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REVIEW ARTICLE

Gene and miRNA expression in giant cell arteritis—a concise systematic review of significantly modified studies

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Abstract

Giant cell arteritis (GCA) is a systemic vasculitis in individuals older than 50 years, characterized by headaches, visual disturbances, painful scalp, jaw claudication, impairment of limb arteries, and systemic inflammation, among other symptoms. GCA diagnosis is confirmed by a positive temporal artery biopsy (TAB) or by imaging modalities. A prominent acute phase response with inflammation is the hallmark of the disease, predominantly targeting large- and medium-sized arteries leading to stenosis or occlusion of arterial lumen. To date, there are no reliable tissue markers specific for GCA. Scarce reports have indicated the importance of epigenetics in GCA. The current systematic review reports significantly changed candidate biomarkers in TABs of GCA patients compared to non-GCA patients using qPCR.

Keywords Giant cell arteritis · Temporal artery biopsy · Vasculitis · miRNA · mRNA expression

Introduction

Giant cell arteritis (GCA) or temporal arteritis is a granulomatous vasculitis affecting large- and medium-sized arteries, predominantly the aorta and its major branches [1]. GCA is the most common systemic vasculitis, occurring in individuals older than 50 years, reaching a peak between 70 and 80 years. GCA classically presents with headache, scalp tenderness, jaw claudication, and visual disturbances accompanied by an intense acute phase response [2]. Patients with GCA mostly exhibit elevated serum erythrocyte sedimentation rate, C-reactive protein, and serum amyloid A. However, no GCA-specific cell or tissue markers have been identified to date. It is projected that by 2050, more than 3 million people will have been diagnosed with GCA in Europe, North America, and Oceania, with 1/6th visually impaired [3].

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Rheumatology in Slovenia: Clinical practice and translational research

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The diagnosis of GCA is confirmed either histologically or by imaging techniques. Temporal artery biopsy (TAB) shows segmental pan-arteritis with non-necrotizing granulomatous inflammation. The arterial wall is infiltrated by T lymphocytes, macrophages, and multinucleated giant cells [4]. Knowledge of GCA pathogenesis and mechanisms has recently progressed considerably. Guevara et al. emphasized three phases of the immune system in GCA patients. The first phase is the activation of adventitial dendritic cells via toll-like receptors, production of cytokines/chemokines that are responsible for the second stage, e.g., recruitment of $CD4^+$ T cells and their subsequent polarization towards Th1 and Th17. Treg cells were reported to be decreased in the blood, suggesting a Th17/Treg imbalance in GCA. Inhibition of IL-6 with tocilizumab corrects the imbalance by decreasing Th17 and increasing Treg cells, as opposed to corticosteroids, which strongly inhibit Th17 cell responses, without affecting Treg cells. Once Th1/Th17 cells infiltrate the arterial wall, they produce large amounts of IFN-γ and IL-17. In the third phase, IFN-γ, via chemokines (such as CCL2, CXCL9, CXCL10, CXCL11) produced by vascular smooth muscle cells (VSMC), recruits monocytes, which merge into multinucleated giant cells, representing the hallmark of GCA. Stimulated monocytes differentiate into macrophages that produce IL-6, IL-1β, and TNF-α, amplifying local inflammation. Activated macrophages and VSMC produce nitric oxide that triggers destruction of cellular matrix proteins via matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9. Lastly, stimulated macrophages and injured VSMCs produce growth factors, leading to intimal hyperplasia [5].

Zhang et al. also reported that transcriptome analysis of GCA-affected temporal arteries exhibited low expression of coinhibitory ligand programmed death ligand-1 on dendritic cells ($PD\text{-}L1^{lo}$), concurrent with enrichment of programmed death-1 (PD-1) receptor on T cells ($PD\text{-}1^{hi}$) [6]. A breakdown of tissue-protective PD-1/PD-L1 checkpoint leads to dysrupted vascular immunoprivilege. Consequently, $PD\text{-}1^+$ $CD4^+$ T cells enter the otherwise immunoprivileged arterial walls and secrete IFN-γ, IL-17, and IL-21, which drive inflammation-associated angiogenesis and facilitate intimal hyperplasia [7, 8].

B cells have also been found in TABs [9] with recent evidence indicating a disturbed distribution of B cells in GCA, suggesting their potential role in the pathogenesis of the disease [10]. Tertiary lymphoid organs are found in up to 50% of GCA-positive arteries and could represent sites where immune responses against viral antigens are organized [11].

