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Antigen Detection: the Method of Choice in Comparison with Virus Isolation and Serology for Laboratory Diagnosis of Herpes Zoster in Human Immunodeficiency Virus-Infected Patients

HELENA DAHL,[1]* JILL MARCOCCIA,[2] AND ANNIKA LINDE[1]

Department of Virology, Swedish Institute for Infectious Disease Control, S-105 21 Stockholm, Sweden,[1] and Bristol-Myers Squibb Company, Pharmaceutical Research Institute, Drève Richelle, 1410 Waterloo, Belgium[2]

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Ninety-two adult human immunodeficiency virus (HIV)-infected patients with suspected herpes zoster were included in a study. The clinical diagnosis of herpes zoster was verified by examination of blister cell and fluid material or serum samples. Antigen detection by a direct immunofluorescence assay with a fluorescein isothiocyanate-labelled monoclonal antibody, virus isolation, and serologic methods (in-house varicella-zoster virus [VZV] immunoglobulin G [IgG] and IgM enzyme-linked immunosorbent assays and the commercial Enzygnost assay) were compared. The direct immunofluorescence assay was found to be the most sensitive method, diagnosing 85 of 92 infections (92%), while the sensitivity of virus isolation was 65% (60 of 92 patients). Despite the use of two different serological methods, only 60 of 92 patients (65%) had significant VZV IgG titer rises, and only 26 of 92 patients (28%) had detectable VZV IgM. The lack of a VZV IgG antibody titer rise was found to correlate with low CD4 counts in peripheral blood and high VZV IgG titers in the acute-phase serum sample. The frequency of IgM-positive sera was lower than that expected from reports of studies with patients without AIDS. This may be related to early antiviral treatment or deficient antibody production due to the HIV-related immunosuppression. There was no significant difference in CD4 counts between VZV IgM-positive and -negative patients.

Herpes zoster (shingles) occurs when varicella-zoster virus (VZV) is reactivated from its latent state. In the immunocompetent host it is characterized by unilateral vesicular eruptions with dermatomal distribution, and some of its most significant clinical manifestations are the associated acute neuritis and the postherpetic neuralgia. It is estimated that approximately 50% of patients older than 60 years of age will experience pain that persists for more than a month (5, 11). Adults with human immunodeficiency virus (HIV) infections are nine times more likely to develop herpes zoster infections than individuals without HIV infection (9). The occurrence of herpes zoster in HIV-infected individuals is not associated with a significant increase in the rate of progression to AIDS (9). Herpes zoster in the immunocompromised host can be severe, but a majority of asymptomatic HIV-infected patients follow a course similar to that seen in immunocompetent individuals (4). In individuals with late-stage HIV infections, the formation of lesions and scabbing take place over a prolonged time period (12). The patients are at risk for cutaneous dissemination, pneumonitis, hepatitis, retinitis and meningoencephalitis.

A synthetic deoxythymidine nucleoside analog, sorivudine (1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)-uracil); BV-araU], has been shown to be extremely effective against VZV in vitro (1). In a multicenter study the effect of BV-araU was compared with that of acyclovir for the treatment of localized herpes zoster in HIV-infected adults. In order to find optimal laboratory methods for the diagnosis of herpes zoster, the results from 31 participating centers in the European Community, Canada, Australia, and New Zealand involved in the study were evaluated. Three laboratory methods were used for verification of the clinical diagnosis of herpes zoster: direct fluorescent-antigen detection (DFA) in smears from vesicle material, culture of virus from vesicles, and enzyme-linked immunosorbent assay (ELISA) serology for VZV immunoglobulin G (IgG) and IgM with acute- and convalescent-phase serum samples. Two different ELISA methods for IgG and IgM were used: a commercial assay and an in-house assay. The criterion for VZV infection was confirmation by one or more laboratory methods: positivity for DFA, positive virus isolation, positivity for VZV IgM, or a twofold or greater increase in the VZV IgG level between the acute- and convalescent-phase samples. The specific antibody response was also related to the CD4 count in the patients.

MATERIALS AND METHODS

Patients. In a multicenter study, a dosage of 40 mg of BV-araU once a day was compared with a dosage of 800 mg of acyclovir five times daily for the treatment of localized herpes zoster in HIV-infected adults. For inclusion in the study the HIV-infected patients had to be at least 18 years of age, with a clinical diagnosis of localized herpes zoster (grouped maculo and/or maculopapular vesicles on an erythematous base unilaterally in the dermatomal distribution of the dorsal nerve) appearing less than 72 h before enrollment. Ninety-two HIV-infected patients with clinical herpes zoster from whom material for all three tests was available were included in this evaluation of laboratory methods for the diagnosis of herpes zoster. At enrollment 6 of 92 patients (7%) had a CD4 count of <10, 26 of 92 patients (28%) had a CD4 count of ≥ 10 to <100, and 60 of 92 (65%) patients had a CD4 count of >100. Thirty-four of the patients had previous histories of herpes zoster infections, and 28 were previously treated with antiviral drugs against herpesvirus infections. None of the patients included in the study were treated with any antiviral agent against herpesviruses within 2 weeks of entry into the study.

