

**Antibody-capture enzyme-linked immunosorbent assays that use enzyme-labelled antigen for detection of virus-specific immunoglobulin M, A and G in patients with varicella or herpes zoster**

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SUMMARY

Antibody-capture enzyme-linked immunosorbent assays (AC-ELISA) which use enzyme-labelled antigen were developed for detection of varicella-zoster virus (VZV) specific IgM, IgA and IgG antibody in patients with varicella or herpes zoster and in sera from healthy individuals. All 18 patients with varicella developed a VZV-IgM and a VZV-IgG response, 17 also a VZV-IgA response. In contrast, all 19 patients with herpes zoster were shown to be positive for VZV-IgA whereas only 13 of these reacted positively for VZV-IgM. A VZV-IgM response was detected in only two sera from 100 healthy individuals and an IgA response in only one. The presence of virus-specific IgA and IgG in the cerebrospinal fluid as determined by AC-ELISA was a useful indicator of VZV infection of the central nervous system.

By AC-ELISA, VZV-IgG was detected predominantly in sera from patients with acute or recent VZV infection. Only 14 sera from 100 healthy individuals were positive for VZV-IgG by AC-ELISA, whereas all were positive by an indirect ELISA. These results indicate that AC-ELISA's may be useful assays for determination for acute or recurrent VZV infection, but are not suitable for determination of past infection with this virus.

INTRODUCTION

The increasing number of immunocompromised patients stresses the need for simple, sensitive methods to determine acute and past infections with varicella-zoster virus (VZV). This is particularly true since in recent years effective antiviral treatment and prevention of VZV infection by vaccination have become available.

So far, the complement-fixation test (CFT) has been widely used for serologic diagnosis of VZV infections. This test, however, is not very sensitive for determination of past infections and requires detection of a significant antibody

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titre rise in paired serum specimens for diagnosis of acute infection. It has been shown previously [1-8] that detection of VZV-specific immunoglobulin M (VZV-IgM) antibody allows rapid diagnosis of acute VZV infection. In all patients with varicella, VZV-IgM was found early in the disease using a variety of methods including the indirect immunofluorescence assay [2], the indirect enzyme-linked immunosorbent assay (ELISA) [3] as well as antibody-capture assays using radioactive [4, 5, 8] or enzyme labels [6, 7]. In contrast, in patients with herpes zoster an IgM response has been reported in between 20% and 98% of patients [1-8]. Therefore the value of VZV-IgM detection for rapid diagnosis of herpes zoster is less clear.

In a previous study [9] we showed that virus-specific IgA antibody as determined by an antibody-capture ELISA (AC-ELISA) was a sensitive marker of a recent recurrent infection with herpes simplex virus (HSV) and could be used for diagnosis of HSV infection.

The present report describes AC-ELISA for detection of IgM, IgA and IgG antibodies to VZV. The assays are evaluated with respect to their ability to allow a rapid, sensitive and specific diagnosis of acute and past VZV infections.

## MATERIALS AND METHODS

### *Serum specimens*

Serum specimens were obtained during the acute phase ( $S_1$ ) and the convalescent phase ( $S_2$ ), between 10 and 30 days later, from 18 patients with varicella and from 19 patients with herpes zoster. The clinical diagnosis was confirmed by detection of a significant antibody titre rise in CFT. Follow-up sera were obtained from 6 patients with varicella and 6 patients with herpes zoster. Single sera from 100 healthy laboratory workers were examined for control purposes.

In addition, serum and cerebrospinal fluid (CSF) samples were obtained from three patients with central nervous system (CNS) involvement of their VZV infection.

### *Viral antigen*

VZV antigen was prepared from the supernatant fluid of human embryonic lung cells (HEL 299, ATCC, Rockville, Md) infected with VZV, strain Inge. When the cells showed a complete cytopathic effect the supernatant fluid was collected and centrifuged at 2000 *g* for 10 min to remove cellular debris. Next, polyethyleneglycol 8000 (Sigma Chemical Co., St Louis, Mo.) was added to a final concentration of 5% and the mixture was incubated for 5 h at 4 °C.