Although obtaining TABs is a relatively small and safe procedure, this is an invasive technique and could be falsely negative. Just recently, evidence-based recommendations and guidelines have been developed and published for the use of imaging modalities, such as ultrasound, in primary large vessel vasculitis, including GCA, advocating their use in daily clinical practice [12].

Genetic, as well as environmental factors are known to be involved in GCA pathogenesis [4]. Until recently, the most important genetic association with GCA has been reported for the human leukocyte antigen (HLA) region, mainly the alleles of HLA-DRB1*04 (DRB1*0401 and DRB1*0404), IL-10 and vascular endothelial growth factor [13]. A recent genome-wide association study confirmed the association of HLA class II with GCA and identified several single-nucleotide polymorphisms (SNPs) in the gene encoding plasminogen [14]. Interestingly, an association of GCA with SNPs in the gene coding for tyrosine phosphatase non-receptor type 22 (PTPN22), involved in T and B cell receptor signaling pathways, was observed in four analyzed populations of GCA patients [15].

At the interface of genetic and environmental factors, there is the growing field of epigenetics, which influences gene expression without involving changes in the underlying DNA sequence. Bird A. refined the definition of epigenetics as "the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states" [16]. Epigenetic events include DNA methylation, histone modifications, nucleosome remodeling [17], and regulation by non-coding RNAs [18]. DNA methylation is an epigenetic mechanism involving the addition of a methyl group to cytosines, primarily within CpG dinucleotides. In general, DNA methylation regulates gene expression by recruiting proteins involved in gene repression or by inhibiting the binding of transcription factors to DNA. This reaction is catalyzed by a family of DNA methyltransferases (DNMTs). DNMT3a and DNMT3b can establish new methylation pattern to unmodified DNA and are therefore known as de novo DNMTs, while DNMT1 is primarily responsible for maintaining DNA methylation pattern from cell to cell [19].

Alterations in cellular epigenetic states are a hallmark of human disease, including cancer and autoimmune diseases [20, 21]. A number of enzymes exists that act as writers, erasers, and readers for DNA methylation and histone marks as reviewed in [22]. This makes chromatin marks reversible and targetable by epigenetic drugs. In 2006, the first epigenetic drugs (decitabine and vorinostat) were approved for the treatment of cancer [22]. Besides, a new class of small molecule inhibitors was developed, which inhibits binding of BET family of histone readers to acetylated histones [22]. These inhibitors have demonstrated potent anti-inflammatory activities [23, 24].

Response to environmental factors is often mediated by non-coding RNAs, in particular, by microRNAs (miRNAs) [25]. miRNAs are short non-coding RNAs with 18–25 nucleotides in length that can regulate gene expression post-transcriptionally by inhibiting translation or inducing mRNA

degradation and can contribute to different physiological and pathological processes. miRNAs target the 3′ untranslated region of their target mRNA molecule and control their stability and protein interactions. A single miRNA can regulate the expression of various different target mRNAs [26]. Deregulated miRNAs have been reported in GCA as well as in other systemic vasculitides, such as in antineutrophil antibody-associated vasculitis, Behcet's disease, Kawasaki disease, and IgA vasculitis [18].

Recently, our group reviewed significantly modified protein levels of serological biomarkers in GCA [27], exposing IL-6 as the most highly elevated analyte in the circulation of GCA patients, as well as confirmed this experimentally, along with serum amyloid A and IL-23 [28].

In the present study, we targeted studies showing altered gene expression, DNA methylation, and miRNA expression in patients with GCA, with the aims to identify significantly changed tissue biomarkers in GCA, to investigate most promising markers using analyte frequency and to elucidate their effects on disease pathogenesis.

Selected studies and inclusion criteria

We performed a systematic review of publications reporting on mRNA expression and epigenetics associated with GCA and ultimately, produced a priority listing of most promising tissue biomarkers involved in GCA. We searched for GCA biomarkers in the electronic databases PubMed and Google Scholar using the following search terms: [('Giant cell arteritis', OR 'GCA' OR 'Vasculitis OR 'Large vessel vasculitis') AND ('Biomarkers' OR 'qPCR' OR 'mRNA' OR 'miRNA' OR 'epigenetics' OR 'DNA methylation' OR 'histone modification')] and cross-checked them. The articles were reviewed in a two-stage process (Fig. 1). In the first stage of the review, abstracts of all identified articles were screened. Editorials, case reports, and notes were excluded. In the second stage of the review process, full texts of the remaining studies were evaluated. A checklist of specified inclusion criteria was used to ensure uniformity in the assessment of the identified manuscripts. The final articles that were selected ($n = 14$ for mRNA, $n = 1$ for miRNA, and $n = 1$ for DNA methylation) all fulfilled the following eligibility criteria: written in English, compared tissues from patients with GCA with a control group or compared tissues from GCA patients before/after treatment, were informative about the type of method used (qPCR studies) and provided details about the biomarkers identified. Articles that did not meet two or more of these criteria were excluded. Two independent reviewers extracted data from the publications. Analyte frequency analyses were performed based on 12 reports comparing mRNA levels in cross-sectional studies of TABs from GCA patients to non-GCA controls, using qPCR.

