Varicella Zoster Virus in Giant Cell Arteritis: A Review of Current Medical Literature

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ABSTRACT

In recent years, the search for the cause of giant cell arteritis (GCA) has led investigators to look to varicella zoster virus (VZV) as the answer. In some ways, the nature of VZV infection makes it an attractive explanation for the pathology observed in GCA. However, studies to date yield a level of inconsistency that still leaves uncertainty as to whether VZV directly causes GCA, and positive findings have not been successfully reproduced.

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Introduction

Giant cell arteritis (GCA) is a vasculitis of medium- to large-sized arteries, which typically involves the vascular bed of the cranial arteries. It can also affect the aorta in addition to its primary and secondary branches. This inflammation leads to a number of clinical manifestations characterised by headaches, scalp tenderness, jaw claudication, fevers, polymyalgia rheumatica symptoms, and stroke. Vision loss may occur due to anterior ischaemic optic neuropathy, central retinal artery occlusion or, less frequently, posterior ischaemic optic neuropathy. Inflammatory markers, such as an elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level can be helpful in making the diagnosis. However, temporal artery (TA) biopsy remains the gold standard for diagnosis. Yet, negative TA biopsies can occur, likely due to the nature of skip lesions observed in GCA.¹

In GCA, a positive TA biopsy confirms the granulomatous inflammation within the walls of the medium- to large-arteries. The inflammation is composed predominantly of CD4 T cells and activated macrophages. These changes cause inflammation within the vessel wall leading to the involvement of T cells and macrophages in an immune privileged site, resulting in vessel inflammation, hyperplasia of the intimal layer, and luminal damage with vessel occlusion.²

While patients with negative TA biopsies may certainly be in the small subset of individuals whose samples involved normal tissue due to the skip nature of the disease, a negative biopsy should raise the suspicion of other diagnoses. Previous medical literature has shown the presence of varicella zoster virus (VZV) in biopsies of the temporal arteries of patients with GCA-like symptoms ranging from headaches and jaw claudication to visual loss.³ These observations raise the question of VZV as possibly being an overlapping infection in GCA patients or of VZV causing an immune-mediated effect mimicking GCA (Table 1).

GCA has long been established as a chronic inflammatory disease, which exclusively occurs in elderly individuals. Its incidence increases progressively after the age of 50, suggesting that an age-related immune alteration plays a role in the disease pathogenesis. Until recently, a simplistic hypothesis for the pathogenesis of GCA had been proposed involving an interaction in the walls of susceptible arteries with an unknown infectious agent, local dendritic cells (DCs), activated CD4T cells, and effector macrophages.⁴ However, the intricacy of the process in play during GCA is much more complex than previously thought. The artery, which is usually an immune-privileged site characterised by the inefficient clearance of virus and the failure of T cells and macrophages to enter the virus-infected

Table 1. Case reports of VZV in GCA.

Number of Patients	Age of patient/sex	Description	Reference
1	70/M	Subarachnoid haemorrhage and granulomatous angiitis of the basilar artery in which electron microscopy demonstrated VZV viral antigen	Fukamoto et al., 1986. ⁵
1	80/F	Right upper facial pain in the V1 distribution of the trigeminal nerve, right eye photophobia and right sided headache. Patient diagnosed with herpes zoster ophthalmicus (HZO) after developed painful rash. 4–6 weeks after HZO treatment she developed classic GCA symptoms with elevated inflammatory markers. Treated with oral valacyclovir, ophthalmic prednisone and oral prednisone 60 mg daily. Pregabalin provided for post-herpetic neuropathic pain.	Kosa et al., 2010. ⁶
1	54/F	54 year old diabetic developed ischaemic optic neuropathy (ION) followed by acute retinal necrosis and TA infection. Clinical symptoms of GCA. Vitreous fluid contained amplifiable VZV DNA but not HSV-1. Pathology negative for GCA, but notable for VZV antigen in the arterial adventitia. One time intravitreal injection of ganciclovir in the left eye. Oral acyclovir 800 mg 5 times daily for 14 days and oral prednisone 60 mg daily for 7 days followed by 20 mg for 7 days. Antiviral therapy altered to IV acyclovir 10 mg/kg every 8 hours for 14 days, and steroids discontinued.	Mathias et al., 2013. ⁷
1	72/M	72, immunocompetent male. Clinically developed features of GCA and ipsilateral ophthalmic distribution zoster, followed within 2 weeks by VZV encephalitis and 2 months later by ischaemic optic neuropathy. TA biopsy was negative for GCA, contained VZV antigen, and VZV DNA in multiple non-contiguous skip areas. CSF contained VZV DNA. -Initially treated with IV methylprednisolone 60 mg once followed by oral prednisolone 80 mg/day. After 4 hours, developed left ophthalmic-distribution zoster with keratitis and was treated with valacyclovir. When clinical status worsened corticosteroids tapered, oral antiviral converted to IV acyclovir for 3 weeks. Encephalitis resolved. After withdrawal of corticosteroids GCA symptoms recurred, with ophthalmologic exam concerning for ION. Doppler ultrasound revealed TA occlusion. He was treated with oral prednisone 60 mg/day which lead to rapid sustained clinical improvement.	Teodoro et al., 2014. ⁸
1	72/M	Elderly man developed ophthalmic distribution zoster followed one month later by multifocal VZV vasculopathy that manifested as ION as well as clinical GCA. CSF anti-VZV IgG and IgM. Vision recovered with IV antiviral therapy.	Gilden, 2014. ⁹
1	75/F	Elderly woman developed ION with no features of GCA. VZV on ipsilateral TA. VZV antigen in the adventitia of clinically asymptomatic and pathologically GCA negative TA. anti VZV IgG CSF.	Gilden, 2014. ⁹

