Write an account on laboratory diagnosis and prevention of chickenpox virus?

The clinical presentations of varicella or zoster are so characteristic that laboratory confirmation is rarely required. Laboratory diagnosis is required only for atypical presentations, particularly in the immunocompromised, and for distinguishing between HSV infection and herpes zoster.

1. Direct Methods

   a. **Cytology** - smears of scrapings of the base of the lesions will reveal characteristic multinucleate giant cells, also known as Tzanck cells. However this technique will not distinguish between HSV and VZV infection.

   b. **Electron microscopy** - herpesvirus particles can be seen in fluid taken from the early vesicles of either varicella or zoster. This technique cannot distinguish between HSV and VZV.

   c. **Immunofluorescence cytology** - smears of the base of lesions can be examined by immunofluorescence cytology, as in the case for HSV. This technique is more sensitive than EM but is more labour intensive and requires greater technical expertise.

   d. **Molecular Methods** - PCR assays for VZV are available and have been reported to be of use in the diagnosis of VZV meningoencephalitis from CSF specimens.

**Virus isolation**

This remains the definitive method for diagnosing VZV infections. Human fibroblasts are used in most laboratories. Vesicle fluid and scrapings form the base of fresh lesions are the most suitable specimens. Virus can rarely be recovered from crusted lesions. Biopsy material can also be cultured. The CPE produced by VZV is so characteristic that most laboratories do not undertake further identification of the isolates. Immunofluorescence of the cell sheet by monoclonal antibodies is the method of choice for identification. Virus isolation for VZV is rarely carried out because of the long length of time required for a result to be available.

![Cytopathic effect caused by VZV in cell culture. (Courtesy of Linda Stannard, University of](attachment:image.png)
Serology

The most important use for serology is the determination of immune status before the administration of prophylactic therapy. Serological diagnosis of primary varicella infection can be reliably carried out using paired acute and convalescent sera. However, this is less reliable in the case of herpes zoster where there is specific antibodies present already. Therefore it is essential to obtain the first sample as soon as possible after the onset of the rash in order to demonstrate a rising titre. The sharing of antigens between HSV and VZV can make the interpretation of results very difficult. Where possible, the serological diagnosis should be backed up by virus isolation.

a. CFT - this is the most frequently used test. It is perfectly adequate for the diagnostic purposes but is too insensitive for immune status screening.
b. IF - immunofluorescence is appreciably more sensitive than CFT and is used for the determination of immune status in many laboratories.
c. RIA and EIA - these are the most sensitive methods available and therefore are the preferred method for determining immune status. EIA are rapidly becoming the most commonly used method for the determination of immune status.
d. Detection of VZV specific IgM - VZV IgM can be determined by IF and capture RIA or EIA. VZV IgM is produced in primary varicella and herpes zoster and thus it is not possible to distinguish between the two. However, the VZV IgM tests are likely to prove invaluable in determining the nature of congenital varicella infections.

Management

1. Varicella

Varicella is normally a mild disease in immunocompetent individuals and no specific treatment is normally required. Varicella in immunocompromised patients can be a serious and potentially fatal disease. Due consideration must be given to the reduction or withdrawal of any immunosuppressive therapy and the administration of antiviral chemotherapy. Acyclovir is now the drug of choice (VZV is not as susceptible to acyclovir as is HSV and requires 10 fold higher concentration of the drug for effective inhibition) for the treatment of varicella in the immunocompromised and also in normal patients with VZV pneumonia. Approval has also been given in the US for the use of acyclovir for the treatment of chickenpox in otherwise healthy children and adolescents. A multicentre trial of acyclovir demonstrated the efficacy of acyclovir in accelerating cutaneous healing, reduced fever and constitutional symptoms. The use of acyclovir in such an instance is highly debatable and should be left to the primary care physician with full knowledge of the medical and economic factors. Acyclovir is also being increasingly used for prophylactic purposes in immunocompromised patients but it is not possible to give guidelines on their use at present. Other drugs are being evaluated at present. A compound
known as 882C had specific activity against VZV but without any significant activity against HSV or CMV. Specificity is achieved by the requirement of the drug for VZV thymidine kinase in order to be converted to the monophosphate and the diphosphate form.

2. Herpes Zoster

Herpes zoster in a healthy individual is not normally a cause for concern. The main problem is the management of the postherpetic neuralgia. Most modern analgesics are ineffective and opiates should not be given for prolonged periods. Steroids have been reported to be effective but should be given carefully because of the immunosuppressive nature of the drug. Heroic surgery involving the removal of the dorsal roots may be required for those with severe intractable pain. More severe forms of zoster, particularly disseminated disease, are seen in immunocompromised individuals. In contrast to primary infection, reactivation rarely results in life-threatening infection with visceral involvement in these patients. Nevertheless, antiviral chemotherapy is increasingly being given in order to reduce the risk of dissemination and the duration of the illness.