*Corbera-Belalta et al. and Visvanathan et al. were excluded due to not comparing TABs from GCA/non-GCA patients, but rather using 5-day cultured biopsies of GCA/non-GCA patients and longitudinal comparison of TABs before/after treatment, respectively.

Overview of the results

In total, we found 14 reports using qPCR with designated mRNA changed levels of analytes in TABs of patients with GCA (Table 1), from which we then excluded the longitudinal and biopsy-culturing reports. From the remaining 12 mRNA studies and 2 reports on DNA methylation and miRNAs, we extracted data for 52 unique analytes. An overview is provided of the significantly changed tissue biomarkers from TABs of GCA patients (Fig. 2), as well as the analyte frequency calculated for the mRNA studies (Fig. 3).

Gene expression

A variety of significantly modified candidate tissue biomarkers important for GCA has been reported in the literature. Significantly changed mRNA expression levels in TABs of GCA patients vs. non-GCA controls were observed for cytokines and chemokines (IL-1β, IL-6, IL-17, IFN-γ, IL-8, CXCL13), enzymes involved in tissue degradation (e.g., MMP-2, MMP-9), their tissue inhibitors (e.g., TIMP-1, TIMP-2), and molecules involved in regulation of lymphocytes, such as a proliferation-inducing ligand (APRIL) and B cell activating factor (BAFF) (Fig. 2 and Table 1).

Cytokines and chemokines

In the pathogenesis of GCA, activated and mature dendritic cells produce chemokines and cytokines, such as CCL18, CCL19, CCL20, CCL20, and IL-18, which recruit $CD4^+$ T cells to the arterial wall. The pro-inflammatory cytokines IL-1β, IL-6, and IL-23 shift T cell differentiation towards the Th17 lineage, whereas IL-12 and IL-18 are responsible for differentiation of Th1 cell lineage. Th17 and Th1 cells produce IL-17 and IFN-γ, respectively. Production of several other chemokines (CCL2, CXCL9, CXCL10, CXCL11) leads to $CD8^+$ T cells and monocyte recruitment [4]. Cytokines and chemokines are extensively produced at the site of inflammation and it is therefore not surprising that most studies identified their deregulated mRNA expression in TABs from GCA patients.

As early as 1994, Weyand et al. reported the presence of IL-1β, IL-2, IL-6, IFN-γ, and TNF-α mRNA in significantly higher percentage of TABs from GCA patients as compared to non-GCA controls. Similar percentages were also shown