Control patients. For evaluation of the specificity of VZV titer changes and the VZV IgM antibody titer in HIV-infected patients, 29 HIV-infected patients without clinical herpes zoster served as a control group. Eight of 29 patients (28%) had a CD4 count of <100, and 21 of 29 (72%) of the patients had a CD4 count of >100. Two samples from each patient, drawn within an interval of 2 months, were assayed by the two different ELISA methods for IgG and IgM antibodies. Detailed clinical data were not available for this group.

* Corresponding author. Phone: 46 8 7351306. Fax: 46 8 27 22 31.

Antigen detection. DFA was performed with scrapings from the lesions at licensed laboratories close to the clinical study centers. The slides for DFA were prepared by selecting a clear vesicle and removing its roof with a sterile scalpel. The vesicle was then blotted with a sterile swab to remove debris. The base of the lesion was scraped gently with the rounded edge of the scalpel blade to collect cells. The cell material was spread over a 10-mm-diameter area in 50 μl of saline on a clean microscope slide and air dried. The slides were fixed in cold acetone for 10 min and were stored at 2 to 8°C until they were stained. The presence of >100 VZV-infected basal layer cells in the smear was essential for a reliable result; otherwise the specimen was inappropriate for DFA. A fluorescein-labelled monoclonal antibody to VZV (Ortho Diagnostics) was used according to the manufacturer's instructions.

Virus isolation. Virus isolation was performed at laboratories near the participating study centers, according to the instructions given in the protocol. Specimens were drawn on days 1, 3, 5, and 7 if complete crusting did not occur prior to that time. Aspirates from new and old vesicular lesions were assayed. Fresh vesicles containing clear fluid were selected as the new lesions, and vesicles more than 1 day old which had not yet pustulated were used as old lesions. Samples were collected by aspirating clear vesicle fluid into a syringe containing viral transport medium, lightly scraping the base of the lesion with the edge of the needle to loosen basal cells. Aspirates from three vesicles were pooled. If direct inoculation of the aspirates onto a tissue culture was not possible, the aspirate from the syringe was injected into a transport vial and the vial was put on wet ice until its contents were inoculated onto a tissue culture. Each participating center used its own method for VZV isolation and verification of the cytopathic effect. Different cell lines were used, depending on the routine of the investigating laboratory. Human foreskin, MRC-5, and human embryonic lung fibroblasts were the most commonly used cell lines. Inoculated tissue cultures were kept for 3 weeks, with a weekly medium change.

Serological assays. All acute- and convalescent-phase serum samples were drawn over a 4-week interval and were sent to the Department of Virology, Swedish Institute for Infectious Disease Control, Stockholm, Sweden (formerly the National Bacteriological Laboratory), for VZV IgM and IgG investigation.

(i) In-house VZV IgG ELISA. The in-house VZV IgG ELISA was performed as described previously (10). Briefly, 96-well plates were coated with an in-house VZV nucleocapsid antigen at a concentration of 2.5 mg/ml. Serum samples diluted 10-fold, from 1/100 to 1/100,000, in ELISA buffer (phosphate-buffered saline without Ca^{2+} and Mg^{2+} [pH 7.4] with 0.05% Tween 20 and 0.5% bovine serum albumin) were incubated for 2 h at 37°C. After washing three times with a washing buffer (0.9% NaCl and 0.05% Tween 20), alkaline phosphatase-conjugated rabbit anti-human IgG (Sigma Chemical Co., St. Louis, Mo.) was incubated overnight at 37°C. The plates were washed three times, and *p*-nitrophenyl phosphate was used as the substrate. The optical density (OD) at 405 nm was measured in a microplate reader when the positive control reached a predetermined OD value. All paired samples were examined in the same assay. The VZV IgG endpoint titers were calculated as the inverted value of the serum dilution giving an OD value of ≥0.2. Previous investigations have shown an intra-assay titer variation of approximately 10% (9).