After centrifugation at 10000 *g* for 30 min at 4 °C the pellet was resuspended in phosphate-buffered saline (PBS) at pH 7.2, layered on 10 ml of 30% (w/w) sucrose in PBS at pH 7.2 and centrifuged at 90000 *g* for 60 min at 4 °C. The pellet containing the VZV antigen was resuspended in 0.01 M carbonate buffer at pH 9.5. The yield was generally between 0.4 and 1.0 mg of protein per bottle (670 cm<sup>2</sup>). Control antigen was prepared by sonication of uninfected HEL cells, followed by centrifugation at 2000 *g* for 10 min to remove cellular debris. The VZV antigen was conjugated with horseradish peroxidase (Sigma Chemical Co., St Louis, Mo.) by the method of Wilson and Nakane [10], modified as previously described [11].

*Antibody-capture enzyme-linked immunosorbent assay (AC-ELISA)*

The previously described AC-ELISA's [11] were used for detection of VZV-specific IgM, IgA and IgG antibody. Briefly, wells of polyethylene terephthalate-glycol (PETG) microtitre plates (Costar no. 6595, Costar Europe, Badhoevedorp, The Netherlands) were coated overnight at 4 °C with 0.12 ml of heavy-chain specific monoclonal anti-human IgM (Sanbio BV, Uden, The Netherlands) or polyclonal anti-human IgA or IgG (Cappel Laboratories, Cochranville, PA) at a dilution of 1:100, 1:1000 and 1:1000, respectively, in 0.01 M Tris buffer at pH 9.0. After washing four times with 0.01 M PBS at pH 7.2 containing 0.05% Tween 20, 0.1 ml of patient's serum diluted 1:50 in PBS-Tween 20 containing 2% fetal bovine serum and 0.005% merthiolate (PFT-M) was added to each of two wells and incubated for 2 h at 37 °C. Thereafter, the plates were washed again four times and 0.1 ml of horseradish-peroxidase-labelled VZV antigen (VZV-HRPO) diluted in PFT-M containing 0.1 mg/ml of control antigen was added to each well and incubated overnight at 4 °C. The dilution of the VZV-HRPO conjugate was determined previously by checkerboard titration and was usually between 1:400 (IgA) and 1:1000 (IgM). A relatively high concentration of (non-labelled) control antigen was added in excess to block possible non-specific reactivity with HRPO-labelled non-VZV protein in the VZV-HRPO solution. After washing again four times, 0.1 ml of substrate solution was added to each well. Substrate solution was prepared immediately before use by dissolving 4 mg of *o*-phenylenediamine per ml in 0.05 M citrate buffer at pH 5.2, followed by addition of 0.15% of a 30% solution of H<sub>2</sub>O<sub>2</sub>.

After 10 min of incubation at room temperature, the reaction was stopped by adding 0.15 ml of 3 N-H<sub>2</sub>SO<sub>4</sub>. The absorbance was determined by photometry (Titertek Multiskan, Flow laboratories, Irvine, UK) using the buffer control as a blank. The positive/negative cut-off value was determined in each plate by examination in quadruplicate of a 'cut-off' serum. Such a serum was selected at the beginning of the study from a dilution range of the positive control serum in the negative control serum on the basis of comparison with the distribution of the OD values obtained with the healthy controls' sera.

*Indirect enzyme-linked immunosorbent assays*

IgG antibodies to VZV in serum were also quantitated by use of an indirect ELISA similar to that previously described [9]. Briefly, a 0.12 ml amount of VZV antigen diluted in 0.01 M carbonate buffer at pH 9.5 was added to each well of a microtitration plate. The concentration of the VZV antigen for coating was determined previously by checkerboard titration and was usually between 4 and 10 µg/ml. Sera were examined in four fourfold dilutions in PFT-M at a starting dilution of 1 in 200. Peroxidase-labelled anti-human IgG ( $\gamma$ -chain specific, Dakopatts, Glostrup, Denmark) was used at a dilution of 1 in 8000 in PFT-M.