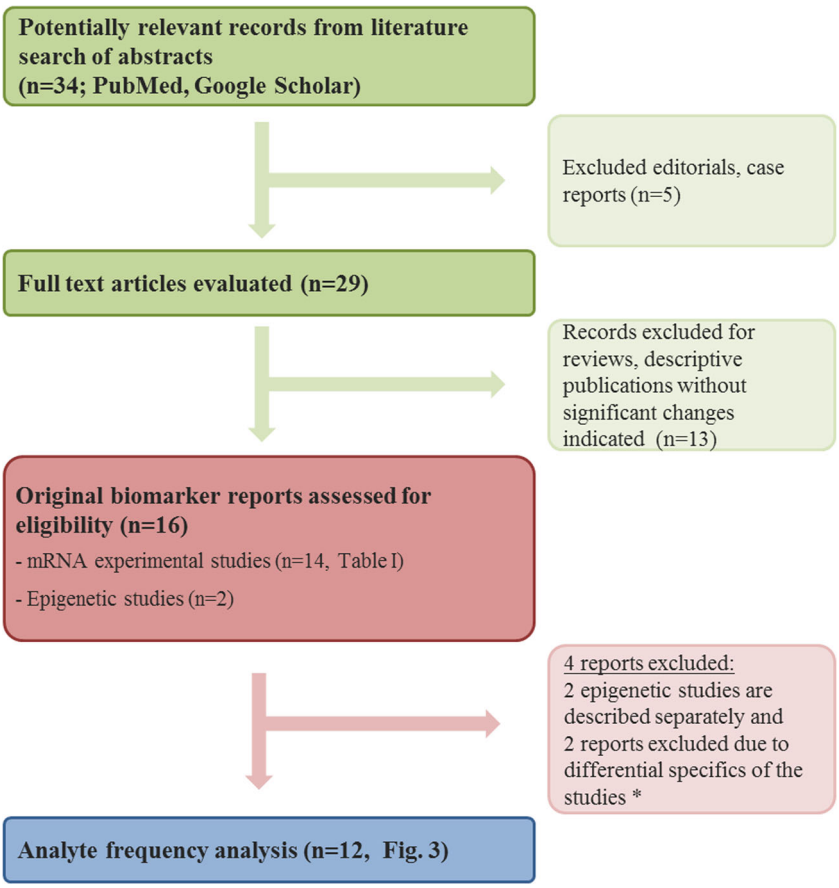

Fig. 1 Selection process for studies analyzing mRNA frequency in GCA patients

for patients with polymyalgia rheumatica, except for IFN-γ, suggesting that this Th1-related cytokine might be involved in the progression of overt vasculitis [29].

Deng et al. reported in 2010 on significantly higher mRNA expression of IL-1β, IL-6, IL-12p35, IL-12p40, IL-23p19, IL-17, IFN-γ, and Foxp3 in TABs of untreated GCA patients compared to controls (suspected GCA with negative TAB). Interestingly, in TABs of GCA patients obtained 3–9 months after glucocorticoid therapy, a marked decrease in the expression of all above mentioned parameters, except for IFN-γ and Foxp3, was observed as compared to the TABs of untreated patients. Moreover, the frequencies of circulating Th17 and Th1 cells were significantly expanded in the circulation of untreated GCA patients with frequencies of only Th17 cells decreasing after therapy. The study therefore concluded that glucocorticoid therapy selectively suppresses Th17 responses in circulation as well as in vasculitis lesions. Th1 responses are spared, indicating that glucocorticoids effectively treat the acute manifestations of GCA, associated with excessive production of Th17 promoting cytokines (e.g., IL-17) while fail to abrogate vasculitis, mediated by Th1 cells and corresponding cytokines [30]. However, this was not confirmed in a study performed by Visvanathan et al., showing a nonsignificant decrease in IL-1, IL-6, IL-23, or IL-17 when comparing matched paired TABs from untreated and treated patients, while confirming persistently elevated IFN-γ expression. The limitation of this study was the low number of included TABs from GCA patients ($n = 4$) [42]. The same authors also showed higher expression of TNF-α in patients with active disease, which was previously also shown for IL-1β and IL-6 [31]. Just recently, Manku et al. reported that IL-6 may contribute to the accumulation of $CD4^+$ T cells in GCA by supporting their proliferation and survival within the arterial wall, through mechanisms that are independent of effects on local Treg expansion. This provides insight both, into how IL-6 contributes to disease pathology, as well as to the importance of tocilizumab therapy in GCA patients [43].

The levels of another cytokine, IL-9, are not influenced by glucocorticoid therapy. Higher expression of IL-9 was accompanied by overexpression of its receptor on neutrophils, thus emphasizing their role in GCA pathogenesis. Moreover, IL-9 could play an important role in GCA chronicity and tissue damage in glucocorticoid-resistant GCA patients [32].

A number of studies later confirmed higher expression of Th17-associated cytokines, e.g., IL-1β, IL-6, and IL-17 [32–34, 36, 37] as well as IFN-γ, produced mainly by Th1 cells [33, 35, 37].

Increased expression of IL-22 and IL-22R1 in inflamed TABs of GCA patients (as opposed to TAB negative controls)