(ii) In-house VZV IgM μ-capture ELISA. The in-house VZV IgM μ-capture ELISA was performed as described previously (10). Briefly, 96-well plates were coated with rabbit anti-human IgM (Dako AG, Copenhagen, Denmark). Serum samples diluted 1/100 and 1/1,000 were incubated for 1 h at 37°C. After washing three times, horseradish peroxidase-labelled VZV nucleocapsid antigen was incubated for 2 h. *ortho*-phenylendiamine dihydrochloride was used as the substrate, and after incubation for 30 min at room temperature and the addition of H_2SO_4, the OD was measured at 450 nm. By this method, VZV IgM was previously found in 84% of immunocompetent patients with herpes zoster (10).

(iii) Enzygnost anti-VZV/IgM and IgG. The Enzygnost anti-VZV/IgM and IgG (Behring, Behringwerke AG, Marburg, Germany) is an enzyme immunoassay for the determination of human IgG and IgM antibodies to VZV. All paired serum samples in the patient and control groups were analyzed for presence of IgG antibodies to VZV. For detection of IgM antibodies to VZV, only the convalescent-phase samples from patients and control patients were investigated, since previous studies have shown that IgM antibodies appear late in patients with herpes zoster (10, 13). The assays were performed according to the manufacturer's instructions.

RESULTS

Comparison of DFA, virus isolation, and the serological assays. VZV infection was confirmed by one or more laboratory methods in 89 of 92 patients (97%) (Table 1). A total of 85 of 92 patients (92%) were DFA positive, and VZV could be isolated in tissue culture from 60 of 92 patients (65%).

In the control group, fourfold or greater VZV IgG titer rises were found in 3 of 29 patients (10%) by the in-house ELISA. A less than twofold titer change was seen in the remaining patients. A greater than twofold titer rise was chosen as significant for herpes zoster. The chosen specificity of the assay was thus 90%. IgM antibodies to VZV were not detected in the control group by the in-house IgM assay. In the Enzygnost IgG assay the antibody level is expressed in milli-International Units per milliliter and refers to the World Health Organization international standard for varicella-zoster immunoglobulin (50 IU). An increase in activity by a factor of at least two in a pair of samples assayed simultaneously is regarded to be a significant change in VZV-specific antibody, according to the manufacturers. More than twofold changes in VZV IgG and VZV IgM were not found in the control group by this assay.

TABLE 1. Laboratory confirmation of VZV infection among 92 patients from whom all types of samples were drawn

Method	No. of positive patients/no. of patients tested (%)
Any method	89/92 (97)
VZV DFA	85/92 (92)
VZV isolation	60/92 (65)
In-house assay for VZV IgG	59/92 (64)
In-house assay for VZV IgM	12/92 (13)[a]
Enzygnost assay for VZV IgG	55/92 (60)
Enzygnost assay for VZV IgM	22/92 (24)[b]

[a] For 9 of 12 patients, only the convalescent-phase serum sample was positive.
[b] Only convalescent-phase serum samples were analyzed.

Significant (twofold or greater) VZV IgG titer rises were measured in 60 of 92 patients (65%; responders) by the in-house ELISA, and the Enzygnost IgG assay showed significant VZV IgG rises in 55 of 92 (60%) of the patients with herpes zoster examined. VZV IgM was found by the in-house μ-capture ELISA in 12 of 92 (13%) of the HIV-infected patients with herpes zoster examined. In 9 of 12 patients, IgM antibodies were detected only in the convalescent-phase serum sample. Only the convalescent-phase blood samples in the zoster patient group with herpes zoster were analyzed by the Enzygnost IgM assay, and VZV IgM antibodies were found in 22 of 92 patients (24%).

A comparison between the in-house and commercial assays showed that for IgG, 52 of 63 samples (83%) were positive by both assays, 8 of 63 (12%) were positive by the in-house ELISA only, and 3 of 63 (5%) were positive by the Enzygnost IgG assay only. Eight of 26 samples (31%) were IgM positive by both assays, while 14 of 26 (54%) were positive by the Enzygnost IgM assay only and 4 of 26 (15%) were IgM positive by the in-house assay only.

No difference between responders and nonresponders for either IgG or IgM could be related to treatments with different drugs. Nineteen of 22 (86%) VZV IgM-positive patients were found in the group of IgG responders, whereas 3 of 22 (14%) VZV IgM-positive patients were found in the group of IgG nonresponders ($P < 0.01$; chi-square test). The serological response was related to the CD4 count. The IgG responders had significantly higher CD4 counts in peripheral blood ($P < 0.001$; Mann-Whitney test) and significantly lower VZV IgG titers by the in-house ELISA in their acute-phase serum samples ($P < 0.001$) than nonresponders. Only one of six patients with a CD4 count of <10 upon enrollment showed a significant VZV IgG titer rise (165 to 1,300), but the titer in the convalescent-phase serum sample was 50-fold lower than the mean titer in the convalescent-phase serum samples of the responders with higher CD4 counts. IgM positivity by the in-house ELISA and the Enzygnost assay was not correlated to CD4 counts. The relationship between CD4 counts and the VZV IgG response is presented in Table 2.

