey C, Martin J, Llorca J (2009) Epidemiology of giant cell arteritis and polymyalgia rheumatica. *Arthritis and Rheumatism* 61: 1454–1461.
- González-Gay MA, Blanco R, Rodríguez-Valverde V, Martínez-Taboada VM, Delgado-Rodriguez M, Figueroa M, Uriarte E (1998) Permanent visual loss and cerebrovascular accidents in giant cell arteritis: Predictors and response to treatment. *Arthritis and Rheumatism* 41: 1497–1504.
- Gribbons KB, Ponte C, Carette S, Craven A, Cuthbertson D, Hoffman GS, Khalidi N, Koening CL, Langford CA, Maksimowicz-McKinnon K, *et al* (2020) Patterns of Arterial Disease in Takayasu Arteritis and Giant Cell Arteritis. *Arthritis care & research* 72: 1615–1624.
- Grigoriou M, Banos A, Filia A, Pavlidis P, Giannouli S, Karali V, Nikolopoulos D, Pieta A, Bertsias G, Verginis P, *et al* (2019) Transcriptome reprogramming and myeloid skewing in haematopoietic stem and progenitor cells in systemic lupus erythematosus. *Annals of the rheumatic diseases* 79: 242–253.
- Hauenstein C, Reinhard M, Geiger J, Markl M, Hetzel A, Treszl A, Vaith P, Bley T (2012) Effects of early corticosteroid treatment on magnetic resonance imaging and ultrasonography findings in giant cell arteritis. *Rheumatology (Oxford)* 51: 1999–2003.
- Hayreh SS (2014) Ocular vascular occlusive disorders: Natural history of visual outcome. *Progress in Retinal and Eye Research* 41: 1–25.
- Hellmich B, Agueda A, Monti S, Buttgereit F, de Boysson H, Brouwer E, Cassie R, Cid MC, Dasgupta B, Dejaco C, *et al* (2019) 2018 Update of the EULAR recommendations for the management of large vessel vasculitis. *Annals of the Rheumatic Diseases* 79: 19–30.
- Hernández-Rodríguez J, Garcia-Martinez A, Casademont J, Filella X, Esteban MJ, López-Soto A, Fernández-Solà J, Urbano-Márquez A, Grau JM, Cid MC (2002) A strong initial systemic inflammatory response is associated with higher corticosteroid requirements and longer duration of therapy in patients with giant-cell arteritis. *Arthritis and Rheumatism* 47:

29–35.

- Hida S, Miura NN, Adachi Y, Ohno N (2005) Effect of Candida albicans cell wall glucan as adjuvant for induction of autoimmune arthritis in mice. *Journal of autoimmunity* 25: 93–101.
- Ifrim DC, Quintin J, Joosten L, Jacobs C, Jansen T, Jacobs L, Gow N, Williams DL, van der Meer JWM, Netea MG (2014) Trained Immunity or Tolerance: Opposing Functional Programs Induced in Human Monocytes after Engagement of Various Pattern Recognition Receptors. *Clinical and Vaccine Immunology* 21: 534–545.
- Jeljeli M, Doridot L, Chêne C, Coelho-Riccio LG, Nicco C, Chouzenoux S, Deletang Q, Allanore Y, Kavian N, Batteux F (2019) Trained immunity modulates inflammation-induced fibrosis. *Nature Communications* 10: 5670.
- Jiemy WF, van Sleen Y, van der Geest KSM, Berge H, Abdulahad WH, Sandovici M, Boots AM, Heeringa P, Brouwer E (2020) Distinct macrophage phenotypes skewed by local granulocyte macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) are associated with tissue destruction and intimal hyperplasia in giant cell arteritis. *Clinical & Translational Immunology* 9: 1164-NA.
- Jung J, Zeng H, Horng T (2019) Metabolism as a guiding force for immunity. *Nature Cell Biology* 21: 85–93.
- Kim SY, Kang SJ, Kim JW, Cho HR, Moon SB, Kim KY, Lee HS, Han CH, Ku SK, Lee YJ *et al* (2012) Effects of Polycan, a β-glucan, on experimental periodontitis and alveolar bone loss in Sprague-Dawley rats. *Journal of Periodontal Research* 47: 800–810.
- Kimura A, Kishimoto T (2010) IL-6: regulator of Treg/Th17 balance. *European Journal of Immunology* 40: 1830–1835.
- Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten L, Jacobs C, van Loenhout J, Xavier RJ, Aaby P, van der Meer JWM, *et al* (2013) Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. *Journal of Innate Immunity* 6: 152–158.
- Kleinnijenhuis J, Quintin J, Preijers F, Joosten L, Jacobs C, Xavier RJ, van der Meer JWM, van Crevel R, Netea MG (2014) BCG-induced trained immunity in NK cells: role for non-specific protection to infection. *Clinical Immunology* 155: 213–219.
- Knockaert D, Vanneste LJ, Bobbaers HJ (1993) Fever of Unknown Origin in Elderly Patients. *Journal of the American Geriatrics Society* 41: 1187–1192.
- Koster MJ, Matteson EL, Warrington KJ (2018) Large-vessel giant cell arteritis: diagnosis, monitoring and management. *Rheumatology (Oxford)* 57: ii32–ii42.
- Koster MJ, Warrington KJ (2017) Giant cell arteritis: Pathogenic mechanisms and new potential therapeutic targets. *BMC Rheumatology* 28: 1–2.