M = Male, F = Female.

elastic media layer, loses this immune-privilege quality with age. Artery tertiary lymphoid organs form and are made up of B cells and T cells organised into functional germinal centres. These structures, potentially triggered by infectious agents, may then drive a series of complex interactions between stromal and endothelial cells, as well as the innate and adaptive immune systems.^{4,10} TA biopsies reveal inflammation and necrosis in the arterial media, with either multinucleated giant cells, epithelioid macrophages, or both. However, skip lesions are common and TA biopsy results are pathologically negative in many patients clinically suspected of having GCA.¹¹

VZV is a human neurotropic alphaherpesvirus that is able to replicate in arteries causing disease. VZV vasculopathy is thought to occur from reactivation from cranial nerve ganglia, followed by a transaxonal spread to arteries, infecting the adventitia first followed by extension transmurally. Whether patients presenting with GCA symptoms who have negative TA biopsies are, instead, manifesting symptoms of

a process driven or triggered by the presence of VZV, has been an explored hypothesis.¹¹

Evidence supporting a role for varicella zoster virus in giant cell arteritis

Investigations into the hypothesis of VZV as a factor for GCA pathogenesis have been reported as early as 2001, when Mitchell and Font set out to determine if there is an association between GCA and VZV on TA histological analysis. Refer to Table 2 for a listing of studies investigating VZV in GCA. In a randomised masked study, 64 specimens were analysed by polymerase chain reaction (PCR) for VZV DNA, including 35 GCA positive and 29 GCA negative biopsies. VZV DNA was found in nine of the 35 GCA positive TA and none of the GCA negative specimens ($p = .010$). Immunohistochemical studies, which were also used to detect the presence of VZV, were positive in several biopsy specimens that demonstrated

Table 2. Studies evaluating VZV in GCA.

Number of Patients	Positive Findings (%)	Negative Findings (%)	Description	Reference
10	0 (0)	10 (10)	PCR and immunohistochemical analyses of formalin-fixed temporal arteries from 10 pathologically verified cases of giant cell arteritis did not reveal varicella zoster virus antigen or DNA.	Nordborg et al., 1998. ¹²
35	9 (26)	26 (74)	PCR was positive in nine (26%) temporal arteries that were histologically consistent with GCA. The remainder of 26 histologically positive temporal arteritis and all 29 of the GCA negative arteries had a negative PCR for VZV, and the differences in these outcomes were statistically significant ($p = .010$). Immunohistological staining did not correlate. Electron microscopy did not detect viral particles. Negative biopsies	Mitchell and Font, 2001. ¹³
30	0	30 (100)	PCR amplification did not detect VZV in any of these patients who were clinically suspected to have GCA. 13 patients had classical GCA, 2 had biopsy negative GCA, 10 had polymyalgia rheumatica	Helweg-Larsen et al., 2002. ¹⁴
15	0	15 (100)	PCR amplification did not detect VZV in 15 patients with histologically proven GCA. 7 controls also did not show VZV.	Kennedy et al., 2003. ¹⁵
50 (total 147, 50 positive for GCA, 97 negative for GCA)	0	50 (100)	PCR amplification did not detect VZV in 50 patients with histologically proven GCA. 97 additional patients with a history compatible with GCA but without histologically proven GCA did not demonstrate VZV on PCR.	Rodriguez-Pla et al., 2004. ¹⁶
57 (total 113, 57 positive for GCA, 56 negative for GCA)	18 (32%) in GCA group had detectable DNA. Median viral load = 0 genomes/ug of DNA	39 (68%) without detectable DNA in GCA group, 38 (68%) in control group	Detectable DNA is as reported in columns 2 and 3, but PCR median viral load for all GCA and control patients was 0 genomes/ug of DNA. Control patients had negative biopsies but were clinically suspect for GCA	Alvarez Lafuente et al., 2005. ¹⁷
37	0	37 (100%)	37 GCA positive biopsies were studied. 66 additional biopsies that were negative for GCA were studied. VZV was not detected by PCR in either group.	Cooper et al., 2008. ¹⁸
24 (all biopsy negative for GCA)	5 (21)	19 (79)	Immunohistochemistry was used to detect VZV gene 63 protein. All patients had negative biopsies for GCA but were clinically suspected to have GCA.	Nagel et al., 2013. ¹⁹
82	61 (74%) by immunohistochemistry 18 by PCR	21 (26%) by immunohistochemistry	Immunohistochemistry of 82 GCA positive biopsies were compared with 13 normal control biopsies. Of the 61 biopsies with detectable VZV antigen, only 45 had amplifiable DNA. On PCR, 18 (40%) of these 45 samples were positive for VZV. Control biopsies were postmortem samples from patients who did not have clinically suspect GCA or VZV. 1 (8%) of the controls had detectable VZV antigen by immunohistochemistry.	Gilden et al., 2015. ²⁰
70	45 (64%)	25 (36%)	45 (64%) of 70 GCA negative biopsies in patients with clinically suspected GCA were positive for VZV on immunohistochemical staining, compared with 11 (22%) of 49 normal biopsies from post mortem controls. PCR was also performed on these samples.	Nagel et al., 2015. ¹¹

