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COMMUNITY RESPIRATORY VIRAL INFECTIONS: AN OVERLOOKED CAUSE OF MORBIDITY AND MORTALITY IN TRANSPLANT RECIPIENTS

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INTRODUCTION

Acute respiratory tract infection is one of the most common human diseases. Viruses are far more frequent etiologic agents of these conditions than bacteria (1,2). Until recently, study of respiratory viruses was restricted to the pediatric population and the role of respiratory viruses in adults was overlooked.
For many years, the interest in the etiology of acute respiratory infections in adult transplant recipients had been focused on opportunistic microorganisms mainly bacterial or fungal, with the exception of viruses of the *Herpesviridae* family. As diagnostic methods for these infections became more accurate and patients were being treated more appropriately, it was clear that more than 30% of pneumonias in the transplant recipient were of unknown etiology and mortality in this group could be as high as 60% (3). After several reports of severe respiratory syncytial virus infections in immunocompromised adults, it was apparent that respiratory viruses should be added to the list of pathogens affecting transplant recipients (4-6).

The objective of this chapter is to discuss the diagnostic methods of respiratory viral infections in critically-ill transplant recipients. The natural history of the infection, except for the frequency of complications, caused by each respiratory virus is the same in

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immunocompetent as in the immunocompromised host, and should be part of the diagnostic process in order to obtain the appropriate specimen at the appropriate time.

Conventional respiratory viruses (CRV) include influenza A, B, and C, respiratory syncytial virus, parainfluenza types 1,2,3, and 4, adenoviruses, picornaviruses (rhinoviruses and enteroviruses), and coronaviruses.

A characteristic feature of influenza viruses is the single-stranded RNA genome that occurs in eight separate segments. As a consequence, influenza viruses exhibit high frequencies of genetic recombination. Of the three types of influenza virus, types B and C are pathogens unique to man. Influenza A (IA) has been isolated from various species of birds and mammals in addition to man. The occurrence of reassortment between two IA viruses in cells simultaneously infected is considered to be the basis for emergence of new subtypes. This phenomenon is called antigenic shift. Minor antigenic changes occur sequentially with time. These are known as antigenic drift and are presumed to be natural mutations selected by population immunity. The constant changes of influenza A virus leads to renewed susceptibility of previously infected persons so that reinfection and illness may occur (7).

As respiratory syncytial virus (RSV) and parainfluenza viruses (PIV) induce a partial immunity, reinfections are frequent at all ages. They are usually associated with the common cold or are asymptomatic. Adenovirus (ADV) infection is associated to species-specific neutralizing antibody production and prevention of symptomatic reinfection. Many adenovirus serotypes cause respiratory disease (8). Although not routinely recognized as respiratory viruses, evidence that enteroviruses cause respiratory disease has been obtained from different sources (9,10) and the majority of enterovirus (EV) serotypes have been related to respiratory illness (11). More than 100 serotypes of rhinovirus (RV) have been recognized. Following rhinovirus infection, a specific humoral response develops in both the serum and in nasal secretions. Serum antibodies remain elevated for a number of years and probably protect individuals from illness during this time, but not from infection with the same serotype. There is little evidence of cross-immunity to other rhinovirus (12). The total number of serological types of coronavirus (CV) which infect man has not been defined. The prototype strains are 229E and OC43. Reinfection with the same or related strains is common (13).

Therefore, due to the great variety of agents capable of affecting the respiratory tract and the feasibility of reinfections, viral respiratory infections occur repeatedly throughout life in the immunocompetent and in the immunocompromised host.

Some significative aspects should be pointed out regarding viral respiratory tract infections besides their frequency, such as the possibility to be acquired in either the community or in the nosocomial setting, the distinct efficiency of each virus to extend the infection from the upper to the lower respiratory tract, the possibility of inducing bacterial or fungal superinfection, and finally the correlation with organ rejection (14,15).