*Other serological methods*

VZV antibody titres were also determined by CFT using the microtechnique described by Casey [12].

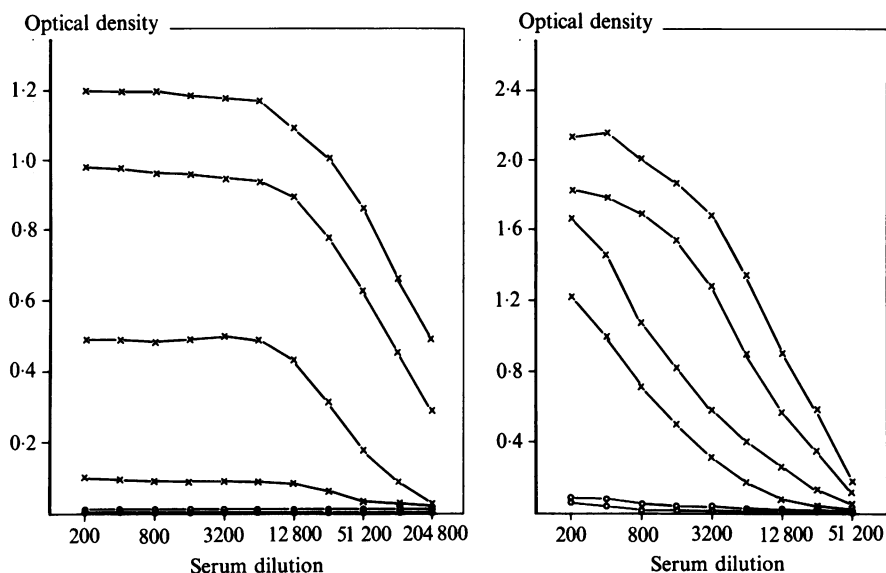


Fig. 1. Dose-response relationship of four positive (x-x) and two negative sera in (left) an antibody-capture ELISA and (right) and indirect ELISA for detection of IgG antibodies to varicella zoster virus.

## RESULTS

### *Dose-response relationship in the AC-ELISA*

Previous studies had shown that the dose-response curves in the AC-ELISA for determination of viral IgM or IgA antibodies level off at high serum concentrations [9]. The same pattern was found in the AC-ELISA for VZV-IgM, -IgA (data not shown) and -IgG (Fig. 1). This pattern is in marked contrast to that of the dose-response curve of the indirect ELISA for VZV-IgG, which has a more sigmoid-like shape (Fig. 1).

### *VZV-IgM, -IgA and -IgG in patients with acute VZV infection and in controls*

Paired sera obtained during the acute phase ( $S_1$ ) or the convalescent phase ( $S_2$ ) from 18 patients with varicella (Fig. 2) and from 19 patients with herpes zoster (Fig. 3) were examined by AC-ELISA for the presence of VZV-IgM, -IgA and -IgG. The results were compared with those of single sera from 100 healthy controls. Furthermore, all sera were also examined by an indirect ELISA for VZV-IgG.

In all patients with varicella, VZV-IgM, -IgA and -IgG was detected with the exception of one patient in whom the reactivity in the VZV-IgA assay remained just below the positive/negative cut-off level. The VZV-IgM reaction was positive in the acute-phase serum from 7 (38.4%) of the 18 patients, the VZV-IgA reaction in 4 (22.2%) and the VZV-IgG was positive by AC-ELISA and by indirect ELISA in 3 (16.7%) and 5 (27.7%) patients, respectively.

