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CASE REPORT

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Fetal varicella syndrome: disruption of neural development and persistent inflammation of non-neural tissues

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Abstract Primary varicella zoster virus (VZV) infection during pregnancy is rare. If it occurs between the 8th and 20th week of gestation, fetal varicella syndrome results in 1–2% of the fetuses. We report about a varicella infection that affected a pregnant mother in the 12th week of gestation. At 33 weeks, a premature girl was born with destruction of neurons in spinal cord, spinal ganglia and plexus myentericus, and secondary developmental disturbance including mummification of one arm and segmental intestinal atresia. The brain did not show any abnormalities. However, VZV DNA could be detected by PCR in tissues from the brain and spinal ganglia. Chronic necrotizing inflammation was found in the placenta, fetal membranes, and one ovary. These locations showed nuclear inclusions which by in-situ-hybridization were proven to be VZV derived. This case demonstrates that in the fetal age, 'neurotropism' of VZV signifies severe destruction but not necessarily persistent inflammation of neural tissue. However, due to the inefficient fetal immune system, inflammation can go on for weeks, preferentially in non-neural tissues.

Keywords Pregnancy · Varicella Zoster Virus · Fetus · Placenta

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Introduction

Chicken pox is one of the most contagious diseases. Therefore, the prevalence of serological immunity is high, and only less than 5% of women of child-bearing age are susceptible to primary infection during pregnancy. If, however, primary infection occurs during weeks 8–20 of gestation, 1–2% of fetuses show a characteristic malformation sequence that originates from disruption of neural tissue development [3, 6]. Pathogenetically, it is the immunological immaturity of a fetus that is of major significance for the development of malformations. Therefore, the term "fetal" varicella syndrome was proposed [1]

We present a case of fetal varicella syndrome which shows the neurotropism in the destruction exerted by the virus. Our search for the virus in fetal tissues revealed a florid virus-related inflammation in non-neural tissues, while within neural tissue the virus was detectable only using polymerase chain reaction (PCR).

Clinical history

A 29-year-old primigravida presented with fever and a rash in the 12th week of pregnancy. Varicella zoster-specific immunoglobulin (Ig)G titer was negative at that time. Pregnancy was uneventful until polyhydramnios occurred in the 25th week. Sonography revealed asymmetric upper extremities, calcifications, and cystic structures in the abdomen. Cells obtained by amniocentesis showed a normal karyotype. Maternal serology at 32 weeks gestational age was highly positive for varicella zoster virus (VZV) IgG and borderline for IgM. In the 33rd week of pregnancy, the mother experienced contractions, cardiotocography was pathological, and a cesarean section was performed. After delivery, the infant required resuscitation, which was discontinued in view of the severe malformations. The patient died at the age of 70 min.

Materials and methods

DNA was extracted from formalin-fixed paraffin-embedded tissues. Ten-micron-thick sections were prepared and, after one section had been stained with hematoxylin and eosin, selected areas of five 10-µm-thick sections were scraped away and placed in a