Acyclovir is the drug of choice and studies have shown that it reduces the duration of virus shedding and accelerates healing but should be given within 48 hours of the onset of symptoms. Acyclovir is usually not justifiable in individuals without underlying disease, except perhaps in the case of ophthalmic zoster when it can be given orally to reduce the risk of ocular complications. The treatment should be continued for 7 days. Previously, it was thought that acyclovir has no effect on zoster-associated but more recent studies suggest that it may be particularly beneficial to those over 50 years of age. Following treatment with the recommended dose of acyclovir (800 mg 5 times daily), these patients become pain-free twice as fast as those receiving placebo. The International Herpes Management Forum now recommends that antiviral therapy should be offered routinely to all patients over 50 years of age presenting with herpes zoster. The advent of newer agents such as valaciclovir and famciclovir will expand the range of antiviral therapies available to the practitioner. In an intent-to-treat analysis of all patients treated within 72 hours of rash onset, famciclovir, the oral pro-drug of penciclovir offered no advantage over acyclovir. However, valaciclovir was reported to resolve zoster-associated pain about one-third faster than acyclovir. There had been no published comparative studies of valaciclovir and famciclovir.

Prevention

Preventive measures should be considered in individuals at risk of serious disseminated varicella infection ie. the immunocompromised and neonates. Immunocompromised individuals should be advised to avoid contact with people with varicella or zoster. If contact has been made, prophylaxis with passive immunization or antiviral chemotherapy should be considered.

1. Passive Immunization

Zoster immune globulin (ZIG) were prepared from patients recovering from shingles. Now ZIG is prepared from blood donors with high titres of anti-VZV. ZIG ii frequently in short supply and
if unavailable, prophylactic acyclovir or HNIG should be given. A reasonable history of varicella from a contact is a reasonable indicator of immunity and obviates the need to administer any prophylactic. Nevertheless, definitive assessment of the immune status can only be made on serological testing. In any case, the administration of HIZG should not be delayed until the results are available although a baseline sample should be obtained. HIZG is not totally effective in preventing infection by VZV. In a study carried out in the UK, 18 out of 27 (67%) seronegative children given HIZG were infected (compared to a normal infection rate of 90%), 14 of whom had symptoms. The rationale of administering HIZG to those at risk is not so much to prevent infection but to prevent the more serious forms of illness with visceral involvement. The actual effectiveness of HIZG in attenuating varicella has yet to be established fully by clinical trials. It is important, particularly in a hospital environment, to bear in mind the shortcomings of HIZG as patients given HIZG are still liable to develop varicella and become a source of infection to others.

HIZG should also be given to susceptible pregnant women in close contact with VZV infection in the hope that it will reduce the risk of transmission to the fetus. Again there is no data on the actual efficacy. In any case, VZV infection is likely to be more severe in pregnant women. HIZG should also be given to the newborn infants of mothers who contract varicella perinatally. It should be noted that acyclovir is not licensed for use in pregnant women or neonates. The types of patients at risk of contracting the severe forms of varicella are listed below:

1. Leukaemias, Hodgkin's disease and other neoplasms of the lymphoreticular system.
2. Other malignancies being treated with cytotoxic drugs or immunosuppressive regimens such as radiotherapy.
3. Primary immunodeficiency syndromes.
4. Bone marrow transplant recipients irrespective of their own or the donor's VZV status.
5. Diseases being treated with high dosage steroids.
6. Susceptible pregnant women in close contact with VZV.
7. Newborn infants of women who contract varicella <= 7 days before or after delivery.
8. Premature infants whose mothers have no history of varicella or any infant whose birth weight was < 1000g.