- Lachmandas E, Boutens L, Ratter JM, Hijmans A, Hooiveld GJ, Joosten L, Rodenburg RJ, Fransen J, Houtkooper R, van Crevel R, Netea MG, Stienstra R (2016) Microbial stimulation of different Toll-like receptor signalling pathways induces diverse metabolic programmes in human monocytes. *Nature Microbiology*, 2: 16246.
- Liozon E, Dalmay F, Lalloué F, Gondran G, Bezanahary H, Fauchais AL, Ly KH (2016) Risk Factors for Permanent Visual Loss in Biopsy-proven Giant Cell Arteritis: A Study of 339 Patients. *The Journal of Rheumatology* 43: 1393–1399.
- Liozon E, Herrmann F, Ly KH, Robert PY, Loustaud V, Soria P, Vidal E (2001) Risk factors for visual loss in giant cell (temporal) arteritis: a prospective study of 174 patients. *The American journal of medicine* 111: 211–217.
- Ma-Krupa W, Jeon M, Spoerl S, Tedder TF, Goronzy JJ, Weyand C (2004) Activation of Arterial Wall Dendritic Cells and Breakdown of Self-tolerance in Giant Cell Arteritis. *The Journal of Experimental Medicine* 199: 173–183.
- Maddur MS, Miossec P, Kaveri S, Bayry J (2012) Th17 Cells: Biology, Pathogenesis of Autoimmune and Inflammatory Diseases, and Therapeutic Strategies. *The American Journal of Pathology* 181: 8–18.
- Mahr AD, Jover JA, Spiera R, Hernández-García C, Fernández-Gutiérrez B, LaValley MP, Merkel PA (2007) Adjunctive methotrexate for treatment of giant cell arteritis: an individual patient data meta-analysis. *Arthritis and Rheumatism* 56: 2789–2797.
- Martínez-Rodríguez I, Jimenez-Alonso M, Quirce R, Jiménez-Bonilla J, Martínez-Amador N, De Arcocha-Torres M, Loricera J, Blanco R, González-Gay MA, Banzo I (2017) 18F-FDG PET/CT in the follow-up of large-vessel vasculitis: a study of 37 consecutive patients. *Seminars in Arthritis and Rheumatism* 47: 530–537.
- Matteson EL, Buttgereit F, Dejaco C, Dasgupta B (2016) Glucocorticoids for Management of Polymyalgia Rheumatica and Giant Cell Arteritis. *Rheumatic diseases clinics of North America* 42: 75–90
- Mekinian A, Biard L, Dagna L, Novikov P, Salvarani C, Espita O, Sciascia S, Michaud M, Lambert M, Hernández-Rodríguez J, *et al* (2021) Efficacy and safety of TNF-α antagonists and tocilizumab in Takayasu arteritis: Multicenter retrospective study of 209 patients. *Rheumatology (Oxford)* 61: 1376–1384.
- Messemaker TC, Mikkers HMM, Huizinga TW, Toes R, van der Helm-van Mil AH, Kurreeman F (2017) Inflammatory genes TNFα and IL6 display no signs of increased H3K4me3 in circulating monocytes from untreated rheumatoid arthritis patients. *Genes and Immunity* 18: 191–196.
- Michailidou D, Duvvuri B, Kuley R, Cuthbertson D, Grayson PC, Khalidi NA, Koening CL,

