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Clinical short communication

Blinded search for varicella zoster virus in giant cell arteritis (GCA)-positive and GCA-negative temporal arteries

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ABSTRACT

Recent analysis of archived temporal arteries (TAs) acquired from 13 pathology laboratories in the US, Canada, Iceland, France, Germany and Israel from patients with pathologically-verified giant cell arteritis (GCA-positive) and TAs from patients with clinical features and laboratory abnormalities of GCA but whose TAs were pathologically negative (GCA-negative) revealed VZV antigen in most TAs from both groups. Despite formalin-fixation, VZV DNA was also found in many VZV-antigen positive sections that were scraped, subjected to DNA extraction, and examined by PCR with VZV-specific primers. Importantly, in past studies, the pathological diagnosis (GCA-positive or -negative) was known to the neurovirology laboratory. Herein, GCA-positive and GCA-negative TAs were provided by an outside institution and examined by 4 investigators blinded to the pathological diagnoses. VZV antigen was found in 3/3 GCA-positive TAs and in 4/6 GCA-negative TAs, and VZV DNA in 1/3 VZV antigen-positive, GCA-positive TAs and in 3/4 VZV antigen-positive, GCA-negative TAs. VZV DNA was also detected in one GCA-negative, VZV-antigen negative TA. Overall, the detection of VZV antigen in 78% of GCA-positive and GCA-negative TAs is consistent with previous reports on the prevalence of VZV antigen in patients with clinically suspect GCA.

1. Introduction

Primary varicella zoster virus (VZV) infection causes chickenpox (varicella), after which virus becomes latent in ganglionic neurons along the entire neuraxis. In aging and immunocompromised individuals, a decline in cell-mediated immunity to VZV manifests clinically as zoster (shingles), dermatomal distribution pain and rash. Zoster may also be complicated by meningoencephalitis, myelitis and VZV vasculopathy, all of which are due to viral replication in infected tissue. Although intracerebral VZV vasculopathy has been recognized for decades, the discovery of VZV infection in extracerebral temporal arteries (TAs) of GCA-positive patients [1,2] and in patients with clinical features and laboratory abnormalities of GCA but whose TAs were pathologically negative [4] is recent. All virologic analyses from 2013 to 15 were conducted on GCA-positive and GCA-negative TAs in which the pathology report was known. In the present study, we searched for VZV antigen and VZV DNA in 10 archived TAs, but were masked to the pathological diagnosis of GCA-positive vs. GCA-negative.

2. Materials and methods

2.1. Human temporal arteries

One investigator (B.J.K.) provided 10 de-identified TAs from 9 subjects to the neurovirology laboratory at the University of Colorado for VZV analysis. Of the 10 TAs, 3 were GCA-positive and 7 were GCA-negative. A portion of all 10 TAs was formalin-fixed and paraffin-embedded (FFPE) for immunohistochemical analysis, and the remainder of 9 TAs was frozen in RNA*later*® Stabilization Solution (ThermoFisher Scientific, Waltham, MA) at −80 °C for nucleic acid analysis. All investigators in the neurovirology laboratory (D.G., M.A.N., T.W. and N.K.) were blinded to the diagnosis of GCA-positive or GCA-negative TA.

2.2. Immunohistochemical analysis of GCA-positive and GCA-negative TA biopsies

One-hundred five-micrometer sections of all FFPE TAs were cut and alternate sections were analyzed immunohistochemically using mouse monoclonal *anti*-VZV gE IgG1 antibody (Santa Cruz Biotechnology, Dallas, TX) and control mouse IgG1 antibody (Dako, Carpinteria, CA). Positive controls consisted of VZV-infected cadaveric cerebral arteries maintained for 14 days *in vitro* and immunostained with mouse *anti*-VZV gE IgG1 antibody, as previously described [3]. Using light

Abbreviations: TA, temporal artery; GCA, giant cell arteritis; VZV, varicella zoster virus; FFPE, formalin-fixed and paraffin-embedded.

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microscopy, 4 blinded readers (D.G., M.A.N., T.W. and NK) examined each section. A TA section was deemed positive or negative for VZV antigen only when all readers agreed.