Infection by respiratory viruses can cause a wide spectrum of clinical manifestations ranging from common cold to life-threatening pneumonia. Respiratory infections can be classified into upper and lower depending on whether the major area involved is above or below the epiglottis. Syndromes associated with one specific agent often overlap greatly with those caused by other, quite different respiratory agents (16,17). However, some viruses, such as IA and RSV, are more likely to cause lower respiratory tract infections (LRTI) than others (18). Respiratory virus infections during infancy often lead to severe lower respiratory tract disease. Re-infection later in life is usually mild except in the high-risk population (elderly persons and patients with underling conditions). Lower respiratory tract infections, particularly in infants and in the elderly, are among the most common reasons for hospitalization in developed areas (19-21). In a significative number of cases infections are simultaneously caused by two or more viruses, producing in these cases more severe respiratory illnesses, and more frequent hospitalization (22,23).

Respiratory viral infections are transmitted from person to person by means of virus-containing respiratory secretions. Infectious particles may be implanted on nasal surfaces from contaminated hands (RV, RSV) or from large droplets (RSV, PIV, some EV, ADV, CV), or may reach the lower respiratory passage from aerosols (IA, IB, CV). Since humans sample the environmental air about 20 times a minute in breathing, and the average person inhales at least 8 microorganisms every minute, or about 10,000 every day (24), it is no wonder that exposure to an infection with respiratory viruses is very common.

The respiratory tract provides a first line of defense against this microbial invasion. The integrity of the mucosa, production of mucus and levels of secretory IgA play an important role. Respiratory viruses produce illness as a consequence of local multiplication. When ciliary action moves the inhaled microorganisms from the initial site of infection in the direction of the throat they can establish fresh sites of infection. Viral infection of the respiratory tract provokes the loss of cilia and ciliar cells, altering the mucociliary scalator and consequently the clearance of mucus, particles and microorganisms (25). If cilia are not functioning properly there is a gravitational flow to other parts of the respiratory tract. Some of the viral proteins expressed in the membrane of cells can act as receptors for bacteria. At the same time, virus replication induces changes in the cell surface, which can also serve as bacterial receptors. It has been demonstrated in experimental infections in humans that 15% of people with influenza A virus are colonized by pneumococcus in 6 days after viral infection. None of them had pneumococcus prior to influenza infection (26).

Association of viral and bacterial infection has been well demonstrated in several clinical conditions, such as in acute otitis media (27) and post-influenzal pneumonia (28). Some studies have even shown the efficacy of influenza vaccination in preventing acute otitis media (29,30)

Each respiratory virus has its own seasonal distribution. Influenza viruses and RSV infections occur during the winter, parainfluenza virus infections throughout the year, rhinoviruses during the autumn, coronaviruses in the winter and spring and adenoviruses all year round. It is noteworthy that nosocomial outbreaks may occur outside of the usual season (31). Different respiratory viruses are in circulation at the same time.

Conventional respiratory viruses may be acquired at any time following transplantation, since these patients are just as susceptible to viral infections of the respiratory tract as the general population. Moreover, nosocomial transmission seems to be very common (32). Over the last decade the high frequency and significant clinical impact of CRVs infections in transplant recipients has been established (33-41). Respiratory viruses are presently considered important causes of pneumonia in these patients. Furthermore, they are now appreciated as the cause of

many of the previously categorized "idiopathic" pneumonias in adult bone marrow transplant patients (3).

Making a diagnosis of viral respiratory tract infection is important in order to take critical decisions on the management of patients. First of all, the possibility to treat some of these infections with antivirals (33,35-38,42-46) makes the diagnosis useful from the point of view of individual patients. Secondly, knowledge that a virus is in circulation in the community or in the hospital allows health personnel to implement preventive measures (32,47). Finally, in some cases, delaying transplantation until the patient recovers could be an option. Laboratory diagnosis of infection in the transplant recipient can be facilitated by the use of established protocols for specific specimen types or specific clinical presentations. The use of established protocols ensures critical specimens will be processed appropriately

Detection or isolation of conventional respiratory viruses usually establishes the etiology of the disease (48) since an asymptomatic carrier state has not been recognized. Only adenoviruses are considered to produce latent infection. However, because pediatric bone marrow transplantation recipients appear to be infected by adenovirus more frequently and earlier in the post-transplant period than adults, detection of adenovirus in children probably reflects mainly primary infection (49). Due to the potential lethality of these infections, irrespective of whether they are primary infection, re-infection or reactivation, close follow-up of adenovirus infected patients is essential (50,51). In immunocompromised patients a more prolonged shedding of respiratory viruses is relatively common and is generally associated with symptoms.