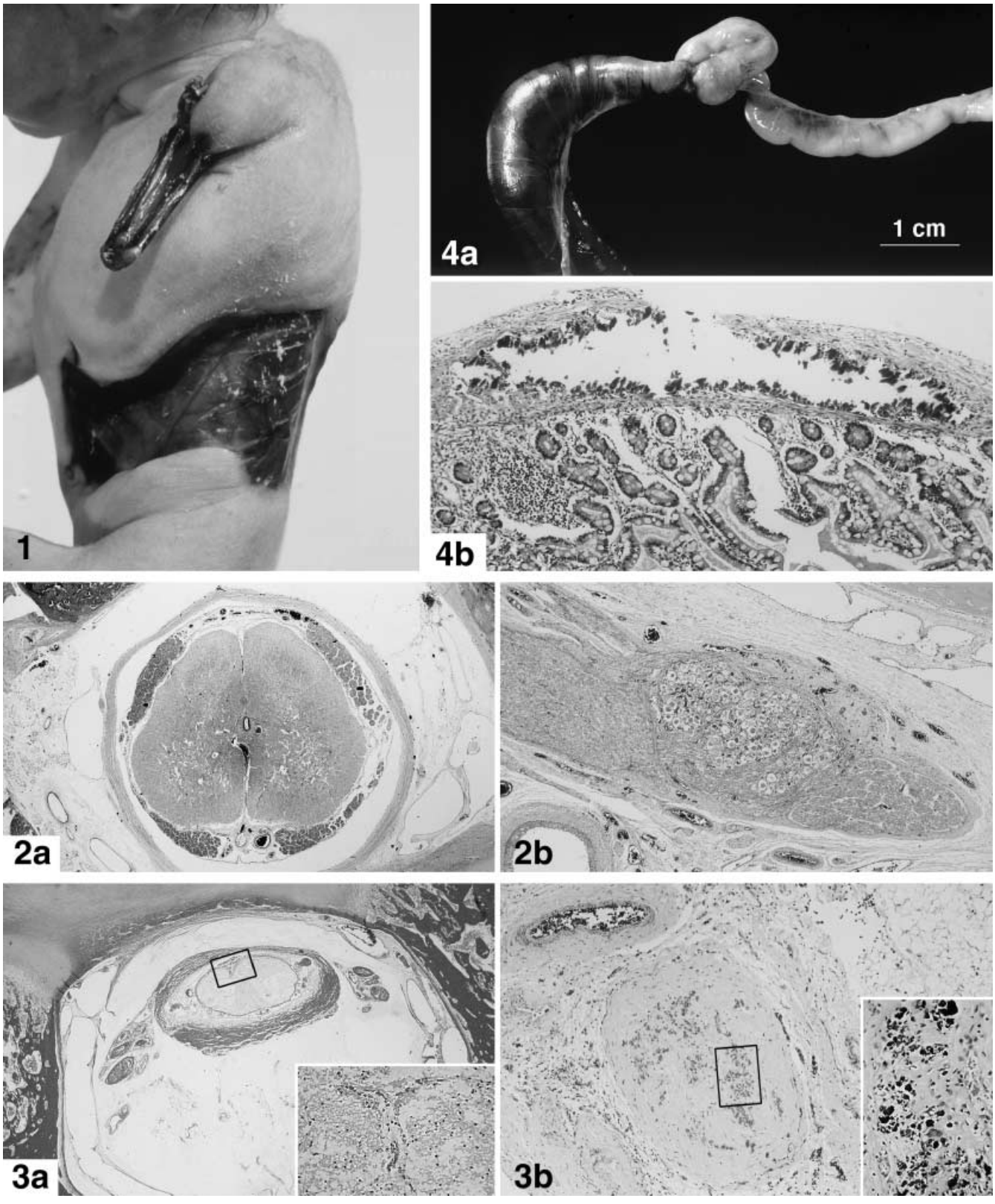

Fig. 1 Premature infant with skin rash on right upper arm, disruption of left arm development, and severe dermatomal scarring

Fig. 2 Intact spinal cord (**a**) and spinal ganglia (**b**) from the lumbar region of the patient. Hematoxylin and eosin, ×12.5 (**a**) and ×36 (**b**)

Fig. 3 Severe atrophy of the spinal cord in the lower cervical and upper thoracic segments (**a**). Many left-sided spinal ganglia with complete destruction of ganglion cells and residual calcification (**b**). Hematoxylin and eosin, ×12.5, inset ×280 (**a**) and ×90, inset ×280 (**b**)

Fig. 4 Atresia of small bowel with cystic dilatation (**a**), caused by selective destruction of the myenteric plexus marked by extensive calcifications (**b**). Hematoxylin and eosin, ×90

