Respiratory Viral Infections in Transplant Recipients

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- Common community-acquired respiratory viruses (**CARVs**) can cause severe and potentially fatal ARF in immunocompromised patients .
- **CARVs** include **influenza virus**, **parainfluenza virus** (PIV), respiratory syncytial virus (**RSV**), **rhinovirus/enterovirus**, and human metapneumovirus (**hMPV**)

- **Community-acquired respiratory viruses** such as **influenza, respiratory syncytial virus (RSV), parainfluenza, adenovirus, rhinovirus, endemic coronaviruses, SARS-CoV-2, and human metapneumovirus** are particularly challenging due to frequent exposures both pre- and post-transplant, as well as the potential for nosocomial transmission

Table 3 Community-acquired respiratory virus (CARV)

Type	Family	Genus	Virus
RNA viruses	Orthomyxoviridae	*Influenza A*	All Influenza A subtypes
		Influenza B	Influenza B
	Paramyxoviridae	*Rubulavirus*	Human parainfluenza virus type 2 (PIV-2) Human parainfluenza virus type 4a (PIV-4a) Human parainfluenza virus type 4b (PIV-4b)
		Respirovirus	Human parainfluenza virus type 1 (PIV-1) Human parainfluenza virus type 3 (PIV-3)
	Pneumoviridae	*Metapneumovirus*	Human metapneumovirus (hMPV)
		Orthopneumovirus	Human orthopneumovirus/Respiratory syncytial virus A (RSV-A) Human orthopneumovirus/Respiratory syncytial virus B (RSV-B)
	Coronaviridae	*Betacoronavirus*	Middle East respiratory syndrome-related coronavirus (MERS-CoV) Severe acute respiratory syndrome-related coronavirus (SARS-CoV) Human coronavirus NL63 Human coronavirus 229E Human coronavirus HKU1 Human coronavirus OC43
	Picornaviridae	*Enterovirus*	Enterovirus A-L Rhinovirus A, B, C

#Nomenclature according to the 2018 International Committee on Taxonomy of Viruses statement

- **Respiratory viral infections (RVIs)** are among the leading causes of morbidity and mortality in pediatric hematopoietic stem cell transplant (HCT) and solid organ transplant (SOT) recipients and have been associated with **chronic graft dysfunction** and **graft failure** in SOT recipients, particularly in **lung transplant** recipients

- **Early detection of RVIs** in transplant recipients may **reduce antibiotic exposure**, prompt timely **initiation of antiviral therapies**, and **allow for appropriate infection control measures** to mitigate nosocomial transmission. Specific RVIs are clinically indistinguishable from one another, and transplant recipients often have **atypical presentations** due to **lifelong immunosuppression**

- **Rates of hospitalization** are also higher among transplant recipients, with data suggesting that **14.5%** of **SOT** recipients had **at least one RVI** that **required hospitalization within 12 months of transplant**, while only 4% of otherwise healthy experienced hospitalization as a result of respiratory viral infection

- Influenza is caused by influenza A and B viruses and characterized by **annual seasonal epidemics and sporadic pandemic outbreaks**. The WHO has estimated that annual influenza outbreaks affect 48.8 million people, of whom 22.7 million see a healthcare provider and nearly a million are admitted to hospital. **Among critically ill patients** with influenza, **12.5%** are **immunocompromised**, and their **mortality** is **2.5** times as high as in non-immunocompromised patients .

- Among patients admitted for influenza, **10%** are **immunocompromised** . RSV infections are typically seasonal and pose similar serious risks to immunocompromised patients as does the influenza virus. **RSV** infection has been found in **up to 12%** of patients undergoing **HCT**, of whom **one-third progressed to lower respiratory tract infection**, which was **fatal** in about **30%** of cases

- **PIV** causes respiratory diseases similar to those seen with **RSV**. **RSV** and **PIV** were found in **11%** and **2.5%** of nasopharyngeal swabs from **critically ill hematology** patients, respectively . In a prospective study of HSCT recipients, **PIV-3** accounted for **71%** of viral respiratory infections .
- The virus is often acquired in the community and brought into the transplant ward by staff, where it may mimic other opportunistic infections, thereby raising diagnostic challenges