In the 19 patients with herpes zoster, VZV-IgM and -IgA was detected in 13 (68.4%) and 19 (100%) patients, respectively. VZV-IgM was found in the acute-phase specimens in two of these patients, VZV-IgA in only one. A positive

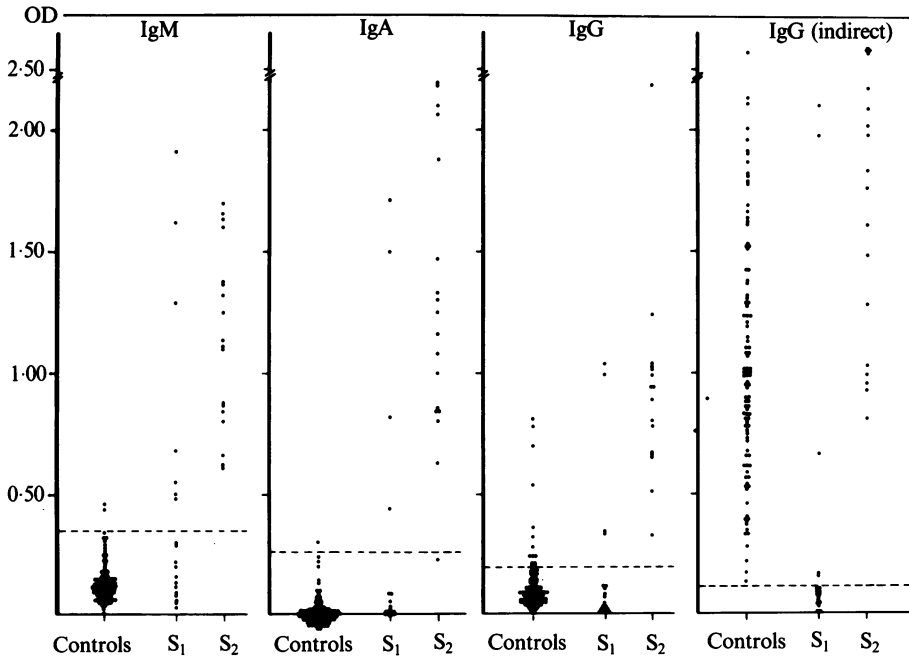


Fig. 2. Detection of IgM, IgA and IgG antibody to varicella zoster virus in acute ( $S_1$ ) and convalescent ( $S_2$ ) sera from 18 patients with varicella and in sera from 100 controls using an antibody-capture ELISA (IgM, IgA, IgG) or an indirect ELISA (IgG).

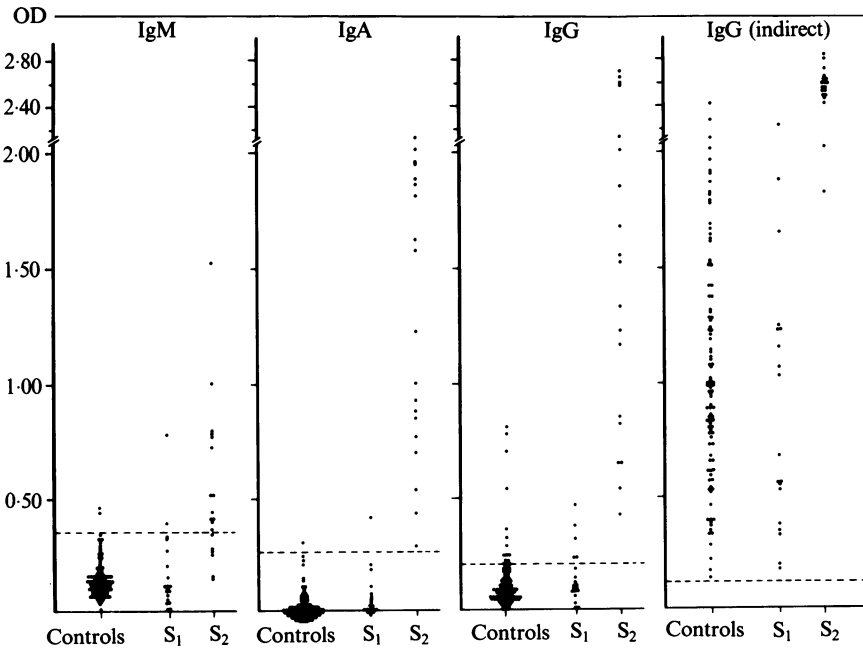


Fig. 3. Detection of IgM, IgA and IgG antibody to varicella zoster virus in acute ( $S_1$ ) and convalescent ( $S_2$ ) sera from 19 patients with herpes zoster and in sera from 100 controls using an antibody-capture ELISA (IgM, IgA, IgG) or an indirect ELISA (IgG).