Langford CA, McAlear CA, Moreland LW, *et al* (2022) Neutrophil activation in patients with anti-neutrophil cytoplasmic autoantibody-associated vasculitis and large-vessel vasculitis. *Arthritis Research & Therapy* 24: 160-NA.

- Mitroulis I, Ruppova K, Wang B, Chen LS, Grzybek M, Grinenko T, Eugster A, Troullinaki M, Palladini A, Kourtzelis I, *et al* (2018) Modulation of Myelopoiesis Progenitors Is an Integral Component of Trained Immunity. *Cell* 172: 147–161.
- Miyabe C, Miyabe Y, Strle K, Kim ND, Stone JH, Luster AD, Unizony S (2016) An expanded population of pathogenic regulatory T cells in giant cell arteritis is abrogated by IL-6 blockade therapy. *Annals of the Rheumatic Disease*s 76: 898–905.
- Molteni R, Biavasco R, Stefanoni D, Nemkov T, Netea MG, Domínguez-Andrés J, Arts RJ, Merelli I, Mazza D, Zambrano S, *et al* (2021) Oncogene-induced maladaptive activation of trained immunity in the pathogenesis and treatment of Erdheim-Chester disease. *Blood* 138: 1554-1569.
- Monti S, Milanesi A, Klersy C, Tomelleri A, Dagna L, Campochiaro C, Farina N, Muratore F, Galli E, Marvisi C, *et al* (2023) Age at diagnosis influences the clinical phenotype, treatment strategies and outcomes in patients with giant cell arteritis: results from the observational GCAGE study on a large cohort of 1004 patients. *Annals of the Rheumatic Diseases* 82: 1098–1106
- Moreel L, Coudyzer W, Boeckxstaens L, Betrains A, Molenberghs G, Vanderschueren S, Claus E, Van Laere K, Blockmans D (2023) Association Between Vascular 18F-Fluorodeoxyglucose Uptake at Diagnosis and Change in Aortic Dimensions in Giant Cell Arteritis: A Cohort Study. *Annals of Internal Medicine* 176: 1321–1329
- Mulder WJ, Ochando J, Joosten L, Fayad ZA, Netea MG (2019) Therapeutic targeting of trained immunity. *Nature Reviews Drug Discovery* 18: 553–566.
- Muratore F, Kermani TA, Crowson CS, Green AB, Salvarani C, Matteson EL, Warrington KJ (2015) Large-vessel giant cell arteritis: A cohort study. *Rheumatology (Oxford)* 54: 463–470.
- Myklebust JT, Gran JT (1995) A prospective study of 287 patients with polymyalgia rheumatica and temporal arteritis: clinical and laboratory manifestations at onset of disease and at the time of diagnosis. *Rheumatology (Oxford)* 35: 1161–1168.
- Nadkarni S, Dalli J, Hollywood J, Mason JC, Dasgupta B, Perretti M (2013) Investigational Analysis Reveals a Potential Role for Neutrophils in Giant-Cell Arteritis Disease Progression. *Circulation research* 114: 242–248.
- Netea MG, Quintin J, van der Meer JWM (2011) Trained Immunity: A Memory for Innate Host Defense. *Cell Host & Microbe* 9: 355–361.
- Netea MG, Dominguez-Andres J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, Joosten

L, van der Meer JWM, Mhlanga MM, Mulder WJ, *et al* (2020) Defining trained immunity and its role in health and disease. *Nature Reviews Immunology* 20: 375–388.