(Continued)

Table 2. (Continued).

Number of Patients	Positive Findings (%)	Negative Findings (%)	Description	Reference
11	11 (100%)	0	VZV antigen detected in 11 of 11 aortas with granulomatous arteritis. Also found in 1 out of 1 case of non-granulomatous arteritis and 5 (28%) of 18 control autopsy obtained aortas. Of the 11 samples with granulomatous arteritis, 10 contained amplifiable cellular DNA, and 7 (70%) of these contained VZV DNA on PCR. PCR also confirmed VZV DNA in the 1 non-granulomatous arteritis sample. 4 (80%) of the control aortas had a positive PCR for VZV.	Gilden et al., 2016. ²¹
204 Temporal Arteries	73/104 (70%)-GCA-positive TA. Viral antigen distribution: -Adventitia- 86% -Media- 67% -Intima- 52% 58- contained cellular DNA 23/58 (40%)- contained VZV DNA.	58/100 (58%)- GCA-negative TA. Viral antigen distribution: -Adventitia- 91% -Media- 82% -Intima- 82% 9/11- contained cellular DNA. 3/9 (33%)- contained VZV DNA. 51/58- contained cellular DNA 9/51 (18%)- contained VZV DNA.	11/61 (18%)- normal TA. Viral antigen distribution: -Adventitia- 91% -Media- 82% -Intima- 82% 9/11- contained cellular DNA. 3/9 (33%)- contained VZV DNA.	Gilden et al., 2016. ²²
10 samples from 9 subjects (3 GCA positive, 6 GCA negative)	3 (100%) of GCA positive biopsies 4 (67%) of GCA negative biopsies	0 (GCA positive) 2 (33%) of GCA negative	Blinded study. VZV antigen found in 3(100%) of GCA positive by immunohistochemical staining. PCR amplification detected VZV DNA in one of these 3 samples. 4 (67%) of GCA negative biopsies were positive for VZV antigen on immunohistochemical staining, and 3 of these 4 samples was positive for VZV DNA on PCR amplification. VZV DNA was detected in one GCA negative, VZV antigen negative biopsy.	Gilden et al., 2016. ²³
34 (34 GCA positive, 25 GCA negative, 30 controls)	1 (3%) of GCA positive 0 of GCA negative 0 of controls	31 (97%) of GCA positive 25 (100%) of GCA negative	1 (3%) of 31 GCA positive biopsies was positive for VZV antigen on immunohistochemistry, but all specimens in all three groups were negative for VZV on PCR	Muratore et al., 2016. ²⁴
5 (5 with GCA, 6 controls). 8 thoracic aortas (8 with GCA, 2 with Takayasu arteritis, 6 with focal idiopathic aortitis, 15 with non-inflammatory aneurysms)	0	30 (100%) of controls 11 (100%) temporal artery biopsies 31 (100%) thoracic aorta biopsies	11 temporal arteries (5 with GCA and 6 controls). 31 thoracic aortas (8 with GCA, 2 with Takayasu arteritis, 6 with focal idiopathic aortitis, 15 with non-inflammatory aneurysms)	Procop et al., 2017. ²⁵
25 (25 with GCA positive biopsies, 25 with negative biopsies)	3 (12%) of GCA positive biopsies 0 of GCA negative biopsies	22 (88%) of GCA positive biopsies 25 (100%) of GCA negative biopsies	25 GCA positive biopsies, and 25 negative biopsies in clinically suspected GCA. Of 3 GCA positive biopsies that stained positive for VZV on immunohistochemical staining, one patient had clinical herpes zoster ophthalmicus diagnosed 3 weeks before onset of GCA symptoms. PCR was not used.	Buckingham et al., 2018. ²⁶

adventitial histiocytes-macrophages, but these did not correlate with either the presence of VZV DNA or histological evidence of GCA. The investigators proposed that a possible explanation for presence of VZV only in GCA positive TAs may be an association of VZV with only a subset of GCA cases, or that viral DNA may have been present in levels below detectability in the GCA negative samples.¹³