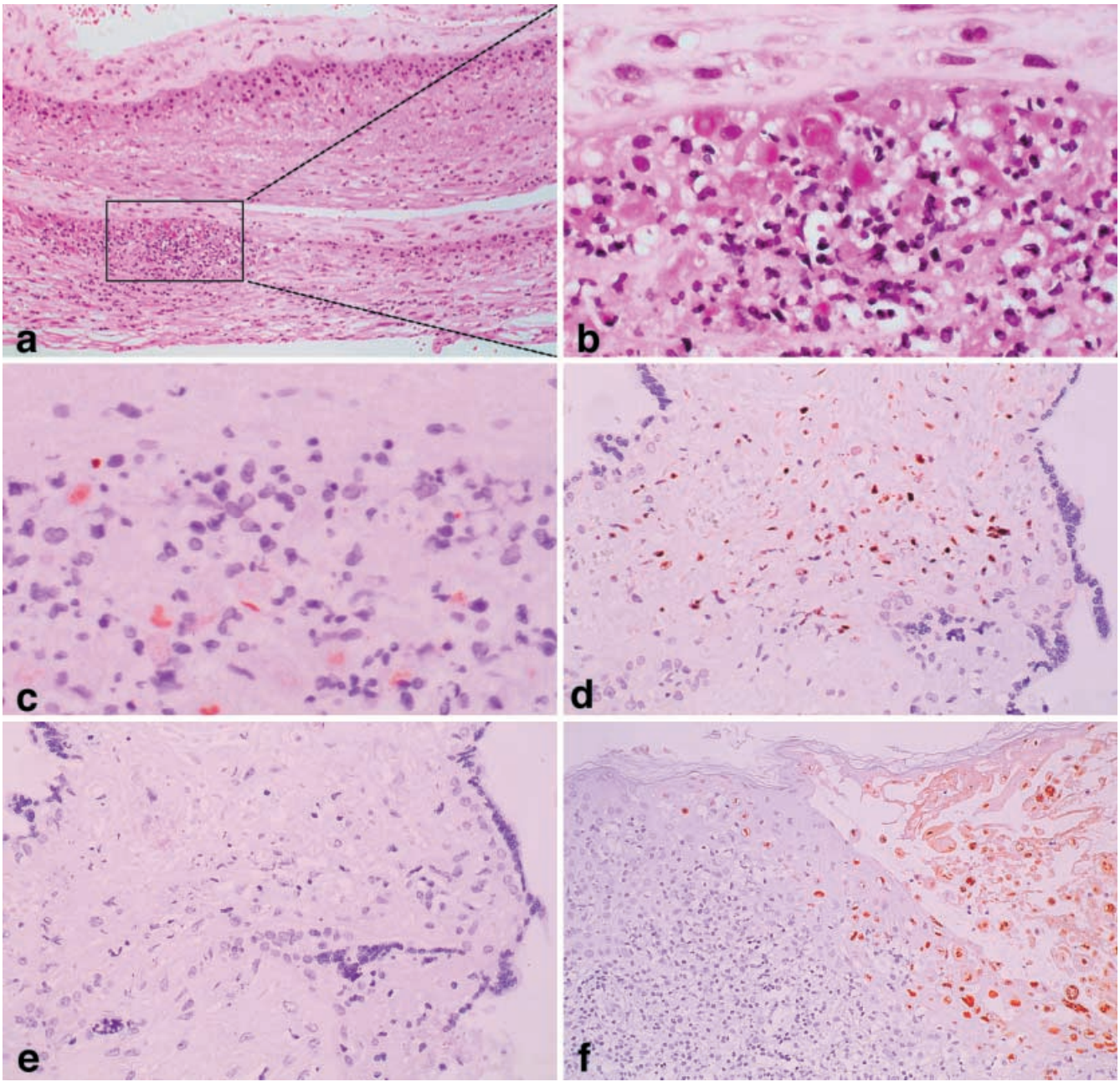

Fig. 5a–f Nuclear inclusions and varicella zoster virus (VZV) in situ hybridization: on the rolled extraplacental membranes, the amnion epithelium is lost; focally, neutrophils are clustered around large eosinophilic inclusions (**a**, **b**), which are positive in the VZV in situ hybridization (**c**). Rare chorionic villi show lymphocytic inflammation and nuclear inclusions in stromal cells with irregular outlines proven to be varicella-induced by in situ hybridization (**d**). Negative control (**e**). Positive control from a varicella blister. Hematoxylin and eosin, x100 (**a**), ×500 (**b**); in situ hybridization ×500 (**c**), ×250 (**d**, **e**), ×150 (**f**)