- Netea MG, Joosten L, Latz E, Mills KHG, Natoli G, Stunnenberg HG, O'Neill LA, Xavier RJ (2016) Trained immunity: A program of innate immune memory in health and disease. *Science* 352: 427–438.
- Nielsen BD, Gormsen LC, Hansen IT, Keller KK, Therkildsen P, Hauge EM (2018) Three days of high-dose glucocorticoid treatment attenuates large-vessel 18F-FDG uptake in large-vessel giant cell arteritis but with a limited impact on diagnostic accuracy. *European Journal of Nuclear Medicine and Molecular Imaging* 45: 1119-1128.
- Nizzoli G, Krietsch J, Weick A, Steinfelder S, Facciotti F, Gruarin P, Bianco A, Steckel B, Moro M, Crosti MC, *et al* (2013) Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. *Blood* 122: 932–942.
- O'Neill LAJ, Pierce E (2015) Immunometabolism governs dendritic cell and macrophage function. *The Journal of Experimental Medicine* 213: 15–23.
- Owen AM, Fults JB, Patil NK, Hernandez A, Bohannon JK (2021) TLR Agonists as Mediators of Trained Immunity: Mechanistic Insight and Immunotherapeutic Potential to Combat Infection. *Frontiers in Immunology* 11: 622614.
- Palamidas DA, Argyropoulou OD, Georgantzoglou N, Karatza E, Xingi E, Kapsogeorgou EK, Anagnostopoulos C, Lazaris AC, Ritis K, Goules AV, *et al* (2021) Neutrophil extracellular traps in giant cell arteritis biopsies: presentation, localization and co-expression with inflammatory cytokines. *Rheumatology (Oxford*) 61: 1639–1644.
- Pearce EL, Pearce EJ (2013) Metabolic Pathways in Immune Cell Activation and Quiescence. *Immunity* 38: 633–643.
- Ponte C, Grayson PC, Robson JC, Suppiah R, Gribbons KB, Judge A, Craven A, Khalid S, Hutchings A, Watts RA, et al 2022 American College of Rheumatology/EULAR Classification Criteria for Giant Cell Arteritis. *Ann Rheum Dis* 81: 1647–1653.
- Prieto-Peña D, Castañeda S, Martínez-Rodríguez I, Atienza-Mateo B, Blanco R, González-Gay MA (2021) Imaging Tests in the Early Diagnosis of Giant Cell Arteritis. *Journal of Clinical Medicine* 10: 3704.
- Prieto-Peña D, Martínez-Rodríguez I, Loricera J, Banzo I, Calderón-Goercke M, Calvo-Río V, González-Vela C, Corrales A, Castañeda S, Blanco R, *et* (2019) Predictors of positive 18 F-FDG PET/CT-scan for large vessel vasculitis in patients with persistent polymyalgia rheumatica *Seminars in Arthritis and Rheumatism* 48: 720 – 727.
- Quinn SM, Cunningham KT, Raverdeau M, Walsh RJ, Curham L, Malara A, Mills K (2019) Anti-inflammatory Trained Immunity Mediated by Helminth Products Attenuates the

Induction of T Cell-Mediated Autoimmune Disease. *Frontiers in Immunology* 10: 1109.

