Laboratory diagnosis of varicella-zoster virus infections

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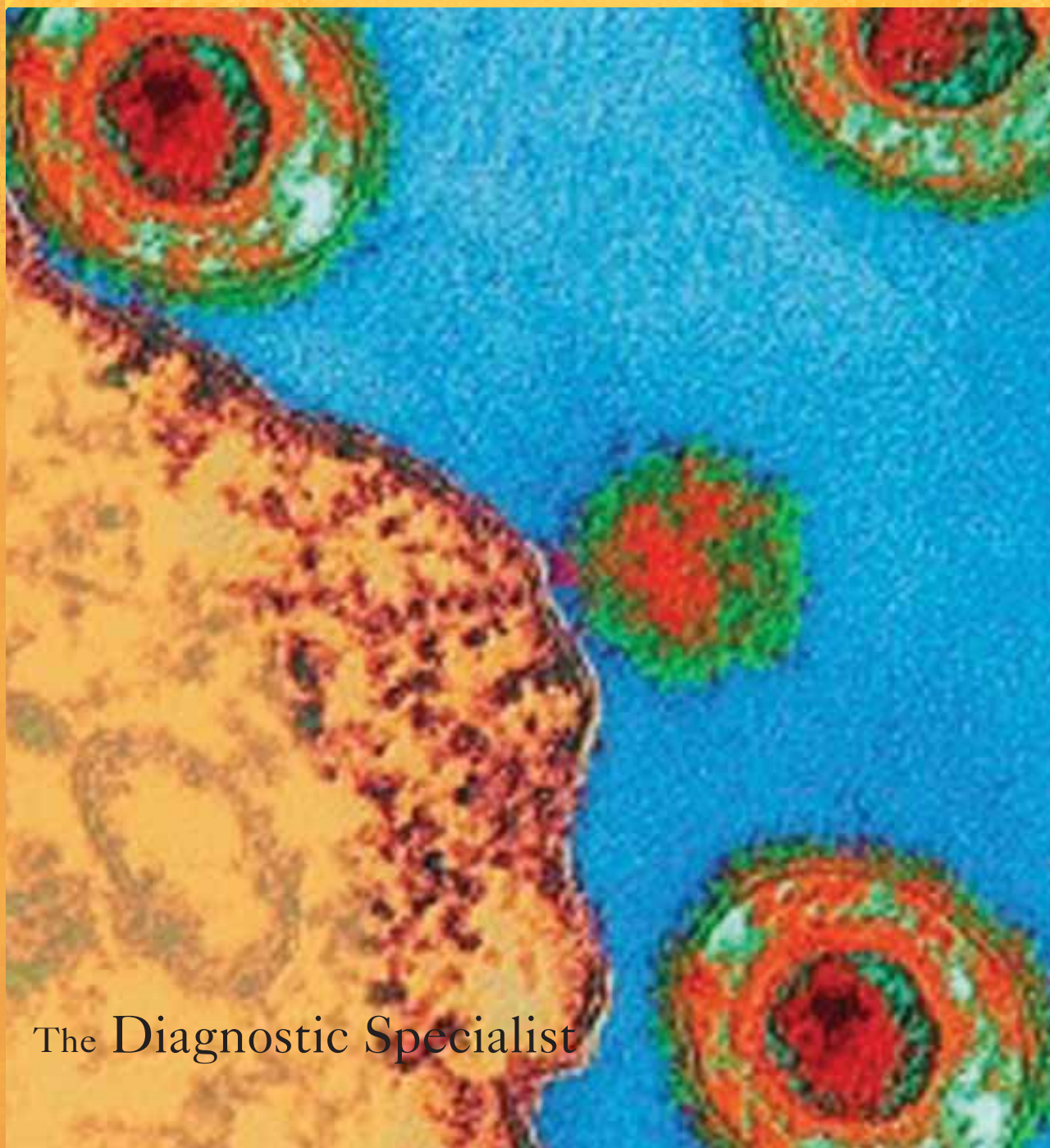

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Summary

Varicella and zoster are generally diagnosed clinically. Laboratory diagnosis is only necessary in atypical course of disease. Since serologic tests do not allow early diagnosis, the presence of varicella-zoster virus (VZV) should be demonstrated. This is best accomplished by detecting viral DNA using polymerase chain reaction (PCR). If PCR is not available, immunofluorescence analysis of skin rashes can be used. Isolation of the virus should only be tried when special investigations such as molecular characterization to distinguish between wild- and vaccine-derived viruses or the evaluation of resistance against antiviral drugs is required. During pregnancy, laboratory testing is indicated when there is a negative or doubtful history of varicella following exposure to VZV, to determine the presence of an intrauterine infection, to confirm suspected congenital varicella syndrome or when neonatal infection occurs. Serologic analyses are useful for determination of immune status to VZV. As methods, immunofluorescence, immunoenzymatic or chemiluminescent assays are appropriate. The detection of VZV-specific IgG antibodies indicates that the patient has immunity against infection. To monitor the success of vaccination, highly sensitive tests based on the detection of IgG antibodies that recognize specific VZV glycoproteins may be necessary.