1.5-ml Eppendorf tube. DNA was extracted with phenol after dissolving the paraffin and proteinase K digestion. Amounts of less than 0.2 µg DNA were used as a template for a highly specific detection of VZV DNA using PCR. A nested protocol was used targeting the gB-gene (orf 31). As amplification controls, 10 and 100 copies of a plasmid carrying the outer 254-bp PCR fragment as insert were included. As an inhibition control, patient's DNA was spiked with 100 copies of the 'ampl. control', and 'mock' amplifications (H) as carry-over control separated the clinical samples. Primer 1 (CATTGAGGAAGTTGAAGCCAG) and primer 2 (GCTTCCAGTTCCAACCAACC) as 'outer' reaction generated a 254-bp product and served as template for the 'inner' reaction with primer 3 (TGGACTTTCCACGGGAGATA) and primer 4 (GCAGGTTCCAGTAATGCTCT), which produced the final 156-bp product. All oligonucleotides were from Microsynth (Balgach, CH). Amplification conditions for the thermocycler PE 9700 (Perkin Elmer, Foster City, Calif.) were: 10 min at 37°C (UDG protocol, Life Technologies, Paisley, UK); then 15 min at 95°C (for activation of "Hotstart" polymerase, Qiagen, Hilden, Germany); followed by 35 cycles of 30 s at 94°C, 30 s at 50°C, 30 s at 72°C, and a final extension of 5 min at 72°C.

In situ hybridization [4, 7] was performed on formalin-fixed and paraffin-embedded tissue using *Hin*dIII A and C fragments of VZV in a PBR 322 vector plasmid.

Results

External appearance

At autopsy, a girl, small and underweight for gestational age, was seen. Inspection showed a left arm with an intact bone structure but mummified soft tissue. The thoracic and abdominal wall showed a defect along the dermatomal segments T7 to T9, covered by a transparent membrane (Fig. 1). The left ankle and foot showed massive flexion deformity (talipes equinovarus), and the left leg was slightly shorter than the right. A maculopapular rash was present on the right upper arm. There was severe scoliosis of the thoracic spine.

Internal findings and microscopy

Analogous to the dermatomal pattern of disruption, histologic examination revealed a segmental pattern of damage to the spinal cord. A few lumbar segments showed intact architecture of the spinal cord with preserved motor neurons of the ventral horns and normal nerve roots (Fig. 2a). In contrast, most of the thoracic and cervical spinal cord was destroyed and reduced to a strand of gliotic scar, surrounded by a rim of collagenous tissue (Fig. 3a). Multiple spinal ganglia showed complete loss of ganglion cells, replacement by fibrous tissue, and residual dystrophic calcifications (Fig. 3b). The abdominal calcifications and cystic structures seen in prenatal sonography corresponded to a short segment of atresia of the small bowel and preatretic dilatation (Fig. 4a). Microscopically, ganglion cells of the myenteric plexus were destroyed and residual dystrophic calcifications were surrounded by multinucleate giant cells of foreign body type (Fig. 4b). The brain was completely normal in size and in its gyrational pattern. On microscopic examination, there was no necrosis and no increase in astrocytic or microglial cells. The eye was not examined. Remarkably, a lymphohistiocytic inflammation was present in the ovary and multiple stromal nuclei contained eosinophilic inclusion bodies. The placenta presented a focal chronic villitis with lymphoplasmocytic and histiocytic