- Quintin J, Saed S, Martens JHA, Giamarellos-Bourboulis EJ, Ifrim DC, Logie C, Jacobs L, Jansen T, Kullberg BJ, Wijmenga C, *et al* (2012) Candida albicans Infection Affords Protection against Reinfection via Functional Reprogramming of Monocytes. *Cell Host & Microbe* 12: 223–232.
- Restuccia G, Boiardi L, Cavazza A, Catanoso M, Macchioni P, Muratore F, Cimino L, Aldigeri R, Crescentini F, Pipitone N, *et al* (2016) Flares in Biopsy-Proven Giant Cell Arteritis in Northern Italy: Characteristics and Predictors in a Long-Term Follow-Up Study. *Medicin*e 95 e3524-NA.
- Restuccia G, Boiardi L, Cavazza A, Catanoso M, Macchioni P, Muratore F, Soriano A, Cimino L, Aldigeri R, Crescentini F *et al* (2016) Long-term remission in biopsy proven giant cell arteritis: A retrospective cohort study. *Journal of autoimmunity* 77: 39–44.
- Rittner KL, Kaiser M, Brack A, Szweda LI, Goronzy JJ, Weyand C (1999) Tissue-Destructive Macrophages in Giant Cell Arteritis. *Circulation research* 84: 1050–1058.
- Roche NE, Fulbright JW, Wagner AD, Hunder GG, Goronzy JJ, Weyand C (1993) Correlation of interleukin-6 production and disease activity in polymyalgia rheumatica and giant cell arteritis. *Arthritis and Rheumatism* 36: 1286–1294.
- Rodriguez-Pla A, Bosch-Gil JA, Rossello-Urgell J, Huguet-Redecilla P, Stone JH, Vilardell-Tarrés M (2005) Metalloproteinase-2 and -9 in Giant Cell Arteritis Involvement in Vascular Remodeling. *Circulation* 112: 264–269.
- Rüegg Stefan, Engelter S, Jeanneret C, Hetzel A, Probst A, Steck AJ, Lyrer P (2003) Bilateral vertebral artery occlusion resulting from giant cell arteritis: report of 3 cases and review of the literature. *Medicine* 82: 1–12.
- Rutz S, Eidenschenk C, Ouyang W (2013) IL-22, not simply a Th17 cytokine. *Immunological reviews* 252: 116–132.
- Ryu S, Kodama S, Ryu K, Schoenfeld DA, Faustman DL (2001) Reversal of established autoimmune diabetes by restoration of endogenous β cell function. *The Journal of Clinical Investigation* 108: 63–72.
- Salvarani C, Crowson CS, O'Fallon WM, Hunder GG, Gabriel SE (2004) Reappraisal of the epidemiology of giant cell arteritis in Olmsted County, Minnesota, over a fifty-year period. *Arthritis and Rheumatism* 51: 264–268.
- Salvarani C, Cimino L, Macchioni P, ConsonniD, Cantini F, Bajocchi G, Pipitone N, Catanoso MG, Boiardi L (2005) Risk factors for visual loss in an Italian population-based cohort of patients with giant cell arteritis. *Arthritis and Rheumatism* 53: 293–297.
- Salvarani C, Pipitone N, Versari A, Hunder GG (2012) Clinical features of polymyalgia

rheumatica and giant cell arteritis. *Nature Reviews Rheumatology* 8: 509–521.