Table 1 Studies investigating mRNA gene expression in TABs of GCA patients

Reference	# GCA/control group	Significantly changed analytes	Determined not significant
Weyand et al. [29]	15/10	IL-1β, IL-6, TGF-β, TCR Cα, IL-2, IFN-γ	TNF-α, GM-CSF, IL-4, IL-5
Deng et al. [30]	8/8	IL-17, IFN-γ, Foxp3, IL-1β, IL-6, IL-12p35, IL-12p40, IL-23p19	–
	8/8[b]	IL17, IL1 β, IL-6, IL-23	IFN-γ, IL-12
Hernández-Rodríguez et al. [31]	36/11	IL-1β, TNF-α	IL-6
Ciccia et al. [32]	35/15	IL-8, IL-9, IL-9R, IL-17, TSLP, TGF-β	IL-4
Ciccia et al. [33]	18/15	IL-32, IL-6, TGF-β, IL-1β, TNF-α, IFN-γ, IL-27p28	–
Ciccia et al. [34]	50/30	CXCL13, CXCR5, LT-β, BAFF, APRIL, IL-7, IL-7R, IL-17	CCL21, CCR7
Ciccia et al. [35]	20/15	IFN-γ, IL-33, STAT6	IL-4, IL-5, IL-25
Espígol-Frigolé et al. [36]	57/19	IL-17A	–
Corbera-Bellalta et al. [37]	28/22[a]	IL-1β, IFN-γ, CCL3, CCL4, CCL5, MMP9	IL-6, TNF-α, CCL2, MMP2, IL-8, TIMP-1, TIMP-2,COL I, COL III, PDGF-A, PDGF-B
Lakota et al. [38]	6/6	Ferritin, MMP2, MMP9, TIMP1, TIMP2, MMP2/TIMP2, MMP9/TIMP1, VCAM-1, MARCO, IL-8, IL-12, ApoA1	MMP12, ICAM-1, IL-6, TNF-α
Segarra et al. [39]	35/12	MMP9, MMP14, MMP9/TIMP1, MMP2/TIMP2, TIMP1, TIMP2	MMP2
Rodríguez-Pla et al. [40]	19/13	MMP9, MMP12	MMP2
Lozano et al. [41]	35/19	ET-1, ECE-1,ET_AR, ET_BR	–
Visvanathan et al. [42]	4/4[b]	PDGF-A, TGF-β, ICAM-1	TNF-α, IL-12, MMP9, PDGF-B, IL-1β, IL-6, IL-23, IFN-γ, IL-17

mRNA expression levels as determined by qPCR. The number of study subjects, the analytes found significantly regulated and the ones showing no statistically significant change are reported. The control group was non-GCA, suspected GCA with negative TAB, unless otherwise indicated. Biopsies were analyzed at the time of diagnosis, except in cases below:

[a] Biopsy cultured for 5 days prior to assay

[b] Longitudinal observation before/after treatment in paired TAB

was recently shown by immunocytochemistry and confirmed by real-time PCR on primary cultures obtained from TABs, as well as peripheral blood mononuclear cells. IL-22 protein was significantly increased, also in plasma of TAB positive GCA patients. All this exposes IL-22, as a potential player in GCA pathogenesis [44].

Significantly higher expression of mRNA for IL-32 was observed for biopsy-proven GCA patients ($n = 11$) compared to non-GCA controls ($n = 5$), which was the first study to show IL-32 overexpression in inflamed arteries of GCA patients. The expression of IL-32 was associated with the Th1 inflammatory response and occurred mainly in neovessels of inflamed arteries indicating a role of IL-32 in the organization of the vascular inflammatory response [33]. The same group also observed increased expression of IL-33, a member of IL-1 cytokine family, in TABs of GCA patients as compared to non-GCA controls, decreasing after glucocorticoid therapy. IL-33 positively correlated with the number of neovessels in TABs and the number of inflammatory parameters, suggesting a role for IL-33 in inflammation and angiogenesis [35].

No elevated expression of Th2-related cytokines, such as IL-4, IL-5, IL-25, was observed in TABs of GCA patients compared to controls [29, 32, 35].

Higher expression of chemokines and chemokine receptors, e.g., CCL3, CCL4, CCL5, CXCL13, CXCR5, and IL-8 has also been reported in different studies [34, 37, 38]. Interestingly, Ciccia et al. observed higher expression of CXCL13 and CXCR5, together with IL-7 and IL-17, which are all involved in lymphoneogenesis in TABs from GCA patients. These patients were characterized by artery tertiary lymphoid organ aggregates with a well-defined organization of separated T and B cell-rich areas. The presence of these aggregates correlated with a strong inflammatory response but not with the duration of clinical symptoms and signs specific for GCA [34].