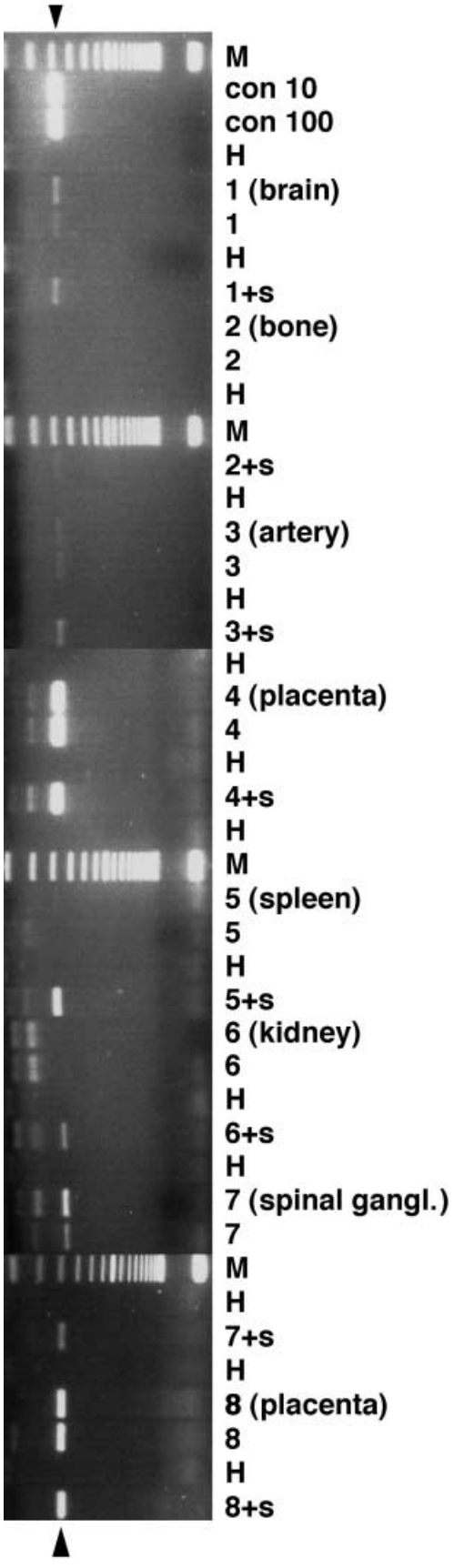

Fig. 6 Polymerase chain reaction (PCR) products analyzed on an ethidium bromide-containing 2% agarose gel. In all PCR lanes, 10 µl amplified material was loaded. *Lanes M* contained a 50-bp DNA ladder; *lanes con10* and *con100* contained 10 or 100 copies of the positive control plasmid. *Lanes* marked *H* contained the H_2O mock control, and the addition *+s* indicates where a spike of 100 copies of positive control DNA was added to the respective patient's DNA sample. All samples were analyzed as independent duplicates (same experiment, separate tubes). *Lane 4* represents DNA from a placental area with inflammation and viral inclusions in hematoxylin and eosin stain and the in situ hybridization. *Lane 8* contained DNA from placental areas without inflammation and without viral inclusions but still reveals a strong signal after PCR. *Arrowheads* indicate the gel position of the expected PCR product of 156-bp length. Amplification signals of smaller size than the expected product correspond to primer dimers and associated nonspecific PCR products

infiltrates; eosinophilic inclusions were seen in large nuclei of stroma cells. In the membranes, chorionic cells were found with inclusions surrounded preferentially by neutrophils (Fig. 5).

Detection of virus

Corresponding to the eosinophilic inclusions, in-situ hybridization detected the presence of VZV DNA within chorionic villi and within the chorionic layer of the extraplacental membranes (Fig. 5). The small focus of cells with viral inclusion bodies in the ovary could not be examined further. Neither spinal cord nor spinal ganglia nor neurons or accompanying cells of the plexus myentericus showed a positive signal in the in situ hybridization. Like others [9, 11], we used a PCR protocol in order to increase the sensitivity for the detection of varicella virus DNA. An amplification signal of appropriate size was detected in positive control and in tissue from the placenta (areas with and areas without inflammation), brain, spinal ganglia, and tissue from great arteries. VZV was not present in kidney, spleen, or bone marrow (Fig. 6).

Discussion

After the age of 14 years, more than 95% of adults show serologic immunity to the VZV. With the acute phase of chickenpox, the virus is not eliminated, but a latent infection is established in the spinal ganglia [4, 13]. With waning immunologic control in an immunosuppressed host, or simply with advancing age, infection reactivates. The disease – then called shingles – brings about a skin rash that occurs in a characteristic distribution following one to several dermatomes. The dermatomal pattern is a striking feature also in the much more severe symptoms of congenital or fetal varicella syndrome [2, 10, 12]. Intestinal lesions as seen in our case have only been reported rarely [5, 8].

The latency of viral DNA in neural tissue has been shown in healthy adults [9]. Also in our patient, PCR proved the presence of virus in neural tissue and it is thus demonstrated that neurons are a sensitive target for varicella virus and readily destroyed. Quite different from the adult, there is an ongoing inflammation within non-neural tissues that contains viral DNA to such an extent that it is revealed by the far less sensitive in-situ hybridization and even by typical viral nuclear inclusions. Few other reports also mention the persistence of VZV DNA in non-neural tissue of fetuses [11, 14].

Immunological control of VZV infection involves predominantly the production of immunoglobulins IgM and above all IgG. The immature immune system of a fetus on its own cannot control VZV infection. Thus, VZV infection of a fetus takes a different time course than infection in later life would. In our case, an active inflammation around viral inclusions went on for more than 20 weeks; in a case reported in the literature, VZV has persisted in the epithelial lining of the esophagus for nearly 2 years after birth [14].

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