Table 1. *Antibody-capture ELISA for VZV-IgM, -IgA and -IgG in follow-up sera from patients with acute VZV infection*

| Patient                 | Days after acute-phase serum | AC-ELISA (OD) |           |           | Indirect ELISA*<br>IgG |
|-------------------------|------------------------------|---------------|-----------|-----------|------------------------|
|                         |                              | IgM           | IgA       | IgG       |                        |
| Varicella               |                              |               |           |           |                        |
| 1                       | -70                          | 0.178         | 0.096     | 0.000     | 0.034                  |
|                         | 0                            | 0.238 (+)     | 0.047     | 0.000     | 0.170                  |
|                         | 8                            | 1.570 (+)     | 2.381 (+) | 1.690 (+) | 2.335                  |
|                         | 15                           | 1.724 (+)     | 1.997 (+) | 1.425 (+) | 2.086                  |
|                         | 97                           | 0.206 (+)     | 0.000     | 0.074     | 0.940                  |
|                         | 170                          | 0.178         | 0.000     | 0.000     | 0.320                  |
| 2                       | -50                          | 0.114         | 0.040     | 0.000     | 0.112                  |
|                         | 0                            | 0.631 (+)     | 0.356 (+) | 0.000     | 0.256                  |
|                         | 7                            | 1.090 (+)     | 1.674 (+) | 0.532 (+) | 1.733                  |
|                         | 27                           | 0.400 (+)     | 0.363 (+) | 0.302 (+) | 1.897                  |
|                         | 118                          | 0.151         | 0.080     | 0.333 (+) | 2.069                  |
| Herpes zoster           |                              |               |           |           |                        |
| 3                       | -42                          | 0.139         | 0.006     | 0.000     | 0.334                  |
|                         | 0                            | 0.120         | 0.000     | 0.000     | 0.319                  |
|                         | 7                            | 0.542 (+)     | 1.226 (+) | 2.301 (+) | 2.531                  |
|                         | 101                          | 0.138         | 0.254 (+) | 1.497 (+) | 2.138                  |
| 4                       | 0                            | 0.058         | 0.035     | 0.130     | 1.258                  |
|                         | 11                           | 0.823 (+)     | 2.229 (+) | 1.962 (+) | 2.549                  |
|                         | 21                           | 0.289 (+)     | 0.796 (+) | 1.574 (+) | 2.985                  |
|                         | 77                           | 0.098         | 0.256 (+) | 0.945 (+) | 2.533                  |
| 5                       | -36                          | 0.168         | 0.000     | 0.000     | 0.654                  |
|                         | 0                            | 0.141         | 0.000     | 0.000     | 0.680                  |
|                         | 38                           | 0.187         | 0.773 (+) | 0.907 (+) | 2.664                  |
|                         | 110                          | 0.178         | 0.209 (+) | 0.388 (+) | 2.532                  |
| Pos./neg. cut-off value |                              | 0.194         | 0.175     | 0.100     | 0.170                  |

\* Sera were examined at a dilution of 1 in 200 (varicella) or 1 in 800 (herpes zoster) in the indirect ELISA.

reaction for VZV-IgA and-IgM was obtained in 1 (1%) and 2 (2%) sera, respectively, from the control group.

The acute-phase serum specimens from patients with herpes zoster showed a significant difference between the reaction for VZV-IgG in the AC-ELISA and the indirect ELISA. As expected, all of these patients had VZV-IgG in their acute-phase serum as detected by the indirect ELISA, but only five were positive by the AC-ELISA. An even larger difference was found in the group of sera from healthy controls. In these, only 14 out of 100 sera were positive by AC-ELISA, whereas all were positive for VZV-IgG in the indirect ELISA.