2. Active Immunization

The currently marketed varicella vaccines are based on the Oka strain of VZV which originated from Japan. It has been modified through sequential propagation in different human and animal cell cultures. Various formulations of such live, attenuated vaccines have been tested extensively and are approved for use in Japan, the Republic of Korea, the United States and several countries in Europe. Some formulations are approved for use at 9 months of age and older. Following a single dose of the above-mentioned vaccines, seroconversion is seen in about 95% of healthy children. From a logistic as well as an epidemiological point of view, the optimal age for varicella vaccination is 12-24 months. In Japan and several other countries 1 dose of the vaccine is considered sufficient, regardless of age. In the United States, 2 doses, 4-8 weeks apart, are recommended for adolescents and adults, in whom 78% were found to have seroconverted after the first, and 99% after the second dose of the vaccine. Children below 13 years receive only 1 dose. Small studies, using formulations different to that currently licensed in the US, show that when the vaccine is administered within 3 days after exposure to VZV, a postexposure protective
efficacy of at least 90% may be expected. Varicella in persons who have received the vaccine ("break-through varicella") is substantially less severe than the disease in unvaccinated individuals. Further studies are needed to clarify the postexposure efficacy of the currently licensed product, especially in outbreak situations.

Live attenuated vaccines are normally contraindicated in immunocompromized individuals. Clinical trials showed that in normal symptomless individuals, symptomless seroconversion is regularly achieved in up to 90% of the vaccinees, but the levels of antibodies are substantially lower than observed after wild virus infection. The seroconversion rate is lower in immunocompromised patients. The vaccine also confers significant protection in immunocompromised children but can cause mild symptoms of rash and fever. The vaccine strain is sensitive to acyclovir should that be required. The vaccine is potentially useful for postexposure prophylaxis. Antibody responses do not appear 3 to 5 weeks after vaccination but CMI responses develop within 4 days of vaccination in approximately 50% of vaccinees and has been shown to confer protection shortly after contact. On the downside though, the vaccine can establish latent infection in some vaccinees and reactivate to cause zoster. However, this occurs far less often than the wild-type virus. The vaccine is transmissible and when it is transmitted, it causes a mild rash in most cases. The prime target for vaccination will be young seronegative children who are immunocompromised. Consideration is also being given to the possibility of vaccinating seropositive adults with the aim of preventing zoster. In the US, the vaccine has now been licensed and is part of the routine universal vaccination programme in children. A single dose of vaccine is given at 12 months of age. For older children and adults over the age of 12, two doses of vaccine are required. Cost benefit analyses which had been carried out were in favour of universal vaccination, especially taking into account the time that parents may have to take off from work as a result of their child’s illness.

In the past, certain lots of the vaccine given to leukaemic children on maintenance chemotherapy resulted in a high incidence of vaccine-associated chickenpox which was sometimes severe. Fortunately, there were no deaths and the lots which were identified contained less-attenuated virus. The newer lots in use appears to be very safe. The prime target for vaccination will be young seronegative children who are immunocompromised. Consideration is also being given to the possibility of vaccinating seropositive adults with the aim of preventing zoster. In view of the efficacy of ZIG and acyclovir in preventing and treating chickenpox in immunocompromized children, caution should be exercised before the vaccine is recommended for immunocompromized children. However, the following criteria should be considered for vaccination.

1. Susceptible to varicella
2. In haematological remission
3. Treatment stopped from 1 week before and 1 week after vaccination

At St Bartholomew’s hospital, UK, 50 leukaemic patients have been vaccinated with a seroconversion rate of 70%. It has been proved to be a safe and effective vaccine. 1 booster dose should be given. The breakthrough infection rate is less than 10% after close contact. The vaccine elicit rapid efficient CMI and can thus be used in postexposure prophylaxis. The problem with this vaccine is that it can remain latent and reactivations can occur, However,
reactivations tend to be less serious. The vaccine is expensive to produce and is relatively unstable, requiring storage at -20°C.

As in the case of mumps, there is a lot of controversy as to whether VZV should be incorporated as part of a universal vaccination program. Several cost-benefit studies had been carried out in different countries which claim to show a benefit. In theory, it should be possible to incorporate VZV into the current MMR vaccine which will increase its attractiveness. At present though, it is still up to the parents to decide whether to vaccinate their children or not. The cost of the vaccine is borne by the parents in most instances.

3. Management of outbreaks in hospital

Patients with uncomplicated chickenpox do not require admission to hospital. If admission is required, then the patient should be put in respiratory isolation. The same applies to patients with other conditions who develop chickenpox while in hospital. For patients with shingles, contact isolation would be sufficient. Other patients on the ward should be assessed for immunity for VZV; usually, a past history of a chickenpox-like illness diagnosed by the General Practitioner is sufficient; however, IgG antibody screening of all patients would be preferable, especially for those individuals who are predisposed to severe chickenpox. Those found to be negative should be discharged home if their condition allows. If they must remain in hospital, they should be cohorted together and put in respiratory isolation for at least 21 days until the incubation period has passed. ZIG should be given to seronegative patients who are susceptible to severe VZV disease such as leukaemic children, immunocompromised individuals. These patients who are given ZIG and isolated should be isolated for at least 28 days since ZIG is known to prolong the incubation period. Likewise, staff should be assessed for past immunity to VZV, if negative, they should either refrain from work for 21 days (US guidelines), or remain in the same ward, or transferred to other wards with immunocompetent patients doing work which requires less patient contact. With the availability of the vaccine, it may be advisable to screen all staff in high risk wards and immunized if found to be negative. The problem is whilst this is easy for staff permanently based on the ward, there is also a high turnover of non-permanent staff such as doctors, domestics, porters etc. There is now active discussion on the possibility of screening and administering the vaccine to medical and nursing students.