Keywords: Chickenpox, Zoster, Varicella-zoster virus (VZV), Laboratory diagnosis, Pregnancy, Immune status

Indications for the laboratory diagnostics

Diagnosis of varicella or zoster is generally based on its typical clinical course and its characteristic clinical picture. From the point of view of differential diagnosis, laboratory analysis is usually required to clarify atypical pathological scenarios in immunodeficient patients, to exclude other types of dermatoses presenting with vesicle formation as well as to detect infections of the central nervous system (CNS) or pneumonia. Other indications for laboratory testing include a negative or uncertain varicella history following exposure to varicella-zoster virus (VZV) during pregnancy, to clarify the presence of intrauterine infection, to confirm cases of congenital varicella syndrome or in neonates who develop varicella. With the introduction of universal varicella vaccination in different countries [4, 5], it is necessary to distinguish between manifestations due to the vaccination and those resulting from naturally acquired varicella. Furthermore, susceptible persons need to be identified before vaccination or immunoprophylaxis. In immunodeficient individuals and healthcare workers, successful vaccination should be confirmed by detection of specific antibodies against the virus [11] (Table 1).

Table 1: Indications for laboratory diagnosis of varicella-zoster virus infections

Differential diagnosis	• Atypical pathological picture in immunodeficient individuals • Other types of dermatosis with vesicle formation • Varicella-zoster virus infections of the central nervous system • Varicella-zoster virus pneumonia
Diagnosis during pregnancy	• Doubtful immunity following exposure to varicella-zoster virus • Intrauterine infection (prenatal diagnosis) • Congenital varicella syndrome • Neonatal varicella
Diagnosis in relation to varicella vaccination	• Discrimination of wild- from vaccine-derived virus • Identifying susceptible individuals • To confirm successful vaccination in healthcare workers and immunodeficient individuals

Laboratory diagnosis of varicella and zoster

For laboratory diagnosis of varicella or zoster, it is necessary to detect the presence of virus, because serologic parameters do not allow early diagnosis. The method of choice is the polymerase chain reaction (PCR, Table 2), which is very sensitive and leads to results within few hours [6]. The virus can be detected not only in the contents of the cutaneous vesicles but also in crusts, which arise from vesicle eruptions, as well as in cerebrospinal fluid, broncho alveolar lavage, blood or tissue samples. Viral DNA is stable at room temperature for several days. So, samples for diagnosis by PCR can be sent by mail without the need for cooling. VZV can also be detected in cutaneous eruptions by means of immunofluorescence [2] provided that the sample contains cells from the base of vesicles.
With a sensitivity of 20%, virus culture is not useful for rapidly clarifying a doubtful diagnosis of varicella or zoster under routine conditions [7]. However, isolation of the virus should be