#### *Temporal course of VZV-antibody production*

Follow-up sera were available from 12 patients with acute VZV infection: 6 with varicella and 6 with herpes zoster. In patients with varicella, VZV-IgM was found to persist for 3-6 months after infection, whereas a VZV-IgM response in patients with herpes zoster was detectable for a period of 1-2 months. VZV-IgA was present in patients with varicella for a shorter period than VZV-IgM, but for a

Table 2. *Antibody-capture ELISA for VZV-IgM, -IgA and IgG in serum and CSF from patients with VZV meningo-encephalitis*

| Patient | Specimen | Days after onset        | AC-ELISA (OD) |           |           |
|---------|----------|-------------------------|---------------|-----------|-----------|
|         |          |                         | IgM           | IgA       | IgG       |
| 1       | Serum    | 19                      | 1·149 (+)     | 0·481 (+) | 1·652 (+) |
|         | CSF      | 9                       | 0·577 (+)     | 1·031 (+) | 2·127 (+) |
|         |          | 19                      | 0·341 (+)     | 0·632 (+) | 2·035 (+) |
| 2       | Serum    | 12                      | 0·150         | 0·389 (+) | 0·194 (+) |
|         | CSF      | 1                       | 0·000         | 0·000     | 0·017     |
|         |          | 12                      | 0·256         | 1·998 (+) | 2·050 (+) |
| 3       | Serum    | 8                       | 0·260         | 1·234 (+) | 0·676 (+) |
|         |          | 17                      | 0·290         | 1·255 (+) | 1·146 (+) |
|         | CSF      | 8                       | 0·000         | 1·295 (+) | 1·326 (+) |
|         |          | 13                      | 0·000         | 1·319 (+) | 1·187 (+) |
|         |          | Pos./neg. cut-off value | —             | 0·272     | 0·260     |

Sera were examined at a dilution of 1:50; CSF at a dilution of 1:5.

longer time in patients with herpes zoster. Representative results are shown in Table 1.

*Intrathecal VZV antibody production in patients with VZV meningoencephalitis*

VZV antibody production in cerebrospinal fluid (CSF) was studied in three patients with trigeminal zoster who developed VZV meningoencephalitis. Since the magnitude of the OD value in an AC-ELISA is determined by the *proportion* of virus-specific antibody in relation to the total concentration of antibody, it follows that OD values of the VZV-IgM, -IgA or -IgG AC-ELISA in CSF exceeding those in serum necessarily indicate intrathecal VZV antibody production and hence VZV infection of the CNS. Correction for blood-brain barrier leakage is not needed. All three patients had a significantly elevated VZV-IgG antibody level in CSF compared to serum, indicating VZV infection of the CNS (Table 2). They all also had increased VZV-IgA reactivity in CSF; in two of them the VZV-IgA reactivity in CSF clearly exceeded that in serum. VZV-IgM was detected in the CSF of only one patient.

DISCUSSION

Specific AC-ELISA were developed for the detection of antibody isotypes to VZV. The advantages of antibody-capture assays for detection of specific IgM and IgA antibody over the indirect type of assays have been well recognized [13, 14] and include the lack of false-positive reactivity due to IgM rheumatoid factor and of false-negative reactivity because of competitive inhibition by high concentrations of virus-specific antibody of the IgG isotype. In an AC-ELISA the magnitude of the reaction is determined by the *proportion* of virus-specific antibody and the total amount of antibody of the given isotype. Therefore, AC-ELISAs are less sensitive than the indirect type of ELISA when this proportion of virus-specific antibody can be expected to be relatively small. Our results show that this is clearly the case for IgG antibodies since the AC-ELISA for VZV-IgG

was far less sensitive than the indirect type of ELISA. Only 14 (14%) out of 100 VZV-IgG positive sera were found positive by the AC-ELISA, whereas all sera were positive by an indirect ELISA. This is in accordance with the results of Forghani and colleagues [7], who also found that an AC-ELISA for VZV-IgG was not sensitive enough for determination of past VZV infections. Therefore the indirect ELISA for VZV-IgG remains the method of choice for determination of VZV immune status.