2.3. PCR amplification of VZV DNA in FFPE TA sections containing VZV antigen

From each of the 7 TAs containing VZV antigen, all VZV antigen-positive sections were scraped with a scalpel and pooled, after which DNA was extracted and analyzed by PCR for the presence of VZV DNA as described [3]. Negative controls were provided by replacing template DNA with PCR-grade water.

2.4. PCR amplification of VZV DNA in frozen TA sections

Nine TAs frozen in RNA*later*® Stabilization Solution were thawed at room temperature until the TA could readily be removed from the RNA*later*® Stabilization Solution, after which the TA was immediately cut into 1-mm sections using a sterile scalpel and weighed in an RNase-free area. DNA was extracted and analyzed by PCR from every other 1-mm section for the presence of VZV DNA as described above. Remaining sections were frozen individually in RNA*later*® Stabilization Solution at −20 °C for future studies.

3. Results

Ten TAs from 9 subjects who underwent TA biopsy to diagnose GCA were examined for VZV by 4 investigators who were blinded to the pathological report of GCA-positive or GCA-negative. VZV antigen was found in 3/3 GCA-positive TAs and in 4/6 GCA-negative TAs (Fig. 1). VZV antigen was present mostly in the adventitia, but was seen in all arterial layers (Table 1). Despite formalin-fixation, VZV DNA was detected in 1/3 VZV antigen-positive GCA-positive TAs and in 3/4 VZV antigen-positive GCA-negative TAs. VZV DNA was also detected in one GCA-negative, VZV antigen-negative TA. VZV DNA was found in one frozen unfixed GCA-negative, VZV antigen-negative TA.

4. Discussion

For the first time in a research study, GCA-positive and GCA-negative TAs were analyzed for VZV by 4 investigators who were blinded to the pathologic report. The detection of VZV antigen in 3/3 GCA-positive