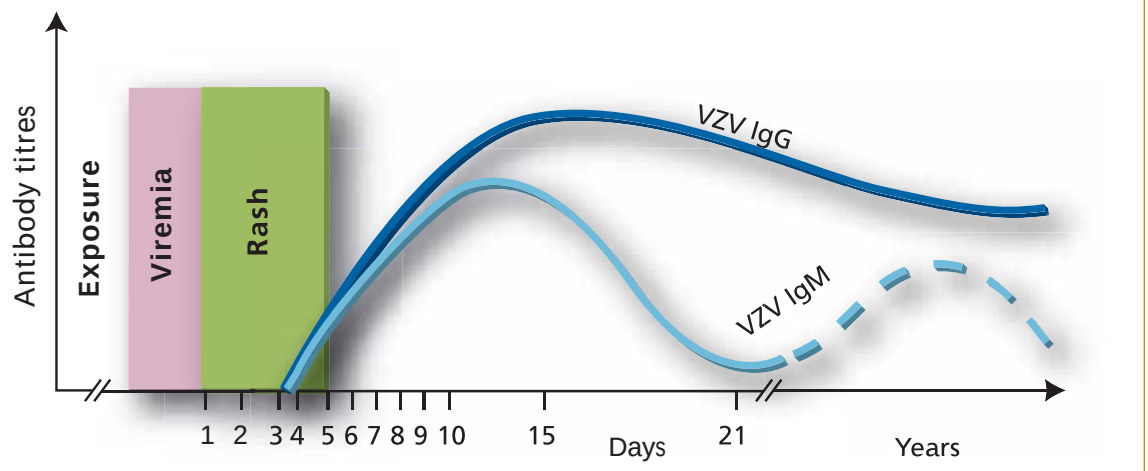
tried when specialized investigations are required. This concerns, for example, VZV genotyping to distinguish between wild-type and vaccine-type viruses [8] or the determination of antiviral activity of drugs against VZV. These methods are only available in specialized virological laboratories. For virus isolation to be successful, samples have to be obtained from fresh vesicles during the first 3-4 days of the disease and immediately transported to the laboratory as the virus is extremely labile. Viral specimens should be collected in cell culture medium supplemented with antibiotics and stored at 4°C no longer than 2-3 days. For longer time of storage, freezing at -20°C or better at -80°C has to be preferred. The restricted spectrum of human cells susceptible to VZV and the prolonged incubation time for up to several weeks required for cytopathic changes to develop are obvious disadvantages of the viral cultivation. Because of the preexisting immune response in cases of zoster, virus isolation from these lesions is more difficult than in chickenpox. Finally, it is important to know that VZV cannot be isolated from the cerebrospinal fluid in CNS infections.

The detection of virus-specific antibodies is not suitable for early diagnosis of varicella or zoster since the result may regularly be interpreted as negative during the first days of the disease. Serological diagnosis is only useful to confirm VZV infections when it is not possible to detect the virus. For this, 2 serum samples should be taken. The first sample can be obtained as early as possible and the second one 7-10 days later. In varicella, the first serum sample is negative when it was taken within the first 4 days of varicella rush (Fig. 1). Generally, chickenpox is associated with the seroconversion of IgG as well as a rise of specific IgM and IgA anti-

Table 2: Principles and methods of the laboratory diagnosis of varicella-zoster virus infections

Principle	Method	Material for analysis/ Transportation	Note
Demonstration of viral DNA	Polymerase chain reaction (PCR)	Liquor, vesicle contents, tissue, broncho alveolar lavage (BAL), EDTA, blood, amniotic fluid	Method of choice
Demonstration of viral antigens	Indirect immunofluorescence	Vesicle contents rich in cells, tissue	Alternative method
Virus Isolation	Isolation in cell culture	Vesicle contents, tissue, BAL, EDTA blood, amniotic fluid/special medium for transportation required	Used when special investigations are required
Differentiation between wild- and vaccine-derived virus	PCR, Restriction fragment length polymorphism analysis, sequencing	Viral isolates, vesicle contents, tissue	Performed in specialized laboratories
Determination of resistance against antiviral drugs	Plaque reduction assay	Viral isolates	Performed in specialized laboratories
Demonstration of antibodies	Immunofluorescence, quantitative immunoassays	Serum, liquor	Determination of immune status, retrospective confirmation of varicella or zoster

Figure 1: Rise of IgM and IgG antibodies after primary infection with varicella-zoster virus



bodies. In dependence of the time interval, zoster leads to a significant rise of IgG and the specific IgA can regularly be detected. By contrast, IgM can only be found in up to 50% of zoster cases [6].

Diagnosis during pregnancy

Varicella is rare during pregnancy. Only 3-4% of German women of childbearing age do not possess protective IgG antibodies and are therefore susceptible to chickenpox [14]. Congenital varicella syndrome occurs in 1-2% of cases when pregnant women contract chickenpox during the first 20 weeks of pregnancy. The mortality is calculated as 30% [3, 10]. Clinical symptoms include skin lesions in dermatomal distribution, neurological defects, eye diseases and skeletal abnormalities. When a maternal varicella infection manifests 4-5 days before to 2 days after birth, there is significant risk of neonatal varicella due to the lack of maternal antibodies.

This is fatal in up to 20% of cases [9] when the child did not receive antiviral treatment. Pregnant women, who contract varicella primarily in the last trimester, are at risk for pneumonia with severe respiratory insufficiency [1]. Normal zoster during pregnancy or the perinatal period is not associated with birth defects or does not cause special problems for pregnant women and newborn infants [12].