The difficulty in diagnosing GCA due to the nature of skip lesions may present a challenge for demonstrating an infectious cause. In cases of negative TA biopsies for GCA, the consideration of a distinct clinical condition such as viral infection should be considered. In 2013, Nagel et al. specifically evaluated TAs in patients with clinical symptoms of GCA, but histopathologically negative TA biopsies, for the presence of VZV antigen.¹⁹ The rationale for this investigation had been supported by observations of multifocal VZV vasculopathy with TA infection and involvement of the ophthalmic, ciliary, or retinal arteries.¹⁹ There had also been reports of patients with clinically suspect GCA and negative TA biopsies who were found to have multifocal VZV vasculopathy.²⁷ In this study, Nagel et al. compared 24 specimens from patients with clinically suspect GCA with 13 postmortem specimens from seven normal subjects. VZV antigen was detected in five out of 24 (21%) of the clinically suspected but TA biopsy negative patients. All five of these patients presented with clinical and laboratory features of GCA, including early visual disturbances. Of these five specimens, VZV was found exclusively in the arterial adventitia in two arteries, in the arterial media in another two, and in both media and intima in one artery. There was no evidence of medial necrosis or multinucleated giant cells. The investigators proposed that GCA and multifocal VZV vasculopathy with TA infection have overlapping clinical and laboratory abnormalities. They also postulated that absence of significant inflammation in VZV infected TA specimens most likely reflects early treatment with corticosteroids. The 13 normal controls did not test positive for any evidence of VZV antigen. The authors of this study recommended that, in the case of patients presenting with clinical symptoms of GCA, particularly early visual disturbances and laboratory abnormalities such as elevated

CRP, the diagnosis of multifocal VZV vasculopathy should be considered. In these patients, the TA biopsies should be evaluated not only for histopathological signs of GCA, but also for immunohistochemical evidence of VZV antigen.¹⁹

In a follow-up publication, Nagel et al. described a patient with clinically suspect GCA and a pathologically negative TA biopsy in which VZV was subsequently detected. Not only were VZV antigen and VZV DNA detected in the TA but they were also detected in the skeletal muscle attached to the TA. Of note, the pattern of detection on virological examination was a skip pattern, akin to the pathological findings seen in GCA. Additionally, the subsequent histopathological examination of sections adjacent to VZV positive areas revealed changes characteristic of GCA. This patient, in particular, had received steroids and developed multiple strokes, suggesting that steroids may have potentiated the viral infection to lead to further strokes consistent with a multifocal VZV vasculopathy. The authors of this report proposed that there may be a spectrum of GCA triggered by VZV reactivation and transaxonal spread to the artery.²⁸

Another study published in 2015 by Nagel et al. evaluated the rate of VZV infection in TA biopsies of patients with clinically suspected GCA in both patients with positive and negative histopathological changes compared with age-matched postmortem controls. Forty-five of the 70 GCA negative TA biopsies (64%) and 68 of the 93 GCA positive specimens (73%) had evidence of VZV antigen in clinically suspected GCA compared with 11 of 49 specimens from normal controls (22%).²⁹ VZV antigen was 2.86 and 3.27 times more likely to be present in GCA negative and GCA positive TA biopsies from patients with clinical GCA compared with controls. Additionally, VZV antigen was more likely to be present in the adventitia of TA biopsies from patients with suspected GCA regardless of whether they were histopathologically positive or negative for GCA. Furthermore, in GCA negative specimens, VZV antigen was predominantly detected around nerve bundles and in the vasa vasorum.

Nagel et al. concluded that, in patients with clinically suspected GCA, the presence of VZV in the biopsies was similar, independent of whether biopsy results were negative or positive for GCA, and that these observations may represent

a continuum of VZV vasculopathy in the TA.²⁹ The investigators also proposed that the findings of VZV antigen around nerve bundles supports the hypothesis that the virus spreads transaxonally, infecting the adventitia first. For individuals with clinically suspected GCA but with negative TA biopsies, lack of histopathological findings may represent early disease in which VZV initially infects the adventitia followed by the development of inflammation that spreads transmurally, with accumulation of lymphocytes, giant cells or epithelioid macrophages, and medial damage.³⁰

Gilden et al. looked more rigorously for VZV antigen in TA biopsies containing histopathological changes of GCA and also showed a high rate of VZV presence. Eighty-two GCA positive TA biopsies were compared with 13 normal TA biopsies obtained from postmortem age-matched controls. The controls did not have any history suggestive of GCA or VZV. VZV antigen was found in 61 (74%) of the GCA positive TA biopsies compared with one (8%) of the control TA biopsies ($p < .0001$, relative risk 9.67). The investigators also noted that VZV antigen was found in 38% (12 of 32) of skeletal muscle samples adjacent to VZV antigen-positive TAs.²⁰ However, as will be discussed later in this review, observations of positive VZV antigen on immunohistochemistry of muscle tissue are likely to be false positives.

Additionally, GCA histopathology was observed in 89% of GCA positive TA biopsies in sample sections adjacent to those containing VZV antigen. However, these findings were not present in adjacent sections from the control group. VZV also demonstrated a skip pattern in most samples. Gilden et al. recommended staining more than 10 sections to improve reliability in detecting VZV antigen, even predicting that analysis of hundreds of sections of each TA will reveal VZV in every GCA positive TA biopsy.¹¹ It is important, however, to note that other studies that failed to reproduce similar trends did use methods of examining at least 10 sections for every 1 cm of TA biopsy. In this study, no clinico-virological correlation could be made regarding the presence of VZV in GCA positive TA specimens and a clinical history of zoster infection, zoster immunisation, or treatment.²⁰

VZV vasculopathy has been well documented in cerebral vessels, but involvement of other large

vessels such as the aorta has also been demonstrated. Gilden et al. published results of VZV antigen detected in all of 11 aortas with pathologically verified granulomatous arteritis, of which nine manifested as aortic aneurysms. In addition, one case of non-granulomatous arteritis, and five of 18 control aortas obtained at autopsy demonstrated VZV antigen. VZV antigen was 3.60 times more likely to be present in aortas with granulomatous arteritis than in control aortas. Ten out of 11 VZV antigen positive granulomatous arteritis contained amplifiable cellular DNA, of which seven (70%) contained VZV DNA. VZV was evident in all arterial layers of the aorta, and the investigators proposed that, similar to the transmural spread seen in GCA, VZV infects the aorta from thoracic sensory ganglia where latent VZV is abundant. In turn, this process can then weaken the structure, leading to aneurysms and strokes.²¹

In a 2016 publication, Gilden and colleagues reported the detection of VZV antigen in both GCA positive and negative TAs of patients. It is important to note that they did acknowledge that VZV reactivates in patients over the age of 50, and this may be a subclinical phenomenon given the lack of inflammatory changes seen on histopathological examination. It is also important to recognise that this investigation was a cumulative study that included results reviewed earlier in this article by both Drs Gilden and Nagel.^{20,29} In this retrospective study, they detected VZV antigen in 73 out of 104 (70%) GCA positive and 58 out of 100 (58%) GCA negative TAs compared with 11 out of 61 (18%) autopsy-obtained control specimens. Fifty-eight of the GCA positive, VZV antigen positive TAs contained cellular DNA; 23 of these specimens (40%) contained VZV DNA. Fifty-one of the 58 GCA negative VZV antigen positive TAs contained cellular DNA, and nine of these 51 (19%) contained VZV DNA. Of the 11 VZV antigen positive controls, nine contained cellular DNA, of which three (33%) contained VZV DNA. VZV antigen was 3.89 times more likely to be present in GCA positive TAs than in normal TAs and 3.22 times more likely to be present in GCA negative TAs than in normal TAs. VZV antigen was detected in multiple arterial layers with a predominance in the adventitia rather than media and intima in all patient groups suggesting transaxonal transport of virus along nerve fibres.²²

Evidence against a role for varicella zoster virus in giant cell arteritis

The question of the role of VZV in GCA pathogenesis is not a new one, and has been in existence as early as the 1990s.³⁰ It should be acknowledged that the current treatment options for GCA carries substantial risk for side effects including osteoporosis, osteonecrosis, diabetes mellitus, weight gain, and increased risk of infection, making it attractive to pursue an alternate treatment strategy such as treatment of an underlying infection. However, despite reports that suggest a role of VZV in the pathogenesis of GCA, the existing medical literature is inconsistent in establishing VZV as a definitive cause of GCA. This lack of data does not justify changing current standard of treatment. However, the reports of VZV in patients suspected of having GCA but who have negative TA biopsies, as described earlier, does raise the possibility of VZV playing a role in GCA.

Decreased cell-mediated immunity to VZV suggests an increased herpes zoster risk in GCA patients compared with an already at risk elderly population. However, similar levels of VZV IgG antibodies are found in GCA patients at the time of diagnosis compared with age-matched healthy controls, indicating that GCA patients do not experience herpes zoster more often in the months preceding diagnosis when compared with controls.³¹ Additionally, epidemiological data has not supported a relationship between the incidence of GCA and VZV reactivation in newly diagnosed GCA amongst patients previously immunised against VZV, compared with non-immunised patients.³² One study in Israel reported a GCA mean incidence of 41.6/100,000/year, varying between 40.9 and 46.7 non-immunised patients per 100,000 per year. For patients previously immunised against VZV, the mean incidence of GCA was 75.2/100,000 in immunised patients. The difference between the two means was not statistically significant ($p = .07$). Another study evaluating the published incidence rates of GCA and VZV in 14 different countries did not find a significant correlation between the incidence of the two conditions.³³

Aside from the inconsistencies in data and the lack of epidemiological support, other questions

remain unanswered regarding the role of VZV in GCA. A neural hypothesis suggests that, with VZV reactivation in the trigeminal ganglia, virus in the ophthalmic division neurons spreads by sensory pathways both to the face and to the cerebral arteries. This phenomenon may explain the restriction of central nervous system arteritis to ipsilateral vessels. However, the presence of VZV in the trigeminal ganglion or trigeminovascular pathways has not yet been demonstrated in arteritis cases. Additionally, the delay of weeks or months between zoster onset and symptoms of arteritis is not clearly explained by the known nature of how neurotropic herpes viruses spread along neural pathways.³⁰

A case report of herpes zoster ophthalmicus with absence of dermatological manifestations has been reported, in which unremitting unilateral eye pain led to PCR testing for VZV, which was positive. Cases like this, in which the symptoms can be confused with those for GCA, can certainly raise the question of whether VZV is the underlying cause in selected cases, but does not demonstrate a causal relationship.³⁴ Similar findings were reported by Fukumoto et al. in a patient with subarachnoid haemorrhage and granulomatous angiitis of the basilar artery, in which electron microscopy demonstrated VZV viral antigen.⁵ However, the distinction here still needs to be determined as to whether these are true GCA cases in which VZV is found to be either the cause, directly or indirectly, of the vasculitis, or whether VZV related vasculitis should be considered a completely separate entity from genuine autoimmune or primary GCA.

There have been several investigations that failed to yield evidence of VZV infection in GCA patients. Nordborg et al. performed PCR and immunohistochemical analyses of formalin-fixed TAs from 10 biopsy confirmed cases of GCA, which did not reveal VZV antigen or DNA.¹²

In a study mentioned earlier in this review, Mitchell and Font observed that PCR for VZV was positive in nine out of 35 (26%) TAs that were histologically consistent with GCA.¹³ Although immunohistochemical analysis demonstrated presence of virus in some of the samples examined, its presence did not correlate with either the presence or absence of VZV DNA on

PCR, nor with histological evidence of GCA. Despite the investigator's findings of positive PCR results in 26% of patients with histological evidence of GCA, there remained 74% of cases that were unaccounted for. This discrepancy suggests that even if VZV might play a role in a subset of GCA patients, it does not explain a majority of GCA cases and that the VZV positivity may be an incidental finding. In this study, there was also a discordance between PCR and immunohistochemical staining results, and electron microscopy did not detect viral particles in several different specimens.

In the study by Gilden et al., which identified VZV in 100% of 11 aortas with pathologically-verified granulomatous arteritis, the antigen was also found in five (28%) out of 18 control aortas obtained at autopsy,¹³ which weakens an argument for its pathogenic role in the non-control specimens.

Other studies have failed to demonstrate VZV in temporal arteries of GCA patients. In a study utilising PCR, Cooper et al. did not detect VZV in 37 TA biopsies that were positive for GCA and 66 that were negative for GCA.¹⁸ Helweg-Larsen et al. also evaluated 30 TA biopsy specimens of patients suspected of having GCA. Of these patients, 13 had classic GCA, two had biopsy-negative GCA, and 10 had polymyalgia rheumatica. PCR assays did not demonstrate any of the eight human herpesvirus DNA in any of the samples. The human herpesviruses they tested for included the herpes simplex viruses HSV-1 and -2, Epstein Barr virus, cytomegalovirus, and VZV.¹⁴ Rodriguez-Pla et al. also searched for evidence of parvovirus B19 and other herpes viruses including HSV-1 and -2, Epstein Barr virus, cytomegalovirus, human herpesvirus 6, and VZV. Of the 50 TA biopsies that were histologically positive for GCA and the 97 that were negative for GCA, all of the herpes virus PCR studies were negative.¹⁶ The results of this relatively large study makes a compelling argument against the role of any of these viruses in the development of GCA.

While there is a possibility that PCR testing for these viruses in TA biopsies is not sensitive enough to detect virus DNA or that technical errors may cause false negative results, these occurrences are highly unlikely, since investigators used control samples to exclude false

negative results. When Kennedy et al. also sought out to find VZV in 15 TA biopsies of patients with histologically proven GCA, they did not demonstrate its presence. In this study, the investigators also evaluated TAs from seven normal control subjects and human trigeminal ganglion tissue from a positive control with latent infection.¹⁵ The authors acknowledged that the most likely reasons for such discrepancies in findings are related to the particular molecular technique used, including the sensitivity of the assays and also the possibility of sampling error. In the study completed by Kennedy et al. *in situ* hybridisation technique used can be expected to detect a minimum of about 10 copies of viral DNA/cell, and the more sensitive *in situ* PCR amplification technique, as little as one copy of viral DNA/cell. The double-PCR assay used by other investigators is reported to be more sensitive, with an estimated two log increase in the sensitivity and the ability to detect one copy of VZV DNA per nanogram of total DNA. However, even the least sensitive of these techniques should be able to detect VZV DNA, if VZV infection plays a role in the histopathological changes found in GCA.¹⁵

In another study conducted by Alvarez Lafuente et al., which looked at parvovirus B19, VZV, and human herpesvirus 6 in GCA, they reported a similar prevalence of detectable VZV DNA in the GCA and control groups. Of 57 samples with biopsy-proven GCA, 18 samples (32%) had detectable VZV DNA compared with 18 samples (32%) in the 56 patients of the control group. However, further testing with PCR showed a mean viral load of 0 genomes/ μ g in both groups.¹⁷ These investigators did report a higher prevalence and median viral load for parvovirus B19 in GCA positive patients, but as is the case with VZV, these findings have not been reproduced consistently in the medical literature.

Serum testing for IgG and IgM antibodies may be another useful aspect in examining for a relationship between VZV and GCA. A positive IgM antibody at the time of the GCA manifestations may point toward a role of VZV in the pathogenesis of GCA. However, in the presence of a positive IgG antibody and negative IgM antibody, VZV as a trigger for an autoimmune process

that persists after the active phase of the infection has resolved, cannot be ruled out.¹⁶ Another possible explanation for inconsistencies in reported findings is that VZV may play a role in the pathogenesis of GCA in some patient populations but not others.²⁶

Despite the positive findings of Gilden et al. and Nagel et al., recent investigations have failed to replicate similar outcomes. Muratore et al. evaluated TA biopsies of GCA positive, GCA negative, and control TA biopsies in an Italian population. Only one out of 33 GCA positive biopsies was positive on immunohistochemistry for VZV antigen. None of the 15 GCA negative biopsies and 30 control samples were positive for VZV antigen, and PCR was negative for VZV DNA in all three groups.²⁴ Procop et al., also seeking to reproduce the findings of Gilden and Nagel, found that none of their surgically sterile samples of TA and thoracic aortic biopsies from patients with GCA, Takayasu's arteritis, focal immune idiopathic aortitis, or non-inflammatory aortic aneurysms, were positive for VZV on PCR, using two validated PCR amplification kits.²⁵ Based on their inability to reproduce previously published positive findings, the investigators advised caution in considering the use of anti-VZV therapy for large vessel vasculitis.

The failure of different groups to reproduce findings of VZV in biopsies of patients with GCA should raise caution about the possibility of false positives when interpreting studies that report a relationship between VZV and GCA.

The most recent study looking at VZV in TA biopsies by Buckingham et al. observed positive immunohistochemical staining for VZV antigen in three out of 25 GCA positive biopsies and none of 25 GCA negative biopsies.²⁶ Interestingly, the investigators observed that the zoster specific immunohistochemical assays appeared to give a false positive result in the presence of TA calcification. They confirmed that these sites also did not contain zoster antigen by performing the assays without the primary anti-VZV antibody and again demonstrated false-positive staining. Additionally, they observed that several TA biopsies contained concentrated areas of erythrocytes within the lumen or in disrupted layers of the arterial tissue, which also demonstrated falsely positive for VZV

antigen. These outcomes are in noteworthy contrast to those of Gilden et al. and Nagel et al. They also suggested an explanation for the observations of positive VZV testing that have been observed in TA biopsies.

Buckingham et al. pointed out several problematic issues with the approach of looking for VZV DNA in TA biopsies. They noted that in prior studies, the percentage of arteries positive for VZV DNA was always lower than the percentage positive by immunohistochemical staining, and that in studies, such as those described earlier by Kennedy et al. and Rodriguez-Pla et al.,^{15,16} TA biopsies were uniformly negative in cohorts of up to 65 patients. Also, TAs are known to be innervated by nerve fibres from ganglia that harbour latent VZV, and therefore, at any time a small number of TAs may be positive for VZV DNA. In their opinion, the immunopathology for which GCA is known could not be elicited by VZV infection without the presence of VZV antigen. In addition, the investigators made a sound argument that the attachment of VZV antibody to skeletal muscle resulting in positive immunohistochemical staining for VZV antigen, is actually a false positive. This claim is based on existing data showing that HSV-1 cannot infect muscle fibres.^{35,36} The inability of HSV-1 viral vectors, and other vectors such as retroviral and adenoviral vectors, to be delivered into muscle cells is due to the physical barrier posed by the basal lamina in mature muscle fibres. HSV-1 has a wider tropism for different tissues than VZV, and it is reasonable to conclude that VZV also cannot infect myofibres. It is also therefore possible that positive findings for VZV antigen in skeletal muscle found on TA biopsies are false positives caused by a non-specific attachment of the monoclonal antibody utilised for testing. Finally, the authors pointed out that prior published studies have been retrospective, with the use of formalin-fixed paraffin-embedded biopsies of TAs, which may be suboptimal for PCR testing. For this reason, their method was to prospectively divide the TA biopsies using a non-formalin fixed specimen to optimise the PCR testing.²⁶

Despite efforts to enhance thorough and systematic methods, thereby minimising false negatives, the results of the study conducted by Buckingham et al. did not demonstrate

a relationship between VZV and GCA. Moreover, these investigators utilised a power analysis based on prior reports of greater than 70% TA biopsies from GCA patients exhibiting positive tests for VZV antigen.¹¹ This required a minimum of 12 TA biopsies from 12 GCA patients, but their study included 25 patients. Notwithstanding this larger sample size, they were still unable to come to the same conclusion as earlier reports.²⁶

Treatment implications of GCA being a VZV vasculopathy

An important question regarding the implications for treatment of GCA is raised in light of these findings. Current practice is to treat patients with clinically suspected, as well as confirmed GCA with high doses of corticosteroids, which are continued for months to years and are associated with their own potential serious side effects. In his 2015 publication, Gilden et al. introduced the idea of dual treatment with intravenous acyclovir and corticosteroids, as they suggested that GCA is a VZV vasculopathy.¹¹ They suggested that steroid refractory cases of GCA may be due to the long-term potentiation of VZV infection by steroid treatment. Currently, however, case reports of patients with GCA treated with antivirals are anecdotal. Whether combination antiviral and steroid agents are as effective as steroids alone in GCA patients is yet to be determined. However, the available data, given the inability to reproduce positive findings, does not warrant a change in the treatment approach for GCA.

Conclusion

There are several plausible reasons to explore the hypothesis that an infectious aetiology may be the cause for a granulomatous arteritis such as GCA. VZV has received some of that attention in recent years, and based on case reports and studies described in this review, it is important to evaluate the claims that VZV may either be a direct cause of GCA as a manifestation of an active infection – or in an alternative scenario, that it may be the trigger for the autoimmune activity observed in GCA. It appears from both

the discussions for and against a role of VZV in GCA pathogenesis, that VZV is unlikely to be responsible for all cases of GCA. The findings reported by Nagel et al. in their GCA biopsy negative cohort suggests that this particular population may represent a clinical scenario in which a more rigorous evaluation for VZV exposure should be pursued.¹¹

Ways in which VZV may trigger vasculitis, as well as reasons for its seemingly evasive nature of detection if VZV is indeed a trigger for GCA, have been proposed by investigators. One mechanism for how VZV may induce an autoimmune-mediated vasculitis lies in the downregulation of programmed death ligand 1 expression in infected cells, particularly immune cells, fostering persistent inflammation in vessels and pathological vascular remodelling during VZV vasculopathy.³⁷ Why substantial inconsistencies exist in the findings reported by clinical researchers, raises its own collection of questions and opportunities for inquiry. As proposed by Mitchell and Font, the absence of viral particles would not necessarily exclude the presence of viral DNA or antigens, because some viral DNA and viral antigens, including the protein IE-63, are present during latent infections when viral replication and production of the virus particles are limited.⁸ In addition, the results reported by Buckingham et al. present an important issue of whether false-positive findings for viral antigen are an artefact of arterial calcification rather than a representation of true VZV infection.

Although some groups such as Nagel et al. and Gilden et al. have been able to show, in multiple investigations, that VZV is detected in a significant proportion of both negative and positive TA biopsies in patients with clinical manifestations of GCA, we present in this review many other studies that fail to replicate their findings. It would be premature to dismiss the positive findings completely, however, because there still remains room for improvement in the consistency of methods used for exploring this hypothesis, as pointed out by Buckingham et al. Prospective studies that incorporate masking of the pathologist interpreting specimens would be highly valuable. In addition, the methods employed for biopsy preparation should account for false positives potentially caused by arterial calcification. It should also be established what specific preparations of the

specimens are most likely to optimise PCR testing, for example, formalin fixed seen in retrospective studies, versus non-formalin fixed.

In their 2001 publication, one of the earliest to look at VZV in GCA, Mitchell and Font wrote, “Whether the association of VZV DNA and GCA is causal or casual cannot be determined from our findings. It is plausible that GCA and its associated granulomatous inflammation are related to the VZV DNA’s becoming detectable. Another possibility is that the VZV DNA is only incidentally associated with GCA”.⁸ The evidence, to date, has not completely ruled out either of Mitchell and Font’s proposed possibilities. However, while a review of the published data does raise the importance of standardised prospective studies to further explore this question, it does present the need to evaluate a patient with possible GCA for an infection such as VZV, particularly in cases where the TA biopsy is negative for GCA and when the patient does not respond to traditional treatment for GCA. In cases, where an evaluation for VZV is positive, and particularly when a patient exhibits clinical symptoms of a VZV infection, anti-viral treatment would be warranted. A recently published expert debate and review on whether anti-VZV treatments should be used in patients with GCA proposed three possibilities on the association between VZV and GCA: 1) that VZV is an ‘innocent bystander’ seen in patients with GCA since nearly all humans carry VZV lifelong; 2) that VZV may possibly trigger GCA though the this relationship is not clear; or 3) that VZV actually causes GCA. However, these authors reached a similar conclusion as we do, that there is insufficient evidence to support a direct causation theory.³² We are in agreement with these authors, that until future investigations can consistently support a role for VZV in GCA, treatment for VZV in patients with GCA would not be supported by the evidence found in current medical literature.

Declaration of interest

No potential conflict of interest was reported by the authors.

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