Enzymes involved in tissue degradation

Ischemic manifestations of GCA are related to the narrowing of the artery and are a consequence of the remodeling process taking place in the arterial wall. In the media, IFN-γ-activated

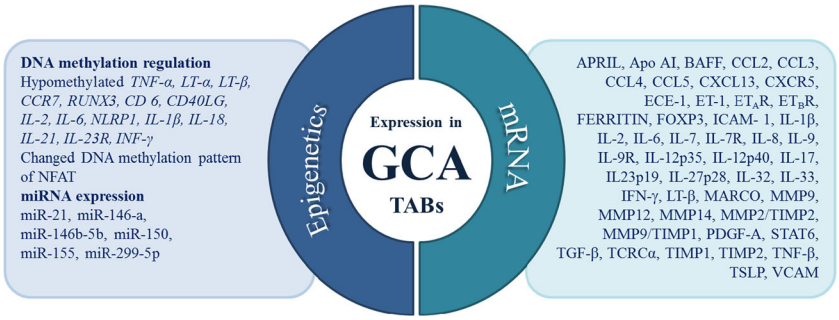

Fig. 2 Significantly modified tissue biomarkers in GCA patients at the mRNA, miRNA, and methylation levels. Legend: biomarkers measured in TABs of GCA as compared to non-GCA patients. Abbreviations: Apo AI, apolipoprotein AI; APRIL, a proliferation-inducing ligand; BAFF, B cell activating factor; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C chemokine receptor; ECE-1, endothelin-converting enzyme-1; ET-1, endothelin-1; ET_AR, endotelin receptor A; ET_BR, endotelin receptor B; FOXP3, forkhead box P3; ICAM, intercellular adhesion molecule-1; IL, interleukin; IFN-γ, interferon γ; LT-β, lymphotoxin-β; MARCO, macrophage receptor with collagenous structure; MMP, matrix metalloproteinase; NFAT, nuclear factor of activated T cells; NLRP3, NOD-like receptor family, pyrin domain containing 3; PDGF, platelet-derived growth factor; RUNX3, runt-related transcription factor 3; STAT6, signal transducer and activator of transcription 6; TCRCα, T cell receptor Cα; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumor necrosis factor-α; TSLP, thymic stromal lymphopoietin; VCAM-1, vascular adhesion molecule-1

macrophages produce mediators, detrimental to the arterial tissue as well as MMP, enzymes with the ability to degrade elastin causing destruction of the media layer and digestion of internal elastic lamina. MMP-2 and MMP-9 both possess gelatinase activity and MMP-9 also plays a role in migration of VSMCs from media to the intima, which leads to intimal hyperplasia [4]. Segarra et al. observed significantly higher expression of MMP-9 and MMP-14 in TABs of GCA patients compared to non-GCA controls, whereas MMP-2 was also expressed in non-inflamed arteries and negatively correlated with MMP-9 expression. Similarly, in GCA TAB samples, tissue inhibitor of MMP-9 (TIMP-1) mRNA was significantly upregulated, whereas TIMP-2 mRNA was significantly decreased compared to controls. Moreover, MMP-9 expression