A lower sensitivity of the AC-ELISA compared to an indirect ELISA appears to be of lesser importance for determination of specific IgM or IgA antibodies since the significance of their determination is given by their transient presence. Actually, the long persistence of specific IgM antibody is increasingly becoming a problem in rapid diagnosis of infections, and extremely sensitive techniques further aggravate this problem [15]. However, a technique should be sensitive enough to detect virus-specific antibody early in the disease. So far, there is no reason to assume that AC-ELISAs lack the required sensitivity for early diagnosis of infection, provided that reagents of sufficient specificity are used. Previously we showed that virus-specific IgM antibody could be detected by AC-assays within a few days after onset of symptoms in patients with primary infections with rubellavirus [16], mumps virus [17], or herpes simplex virus [9]. Others have obtained similar results in patients with hepatitis A virus [18] and measles virus [19] infection. In the present study VZV-IgM was detected in all 18 patients with varicella, in 7 of these (38.9%) in the acute-phase serum obtained within a few days after onset of symptoms. This is in agreement with the results of others [2, 4, 8]. Furthermore, VZV-IgM was detected in serum from 13 out of 19 (68.4%) patients with herpes zoster. Similar results have been obtained by others using both indirect assays as well as antibody-capture assays. In these reports VZV-IgM in patients with herpes zoster was detected by indirect radioimmunoassay in 69% [4], by indirect immunofluorescence in between 50% and 78% [1, 2, 8] and by AC-ELISA in between 50% and 84% [6, 7] of patients. In all studies the VZV-IgM response in patients with herpes zoster was found to be of a lesser magnitude compared to the response in patients with varicella and to be relatively short-lived. The differences in the frequency of a VZV-IgM response in herpes zoster patients in these studies may well be explained by the use of different techniques and reagents [20]. Moreover, in view of the short duration of the VZV-IgM response in these patients, differences may also be due to varying sampling intervals.

Recently, an antibody-capture radioimmunoassay (AC-RIA) for detection of VZV-IgM was described which detected VZV-IgM in 98.2% of herpes zoster patients [8]. A considerable number of patients in that study were still VZV-IgM-positive at more than 7 months after onset of rash. The enhanced sensitivity of that method may be due to differences in the antigen preparation or to the use of radiolabelled monoclonal antibodies as the conjugate, since the use of monoclonal antibodies in general ensures less background and therefore allows the derivation of a lower positive/negative cut-off value. In that respect it would be interesting to know more about the VZV-IgM results of the AC-RIA in sera from a population of healthy persons.

VZV-IgA was detected in all but one patient with varicella and in all patients



with herpes zoster. The persistence of VZV-IgA was comparable to that of VZV-IgM. Only one serum from the control group was positive for VZV-IgA, further indicating the transient nature of the VZV-IgA response. Similar results have been obtained by others [2, 21, 22] and indicate the suitability of VZV-IgA as a diagnostic criterion for the diagnosis of VZV infection, particularly in herpes zoster patients, in whom VZV-IgM cannot always be detected. Moreover, as in patients with herpes encephalitis [9], detection of VZV-IgA (and -IgG) by AC-ELISA in cerebrospinal fluid appears to be a useful method for establishing CNS involvement of a VZV infection. Low levels of VZV-IgM were detected in two sera from the control group and of VZV-IgA in only one of these. Both sera were positive in the AC-ELISA for VZV-IgG. We consider these results to indicate 'silent' reactivations or reinfections with VZV as these have been noted before to occur regularly [23-25].

In conclusion, our data indicate that the AC-ELISA for VZV-IgM and -IgA may facilitate a more rapid diagnosis of acute VZV infection in single sera, both in patients with varicella and with zoster. For determination of VZV immune status an indirect ELISA for VZV-IgG should be used.

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