- Samson M, Audia S, Fraszczak J, Trad M, Ornetti P, Lakomy D, Ciudad M, Leguy V, Berthier S, Vinit J, *et al* (2012) Th1 and Th17 lymphocytes expressing CD161 are implicated in giant cell arteritis and polymyalgia rheumatica pathogenesis. *Arthritis and Rheumatism* 64: 3788–3798.
- Saravia J, Chapman NM, Chi H (2019) Helper T cell differentiation. *Cellular & Molecular Immunology* 16: 634–643.
- Schmidt WA, Kraft HE, Vorpahl K, Völker L, Gromnica-Ihle EJ (1997) Color Duplex Ultrasonography in the Diagnosis of Temporal Arteritis. *The New England journal of medicine* 337: 1336–1342.
- Schmidt WA (2013) Role of ultrasound in the understanding and management of vasculitis. *Therapeutic Advances in Musculoskeletal Disease* 6: 39–47.
- Schvartzman JM, Thompson CB, Finley LWS (2018) Metabolic regulation of chromatin modifications and gene expression. *The Journal of Cell Biology* 217: 2247–2259.
- Sebastian A, Tomelleri A, Dasgupta B. (2021) Current and innovative therapeutic strategies for the treatment of giant cell arteritis. *Expert Opinion on Orphan Drugs* 9: 161–173
- Shehadeh I, Vardi P, Calcinaro F, Lafferty K (1994) Effect of adjuvant therapy on development of diabetes in mouse and man. *Lancet* 343: 706–707.
- De Silva M, Hazleman BL (1986) Azathioprine in giant cell arteritis/polymyalgia rheumatica: a double-blind study. Annals of the rheumatic diseases 45: 136–138.
- Slart RHJA, Glaudemans AWJM, Chareonthaitawee P, Treglia G, Besson FL, Bley TA, Blockmans D, Boellaard R, Bucerius J, Carril JM, *et al* (2018) FDG-PET/CT imaging in large vessel vasculitis and polymyalgia rheumatica: joint procedural recommendation of the EANM, SNMMI, and the PET Interest Group (PIG), and endorsed by the ASNC. *European Journal of Nuclear Medicine and Molecular Imaging* 45: 1250 – 1269.
- Song GG, Choia SJ, Ji JD, Lee YH (2012) Toll-like receptor polymorphisms and vasculitis susceptibility: meta-analysis and systematic review. *Molecular Biology Reports* 40: 1315–1323.
- Soriano A, Muratore F, Pipitone N, Boiardi L, Cimino L, Salvarani C (2017) Visual loss and other cranial ischaemic complications in giant cell arteritis. *Nature Reviews Rheumatology* 13: 476–484.
- Soto JA, Galvez NM, Andrade CA, Ramírez MA, Riedel CA, Kalergis AM, Bueno SM (2022) BCG vaccination induces cross-protective immunity against pathogenic microorganisms. *Trends in Immunology* 43: 322–335.
- Stone JH, Han J, Aringer M, Blockmans D, Brouwer E, Cid MC, Dasgupta B, Rech J,

Salvarani C, Spiera R, *et al* (2021) Long-term effect of tocilizumab in patients with giant cell arteritis: open-label extension phase of the Giant Cell Arteritis Actemra (GiACTA) trial. *The Lancet Rheumatology* 3: e328–e336.

- Stone JH, Tuckwell K, Dimonaco S, Klearman M, Aringer M, Blockmans D, Brouwer E, Cid MC, Dasgupta B, Rech J, *et al* (2017) Trial of tocilizumab in giant-cell arteritis. *New England Journal of Medicine* 377: 317 – 328.
- Sun JC, Beilke JN, Lanier LL (2010) Immune memory redefined: characterizing the longevity of natural killer cells. *Immunological Reviews* 236: 83–94.
- Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, Frezza C, Bernard NJ, Kelly B, Foley N, *et al* (2013) Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* 496: 238–242.
- Terrier B, Guillaume G, Chaara W, Allenbach Y, Rosenzwajg M, Costedoat-Chalumeau N, Fouret P, Musset L, Benveniste O, Six A, *et al* (2011) Interleukin-21 modulates Th1 and Th17 responses in giant cell arteritis. *Arthritis and Rheumatism* 64: 2001–2011.
- Tomelleri A, Campochiaro C, Farina N, Mariotti L, Baldissera E, Grayson PC, Matucci-Cerinic M, Dagna L (2023). Effectiveness of a two-year tapered course of tocilizumab in patients with giant cell arteritis: A single-centre prospective study. *Seminars in Arthritis and Rheumatism* 59:152174.
- Tomelleri A, Campochiaro C, Sartorelli S, Farina N, Baldissera E, Dagna L (2021) Presenting features and outcomes of cranial-limited and large-vessel giant cell arteritis: a retrospective cohort study. *Scandinavian journal of rheumatology* 51: 1–8.
- Tomelleri A, Coath F, Sebastian A, Prieto-Pena D, Kayani A, Mo J, Dasgupta B (2021) Long-Term Efficacy and Safety of Leflunomide in Large-Vessel Giant Cell Arteritis: A Single-Center, 10-Year Experience. J*ournal of Clinical Rheumatology* 28: e297–e300.
- Tomelleri A, van der Geest KSM, Khurshid MA, Sebastian A, Coath F, Robbins D, Pierscionek B, Dejaco C, Matteson EL, van Sleen Y *et al* (2023) Disease stratification in GCA and PMR: state of the art and future perspectives. *Nature reviews Rheumatology* 19: 446–459
- Tomelleri A, van der Geest KSM, Sebastian A, van Sleen Y, Schmidt WA, Dejaco C, Dasgupta B (2021) Disease stratification in giant cell arteritis to reduce relapses and prevent long-term vascular damage. *The Lancet Rheumatology* 3: e886–e895
- Torchinsky MB, Blander JM (2010) T helper 17 cells: discovery, function, and physiological trigger. *Cellular and Molecular Life Sciences* 67: 1407–1421.
- Vodopivec I, Rizzo JF (2018) Ophthalmic manifestations of giant cell arteritis. *Rheumatology (United Kingdom)* 57: ii63–ii72.
- Wadström K, Jacobsson LTH, Mohammad A, Warrington KJ, Matteson EL, Jakobsson ME,