Pregnant women with doubtful or negative history of varicella must immediately be tested for VZV IgG to determine their immune status. If no antibodies can be detected or there is an indeterminate or unknown status of immunity, the woman must be considered susceptible to varicella. When varicella occurs during the first or second trimester, the pregnancy should be carefully monitored. Regarding prenatal diagnosis, fetal ultrasound has to be performed at first to identify signs of congenital varicella syndrome. If anomalies can be detected between the 20th and 22nd gestational week laboratory investigations for VZV DNA in placental villi, fetal blood or amniotic fluid are indicated. Alternatively, it is possible to detect virus-specific IgM in umbilical cord blood, but its presence has been reported in rare cases. For the interpretation of the results, it should be kept in mind that a positive result does not necessarily correlate with fetal disease.

Diagnosis in newborns

In newborns with suspected congenital varicella syndrome, the causal relationship between maternal varicella infection and the congenital abnormalities should be verified by detection of the virus in tissue samples or cerebrospinal fluid using PCR. The detection of virus-specific antibodies in the neonate can also prove prenatal infection. However, since specific IgM can only be observed in 25% of the cases, serologic diagnosis is mostly based

on the persistence of VZV-specific IgG class antibodies beyond 7 months of life when maternal antibodies should normally have disappeared. Unlike intrauterine rubella or cytomegalovirus infection, VZV has not been isolated from neonates with congenital varicella syndrome. Differential diagnosis of congenital varicella syndrome must consider infections by rubella virus, cytomegalovirus, herpes simplex virus, coxsackie virus and Toxoplasma gondii. In addition, the genetic disorder MIDAS (microphthalmia, dermal aplasia and sclerocornea), which presents with primary symptoms of cutaneous lesions in dermatomal distribution and microphthalmia, must be considered.

The diagnosis of intrauterine acquired neonatal varicella is usually based on the typical clinical picture and the characteristic point in time of the infection, both in the newborn and the mother. To confirm the diagnosis with laboratory tests, PCR analysis of cutaneous swabs, biopsies, liquor and tissue samples should be performed. The differential diagnosis of neonatal varicella includes herpes simplex virus and enterovirus infections.

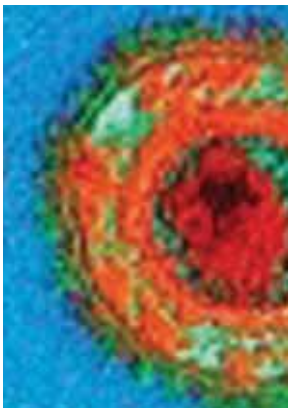
Determination of specific immune status

To clarify the immune status, VZV-specific IgG has to be analyzed. Generally, IgG class antibodies correlate with the cellular immunity, which plays the key role in protection against VZV infections, but is technically difficult to detect. Standard methods such as immunofluorescence, immunoenzymatic or chemiluminescent assays are routinely used to detect antibodies. Independently of the test used, each result which is interpreted as positive (presence of VZV-specific IgG) can be regarded as proof of immunity, while patients with borderline results should be considered "non-immune".

The tests available on the market differ in sensitivity, for which very low titres may escape detection. To monitor the success of vaccination and for vaccination studies, highly sensitive tests such as the fluorescent antibody to membrane antigen test (FAMA) [7, 15] and a special glycoprotein (gp)-ELISA [13] have to be used. It is recommended that apparent "non-responders" to varicella vaccination should be re-tested with one of these assays. However, most tests employed are restricted to a small number of research laboratories and are not commercially available.

Practical Essentials

- **Laboratory analysis is needed for atypical clinical manifestations of varicella and zoster. In these cases, the virus should be detected by PCR.**
- **Varicella caused by the vaccine virus can be distinguished from naturally contracted infection using molecular biological methods.**
- **During pregnancy, laboratory diagnosis is indicated (1) when there is a negative, indeterminate or unknown history of varicella following exposure to the virus, (2) to diagnose intrauterine infection and (3) in case of suspected congenital varicella syndrome or neonatal chickenpox.**
- **Serological analyses are primarily justified for the determination of VZV immune status. Methodically, the most suitable tests are immunofluorescence assays and quantitative enzyme or chemiluminescent immunoassays. Independently of the laboratory, each positive result can be interpreted as immunity against infection.**
- **Serological methods are not useful for early diagnosis of varicella and zoster. In most cases, they only allow retrospective diagnosis.**



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