

CONCISE REPORT

Human parvovirus B19, varicella zoster virus, and human herpes virus 6 in temporal artery biopsy specimens of patients with giant cell arteritis: analysis with quantitative real time polymerase chain reaction

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Objective: To evaluate the role of parvovirus B19 (B19), varicella zoster virus (VZV), and human herpes virus 6 (HHV-6) in the aetiopathology of giant cell arteritis (GCA).

Methods: Temporal artery biopsy specimens from 57 patients with GCA and 56 controls were investigated. DNA was obtained by biopsy, and quantitative real time polymerase chain reaction assay performed to establish the prevalence and viral load of B19, VZV, and HHV-6. Amplification of the human β -globin gene was used as internal positive control.

Results: (a) B19 was detected in 31/57 (54%) patients (median viral load 45.2 (25th–75th centiles 0–180.2) copies/ μ g DNA) v 21/56 (38%) controls (median viral load 0 (0–66.7) copies/ μ g of DNA; $p=0.07$ for DNA prevalence, $p=0.007$ for viral load. Among 31 B19 positive samples, 21 (68%) patients with biopsy proven GCA had $>10^2$ B19 copies/ μ g of DNA v 5/21 (24%) controls; $p=0.001$. (b) No significant difference was found for VZV ($p=0.94$ for DNA prevalence; $p=0.76$ for viral load) and HHV-6 ($p=0.89$ for DNA prevalence; $p=0.64$ for viral load) in the GCA group compared with controls.

Conclusion: B19 may have a role in the aetiopathology of GCA, particularly in those patients with high viral load; no evidence was found for VZV and HHV-6.

PATIENTS AND METHODS

Patients and specimens

We examined TAB specimens from 113 patients suspected of having GCA. Patients, before biopsy, had taken either no drugs for GCA or corticosteroids for less than 2 weeks. Fifty seven patients (38 women) had biopsy proven GCA (GCA group) and 56 patients (40 women), had no histological evidence of vasculitis (control group). The mean (SD) age of the GCA group was 78 (8.7) years and of the controls 77.6 (7.9) years; $p=0.75$. Patients were recruited from the rheumatology service of Hospital Clinico San Carlos (HCSC), Madrid. TAB specimens were collected at the pathology service of HCSC. The investigators who carried out the DNA analysis did not know the clinical and histological diagnoses. The study was approved by the local ethics committee.

DNA extraction

Ten sections 4–6 μ m thick taken from each biopsy specimen were deparaffinised by incubating for 5 minutes each in two changes of xylene and for 5 minutes in absolute ethanol. The specimens were dried and rehydrated in distilled water. The DNA was then extracted using Qiagen columns (QIAamp DNA Mini Kit; Qiagen Inc), according to the protocols supplied by the manufacturer; then was quantified by spectrophotometry. In all samples, DNA extraction was successful, as confirmed by the presence of sufficient and non-degraded human DNA.

Quantitative real time PCR

The DNAs were analysed by quantitative real time polymerase chain reaction (q-rtPCR) for the presence of B19, VZV, and HHV-6 genomes and the human β -globin gene as internal control (to ensure that DNAs were suitable for DNA amplification). Primers, TaqMan probes, and reaction conditions for the q-rtPCR assay for VZV,⁹ HHV-6,¹⁰ and β -globin¹¹ have been described previously. A commercial kit (Artus B19 RG RealArt PCR kit) was used for the B19 assay. For each virus three reactions were carried out. The quantitative assessment was performed in a Rotor-Gene 2000 real time cyler (Corbett Research, Sydney, Australia). Cycling conditions were preincubation at 95°C for 15 minutes to activate AmpliTaq gold DNA polymerase, then two step thermocycling (denaturation and anneal/extension) was performed for 40 cycles. The q-rtPCR for B19 was carried out according to the manufacturer's protocol. With these