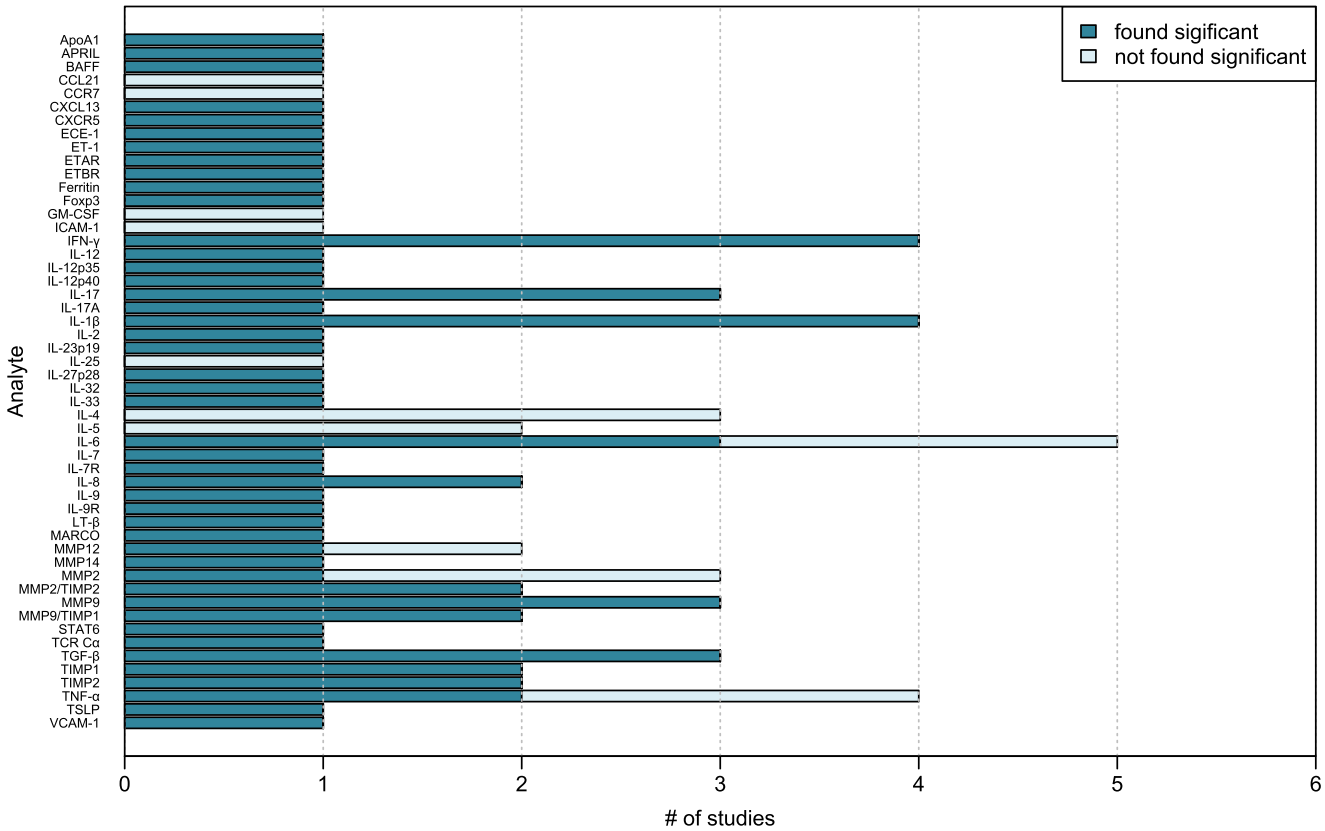

Fig. 3 Analyte frequencies in 12 GCA tissue biopsy studies (52 unique analytes)

tended to decrease after therapy, but was not significantly modified. The authors have also proposed to calculate mRNA expression ratios of MMPs/TIMPs, specifically MMP-9/TIMP-1 and MMP-2/TIMP-2, which were significantly changed in GCA patients compared to controls [39]. This has been confirmed by Lakota et al. in 2015 [38]. MMP-9 mRNA expression levels were also found to be significantly elevated in GCA patients in a different study in 2014 [40] and in supernatants of cultured arteries of GCA patients [37]. Interestingly, the MMP-12 gene has also been shown to be overexpressed in TABs of GCA and could also be important in disease pathology [40].

Endothelial-related proteins

Lozano et al. aimed to prove that endothelin axis might play a role in inflammation-induced vasospastic phenomena that can lead to development of disease-related ischemic complications. In their study, decreased mRNA expression of endothelin-1, endothelin-converting enzyme-1, and endothelin receptors A and B were observed in TABs of GCA patients compared to non-GCA controls; however, no differences were found between patients with or without ischemic complications [41].

Analyte frequency

Frequency of analytes was calculated from the 12 cross-sectional studies indicated in Table 1 (excluding longitudinal and culturing studies), based on the number of reports available for a particular analyte (Fig. 3). The highest analyte frequency ($n = 5$) was obtained for IL-6; however, only 3/5 reports indicated significantly changed levels of IL-6. Three analytes (IFN-γ, IL-1β, and TNF-α) were measured in four reports; however, only IFN-γ and IL-1β were found consistently significantly elevated in all reports, while TNF-α was found without a significant change in two reports.

IL-17, TGF-β, and MMP-9 were all found to be significantly changed in three studies in TABs of GCA patients; on the other hand, both IL-4 and IL-5 consistently showed no significant change in 3/3 and 2/2 studies in TABs, respectively.

Microarray studies

Published transcriptome studies reporting on microarray gene expression data in TABs of GCA patients are scarce, with a report in GEO DataSet repository (#GSE63425) on VSMCs isolated from TABs of suspected GCA patients [45] which highlighted increased VSMC proliferation during GCA contributing to vessel wall remodeling and arterial obliteration. Inhibition of endothelin-1 and specifically, its receptor with macitentan could reverse this phenotype, and was offered as promising therapy, in addition to glucocorticoids. Another report compared two GCA positive TABs versus 2 GCA negative TABs, with differential expression of over 2000 genes and chitinase-3-like protein 1 as most up-regulated, followed by macrophage receptor with collagenous structure (MARCO) and the serine proteinase inhibitor SERPIN A1 [40]. MARCO was also confirmed to be significantly upregulated in TABS of GCA versus non-GCA patients [38]. Among the MMPs, only MMP-12 and MMP-9 showed significant upregulation in microarrays [40].