H. VZV infection during pregnancy

Varicella is one of the classical diseases of childhood. The majority of individuals has been infected before reaching adulthood so that varicella is uncommon during pregnancy. The actual incidence of varicella during pregnancy is not known but is now thought to be more common than rubella because of rubella vaccination. Pregnant women who contract VZV are at risk for the more serious complications such as pneumonia and encephalitis, which may be fatal. There is controversy surrounding the administration of ZIG to susceptible women in contact with chickenpox. There is evidence to suggest that ZIG does reduce the attack rate and the severity of the disease and thus should be given to pregnant women if supplies permit. Intrauterine and
perinatal infection may occur with three possible clinical expressions:

1. Maternal chickenpox during the first 5 months of pregnancy may be followed by congenital varicella syndrome in the fetus
2. Maternal chickenpox during the second and third trimester may lead to the appearance of zoster in a healthy child
3. Maternal chickenpox just before or after delivery may cause severe neonatal disease

1. Congenital varicella syndrome

Congenital varicella syndrome comprises of a number of abnormalities, some of which scarring of the skin and hypoplasia of one or more limbs are characteristic. Other abnormalities include CNS and eye abnormalities, abnormalities of the GI and GU tract. Death occurs in infancy in many cases. It had been postulated that the principal manifestations of varicella embroopathy are not caused by primary infection of the fetus but by in utero reactivation of virus whose period of latency in the fetal nervous system has been shortened by immunological immaturity.

The incidence of embryopathy is difficult to determine. For a start, the syndrome is ill defined. Scarring of the skin and hypoplasia of a limb are characteristic but CNS and eye abnormalities may be due to other causes. The risk had been reported to be 3% but this is likely to be an over-estimate. There are at least eight case reports of infants suggestive of congenital infection born to mothers with a history of zoster during the first 4 months of pregnancy, however this association may be coincidental and to date, there is no firm data linking maternal zoster to congenital infection. Specific IgM can be detected from the cord blood in many, but not all cases of suspected congenital varicella syndrome.

2. Zoster in childhood

Cases of childhood zoster had been reported from infants whose mothers contracted chickenpox during the second and third trimester of pregnancy. Other cases of childhood zoster occurs in children who contracted chickenpox very early in life. In general, case reports of childhood zoster, whether resulting from pre or post-natal infection, supports the hypothesis that the earlier the child gets chickenpox, the earlier it is likely to get zoster because of the immaturity of the immune system.

3. Neonatal chickenpox

Chickenpox during the first few months of life is uncommon, partly because of the lack of exposure and because of the presence of maternal IgG. The newborn child of a non-immune mother may contract the disease if the mother develops the rash at the time of delivery. The severity of the disease may vary from a few spots to a severe fatal disease with pneumonia and general dissemination involving the lungs, spleen, heart, pancreas, kidneys and suprarenal glands. The fatality rate of 30% that had been reported is probably exaggerated. The risk of a child acquiring varicella is dependent on the rapidity with which the mother develops and transfers humoral immunity across the placenta and that depends on the time interval between the date of onset of the rash in the mother and the date of delivery. If the onset of the rash in the
mother occurred seven days or more before delivery, sufficient immunity will have been transferred so that even if infection has occurred in utero, the infection will be mild or inapparent. Thus the period of high risk is the development of maternal rash between 5 days before delivery and 2 days after delivery. ZIG should be given to all infants whose mothers develop chickenpox during the last seven days of pregnancy or the first 14 days after delivery but its effect is uncertain. In all probability, it probably does reduce severity.

The diagnosis is made by observing the symptoms and the typical appearance of the rash. The blisters are like dew drops on the petal of a flower. Except that the dew drop is on a background of red rash.

Laboratory diagnosis is seldom required because of clear-cut clinical signs. Examination of the fluid present within the lesion under the microscope shows characteristic round particles. Scrapings from the floor of the lesion show, what are called, Giant cells.