Recent studies have suggested that persistent infections may be involved in the pathogenesis of giant cell arteritis (GCA), although evidence has been inconclusive. GCA is a disease of unknown aetiology characterised by acute inflammation affecting medium and large arteries in elderly people, with a predilection for branches of the temporal arteries. Multiple environmental and genetic factors probably play a part in susceptibility to GCA.¹ Although certain HLA-DR alleles are correlated with GCA,² genetic factors are insufficient to explain the marked variation in geographic prevalence³ and the temporal fluctuation in the incidence of the disease.¹ Different findings suggest that infectious agents may be involved in the pathogenesis of the disease: parvovirus B19 (B19),^{4,5} human herpes virus 6 (HHV-6),⁶ and varicella zoster virus (VZV).^{7,8}

This study aimed at determining B19, VZV, and HHV-6 DNA prevalence and viral loads in temporal artery biopsy (TAB) specimens of patients with GCA, to analyse the hypothesis that an environmental factor may have a role in the pathogenesis of GCA.

Abbreviations: B19, parvovirus B19; GCA, giant cell arteritis; HHV-6, human herpes virus 6; q-rtPCR, quantitative real time polymerase chain reaction; TAB, temporal artery biopsy; VZV, varicella zoster virus

Table 1 DNA prevalence of B19, VZV, and HHV-6 in TAB specimens of patients with GCA and in control group

	GCA (n=57)	CG* (n=56)	p
B19	31 (54)	21 (38)	0.07
HHV-6	20 (35)	19 (34)	0.89
VZV	18 (32)	18 (32)	0.94

Results are shown as No (%).

*CG, control group

conditions, the sensitivity of the assay was one copy for all the viruses and the internal control.

The final DNA copy number was determined by calculation of the number of PCR cycles necessary for a standard curve of known amounts (10 000, 1000, 100, 10, and 1 DNA copies) of purified and quantified B19, VZV, and HHV-6 DNA to cross a fluorescent threshold and interpolation of the unknowns. Each point was obtained in triplicate and we repeated the standard curve for each of the PCRs.

These data were sent to a desktop PC and were displayed in real time on the screen as fluorescence versus cycle number. Threshold cycle or computed tomography and quantification of DNA for each sample (standard curve points and unknowns) were performed by the software provided with the Real-Time Cycler Rotor-Gene 2000.

Data analysis

Categorical variables are presented as a frequency distribution and quantitative variables as a median. The χ^2 test was used to compare categorical variables. Differences between groups were analysed using the Mann-Whitney U test for quantitative variables. A two sided p value of 0.05 was the criterion for significance in all cases.

RESULTS

Comparative study of B19, VZV, and HHV-6 DNA prevalence

Table 1 shows the DNA prevalence of B19, VZV, and HHV-6 in TAB specimens collected from patients with biopsy proven GCA and from controls. B19 DNA was detected in 31/57 (54%) TAB specimens with histological evidence of GCA, and in 21/56 (38%) TAB specimens with no histological evidence of GCA; this difference was almost significant ($p = 0.07$). The DNA prevalence of VZV and HHV-6 was similar in the GCA and control groups.

Comparative study of B19, VZV, and HHV-6 viral load

There was a significant difference (table 2) in B19 median viral load between TAB specimens in patients with biopsy proven GCA (45.2 genomes/ μg of DNA) and TAB specimens in the control group (0 genomes/ μg of DNA; $p = 0.007$). Among the patients with GCA we found two TAB specimens with $>10^3$ B19 genomes/ μg of DNA (table 3). In 19 (61%) patients with biopsy proven GCA the viral load ranged between 10^2 and 10^3 B19 genomes/ μg of DNA, compared with only five (24%) patients in the control group; $p = 0.001$.

For HHV-6 and VZV, we found a median viral load of 0 genomes/ μg of DNA for both viruses, in both groups. We did not find any TAB specimen with more than 10^3 genomes/ μg of DNA for HHV-6 or VZV (table 3).