Good diagnostic virology depends on the quality of specimens and the information supplied to the laboratory. Since all viruses multiply within living-cells, the material for diagnosis should be taken from the principal site of infection and contain as many whole cells as possible. For recovering viruses from the respiratory tract, swabs from both throat and nasal passage should be collected. For the appropriate collection of throat swabs a vigorous rubbing of the posterior pharynx and tonsils should be done. Some studies showed that nasopharyngeal washes produced better specimens than nasopharyngeal swabs probably because larger quantities of secretions were obtained (52,53). If antigen detection tests are to be done in addition to, or instead of, virus isolation,

nasopharyngeal secretions by washing or aspirating the posterior nasopharynx should be collected. Cells obtained by bronchoalveolar lavage (BAL) can also be used (54).

At Hospital de la Santa Creu i Sant Pau (HSCSP), nasopharyngeal secretions are collected by inserting a plastic catheter, previously connected to a syringe containing 1-2 ml of sterile saline solution, into one nostril, introducing it to the nasopharynx, and aspirating by gentle suction. Mucus is transferred to a sterile tube by means of passing the saline solution from the syringe through the catheter.

Specimens should be withdrawn early in relation to onset of symptoms. For respiratory infections, virus shedding varies from 3 to 7 days, and viruses are present in a higher titer at the onset. Rapid delivery to the laboratory is also essential since the majority of respiratory viruses are fragile, survive poorly outside the human host, and are particularly susceptible to drying, heat and pH change. For optimal recovery of viruses, specimens should be transported on wet ice or placed in a refrigerator at 4°C. If the specimen is not going to be processed within a maximum of 5 days, freezing at –70°C is advised (55).

Exfoliated epithelial cells recovered from the respiratory tract may present with a variety of cytological features such as multinucleation, smudged nuclei, and nuclear or cytoplasmic inclusions but these changes are found only in a small proportion of cells (56). Respiratory viral infections are almost never diagnosed on the basis of cytologic specimens only.

The gold standard for laboratory diagnosis of viral infections is the recovery of a virus. Because viruses are able to recognize and enter only appropriate target cells, each specimen should be inoculated in various culture systems in order to isolate different viruses. Cell cultures permit the detection of a single infectious particle and also the replication of a wide number of viruses. They also provide confirmation of the results obtained by other techniques. Viral isolation techniques enable identification of those viruses for which rapid methods of detection have not yet been developed. It is important to isolate the virus to perform genetic and antigenic studies. Most CRV grow readily in cell cultures (57).
Furthermore, the only option to detect rhinoviruses is by means of isolation in cell culture. The most common cell lines used are MRC5 or WI-38 human embryonic fibroblasts or fetal tonsil cells.

Presumptive identification of an isolate as a rhinovirus is made on the basis of cytopathic effect which is detected two or three days after inoculation, and demonstration of lability at pH 3 (58). Respiratory coronaviruses are not grown in tissue culture. Most diagnoses in the past have been made by serologic methods and only for strains 229E and OC43 group.

Because viral isolation in cell cultures is, in many cases, not sufficiently rapid to influence patient management greater emphasis has been placed on the development of tests capable of detecting virus in clinical specimens within a few hours.

The application of shell-vial technology for the detection of respiratory viruses using multiple cell lines can provide a relatively rapid diagnosis. However, is labor intensive and requires immediate inoculation of specimens in the cell culture since it relies on virus viability (59,61). Furthermore, sensitivity of this technique for ADV and PIV is not sufficiently high (62).

The immunofluorescence (IF) technique has been recommended by the World Health Organization as an efficient and rapid method for detection of viral antigen in clinical specimens (63). This method allows diagnosis two hours after specimen arrival in the laboratory. It requires the use of high-quality reagents, accurate obtention and processing of the specimens and technical expertise for interpretation. Each reagent employed must be carefully tested for specific and non-specific activity and must be accurately assessed before it can be used for diagnostic purposes. Commercially available reagents provide acceptable sensitivity and specificity. The type of specimen and the preparation technique for its examination by immunofluorescence are of fundamental importance for obtaining successful results in the diagnosis of virus infections. Staining may be direct (IFD), using a specific antiviral antibody conjugated to fluorescein, or indirect (IFI), with the use of unlabeled specific antiviral antibody followed by fluorescein labeled antibody directed against the initial antibody. The criteria for making a positive diagnosis of a virus infection by IF should be the observation of fluorescence of virus antigen, in a characteristic distribution within whole cells collected from the site of infection, in parallel with complete absence of intracellular fluorescence in control tests (64). Specimens which contain mucus, leukocytes, or yeasts may lead to false-positive identifications if strict criteria for reading are not observed. Only fluorescence in intact cells is acceptable. The positive cells are

predominantly cylindrical epithelial cells with or without cilia. Smears made on the second and third days of illness yield the best immunofluorescence results. The presence of mucus can originate false-negative results. An additional benefit of IF is that the quality of the specimen can be assessed. IF is often more sensitive than cell culture because it does not require viable virus (64). Sensitivity of IFD or IFI is comparable (65).

As early as 1963, Hers (66) reported the results of his investigations on the rapid diagnosis of infection with IA virus. He found that IF had a sensitivity of 87%. He emphasized the importance of using fresh specimens because of the difficulties experienced with non-specific staining arising from autolytic cells. Gardner and Mc Quillin (67) developed IF for routine diagnosis of RSV infection and used the staining of cells in nasopharyngeal aspirates. The results gave a good correlation with isolation in cell culture with an overall agreement of 94%. Furthermore, they have clearly demonstrated that immunofluorescence is far more sensitive than isolation techniques, especially when infection has been in progress for a number of days. Of 201 specimens taken during the virological follow-up of patients with RSV infection, as demonstrated by isolation in cell culture and detection by direct immunofluorescence, infectivity had been lost (only 29% of specimens positive by culture) but specific fluorescence was still observed (77% specimens positive). The difficulty in isolating virus after the early days of the illness is mainly due to the coating of cells by antibodies. This situation occurs for most respiratory viruses. Moreover, in most specimens found to be positive by culture and negative by immunofluorescence, the authors stated that too few respiratory cells were available for examination.
Sensitivity of the assay varies depending on the viral antigen studied. Reported RSV sensitivity ranged from 79-95%, between 43-92% for IA, from 43-100% for PIV 1, 25-71% for PIV 2, from 0-76% for PIV 3, and 17-69% for ADV (68-74). The differences in sensitivity are probably due to the variable quality of specimens and reagents. Applying the specimen to slides by cytocentrifugation and staining with IA reagent, Landry et al reported a sensitivity of 90% compared to cell culture (53). Various commercial reagents offer pooled monoclonal antibodies directed against several respiratory viruses that are fairly sensitive and highly specific. They enable a rapid screening technique. Thereafter, individual antiviral antibodies are used to identify specific viruses from positive specimen (72).

The results obtained at the Microbiology Laboratory of HSCSP applying IF techniques for detection of RSV show a sensitivity of 93% (37/40) in comparison with isolation in cell culture, or 99% (110/111) considering IF as the reference technique. For IA virus sensitivity of IF technique ranges from 60-92%. Sensitivity of IF for ADV is 43% (62/144), for PIV it ranges from 17% (1/6) for PIV 2, to 54% (13/24) for PIV 3. Adding the non-typed PIV the global sensitivity for PIV group is 55-77% (n° 65) in comparison with isolation techniques.

The enzyme-immunoassay (EIA) membrane technique takes minutes to perform and does not require special equipment or trained personnel. Membrane EIA is currently available for RSV and IA. The major advantages of EIA are simplicity, speed, objectivity and simple interpretation. They are less sensitive than culture or molecular methods (53,75). Sensitivity for IA detection varies between 67-90% (73,74,76) and for RSV from 75-90% (60,75,77). Membrane EIA can be used as a screening assay but negative results should be confirmed by another test. The need to confirm every negative result limits the value of the test.

In our center (HSCSP), of 130 nasopharyngeal specimens from children found to be negative by means of a commercial membrane EIA for RSV detection, 20 (15%) were positive by IFD to RSV. It is also interesting to note that in negative specimens, 2 IA, 2 IB, 11 EV, 10 ADV, and 6 PIV were detected. It is therefore worthwhile investigating viruses other than RSV in children.