TABLE 2. CD4 counts upon enrollment and VZV IgG titers in acute- and convalescent-phase serum samples from responders and nonresponders measured by the in-house ELISA

Group	Mean (range) CD4 count	VZV IgG titers[a]	
		Acute-phase serum sample	Convalescent-phase serum sample
Responders	270 (4–740)	9,400 ± 17,000	56,800 ± 30,000
Nonresponders	140 (0–612)	24,100 ± 23,100	26,000 ± 23,000

[a] Values are means ± standard deviations.

DISCUSSION

The clinical diagnosis of herpes zoster in this study seems reliable, since in 97% of the patients a laboratory confirmation of VZV infection was obtained. As in previous studies (6–8), DFA had the highest sensitivity. The assay is easy to perform and takes approximately 1 h. The only limitations are that experienced staff is needed for the microscopic evaluation and that the epithelial cell deposits on the slide must contain sufficient numbers of cells. The problems of VZV isolation due to rapid inactivation of the virus are well known. The use of different types of cells and different methodologies at the different centers in the study may have affected the results, but no significant differences in positive results were noted among the centers. The results probably depict well what is expected to be found by routine laboratory investigation. The sensitivity of virus isolation perhaps could have been increased by using shell vial culture instead of tube culture for VZV isolation, but when the study started, shell vial cultures were not evaluated for VZV isolation (3).

The number of samples with significant VZV IgG titer rises and the number of IgM-positive samples were fewer than expected (10), despite examination by both the in-house and the commercial Enzygnost assays. IgG titer rises were demonstrated in 65 and 60% of patients with a clinical diagnosis of herpes zoster by the in-house and Enzygnost assays, respectively. A more pronounced difference between the assays was seen for IgM. The in-house ELISA detected IgM in 12 of 92 (13%) of the convalescent-phase serum samples, while the Enzygnost assay detected IgM in 22 of 92 (24%) such samples. The indirect IgM ELISA method probably has a higher sensitivity than the in-house μ-capture ELISA with a labelled antigen.

The number of IgG responders decreased with decreasing CD4 count. Of the patients with a CD4 count of <10, one of six (17%) responded. The numbers were 16 of 26 (62%) and 45 of 60 (75%) for those with CD4 counts of 10 to 100 and >100, respectively. We also found that the acute-phase serum samples of the nonresponders had significantly higher VZV IgG titers than those of the responders. One reason for a lack of increase in titers is probably the absence of T-helper cells. Thirty-four of the patients included in the study had a history of previous VZV reactivations, which might explain the high IgG titers in the acute-phase serum samples. The IgG titers seen in the acute-phase serum samples from the control group were similar to those seen in serum samples from the group of nonresponders with herpes zoster. The IgG titer in an acute-phase serum sample thus cannot be used to diagnose a herpes zoster infection in AIDS patients. A twofold or greater rise in IgG titer to VZV was found in three of the control patients by the in-house ELISA, indicating a subclinical reactivation in certain patients or a possible nonspecific reaction by this assay.

Previously, we have found VZV IgM responses in 80% of patients with herpes zoster, using the same μ-capture ELISA used in this study (10), and other studies have shown similar frequencies (2). The low number of IgM-positive individuals found in this study by both ELISAs may indicate that the specific production of IgM is severely affected in HIV-infected patients. However, previous studies in our laboratory as well as studies conducted by others have shown that the VZV IgM response in patients with herpes zoster often occurs after the IgG booster response (10, 13). In accordance with this, VZV IgM was detected only in the convalescent-phase serum samples from 9 of 14 patients. A diminished viral load due to the early antiviral treatment, resulting in a lack of antigen stimulus, is therefore another possible explanation for the low rate of VZV IgM-positive serum samples in this study.

In conclusion, it seems that laboratory confirmation of the clinical diagnosis of herpes zoster is not always necessary, since the specificity of the clinical diagnosis is 91%. If a laboratory verification is desirable, DFA is the method of choice. Virus isolation has the second best sensitivity and should always be performed if assays for antiviral resistance are indicated. Neither IgG nor IgM serology is sensitive enough to be the first choice for use in the routine diagnosis of herpes zoster in HIV-infected patients.

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