DISCUSSION

Parvovirus B19 is a non-enveloped icosahedral DNA virus discovered fortuitously in 1975 in the United Kingdom. In recent years, there has been controversy about the

Table 2 B19, VZV, and HHV-6 viral load in patients with GCA and control group

Viral load*	GCA	CG	p
B19	45.2 (0–180.2)	0 (0–66.7)	0.007
HHV-6	0 (0–47.8)	0 (0–37.7)	0.64
VZV	0 (0–25.8)	0 (0–26.4)	0.76

*Viral loads are expressed in genomes/ μg of DNA as the median (25th–75th centiles).

CG, control group.

epidemiological data of studies that analysed the role of B19 in the aetiology of GCA, the most common form of systemic vasculitis in adults, which preferentially affects large and medium sized arteries in patients over the age of 50. One case of B19 persistent infection in a patient with GCA with high titres and presence of viral genomes in the blood was reported by Staud and Corman in 1996.¹² In 1999 a study by Gabriel *et al* was published supporting the potential role of B19 in the disease.⁴ They examined TAB tissue from 50 patients using PCR and their results indicated a statistically significant association between histological evidence of GCA and the presence of B19 DNA in TAB tissue ($p = 0.0013$). However, other authors did not reach the same conclusions^{13–14}: Salvarani *et al* reported that B19 seems to be an innocent bystander commonly present in the arteries of elderly people,¹³ and Helweg-Larsen *et al* investigated 30 temporal artery biopsy specimens and the result was negative in all of them.¹⁴

In this study we describe a significant association between the high viral load of B19 in temporal arteries and histological evidence of GCA. To our knowledge, this study is the first to investigate the B19 viral load in the temporal arteries of patients with GCA by q-rtPCR. Although a clear tendency was observed for a higher prevalence of B19 DNA in patients with GCA (54%) compared with controls (38%), it did not reach significance ($p = 0.07$). However, when we analysed the B19 viral load of the 31 patients with biopsy proven GCA with B19 genomes in their TAB specimens (table 3), two (6%) had high levels of viral load ($>10^3$ copies/ μg of DNA), 19 (61%) had 10^2 – 10^3 B19 copies/ μg of DNA, and 10 (32%) 10^1 – 10^2 copies/ μg of DNA. Among 21 patients without histological evidence of vasculitis, only five (24%) had 10^2 – 10^3 B19 copies/ μg of DNA. The difference in the number of specimens with more than 10^2 copies between both groups is significant ($p = 0.001$). Nevertheless, our findings did not confirm any association between histological evidence of GCA and the presence of VZV and HHV-6 DNA in TAB tissue. Interestingly, we found that about 30% of TAB specimens, with the exception of 54% in the case of B19 in the GCA group, were positive for some viral DNA. This high prevalence of viral DNA might be related to the advanced age of patients and/or to the particular characteristics of arterial tissue but, in most cases, viruses seem to be only innocent bystanders.

In any event, B19 DNA seems to be commonly present in TAB specimens of elderly people with GCA, where it probably persists for years or decades after primary infection; this hypothesis is further supported by the finding that the cellular receptor for B19 (erythrocyte P antigen) is also expressed on endothelial cells.¹⁵ However, although the virus is probably latent in the control group, among patients with biopsy proven GCA a reactivation of B19 latent infection may be a possible disease trigger, particularly in those patients with high viral load.

More effort is needed to understand the mechanisms of the viral reactivation and their relationships with the inflammatory process of the disease: does B19 reactivation lead to

Table 3 B19, VZV and HHV-6 viral load ranges in positive patients with GCA and controls

Viral load range*	B19		HHV-6		VZV	
	GCA (n=31)	CG (n=21)	GCA (n=20)	CG (n=19)	GCA (n=18)	CG (n=18)
10 ¹ -10 ²	10 (32)	16 (76)	15 (75)	16 (84)	14 (78)	18 (100)
10 ² -10 ³	19 (61)	5 (24)	5 (25)	3 (16)	4 (22)	0 (0)
>10 ³	2 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

*Viral loads are expressed in genomes/ μ g of DNA, No (%).
CG, control group.

inflammation or inflammation leads to B19 reactivation? Further studies are required to evaluate the exact role of B19 in the aetiopathology of GCA.

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