Rapid methods for coronavirus (229E and OC43) detection have been described such as IF (78) and enzyme-immunoassay (79), but they are not widely used and sensitivity and specificity remain unknown. Unfortunately, no rapid detection systems for clinical specimens utilizing fluorescence or enzyme-immunoassay are currently available for the diagnosis of rhinovirus infection.

The use of nucleic acid amplification methods has not become routine in clinical laboratories due to the lack of commercial kits and the expense of the method as compared to the other available tests (62). Furthermore, PCR does not represent a significant improvement over existing methods for detection of conventional respiratory viruses (80). An exception of this affirmation is the detection of rhinoviruses and enteroviruses (81). Reverse transcription PCR afforded a 15% increase in the detection of picornaviruses in nasal washes compared to cell culture (82).

Detection of nucleic acid of coronaviruses by PCR has been developed but no extensive studies have been undertaken (80). The polymerase chain reaction improves the sensitivity of viral detection in clinical specimens. However, we do not know the meaning of results using this test (83).

Serological tests for all these agents are available but are not useful for the clinical management of the individual patient because they require an acute and convalescent-phase serum, two to three weeks later, in order to demonstrate a fourfold titer rise. Detection of virus-specific IgM antibodies is possible but has not demonstrated sufficient sensitivity (84-86). The complement fixation, hemagglutination inhibition and EIA tests are the most commonly used methods for the serological diagnosis of viral respiratory infections but the sensitivity is only about 70-80% (87,88). Moreover, children may fail to show rises in serum antibody, and this may also occur in some immunocompromised patients.

At HSCSP, a prospective study of conventional respiratory virus infections in solid tumor patients treated with high-dose chemotherapy and autologous peripheral blood stem cell transplantation (ABPBSCT) was carried out. From September 1998 to November 1999 64 patients were consecutively included. Sixty patients developed a total of 75 febrile episodes. Viral cultures and rapid antigen detection by immunofluorescence were performed in nasopharyngeal secretions and/or bronchoalveolar lavage of patients with a respiratory tract infection. Of 32 documented infections 9 (14% of all patients) were respiratory viral infections, 7 were upper respiratory tract infections (4 IA, 1 RSV, 1 IA+EV, 1 IA+IB), and two were pneumonia (1 IA, 1 PIV 2). Amantadine was administered to patients with IA infection and aerosolized ribavirin to the patient with PIV 2 infection. No deaths were related to CRV infection. CRV infection was second after catheter related infection. A surveillance program for detection of respiratory viral infections in patients with hematological malignancies was later instituted in HSCSP. Between August 1999 and February 2000, 47 consecutive patients (30 of them bone marrow transplanted) presented with respiratory symptoms and were included in the study. A nasopharyngeal aspirate was taken from all patients at presentation. Forty-three episodes of viral infection were identified in 35 (74%) patients. Viruses related with those episodes were 34 IA, 1 RSV, 3 IA+RSV, 2 EV, 1 PIV 1, 1 PIV 3, 1 ADV. Ten patients developed lower respiratory tract infection and a BAL specimen was obtained. Viruses were

detected in six (60%) of them (4 IA, 1 PIV 3, 1 EV). Three patients with influenza A LRTI infection died (all bone marrow transplanted); in two, IA virus was detected in the pulmonary necropsy and in one, CMV and *Aspergillus* infection were established. Progression of IA infection of upper respiratory tract to LRT occurred in 12.5% (4/32) and mortality attributed or related to IA was of 75% (3/4). Three (13%) of 23 bone marrow transplanted patients died after a IAV infection, two with viral pneumonia and one with superinfection. It is important to note that none of 7 patients in the APBSCT study and none of 12 patients with hematological malignancies from whom initial specimens did not yield any virus, had a subsequent LRTI of viral origin.

CONCLUSIONS

1.- CRV infections are very frequent. Impaired defense mechanisms present in transplant recipients increase the likelihood of more severe infection in these patients. Etiologic diagnosis is therefore desirable.
2.- Rapid diagnosis helps the instauration of early therapy. This reduces the extension of the infection and probably restricts superinfection.
3.- Obtaining nasopharyngeal secretions is a simple non-aggresive procedure and is applicable to both the immunocompetent and the immunocompromised host.
4.- Antigen detection by IF on cells of the nasopharyngeal secretions is a simple and rapid technique to diagnose respiratory viral infections. It is also applicable to cells of BAL specimens.

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