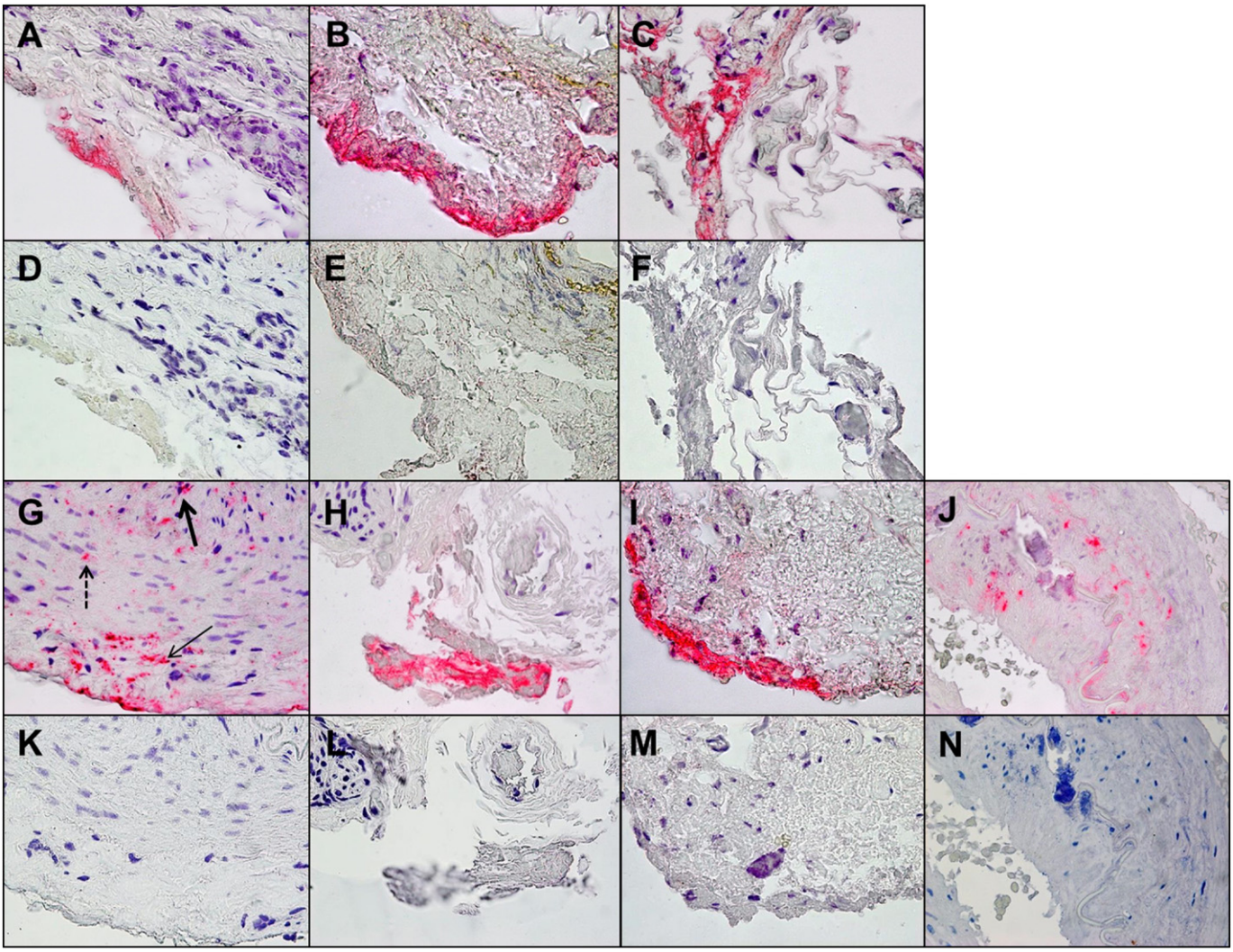

Fig. 1. Varicella zoster virus (VZV) antigen in temporal arteries (TAs) of patients with pathologically-verified giant cell arteritis (GCA) and patients with clinical features and laboratory abnormalities characteristic of GCA whose TAs were pathologically negative. Immunohistochemical analysis of TAs from 3 GCA-positive subjects revealed VZV antigen in the adventitia of all 3 TAs (A–C, pink color). VZV antigen was seen in the TAs of 4/6 GCA-negative subjects in the adventitia (G, thin arrow), media (G, dashed arrow) and thickened intima (G, thick arrow), in the adventitia alone (H and I) and in the media and thickened intima (J). No staining was seen in sections adjacent to those containing VZV antigen when mouse isotype IgG1 was substituted for mouse *anti*-VZV gE antibody (C–E, I–K). Magnification 600×.

Table 1
Localization of VZV antigen in GCA-positive and GCA-negative TAs.

Subject	Diagnosis	Localization of VZV antigen
1	GCA-positive	Adventitia, adjacent skeletal muscle
2	GCA-positive	Adventitia
3	GCA-positive	Adventitia
4	GCA-negative	Adventitia, intima
5	GCA-negative	Adventitia
6	GCA-negative	Adventitia, media, intima
9	GCA-negative	Adventitia, media, intima

TAs and in 4/6 GCA-negative TAs supports earlier larger studies that found VZV antigen in most GCA-positive and GCA-negative TAs [3,5]. Similarly, VZV DNA was detected by PCR in VZV antigen-positive sections from 1/3 GCA-positive TAs and from 3/4 GCA-negative TAs.

The methods employed here are not yet practical for routine diagnostic evaluation of TA biopsies. In the future, it may be reasonable to obtain fresh-frozen tissue at the time of biopsy for virological analysis. PCR analysis of DNA extracted from fresh-frozen unfixed tissue could eventually become part of the protocol. Future larger long-term studies will be required to determine if analysis of TA biopsies for VZV leads to changes in the management of GCA patients.

5. Conclusions

The work presented here further supports our laboratory's findings that VZV plays a role in the pathogenesis of GCA. Future studies will be required to determine if routine testing of TA biopsy specimens for VZV is warranted and if antiviral treatment confers additional benefit to corticosteroid treatment.

Conflict of interest statement

All authors report no conflicts of interest.

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