- The **hMPV** is closely related to **RSV** and often causes severe infections requiring **mechanical ventilation** in patients who are **elderly and/or have comorbidities**. **Rhinoviruses/ enteroviruses** are Picornaviridae that circulate throughout the year and are increasingly recognized as a cause of lower respiratory tract infection in immunocompromised patients . **In critically ill hematology patients**, **rhinoviruses/enteroviruses** were the **most prevalent** viruses detected **at ICU** admission (56%)

- **Risk factors** for viral pneumonia overlap those for bacterial pneumonia, and co-infection is common in patients with severe pneumonia .
- **Steroid therapy, hematological malignancies, lymphopenia, older age, and HSCT** are strongly associated with viral infections . There is a seasonal distribution with peaks in the winter and spring

- The symptoms and imaging study findings **are not specific** for viral infections, and overlap occurs with the changes seen in bacterial infections, although a **diffuse airspace pattern** is more common in **bacterial pneumonia** . The **main findings** are the **tree-in-bud** and **ground-glass** patterns.

NODULAR LESIONS

- Bacterial pneumonia
- Aspergillosis
- Nocardiosis
- Mucormycosis

- Sputum and blood culture
- Antigenuria
- Sputum with mycologic culture
- PCR Aspergillus and mucormycosis
- Galactomannan, BD-glucan
- Bronco-alveolar lavage

SEPTAL THICKENING

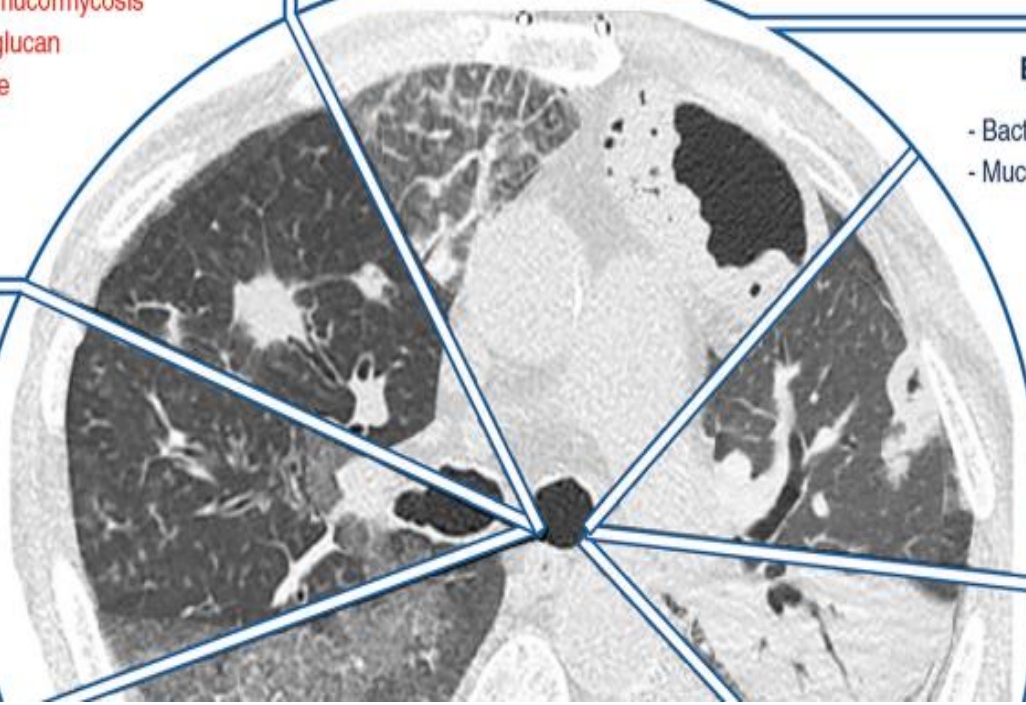
- Atypical bacterial pneumonia

- Sputum and blood culture
- Antigenuria
- Broncho-alveolar lavage

CAVITATION

- Mycobacteria
- Histoplasma
- Bacterial pneumonia *(S. aureus)*

- Sputum and blood culture
- Mycobacteria culture
- Histoplasma PCR



EXCAVATED NODULES

- Bacterial pneumonia
- Mucormycosis
- Nocardiosis
- Actinomyces
- Aspergillosis

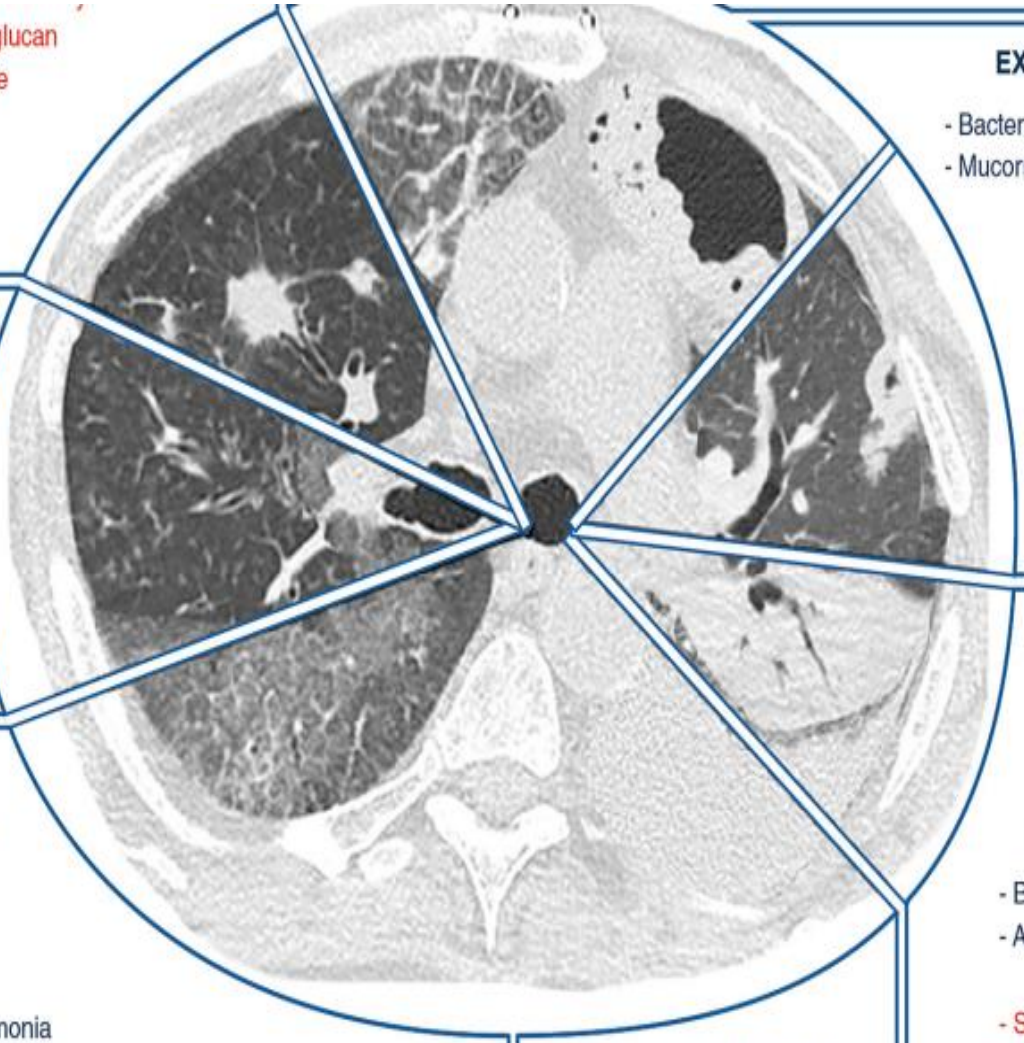
- Sputum and blood culture
- Mucor PCR
- Nocardia PCR
- BD-glucan
- Galactomannan
- Aspergillus PCR

MICRONODULES

- Bacterial pneumonia
- Viral pneumonia
- Mycobacteria

- Sputum and blood culture
- Multiplex virus PCR
- Mycobacteria culture
- CMV, VZV, HSV, PCR

- Galactomannan, BD-glucan
- Bronco-alveolar lavage



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GROUND GLASS OPACITIES

- Pneumocystosis
- Viral pneumonia
- Atypical bacterial pneumonia

- Sputum and blood culture
- Legionella antigenuria
- Induced sputum for Pneumocystis search
- BD-glucan
- Multiplex virus PCR

PLEURAL EFFUSION

- Bacterial pneumonia
- Tuberculosis

- Sputum and blood culture
- Antigenuria

CONSOLIDATION

- Bacterial pneumonia
- Aspergillosis

- Sputum and blood culture
- Antigenuria
- Mycologic sputum culture
- GM, BD-glucan

- **CARVs** can be identified by **cultures, serology, or rapid diagnostic tests based on enzyme immunoassay (EIA), immunofluorescence, or PCR.**
- **PCR** is now the reference standard diagnostic test .
- The IDSA recommends that **all immunocompromised patients** presenting with **acute onset** of respiratory symptoms be tested for **influenza**

- In patients receiving mechanical ventilation, **endotracheal aspirates or BAL fluid should be collected**, **even when influenza tests on upper respiratory tract specimens are negative** .
- In a study of pulmonology ward patients that used BAL as the reference standard, **nasopharyngeal PCR** testing had positive and negative predictive values of **88%** and **89%**, respectively .

- Uncertainty still surrounds **the type of sample most appropriate** for detecting each type of virus (nasal/ throat swab, BAL, mini-BAL, cytopathology, or even lung biopsy when performed) .
- An important consideration when **choosing the sampling technique is the clinical condition of the patient**.

- When a virus is identified in the respiratory tract, **differentiating colonization from infection** may be challenging . However, presence of the **influenza virus** usually indicates **infection**.
- In **RSV** infection, **blood testing** may be helpful, as **RSV-RNA** was detected in plasma samples of **one-third of HSCT** patients with pulmonary RSV infection and was associated with a **poor outcome**

- Both WHO and CDC recommend **oseltamivir** as the first-line agent for influenza. **Systemic steroids should not be used** unless strongly indicated for another condition . In patients with **severe illness**, **prolonged treatment** may be in order, although the optimal duration is uncertain. **Testing for antiviral resistance** at this stage should be considered, as immunocompromised patients are at higher risk of developing resistance and prolonged viral shedding

- **RSV treatment** with **intravenous immunoglobulins** and **ribavirin** has been suggested, but there is **no published evidence** that this **treatment can benefit** to the patient . In recent epidemiologic studies, the prevalence of CARV in critically ill hematological patients was similar to that in the general population with CAP; however, **the presence of CARV doubled the mortality rate** . **Allogeneic HSCT recipients** are at particularly high risk of death from CARV infection .

- **In immunocompromised** patients, the viruses most commonly responsible for systemic viral infections are **DNA viruses**. **The herpes viruses** responsible for pneumonia include herpes simplex viruses 1 and 2 (**HSV-1, HSV-2**), varicella–zoster virus (**VZV**), and cytomegalovirus (**CMV**). Herpes viruses are known to establish lifelong infections and can often reactivate during episodes of immunosuppression

Table 4 Systemic viruses responsible for pneumonia in immunocompromised patients

Virus type	Source	Extra-respiratory manifestations	Diagnosis
HSV (HSV-1, HSV-2)	Donor transmission to transplant recipient Reactivation in T-cell defects	Skin and genital eruption Encephalitis, esophagitis, Keratitis	PCR (blood, BAL, tissue) Tissue culture Serology Histopathology
VZV	Donor transmission to transplant recipient Reactivation in T-cell defects	Varicella, herpes zoster Encephalitis, cerebellitis, hepatitis, myelitis Herpes zoster ophthalmicus	PCR Direct fluorescent antibody testing Viral culture Histopathology
CMV	Donor transmission to transplant recipient Reactivation in T-cell defects	Esophagitis, gastritis, colitis Retinitis, encephalitis, myelitis, polyradiculopathy Neutropenia	PCR (blood, BAL) Histopathology Serology
Adenovirus	Reactivation	Hemorrhagic cystitis, nephritis Colitis, hepatitis, encephalitis	Viral culture (nasal, blood, urine, CSF, tissues) EIA, Immunofluorescence, PCR, serology Histopathology

HSV herpes simplex virus, *VZV* varicella–zoster virus, *CMV* cytomegalovirus, *PCR* polymerase chain reaction, *BAL* bronchoalveolar lavage, *CSF* cerebrospinal fluid, *EIA* enzyme immunoassay

- Adenoviridae include **human adenoviruses (HAdV) A to G**, each of which produces a different clinical pattern. In immunocompromised patients, HAdV can cause life-threatening multiorgan damage . Risk factors change over time with the changes in immunosuppression

- **Viral infections** are most common in patients with **T-cell deficiencies** and are of particular concern in those taking **high-dose steroids (≥20 mg/day for≥4 weeks)** or having received **T-cell-depleted allogeneic HSCT** or treatment with **alemtuzumab** or **fludarabine** .

- When the lungs are involved, the respiratory symptoms are **nonspecific** (tachypnea and/or dyspnea, hypoxia). The lung infiltrates typically appear as a **crazy-paving pattern, ground-glass opacities, micronodules, and/or consolidations.**
- A **definite diagnosis of CMV pneumonia** requires **clinical symptoms of pneumonia** and **identification of CMV** in **lung tissue** by virus isolation, rapid culture, histopathology, immunohistochemistry or DNA hybridization techniques

- **Probable CMV pneumonia** is defined as **clinical symptoms and/or signs of pneumon**ia combined with **CMV detection** by viral isolation, rapid BAL fluid culture, or CMV DNA quantitation **in BAL fluid**. **No reliable cut-off** for the CMV DNA load has been established, however. Furthermore, CMV shedding may occur in the lower respiratory tract, and the CMV DNA load may, therefore, be low in patients with asymptomatic infection .

- A cut-off **CMV DNA level >500 IU/ml** was proposed to serve that purpose, this displaying a positive predictive value of roughly 50% for probable CMV pneumonia using current prevalence rates

- **Higher CMV DNA loads in BAL** fluid specimens was observed in episodes in which **CMV DNAemia was detected concurrently**

hemato

MDPI

Review

Quantitative PCR for the Diagnosis of HCMV Pneumonia in HSCT Recipients and Other Immunocompromised Hosts

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Abstract: Pneumonia is among the most serious manifestations of HCMV infection, with high morbidity and mortality. Probable pneumonia is defined as the detection of HCMV in bronchoalveolar lavage (BAL) by viral isolation or DNA quantification (qPCR) combined with symptoms and/or signs of respiratory infection. However, currently, there is no reproducible and well-defined viral load (VL) from BAL that can reliably differentiate patients with pneumonia from the much more common detection of viral DNA in seropositive patients without true HCMV pneumonia. Several studies have been published with the aim of establishing an optimal VL for differentiating pneumonia from viral lung shedding. The aim of this review is to collect and analyze the methodology and the conclusions obtained in studies whose objectives included the correlation between HCMV VL in BAL and/or the plasma and the occurrence of HCMV pneumonia. For this purpose, a total of 14 articles have been included. There are some conclusions on which they all agree. PCR techniques were more sensitive and had a higher NPV than culture techniques but were less specific and had a low PPV. The mean HCMV loads in both BAL and the plasma were significantly higher in patients

Examples of trials

- **Optimal cut-off** HCMV VL in BAL for diagnosing HMCV pneumonia is **4545 IU/mL ,**91% sensitivity and 77% specificity. (Lodding et al., 2017)

- Cut-off value of **28,774 copies/mL (16,729IU/ml)** HCMV in BAL was correlated with HCMV pneumonia.(Young Lee et al., 2017)

- On the other hand, a **negative CMV DNA test in BAL fluid has nearly 100% negative predictive value and, therefore, excludes CMV pneumonia**, assuming satisfactory sampling.

- **VZV pneumonia** is usually readily diagnosed based on the **typical skin rash**, although it may fail to develop in patients with severe immunosuppression . **Replicating VZV is almost always found in BAL fluid**

- **HSV pneumonia** is more **challenging to diagnose**, as reactivation in blood, saliva, or the throat is frequent in critically ill patients . Thus, **HSV detection in the lower airways may merely indicate airway contamination without parenchymal involvement**. The diagnosis rests on HSV detection in BAL fluid and on the demonstration of specific **nuclear inclusions in BAL cells** . Macroscopic bronchial lesions may be seen during fiberoptic bronchoscopy, albeit only rarely

Nucleic acid amplification assays are the preferred diagnostic test for immunocompromised patients due to their high sensitivity, specificity, and rapidity of results. **Multiplex polymerase chain reaction** (**PCR**) assays are commercially available and allow simultaneous detection of a variety of recognized viral respiratory pathogens, although specific assays differ in sensitivity and specificity

- **Timing of sample collection** is crucial as poorly collected specimens can yield false negative results . Likewise, **anterior nasal swab testing** may be **negative** in patients **with lower respiratory tract infections**

- Guidelines suggest that patients suspected to have a RVI should have a **nasal swab sent for testing**. If there is clinical concern for lower respiratory tract infection, including with other non-RVI pathogens, **bronchoalveolar lavage** can be considered after weighing risks and benefits in a given patient .

- **Viral shedding** can be prolonged in immunocompromised patients despite use of appropriate antivirals, but the clinical and epidemiologic importance of prolonged excretion of virus is unclear . **PCR testing does not distinguish between viable and nonviable virus**, which can lead to challenges in interpreting a positive result . **Monitoring of viral replication by PCR should generally not be used to guide duration of antiviral therapy.**

- continuation of antiviral therapy depends on clinical symptoms . Recent data indicate that **PCR cycle threshold values correlate with infectivity for SARS-CoV-2**, however, data are limited regarding the clinical correlation for SARS-CoV-2 cycle threshold in immunocompromised hosts.

- Further investigation is needed to determine whether **cycle threshold data** can help inform strategies for prevention and treatment of RVIs in transplant recipients, especially in the context of prolonged viral shedding .

- **Rapid antigen detection** directly identifies proteins produced by viruses in respiratory secretions. This method is available for **influenza, RSV, and SARS-CoV-2**. Rapid antigen detection offers a number of advantages over molecular assays, as it is relatively **inexpensive, easy to perform, and allows for rapid results within minutes**

- **Rapid antigen detection** tests have **suboptimal sensitivity**, with reports varying between **50 and 60% for RSV and influenza** . The sensitivity of an antigen test for **SARS-CoV-2 is 30-40%** lower when compared with PCR .
- Despite the lower sensitivity, rapid antigen detection can be helpful in guiding patient management decisions as well as large-scale public health interventions.

- **Serology** is **no longer** used for diagnosis as it is slower and **less specific** than rapid antigen testing and molecular assays. **Serological testing for RVI in transplant patients is rarely used** and is especially unreliable in the setting of poor antibody response to infection.
- **Viral culture is rarely used** in clinical practice as secondary to **low sensitivity** for some viruses, **inability to test for multiple viruses** at one time, need for technical expertise, and **prolonged time to diagnosis** compared to rapid diagnostic techniques

RVI in HSCT

- **HSCT recipients** are at increased risk for morbidity and mortality from RVIs due to the extent and duration of their immunosuppression. Small, single-center cohorts have reported **incidence rates of RVIs** in this population between **5.1 and 21%** .

- A large, multicenter retrospective study followed a cohort of **1560 HCT recipients** reported an incidence rate of inpatient **symptomatic RVI as high as 16.6% within 1 year post-transplant, with no significant differences reported between allogeneic and autologous HSCT recipients (17.4 vs 14.2%, respectively)**

- **Human rhinovirus** was the most commonly detected pathogen, followed by **parainfluenza virus** and **RSV**. **Seasonality of RVIs in pediatric HCT recipients is similar** to that observed in the general pediatric population, with most RVIs occurring **between October and March**

- **Significant mortality** due to acquisition of a RVI **in the first year following transplant** has been documented, with RVI-attributable mortality rates between **0.6 and 10%** .
- **Risk factors** for poor outcomes include **allogeneic transplant, graft versus host disease (GVHD), use of immunosuppressive agents, and steroid exposure**

RVI in Renal Transplantation

- RVIs have been reported to occur at a rate of **5.5% in the first year after transplant** . Unlike other pediatric solid organ transplant recipients, symptomatic RSV infection is not commonly diagnosed in pediatric renal transplant patients . Furthermore, the course of RSV infection did not differ from that reported in otherwise healthy children, with **no increased mortality** observed in renal transplant recipients

- One retrospective study examining the incidence and outcome of RSV in 173 pediatric renal transplant recipients noted that of the 5 patients (**3%) with RSV, 3 developed biopsy-proven acute rejection during** or immediately following RSV diagnosis**.**
- **Allograft dysfunction and acute rejection** have also been described after **severe cases of influenza** in adult renal transplant recipients.

RVI in Liver Transplantation

- A large multicenter consortium of 448 pediatric liver transplant recipients described a RVI rate **of 15.6% within the first year after transplant**. **No deaths** were attributable to RVI in isolated liver transplant recipients, and only one recipient developed a respiratory complication (pulmonary hemorrhage) within three months of RVI onset

- Contrarily, RSV infection in particular has been associated with **significant morbidity** in liver transplant recipients and is associated with an **increased rate of hospitalization** compared to the general population, with a **death rate of 4.5%**. **Factors** associated with a more severe RSV course included **preexisting lung disease and RSV diagnosis within 20 days of transplant .**

- RSV infection in liver transplant recipients occurs during peak epidemic months, with nosocomial transmission accounting for a significant proportion of cases.

- **Studies have not reported severe SARS-CoV-2 disease in pediatric liver transplant recipients**. In a multicenter observation registry including 180 pediatric liver transplant recipients with confirmed SARS-CoV-2 infection, **no recipient required mechanical ventilation**

- **Disseminated adenovirus infection** has been documented to occur in **3.5-38% of pediatric liver transplant recipients,** with clinical manifestations ranging from asymptomatic to fulminant disease [32]. In liver transplant recipients, adenovirus can affect **the respiratory tract** as well as the **gastrointestinal** and **urinary trac**ts. However, **hepatitis** is the most common manifestation in this population.

RVI in Lung Transplantation

- **Infection** accounts for **nearly 40% of post-transplant mortality in lung transplant** recipients, with RVIs reported in 1.4-66% of recipients. RVIs occur frequently in the **early post-transplant** period, with reported rates up to **13.8% within the first year after transplant .**

- Lung transplant recipients are particularly prone to complications related to RVIs. Studies in adult lung transplant recipients have linked **RVIs to bronchiolitis obliterans syndrome** , but the **relationship between graft dysfunction and RVIs** in the pediatric population remains **less clear**

- **adenovirus respiratory infection** was associated with **graft failure and d**eath in a separate cohort . Similarly, a large retrospective analysis of RVIs in a cohort of nearly 600 pediatric lung transplant recipients reported that development of a **RVI within the first year of transplant was a predictor of death or re-transplantation due to graft failure**

- In comparison to adult lung transplant recipients, **pediatric lung transplant recipients** seem **less likely to develop severe disease secondary to SARS-CoV-2**

RVI in Heart Transplantation

- RVIs occur frequently after pediatric heart transplantation and are associated with **significant rates of hospitalization and high health care costs** .
- A retrospective study of 251 pediatric heart transplant recipients documented a RVI **rate of 18.3%** within the first year after transplant

- A study using the Pediatric Health Information System (PHIS) database found similar rates of infection in 3815 pediatric heart transplant recipients, with **RSV and influenza being the most commonly identified infections in the post-transplant period.** Patients who were received an induction regimen containing 2 immunosuppressive agents had an increased incidence of RVI in the first year after transplant. D

- infection with respiratory viruses has not **been significantly associated with graft rejection** . As seen with pediatric lung transplant recipients, heart transplant recipients who contract SARS-CoV-2 infection tend to have quick resolution of their illness and with no reported long-term sequelae