Epigenetics

Only one study aimed to assess DNA methylation status [46]. Equally, only one report investigating miRNA expression in TABs of GCA patients exists to date [47]. Identified factors were mainly associated with Th1 and Th17 cell responses, inflammation, and cellular senescence. An overview is provided in the left panel of Fig. 2.

DNA methylation

Coit et al. identified 1555 hypomethylated CG sites in 853 genes in TABs of GCA patients ($n = 12$) compared to non-GCA age-, sex-, and ethnicity-matched controls ($n = 12$), many of which were associated with Th1 and Th17 cells [46]. Pro-inflammatory hypomethylated genes included TNF-α, lymphotoxins-α and -β, chemokine receptor CCR7, runt-related transcription factor 3 (RUNX3), cluster of differentiation (CD) 6, CD40LG, IL-2, IL-6, inflammasome component NOD-like receptor P1 (NLRP1), IL-1β, IL-18, IL-21, IL-23R, and IFN-γ. Changes in DNA methylation patterns were also shown for the nuclear factor of activated T cells (NFAT), a critical factor mediating production of proinflammatory cytokines, including IL-23. A role was suggested for increased activity of NFAT signaling pathway in GCA. This was confirmed by immunohistochemistry, which showed increased expression and nuclear localization of NFAT1. NFAT signaling downstream targets, such as interleukin (IL)-21/IL-21R and CD40L, were found overexpressed in GCA-affected arteries. Therefore, the regulation of NFAT expression at the DNA methylation level could promote activation of the Th17 cell response in GCA. It was proposed that a specific NFAT inhibitor (e.g., dipyridamole) would be well tolerated and, with added beneficial anti-platelet activity, could have therapeutic potential in GCA [46].

miRNA expression

One report of dysregulated miRNAs in GCA has been published by comparing inflamed TABs from GCA patients, non-inflamed TABs from GCA patients, and non-inflamed TABs

from non-GCA patients [47]. miR-21, -146-a, -146b-5p, -150, -155, and -299-5p were found to be significantly more expressed in inflamed TABs from GCA patients. On the other hand, negative TABs from patients with GCA or non-GCA had a similar miRNA expression profile. Expression of miR-146b-5p was the most promising diagnostic biomarker, discriminating inflamed from normal TABs with 100% specificity and sensitivity. However, mRNA expression levels of the known protein targets did not negatively correlate with miRNA expression levels. Interestingly, a comparison of the same miRNAs using peripheral blood mononuclear cells derived from the same donors across the groups demonstrated no differences in their miRNA profiles. These data suggest that the deregulation of these miRNA levels is tissue specific. miR-146-a, -146b-5p, -21, and -155 can be induced by cellular senescence. Indeed, low-grade, systemic inflammation persistent in the elderly and age represent considerable risk factors for GCA development. The observed miRNA profiles might indicate a premature cellular senescence, contributing to inflammation, and might be involved in disease pathogenesis. The main limitation of this study is that most patients were not steroid-naive when miRNA analysis was performed [47].

Conclusion

Judging from the analyte frequency data, IFN-γ, IL-1β, IL-17, TGF-β, and MMP-9 are the most promising, significantly elevated tissue markers that could aid clinicians in their evaluation of GCA pathogenesis, as well as potential therapy. This also goes well in line with the experimental data shown recently by Weyand et al. that PD-1$^+$ CD4$^+$ T cells in GCA secrete IFN-γ, IL-17, and IL-21, thereby driving inflammation-associated angiogenesis and intimal hyperplasia [7].

The current report also exposes 3/5 studies showing IL-6 mRNA to be significantly elevated in TABs of GCA patients as compared to non-GCA patients, while 5/6 studies reported significantly elevated circulatory IL-6 in GCA patients versus healthy blood controls [27]. Studies to date have shown efficacy of anti-IL-6 antagonists in GCA [48].

There is a limited number of studies on epigenetics in GCA. To date, only a few reports have addressed the epigenome in GCA indicating that epigenetics could underpin the variable clinical course of GCA [49]. So, investigating epigenetic mechanisms in GCA could crucially contribute to the understanding of disease complications, as well as promote the development of novel targets for therapies in the future. Taken together, the data presented requires validation by multiple groups on larger populations of patients.

Data Availability: Not applicable.

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Compliance with ethical standards

Disclosures None.

Ethical approval and consent to participate Not applicable.

Consent for publication All authors give consent for publication.

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