Turesson C (2022) Analyses of plasma inflammatory proteins reveal biomarkers predictive of subsequent development of giant cell arteritis: a prospective study. *Rheumatology (Oxford)* 62: 2304–2311.

- Wagner AD, Goronzy JJ, Weyand C (1994) Functional profile of tissue-infiltrating and circulating CD68+ cells in giant cell arteritis. Evidence for two components of the disease. *The Journal of Clinical Investigation* 94: 1134–1140.
- Wang L, Ai Z, Khoyratty TE, Zec K, Eames HL, van Grinsven E, Hudak A, Morris SJ, Ahern D, Monaco C, *et al* (2020) ROS-producing immature neutrophils in giant cell arteritis are linked to vascular pathologies. *JCI insight*, 5: e139163.
- Watanabe R, Berry GJ, Liang D, Goronzy JJ, Weyand C (2020) Cellular Signaling Pathways in Medium and Large Vessel Vasculitis. *Frontiers in immunology* 11: 587089-NA.
- Watanabe R, Maeda T, Zhang H, Berry GJ, Zeisbrich M, Brockett R, Greenstein AE, Tian L, Goronzy JJ, Weyand C (2018) MMP (Matrix Metalloprotease)-9–Producing Monocytes Enable T Cells to Invade the Vessel Wall and Cause Vasculitis. *Circulation Research* 123: 700–715.
- Watts RA, Hatemi G, Burns JC, Mohammad AJ (2022) Global epidemiology of vasculitis *Nature reviews Rheumatology* 18: 22 – 34
- Weyand C, Goronzy JJ (2013) Immune mechanisms in medium and large-vessel vasculitis. *Nature Reviews Rheumatology* 9: 731–740.
- Weyand C, Liao YJ, Goronzy JJ (2012) The immunopathology of giant cell arteritis: Diagnostic and therapeutic implications. *Journal of Neuro-Ophthalmology* 32: 259–265.
- Weyand C, Goronzy JJ (2014) Giant-Cell Arteritis and Polymyalgia Rheumatica. *New England Journal of Medicine* 371: 50–57
- Widico CR, Newman DH (2005) Evidence-based emergency medicine/rational clinical examination abstract. Does this patient have temporal arteritis? *Annals of Emergency Medicine* 45: 85–7
- Wiszniewska M, Devuyst G, Bogousslavsky J (2007) Giant Cell Arteritis as a Cause of First-Ever Stroke. *Cerebrovascular Diseases* 24: 226–230.

Firma dottorando: