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# Clonal hematopoiesis across the age spectrum of vasculitis patients with Takayasu's arteritis, ANCA-associated vasculitis, and giant cell arteritis

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## Abstract

**Objectives:** Aging and inflammation are associated with clonal hematopoiesis (CH), the emergence of somatic mutations in hematopoietic cells. This study details CH in patients with systemic vasculitis in association with clinical, hematologic, and immunologic parameters.

**Methods:** Patients with three forms of vasculitis were screened for CH in peripheral blood by error-corrected sequencing. Relative contributions of age and vasculitis on CH prevalence were calculated using multivariable logistic regression. Clonal hierarchies were assessed by proteogenomic single-cell DNA sequencing, and functional experiments were performed in association with CH status.

**Results:** Patients with Takayasu's arteritis (TAK; n=70; mean age=33.2 years), ANCA-associated vasculitis (AAV; n =47; mean age=55.3 years), and giant cell arteritis (GCA; n=59; mean age=71.2 years) were studied. CH, most commonly in *DNMT3A* and *TET2*, was detected

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Competing interests

Authors declare no competing interests.

Contributorship:

FGR, KVW, AIJ, BAP, NSY, PCG conceptualized and designed the study, and interpreted the results, wrote, and edited the manuscript. FGR, DH, LA, performed and analyzed ECS experiments. FGR interpreted the scDNA results. AIJ, CR, KAS performed functional experiments. RTC provided healthy controls data for ECS analysis. KVW, AIJ, KAQ, PCG performed statistical analysis for clinical outcomes. KAQ, PCG provided clinical care.

Ethical approval information

This work was approved by the Institutional Review Board at the National Institutes of Health (#14-AR-0200).

Patient and Public Involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

in 34% (60/176) of patients vs. 18% (28/151) of age-matched controls (p<0.01). Prevalence of CH was independently associated with age (standardized B=0.96, p<0.01) and vasculitis (standardized B=0.46, p<0.01), occurring in 61%, 32% and 13% of GCA, AAV, and TAK patients, respectively. Both branched and linear clonal trajectories showed myeloid-lineage bias, and CH was associated with markers of cellular activation. In GCA, mutations were detected in temporal artery biopsies, and clinical relapse correlated with CH in a dose-dependent relationship with clone size.

**Conclusions:** Age was more strongly associated with CH prevalence than inflammation in systemic vasculitis. Clonal profile was dominated by *DNMT3A* mutations which were associated with relapse in GCA. CH is not likely a primary causal factor in systemic vasculitis but may contribute to inflammation.

### Keywords

vasculitis; Takayasu's arteritis; ANCA-associated vasculitis; giant cell arteritis; clonal hematopoiesis

## Introduction

The systemic vasculitides are a family of diseases characterized by inflammation of blood vessels. Takayasu's arteritis (TAK), antineutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV), and giant cell arteritis (GCA) are forms of systemic vasculitis that in aggregate affect patients across a broad age spectrum. TAK is a large-vessel vasculitis that primarily affects younger patients, while AAV is a small-vessel vasculitis that most commonly occurs in middle age, and GCA is a large-vessel vasculitis that is exclusive to later life.[1–3] Although clinical and demographic differences exist between these forms of vasculitis, severe and organ-threatening disease due to vascular inflammation is common.

Systemic inflammation and aging have been linked to increased prevalence of clonal hematopoiesis (CH), a phenomenon characterized by the emergence and subsequent expansion of somatic mutations in blood cells that associates with all-cause mortality.[4,5,6]. In healthy individuals, CH frequency in peripheral blood increases with age and is prevalent in >10% individuals older than 70 years at a variant allele fraction (VAF) ≥ 2%, but ubiquitous in people older than 65 of age when more sensitive sequencing techniques are used for screening.[5,7,8] *DNMT3A, TET2* and *ASXL1* mutations are most common in healthy. A preferential selection of *DNMT3A* and *TET2* clones has been described in many inflammatory disorders. *DNMT3A* mutations are preferentially selected in rheumatoid arthritis, ulcerative colitis, small cross-sectional studies in vasculitis, and chronic infection,[8–12] while *TET2* mutations have been linked with low-grade inflammation and increased risk of cardiovascular disorders.[13]

Whether age and chronic inflammation are synergistic or independent contributors to CH is not well established. CH in myeloid cells has been hypothesized to fuel a vicious inflammatory feedback loop via increased production of pro-inflammatory cytokines.[6,14–22] Conversely, CH may be a molecular marker of an underlying inflammatory environment with minimum modulatory impact on cellular function.

In this study, we characterized the CH landscape of a unique cohort of patients with vasculitis representing the human lifespan using error-corrected sequencing and single-cell proteogenomic sequencing. We modeled the relative contribution of age versus systemic inflammation on CH prevalence in patients with vasculitis and correlated results to clinical outcomes and functional assays.

## Methods

### Study Population

Patients with vasculitis were recruited across North America into a prospective, observational cohort at the National Institutes of Health (NIH) in Bethesda, MD (NCT02257866). All patients included this study fulfilled the 2022 American College of Rheumatology (ACR) Classification Criteria for TAK, AAV, or GCA.[1–3,23] Patients were enrolled at any point during their illness, regardless of treatment. All patients underwent standardized clinical assessment at each study visit. Relapsing disease was defined as recurrence of clinical symptoms attributed to active vasculitis requiring increase in glucocorticoid therapy ≥50% from baseline dose or addition of steroid-sparing therapy. Age-matched healthy individuals were used as controls; up to two patients with vasculitis were matched to each healthy control within 5 years of age at the time of baseline DNA sample collection. The same control could be matched across different forms of vasculitis depending on age. All subjects provided informed consent. Samples were collected according to the Declaration of Helsinki. Patients were not directly involved in study design.

### Bulk and single-cell DNA sequencing

Patients and controls were screened for CH using a customized error-corrected sequencing (ECS) panel covering common myeloid-related genes and *UBA1*, the causal gene for VEXAS syndrome,[24] as previously described.[25] DNA libraries were sequenced on the NovaSeq6000 (average coverage of 600x deduplicated reads), and variants with a de-duplication ratio >3:1 and minimum VAF ≥ 0.5% were included in the analysis. When available, mutations were tracked over time in blood and quantified in DNA from sorted monocytes, neutrophils, and temporal arterial biopsy (TAB). *De novo* variants (VAF ≥ 0.5%) identified at any timepoint were serially tracked in all other samples and retrospectively included in the analysis if detectable at VAF≥0.1%. Single-cell proteogenomic DNA (scDNA) sequencing (genotype coupled with immunophenotyping) was performed in total blood cells from three GCA patients, two with multiple CH mutations, according to the Mission Bio Tapestry platform protocols as previously described.[25] scDNA data derived from healthy controls were used as reference for cell cluster analysis based on the expression of 45 protein surface markers. Details are shown in supplemental material.

### Isolation of blood subpopulations and CD14+ monocyte stimulation experiments

Peripheral blood mononuclear cells (PBMCs) and neutrophils were first isolated from EDTA blood samples by Ficoll density gradient. Briefly, after PBMCs were isolated, the granulocyte layer was resuspended in half of the blood volume of 20% Dextran for 15 minutes. PBS was added to a total volume of 30 mL. After 30 minutes, the top layer of the separation (neutrophils) were moved to a new tube then washed in PBS. Monocytes

and T lymphocytes were purified from PBMCs by positive selection using CD14 and CD3 beads (Miltenyi Biotech #130–050-201) following manufacturer's instructions. For each patient and control, $1\times10^6$/mL CD14+ cells were seeded in triplicate in 12-well plates for stimulation with (100 ng/mL). After an overnight IFN-g stimulation, CD14+ cells were stimulated with LPS (100 ng/mL) for 4.5-hours and ATP (5 mM) for 30 minutes. Media from each condition was harvested for cytokine/chemokine profiling using a custom designed Luminex Multiplex array with the following targets: IL-1β, IL-1RA, IL-8, IL-10, IL-12p70, IL-23, IP-10, MCP-1, MIP-1α, MIP-1β, TNF. Subsequent comparative analyses were considered exploratory and were not adjusted for multiple comparisons.

### Statistical Analyses

The prevalence of CH detected in peripheral blood was compared between patients with each form of vasculitis to age-matched controls using a VAF ≥ 0.5% threshold. To enable comparisons to prior studies that used lower depth of sequencing, results using a VAF threshold of ≥2% are also reported. Multivariable logistic regression was used to assess the independent association of age and vasculitis with presence of CH. Standardized beta coefficients were calculated to compare the relative strength of association. Clinical associations between categorical and continuous variables were compared using Fisher exact test and Wilcoxon rank sum test.

## Results

### Study Population

Patients with vasculitis (n=176) ranged in age from 5–88 years. TAK patients (n=70) were the youngest (mean age=33.6 ± 14.8 years) followed by AAV (n=47; median age=55.3 ± 14.7 years) and GCA patients (n=59; mean age=71.1 ± 8.6 years; Table 1). The majority of patients were female (71%) and disease duration was variable at time of assessment (Table 1). On average, patients with GCA were initially evaluated earlier in the disease course on higher doses of daily prednisone compared to patients with TAK and AAV.

### Clonal landscape of systemic vasculitis

CH incidence in TAK, AAV, and GCA was 13% (9/70), 32% (15/47), and 61% (36/59) respectively (Figure 1A–B; Supplemental Table 1). These frequencies decreased to 3% in TAK, 13% in AAV and 24% in GCA when the VAFs cut-off >2% were used (Figure 1B). In all cohorts, *DNMT3A* was the most commonly mutated gene followed by *TET2. DNMT3A* mutations were seen in 10% of TAK, 19% of AAV, and 43% of GCA patients. *TET2* mutations were absent in the TAK cohort but found in 11% AAV and 21% GCA patients (Figure 1C). Most patients with somatic mutations (65%) had variants at VAF < 2% (median VAF = 1%), regardless of the mutated gene and type of vasculitis (Figure 1C). The frequency of patients with mutations at VAF ≥ 2% increased with age (Figure 1D). Somatic mutations at VAF ≥ 10% were only seen in a minority of patients (10%), all were ≥ 58 years, had GCA (n = 5) or AAV (n =2), and had ≥ 2 mutations (Figures 1A, 1C). The frequency of patients with ≥ 2 CH mutations was similar among different forms of vasculitis (3/9 [33%] of TAK, 6/15 [40%] of AAV, 14/37 [38%] of GCA), and the number of concurrent CH mutations increased with age (Figure 1D). *TET2* mutations were

all truncated and most *DNMT3A* mutations were located in the MTase domain, including the p.R882 hotspot mutations seen in four patients (1 TAK, 1 AAV, and 2 GCA) at VAFs ranging from 1%–30%.

When compared to age-matched controls, CH was significantly enriched in GCA (61% vs. 35%; p<0.01) but not in TAK (13% vs. 6%; p=0.25) or AAV (32% vs. 17%; p=0.15; Figure 1E). This difference was not observed when a VAF ≥ 2% was used for analysis. Preferential selection of *DNMT3A* clones was associated with aging and vasculitis type. *DNMT3A* mutations were enriched in middle age and older patients (30 to 69 years) with GCA and AAV compared to age-matched healthy controls (44% in GCA vs. 24% in AAV vs. 15% in TAK vs. 12% in controls) but found at similar frequencies among patients and controls older than 70 years (44% in GCA vs 50% in AAV and 40% in controls; Figure 1E). *TET2* mutations were mostly enriched in AAV patients (7% in GCA vs. 13% in AAV vs. 3% in controls) (Figure 1E).

In multivariable regression analysis, both age (B=0.05, p<0.01) and vasculitis (B=0.50, p<0.01) were independently associated with CH at VAF ≥ 0.5%. Using standardized beta coefficients, age (Beta=0.96) was 2.09 times more strongly associated with CH than vasculitis (Beta=0.46). Only two patients younger than 30 years were found with CH, both had TAK; no controls at the same age range had CH (Figures 1A, 1D).

## Clonal dynamics, trajectories, and functional impact

Clonal dynamics were tracked in 35 patients, including 17 with CH at baseline. All patients with serial samples were on immunosuppressant treatment at time of initial sampling, and 10 patients successfully discontinued therapy during follow up due to established disease remission. In 16 GCA and 1 TAK, clone sizes were stable or slightly changed in blood over a mean follow up of 3.1 (± 2.1) years (Figure 2A and Supplemental Figure 1). During follow-up, a new mutation was detected in one patient (V031) and a preexistent clone disappeared in another (V669); CH was not detected in 17 patients at baseline or after a median of 2 years of follow-up (ranging from 0.5–5.5 years).

To better investigate the clonal trajectories and composition of mutated cells, we performed scDNA sequencing in peripheral cells from three GCA patients, two with multiple mutations in *DNMT3A/TET2*. In V053, a branched trajectory with 4 independent clones was seen: the *DNMT3A* p.R882H and p.R882S were independent driver mutations, and two *TET2* mutations were sub-clonal to the p.R882H (Figure 2B). In contrast, a linear trajectory was seen in V639; a driver mutation in *DNMT3A* preceded the acquisition of the *TET2* mutation seen in similar VAFs in bulk ECS. In both patients, *TET2* mutations co-occurred with *DNMT3A* in single cells, which were mostly of myeloid lineage; up to 15–20% of CD4+ and CD8+ T cells, B cells, and NK cells were also mutated (also carrying the classical myeloid-related *DNMT3A* p.R882 mutations). The fractions of monocytes ($Lin^{-}CD14^{+}$), neutrophils ($Lin^{-}CD16^{+}CD62L^{+}$), and myeloid progenitor cells ($CD117^{+}CD38^{+}CD33^{+}$) were mostly CH mutated and increased in comparison to healthy controls (Figure 2B). Also, myelopoiesis was shifted towards increasing counts of intermediate monocytes ($CD14^{+}CD16^{+}$) and neutrophils expressing $CD11b^{high}$, a phenomenon reported in systemic inflammation and associated with pro-inflammatory phenotype.[26,27] Similar results were

seen in a single GCA patient without CH (V421), suggesting that cell composition may not be primarily driven by CH presence; a significant B cell clonal expansion, all wild-type, was also seen in this patient (Supplemental Figure 2 and 3A).

We next compared whether mutated *DNMT3A/TET2* cells were associated with increased expression of classical protein surface markers linked to inflammatory and cytotoxic charcateristics.[28–30] We found no differences in the expression of CD10, CD11b, CD62L, and CD64 in neutrophils, regardless of mutation status, among patients and controls (Supplemental Figure 3B). In contrast, CH presence associated with an inflammatory CD16+ phenotype in monocytes and higher expression of cytotoxic markers in NK cells, and T lymphocytes (Figure 2C). Higher CD14+/CD16+ expression was seen in monocytes from V053 and V639 but not V421 when compared to controls; among these, cells with CH had significantly higher expression of these markers. Higher expression of CD10, CD16 and CD11b linked to high cytotoxic activity was significantly increased in NK cells, CD4+ and CD8+ T cells harboring a CH mutation but not in wild-type cells.

To investigate whether CH impacted monocyte function, we stimulated purified cells from 13 and 10 GCA patients with and without CH, respectively. Results were compared to data derived from 6 and 9 age-matched controls with and without CH, respectively. Monocytes from GCA patients with and without CH upon stimulation with LPS and interferon gamma (IFN-ɣ) produced similar levels of cytokines, however, chemokine production of MCP-1, MIP1α, and MIP1β were significantly higher in monocytes from CH patients after IFN-ɣ stimulation (Figure 3A). Monocytes from healthy controls with CH also produced more MIP-1α compared to those without CH across a series of different experimental conditions (Figure 3A).

## CH associated with increased myeloid counts and lineage bias in peripheral blood

Clinically, presence of CH at VAF ≥ 0.5% was associated with higher absolute neutrophils counts (ANC; $p<0.02$) while CH at VAF ≥ 2% was significantly associated with higher ANC and absolute monocyte counts (AMC), higher neutrophil frequency and neutrophil to lymphocyte ratio, and lower lymphocyte frequency compared to patients without CH (Table 2). There were no significant differences in hemoglobin levels, red blood cell count, or daily prednisone dose at the time of hematologic assessment between patients with and without CH defined at both VAF thresholds. Overall, presence of CH did not associate with cytopenias but with increased number of myeloid cells in peripheral blood. No patients developed hematologic malignancies.

## CH associated with relapse in patients with GCA

Given the high prevalence of CH in GCA relative to the other forms of systemic vasculitis, associations between CH and clinical features of vasculitis were only studied in GCA patients. GCA patients with CH defined at VAF ≥ 0.5% only had lower erythrocyte sedimentation rate (ESR) values at diagnosis compared to those without CH (51 vs 85 mm/hr; $p=0.03$). However, patients with CH defined at VAF ≥ 2% were more likely to have relapsing disease (67 vs 25%, $p<0.01$), were older at time of CH assessment (74 vs 71 years, $p=0.07$), had a lower maximum ESR value at diagnosis (28 vs 83 mm/hr; $p=0.03$), and

were less likely to experience clinical response when treated with tocilizumab (63% vs 93%; p=0.06; Table 3). A dose response was observed between CH VAF% and relapse (Figure 3B). The association of CH and relapsing disease was mediated by *DNMT3A*-defined CH (82% of relapsed patients) more strongly than *TET2*-defined CH (24% of relapsed patients). Survival outcomes were not assessed as only one death occurred during the study period in a patient with GCA and CH.

To determine whether CH mutations from circulating immune cells could be detected in arterial tissue, we sequenced TAB specimen material from five patients with GCA at diagnosis in parallel with peripheral blood sequencing. Four of five patients had mutations detected in TAB and peripheral blood. In two patients with TAB results positive for transmural inflammation, CH mutations were detected in both peripheral blood and TAB tissue: in one, *TET2* mutations were detected at VAF of 0.5% in both blood and TAB, while in the second, a *DNMT3A* mutation was enriched in blood over TAB (1% vs 0.4% VAFs, respectively). Among three patients with negative TAB results but a clinical diagnosis of GCA confirmed by vascular imaging studies, two were found with CH in blood at VAFs of 1% that was detected in TAB at very low levels (VAF<0.5%; Supplemental Table 2).

### UBA1 somatic mutations in systemic vasculitis

Somatic mutations in *UBA1* are now considered within the spectrum of CH and define the VEXAS syndrome, which is associated with different clinical forms of vasculitis. We therefore screened all study participants for *UBA1* mutations. No patient had a detectable mutation in *UBA1* at a VAF consistent with VEXAS syndrome; however, one female patient with GCA had a pathogenic *UBA1* mutation (c.118-G>T) in blood on two separate occasions over a four-year interval, at VAFs of 0.3% and 0.9%. This patient had negative bilateral temporal artery biopsies in the setting of frontotemporal headaches, constitutional symptoms, polymyalgia rheumatica, and elevated acute phase reactants. Her disease was also defined by acute onset and rapidly progressive upper and lower extremity claudication with severe arterial damage restricted to the bilateral axillary arteries and femoral arteries. She had no cytopenia but did have chronic unexplained macrocytosis (maximum mean corpuscular volume 99 fL). She responded well to tocilizumab and tapered glucocorticoids, and her vasculitis has been in stable remission off treatment for five years.

## Discussion

In three forms of systemic vasculitis representing a broad age spectrum of patients, both age and inflammation were independent predictors of CH. The relative association was twice as strong for age compared to a diagnosis of systemic vasculitis. Thus, while systemic inflammation may be associated with increased risk for CH, age remained the strongest predictor of CH in patients with vasculitis. When compared to age-matched controls, increased CH frequency was most prominently seen during middle age, with no differences in CH prevalence observed in study subjects younger than 30 years or older than 70 years. However, most clones were small and when the traditional VAF cut-off of 2% was used for CH detection, there was no difference between the controls and patients with vasculitis. The largest VAFs in this study were restricted to older patients with AAV or GCA, further

supporting the importance of age in the generation and expansion of CH clones. Of note, most mutated cells were myeloid but unexpected frequencies (up to 20%) of mutated lymphocytes and NK cells were also seen, suggesting that clonal selection occurred at the hematopoietic stem and progenitor cell level.

CH has been studied in a few rheumatologic diseases. CH prevalence was reported to be 15% in rheumatoid arthritis and systemic sclerosis and 30% in case series of AAV and GCA.[8,11,12,31] Similar to this study, *DNMT3A* followed by *TET2* mutations dominated the clonal landscape, mostly detected at VAFs of 2–10%. In these studies, the traditional VAF cut-off of 2% was often used to define CH, which would not have comprehensively characterized the mutational burden observed in this study with variants predominantly found at VAFs <2%. Indeed, at VAF >1%, CH incidence in a small GCA cohort and population-based controls was similar.[32] CH has also been recently characterized in the newly discovered VEXAS syndrome. A clonal landscape dominated by *DNMT3A* (including the *DNMT3A* p.R882 hotspot mutations known to be highly associated with myeloid malignancies) and *TET2* mutations with skewed hematopoiesis towards myeloid production was observed.[24,25] VEXAS provides an excellent comparator disease to GCA, as both diseases are exclusive to adulthood. Interestingly the prevalence of CH in GCA reported here (61%) was nearly identical to the previously reported prevalence of CH in VEXAS (60%) using the same sequencing methods and CH definitions. These comparisons highlight that CH mutations, particularly those in *DNMT3A* and *TET2*, are not specific to vasculitis, but rather are associated with systemic inflammation in older patients across a spectrum of inflammatory diseases.

Population-based studies suggest that CH in *TET2*, but not *DNMT3A*, may increase risk for specific cardiovascular and inflammatory diseases.[33 6,34,35] A recent study demonstrated a 1.48 fold increased risk of incident GCA and *TET2-CH*.[36] Corresponding functional studies of *TET2* knockout hematopoietic cells reveal increased myeloid-mediated inflammation in murine models of atherosclerosis and gout[6,33,37] While these studies show an association between CH and various inflammatory diseases, they do not establish causality. In contrast, our findings support the hypothesis that CH mutations are likely a consequence more than a cause of inflammation in systemic vasculitis. More specifically, *DNMT3A*-CH is preferentially associated with aging whereas *TET2-CH* seems to be preferentially selected in an aged inflammatory environment. Indeed, multiple *TET2* mutations in two GCA patients were sub-clonal to *DNMT3A* in single cells. The increased risk of inflammatory diseases inferred by large association studies likely reflects an association between CH and inflammation in older populations.

Our findings support the concept that CH primes myeloid cells to a pro-inflammatory phenotype,[37] which potentially may modulate an underlying inflammatory process and alter the course of disease. GCA patients with CH had increased myeloid cell burden, and monocytes, NK cells, and T lymphocytes displayed an activated phenotype marked by high expression of CD10, CD11b, and CD16. CH mutations were found in affected arteries at similar levels found in peripheral blood, similar to the finding that CH clones can be also detected in atherosclerotic plaques.[38] Monocytes from GCA patients with CH secreted higher levels of chemokines that modulate macrophage proinflammatory signaling pathways

(MCP1, MIP1b and MIP1a), findings consistent with recently reported study that identified pathways involved in macrophage function as a mediator of inflammation linked to *TET2* mutations in monocytes.[37] These chemokines regulate monocyte/macrophage migration from blood across vascular endothelium during immunologic surveillance of tissue in response to inflammation, suggesting CH may contribute to vascular inflammation.[39,40] A bidirectional association between CH and vascular inflammation may exist and explain the higher likelihood of GCA patients with CH to experience clinical relapse. However, a casual directionality with relapse remains uncertain, as patients were sampled at different points in the disease course, and the association may simply reflect the burden of recurrent inflammation on CH prevalence in patients with vasculitis. Although sample size was modest, less frequent clinical response to tocilizumab was observed in GCA patients with CH at VAF ≥2%. Larger, prospective observational cohort studies are needed to further define potential associations of CH and clinical features of disease in patients with vasculitis, including risk relapse risk, treatment response, cardiovascular events, and risk for hematologic malignancy.

With the recent discovery of the VEXAS syndrome, *UBA1* is now included in a panel of genes linked to CH. Accordingly, we sequenced all patients in this study to detect variants in *UBA1*, especially since patients with VEXAS can be clinically diagnosed with various forms of vasculitis, including biopsy-proven GCA. Another recent study sequenced a large cohort of patients with GCA and found no *UBA1* mutations,[41] suggesting that prevalence of VEXAS in GCA is not common. Similarly, in this study, only one patient with vasculitis was found to have a *UBA1* VEXAS-defining mutation in blood. The patient was female with GCA who had a very small *UBA1* clone (VAF <1%) that persisted for years. She had unexplained macrocytosis and somewhat atypical features of GCA including severe stenosis in the femoral arteries, an arterial bed not typically damaged in GCA. In parallel to this observation, a prior study from Japan also detected *UBA1* mutation at VAF of 0.1% in a single female patient with a clinical diagnosis of relapsing polychondritis.[42] Whether low prevalence *UBA1* mutations detected in peripheral blood influence clinical phenotypes in systemic inflammatory diseases, particularly in female patients, warrants further investigation.

Study limitations include small sample sizes that precluded robust analyses to detect clinical associations with CH at both bulk and single-cell levels, and the lack of cytokine profiles and marrow biopsies for morphologic analysis prior any treatment. Although monocyte stimulation experiments were not adjusted for multiple comparisons and were performed on bulk cell populations rather than purified mutant cell populations, a recent study showed that single-cell transcriptome of wild-type *DNMT3A* cells were similar to mutated cells from the same environment, suggesting that these mutations may globally affect cell function and are not restricted to mutated cells.[43] CH was only screened in small set of previously identified myeloid-related genes. Whether mutations in lymphoid lineages across a different set of genes are present and are clinically relevant in patients with vasculitis remains to be determined. Clinical associations were only studied in patients with GCA due to sample size restrictions.

In summary, CH is frequently detected in patients with systemic vasculitis with increased prevalence in association with aging. Inflammation accelerates age-related CH, dominated by *DNMT3A* and *TET2* mutations. CH clones are mostly stable regardless of treatment, biased towards myeloid differentiation, and associated with increased counts of pro-inflammatory monocytes, T lymphocytes, and NK cells with high cytotoxic activity. Clinically, CH is a marker of relapse in GCA and correlates with increasing clone size. To what extent CH mutations are a consequence or cause of inflammation in patients with vasculitis requires further study; however, findings from this study suggest on balance that these mutations do not have a strong primary effect on risk to develop vasculitis but may modify disease course. Exploration of a wider set of genes, beyond those commonly associated with CH, may yield further insight into causal mechanisms of disease in systemic vasculitis.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Data sharing statement

Data are available on reasonable request.

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## Key messages

**WHAT IS ALREADY KNOWN ON THIS TOPIC:**

Although clonal hematopoiesis (CH) has been linked to chronic inflammation and age-related immune dysregulation (“inflammaging”), the causal role of inflammation and its synergism with aging for CH selection are uncertain.

**WHAT THIS STUDY ADDS:**

This study characterizes the CH profile of blood from patients with three forms of vasculitis, demonstrates that age is a stronger predictor of CH than inflammation, and shows an association between relapse and CH in patients with GCA.

**HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY:**

Somatic mutations in blood are common in patients with vasculitis; however, CH is likely a biomarker of “inflammaging” rather than an etiologic risk factor for vasculitis.

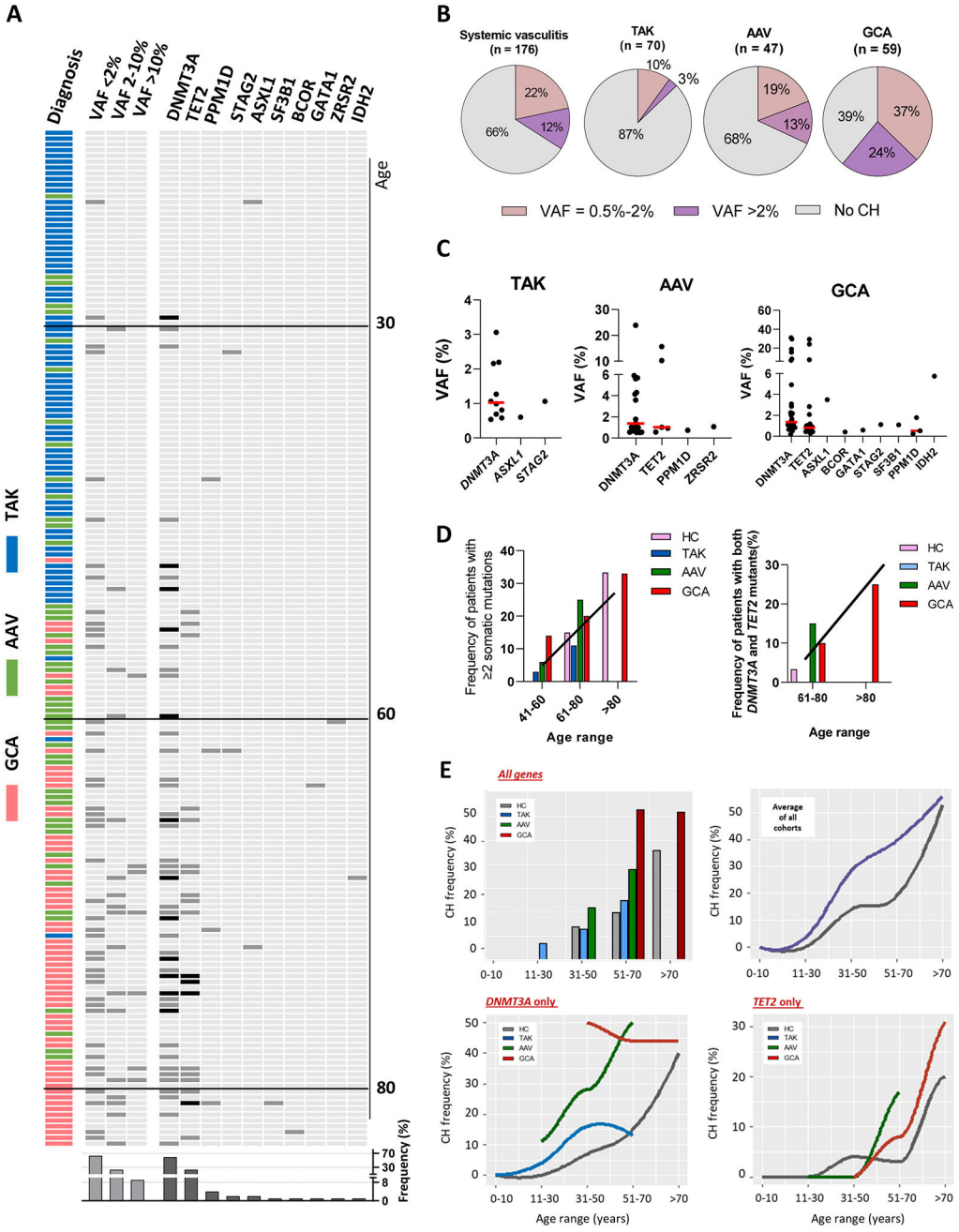


**Figure 1. Clonal landscape of systemic vasculitis.**

**A)** Oncoprint with somatic mutations identified in 176 patients with systemic vasculitis according to their age. Patients with Takayasu's arteritis (TAK; n = 70), ANCA-associated vasculitis (AAV; n = 47), and giant cell arteritis (GCA; n= 59) are colored in the graph. The mutated gene and range of variants allele frequency (VAF), as well as their frequencies, are shown in the figure. **B)** Frequency of patients with clonal hematopoiesis according to their vasculitis type and variants VAFs. **C)** Variant allele frequency (VAF) of somatic mutations identified in peripheral blood (PB). The median VAFs and ranges are shown in the figure according to the mutated gene. **D)** Frequency of patients with vasculitis (TAK, AAV, and

GCA) and healthy controls (HC) with multiple variants (>2 somatic mutations), or with both DNMT3A and *TET2* mutations. **E)** CH frequency in TAK, AAV, GCA, and healthy controls (HC) according to age range and the two most commonly mutated genes, *DNMT3A* and *TET2*.

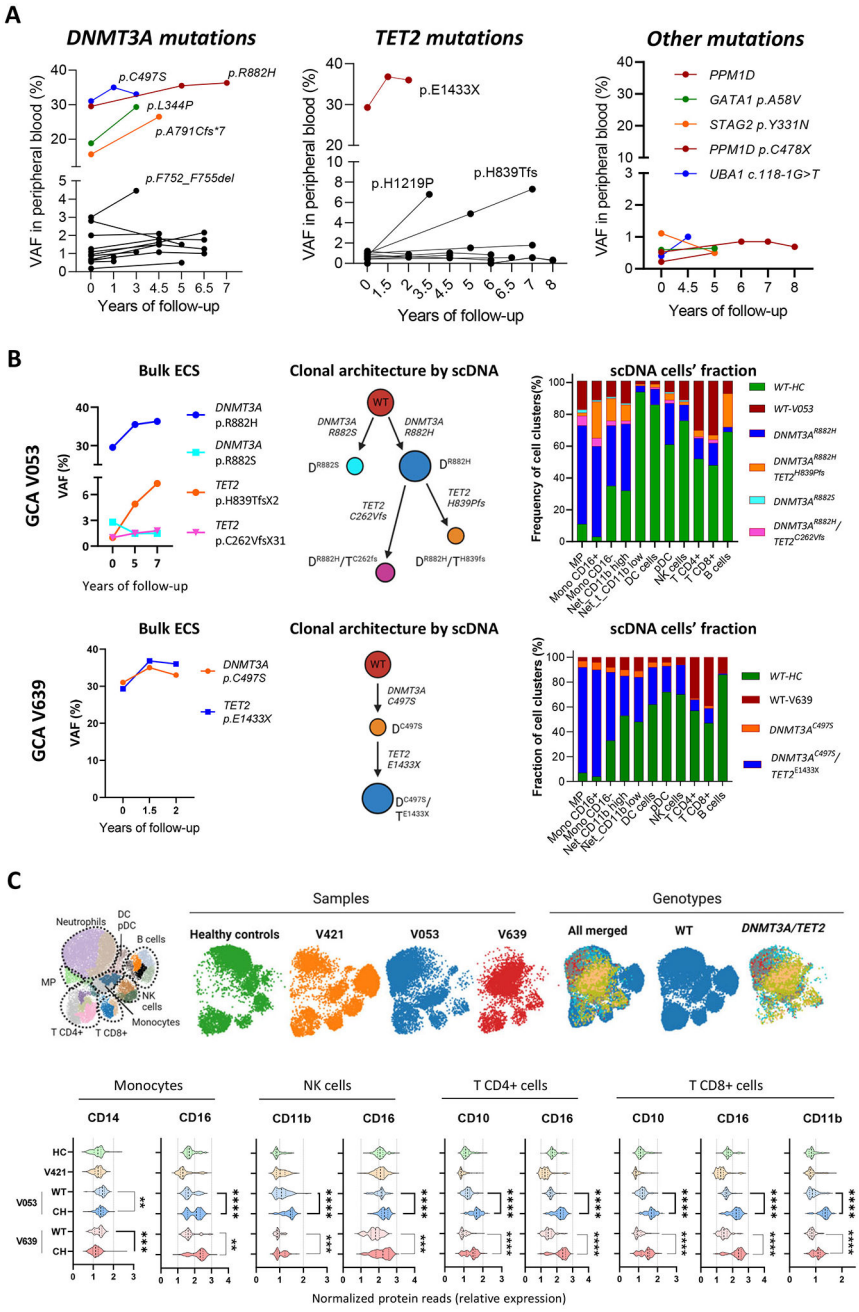


**Figure 2. Clonal dynamics and trajectories in systemic vasculitis.**

**A)** Longitudinal analysis of clonal hematopoiesis. Somatic mutations in *DNMT3A, TET2*, and other genes identified in 17 patients at first visit were tracked over a median follow-up of 3.1 (± 2.1) years; all variants were mostly stable. Variants at VAFs> 5% are colored and their location is shown in the graph. **B)** Clonal trajectories of two patients (V053 and V639) with GCA with multiple somatic mutations in *DNMT3A/TET2*. Clonal hierarches, both linear and branched, of variants detected by bulk error-corrected sequencing (ECS) were assessed by single-cell proteogenomic analysis (scDNA). The frequency of different cell clusters based on the expression of protein surface markers are shown in

figure according to cells' genotype. Representative data from peripheral blood cells derived from a healthy control (HC) were used as a reference for analysis. Single cells were labeled according to their genotypes; wild-type (WT) cells were labeled as either from the healthy control (WT-HC) or V053 and V639 patients. Cells clusters were characterized according to their differentially expressed protein surface markers (normalized reads) when stained with the cocktail of 45 antibodies targeting common blood protein surface markers (TotalSeq-D Heme Oncology Cocktail antibody-oligo conjugate) and processed with the Tapestry protocol (Mission Bio). Cell populations were characterized based on a reference data from healthy samples and available Mission Bio datasets as: myeloid progenitor cells (MP; CD34lowCD38+CD117+CD123+CD45RA-CD141+CD71+CD7+CD33+CD64+), neutrophils (Net; CD16+CD62L+Lin-) with high or lower CD11b expression, monocytes (Mono; CD14+CD16+/−), plasmacytoid dendritic cells (pDC; $CD14^{-}CD123^{+}Fc\varepsilon RIa^{+}$), and conventional DC (DC; $CD14^{-}CD141^{+}CD11c^{+}CD11b^{+}$), T lymphocytes (CD3+CD8+ or CD4+), B lymphocytes (CD19+), natural killer (NK; CD3-CD56+CD7). **C) scDNA profiles and relative expression of specific protein markers in blood subpopulations.** The Uniform Manifold Approximation and Projection (UMAP) scDNA profiles for healthy and vasculitis samples are shown in the graph. Cells from healthy and V421 were all wild-type while a subset of cells from V053 and V639 were *DNMT3A/TET2* mutated. Cells' genotypes according to their UMAP projection are shown in the figure. Bottom panels show the relative expression of markers linked to activation of monocytes, NK and T cells in cells from controls (HC), V421, V053, and V639 according to their genotype, wild-type (WT) or with CH mutations (CH).

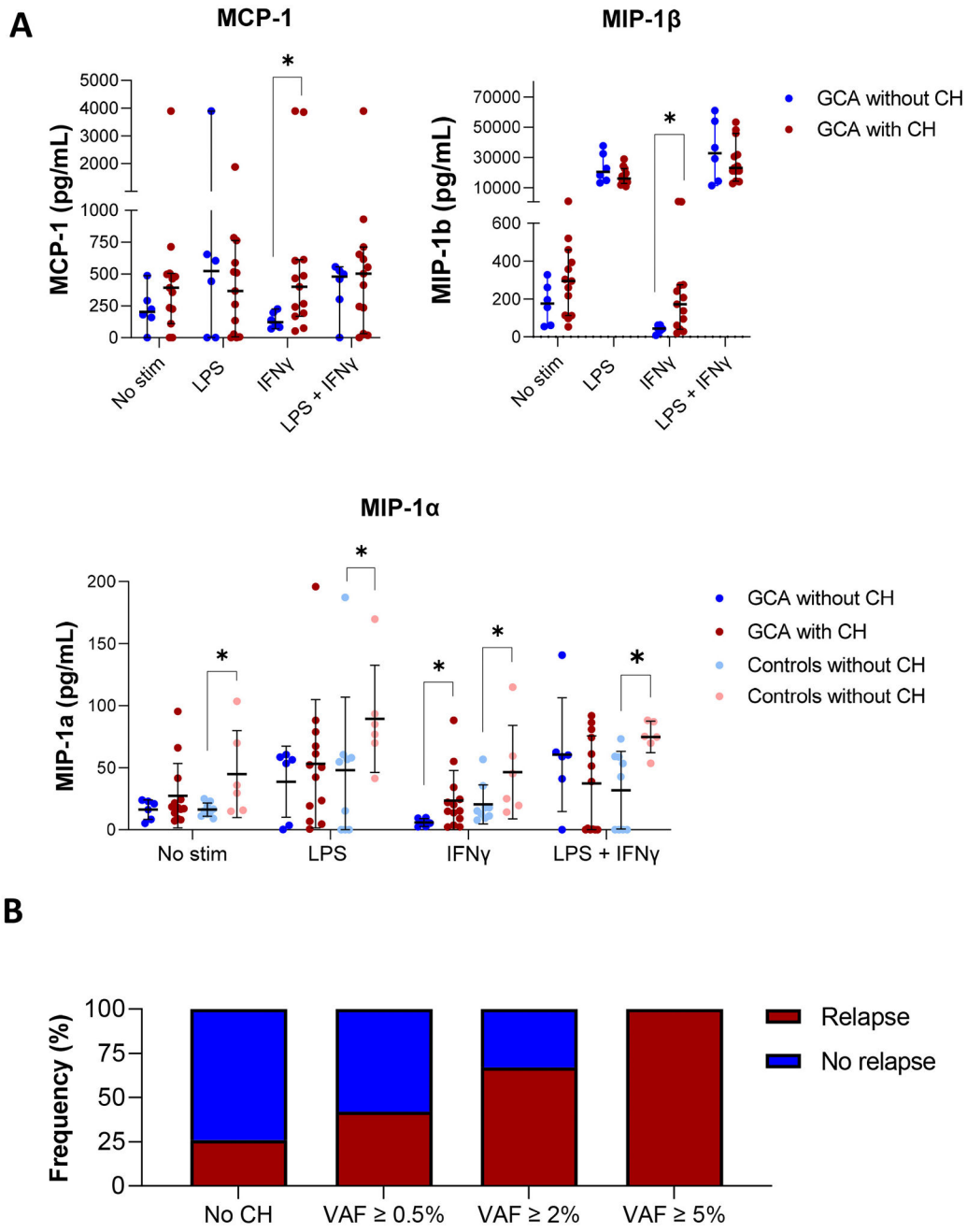


**Figure 3. Clinical and functional impact of clonal hematopoiesis.**

**A) CD14+ cell stimulation experiments.** Protein levels of MCP-1, MIP1α, and MIP1β were increased in supernatants of CD14+ cells from GCA patients with CH stimulated with INF-γ in comparison to stimulated non-mutated cells. MIP1α levels were also increased in supernatants of CD14+ cells from healthy controls with CH in comparison to non-mutated cells stimulated under different experimental conditions. **B) Dose response association of CH variant allele frequency (VAF) and relapse risk of GCA patients.** Higher mutations

VAFs correlated with relapse in GCA. All GCA patients with CH at VAF ≥ 5% experienced relapse.

**Table 1.**

Baseline Characteristics of Patients with Vasculitis.

| | TAK (n = 70) | AAV (n = 47) | GCA (n = 59) |
|---|---|---|---|
| **Mean age, years** (SD) | 33.2 (10.2) | 55.3 (14.7) | 71.2 (8.6) |
| **Median age** (range) | 34 (5 – 71) | 60 (19–77) | 72.5 (50 – 88) |
| **Female sex** | 59 (84%) | 25 (53%) | 41 (69%) |
| **Median disease duration**, months (Interquartile range) | 96.8 (21.8–325.5) | 45.3 (10.1–336.8) | 38.2 (15.6–77.5) |
| **Mean prednisone dose, mg/day** (SD) | 8.7 (14.3) | 8.5 (16.8) | 16.3 (18.9) |
| **DMARD use** | 63 (90%) | 41 (87%) | 48 (81%) |
| **Cyclophosphamide use** | 0 (0%) | 12 (26%) | 0 (0%) |
| **Hematologic parameters** | | | |
| Hemoglobin (g/dL) (SD) | 12.8 (1.5) | 13.3 (1.63) | 13.04 (1.52) |
| Absolute Lymphocyte count ($\times 10^3$/mL) (SD) | 2.24 (1.0) | 1.42 (0.72) | 1.86 (1.07) |
| Relative Lymphocyte counts (SD) | 28.2 (12.3) | 19.0 (10.9) | 20.6 (12.0) |
| Absolute Neutrophil Count ($\times 10^3$/mL) (SD) | 5.6 (2.8) | 5.97 (2.62) | 7.07 (3.09) |
| Relative Neutrophil counts (SD) | 62.8 (14.1) | 70.5 (13.2) | 70.5 (14.9) |
| Absolute Monocyte Count ($\times 10^3$/mL) (SD) | 0.24 (0.03) | 0.57 (0.21) | 0.57 (0.23) |
| Relative Monocyte counts (SD) | 6.26 (2.50) | 7.43 (2.75) | 6.32 (2.79) |
| Platelet Counts ($\times 10^3$/mL) (SD) | 311 (82) | 259 (63) | 273 (78) |
| Median Neutrophil / Lymphocyte Ratio (Interquartile range) | 2.27 (1.38–3.40) | 3.90 (2.61–7.49) | 3.17 (1.86–8.85) |

Abbreviations: SD, standard deviation; IQR, interquartile range; DMARD, Disease-modifying antirheumatic drugs; %, relative

**Table 2.**

Association of clonal hematopoiesis (CH) and hematologic parameters in vasculitis

| | VAF ≥ 0.5% | | | VAF ≥ 2.0% | | |
|---|---|---|---|---|---|---|
| | **With CH n = 60** | **No CH n = 116** | ***P*-value** | **With CH n = 24** | **No CH n = 152** | ***P*-value** |
| **Hemoglobin (g/dL)** | 13.1 | 13.0 | 0.46 | 13.3 | 13.0 | 0.31 |
| **Lymphocyte count (×10$^3$/mL)** | 1.75 | 1.92 | 0.20 | 1.65 | 1.92 | 0.16 |
| **Lymphocyte %** | 20.8 | 24.0 | 0.07 | 17.9 | 24.0 | **0.01** |
| **Neutrophil Count (×10$^3$/mL)** | 6.76 | 5.79 | **0.02** | 7.11 | 6.04 | 0.04 |
| **Neutrophil %** | 70.1 | 66.5 | 0.07 | 72.8 | 66.5 | **0.02** |
| **Monocyte Count (×10$^3$/mL)** | 0.57 | 0.54 | 0.32 | 0.63 | 0.54 | **0.04** |
| **Monocyte %** | 6.46 | 6.56 | 0.80 | 6.9 | 6.6 | 0.50 |
| **Platelet Count (×10$^3$/mL)** | 272 | 288 | 0.15 | 262 | 288 | 0.09 |
| **Neutrophil /Lymphocyte Ratio** | 9.5 | 11.0 | 0.22 | 6.81 | 5.00 | <**0.01** |

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**Table 3.**

Associations of Clonal hematopoiesis (CH) Defined at Different Variant Allele Frequency (VAF) Thresholds and Clinical Features of Giant Cell Arteritis (GCA)

| | | CH Defined by VAF ≥ 0.5% | | | CH Defined by VAF ≥ 2.0% | | |
|---|---|---|---|---|---|---|---|
| **Variable** | **Patients with GCA (n = 59)** | **Clonal Hematopoiesis (n = 36)** | **No Clonal Hematopoiesis (n = 23)** | **P-value** | **Clonal Hematopoiesis (n = 15)** | **No Clonal Hematopoiesis (n = 44)** | **P-value** |
| Age, years | 72 (65–76) | 72 (65–80) | 71.5 (63–75) | 0.44 | 74 (70–83) | 71 (63–75) | 0.07 |
| Female | 41 (69%) | 27 (75%) | 14 (61%) | 0.26 | 10 (67%) | 31 (70%) | 0.75 |
| Disease duration, days | 458 (188–930) | 413(188–894) | 539 (206–1080) | 0.69 | 495 (257–1045) | 405 (182–857) | 0.23 |
| Max CRP, mg/L | 49 (19–104) | 47(33–113) | 55 (11.5–84) | 0.56 | 55 (40–105) | 44 (15–105) | 0.57 |
| Max ESR, mm/hr | 79 (40–104) | 51 (24–100) | 85 (69–109) | **0.03** | 28 (19–85) | 83 (45–108) | **0.03** |
| History of relapse | 21 (36%) | 15 (42%) | 6 (26%) | 0.27 | 10 (67%) | 11 (25%) | **<0.01** |
| Cranial Symptoms * | 41 (69%) | 24 (67%) | 17 (74%) | 0.77 | 11 (73%) | 30 (68%) | 1.00 |
| Vision Loss | 7 (12%) | 3 (8%) | 4 (17%) | 0.41 | 0 (0%) | 7 (16%) | 0.17 |
| Constitutional # | 30 (51%) | 15 (42%) | 15 (65%) | 0.11 | 8 (53%) | 22 (50%) | 1.00 |
| Polymyalgia rheumatica | 26 (44%) | 17 (47%) | 9 (39%) | 0.60 | 8 (53%) | 18 (41%) | 0.55 |
| Limb claudication | 20 (34%) | 15 (43%) | 5 (22%) | 0.16 | 7 (47%) | 13 (30%) | 0.35 |
| TAB Performed | 45 (76%) | 26 (72%) | 19 (83%) | 0.53 | 12 (80%) | 33 (75%) | 1.00 |
| TAB Positive | 32 (71%) | 21 (81%) | 12 (63%) | 0.31 | 10 (83%) | 23 (70%) | 0.46 |
| Large-artery damage % | 30 (51%) | 21 (58%) | 9 (39%) | 0.19 | 9 (60%) | 21 (48%) | 0.55 |
| Methotrexate ever | 29 (49%) | 19 (53%) | 10 (44%) | 0.60 | 9 (60%) | 20 (45%) | 0.38 |
| Tocilizumab ever | 37 (63%) | 23 (64%) | 13 (57%) | 0.58 | 8 (53%) | 28 (64%) | 0.56 |
| Tocilizumab responder | 30 (81%) | 19 (83%) | 12 (92%) | 0.63 | 5 (63%) | 26 (93%) | 0.06 |

*Defined as headaches, scalp tenderness, or jaw claudication.

#Defined as fever, weight loss, or severe fatigue. TAB = temporal artery biopsy.

%Defined as stenosis, occlusion, or aneurysm of aorta or primary branch artery.