ABSTRACT Adenoviruses are a highly prevalent infection that can cause a range of clinical syndromes in immunocompromised patients, ranging from localized disease of the respiratory tract, gastrointestinal tract, or urinary tract to disseminated disease. Adenovirus infections may develop in this unique population as the result of primary infection or reactivation of latent virus. Disease can be potentially progressive with high rates of mortality in patients with pneumonia and disseminated disease. Fortunately, cidofovir and its lipid ester, brincidofovir, appear to be effective for the treatment of adenovirus, although neither is specifically approved for this indication. Adenovirus should always be considered when immunocompromised patients present with any clinical syndrome that could be compatible with adenoviral infection. Once disease is suspected, cultures or molecular testing of appropriate specimens should be obtained and blood should be sent for adenovirus polymerase chain reaction (PCR) whenever adenovirus is suspected. Monitoring of quantitative viral loads in blood is helpful in predicting response to therapy with a significant drop (>1 log) associated with a higher probability of clinical response.

INTRODUCTION

Adenoviruses are non-enveloped, double-stranded deoxyribonucleic acid (DNA) viruses associated with a wide range of clinical syndromes in humans (1, 2). To date, 67 immunologically distinct serotypes of adenoviruses have been described and further classified into one of seven (A–G) species based on hemagglutinin properties, DNA homology, oncogenic potential in rodents, and clinical disease (see Table 1)(1, 3–6).

Without significant seasonal variation, adenoviruses cause mostly self-limited respiratory, gastrointestinal (GI), or conjunctival disease throughout the year. Transmission can occur via inhalation of aerosolized droplets, direct conjunctival inoculation, fecal-oral spread, or exposure to infected tissue or blood (7). Incubation period is dependent on the viral serotype and the mechanism of transmission and can range from 2 days to 2 weeks (7). Viral shedding in immunocompetent patients occurs for approximately 1 to 3 days from the throat of adults with common colds; 3 to 5 days from nose, throat, stool, or eyes in patients with pharyngoconjunctival fever; 2 weeks from eye cultures for keratoconjunctivitis; and 3 to 6 weeks from throat or stool from children with respiratory or generalized illness. Although detailed information on viral shedding is limited in immunocompromised patients, shedding is typically prolonged. Irrespective of the primary site of infection, stool cultures are commonly positive for most clinical syndromes over the first two weeks of illness (7). Latency may occur, with various groups documenting intermittent detection for months or even years, particularly when highly sensitive detection techniques, such as nucleic acid amplification, are used (2). Proposed sites of viral latency include pharynx (tonsils and adenoids), intestine, urinary tract, and lymphocytes, although some of these remain controversial (5, 8).

Adenoviruses have a worldwide distribution and infections occur throughout the year without significant seasonal variability. Most infections occur as sporadic events, although local or regional epidemics have been
Adenovirus infections are most commonly described (9, 10). Adenovirus infections are most common among children, people living in close quarters or closed populations, such as college students and military recruits, and among immunocompromised patients (1, 2, 11). Although disease among immunocompetent patients is almost always self-limited, adenoviruses cause a wider spectrum of clinical disease in immunocompromised patients, with more end-organ involvement, disseminated disease, and higher mortality (1, 2). Among immunocompromised patients, infection is most commonly described in hematopoietic stem cell transplant (HSCT) and solid organ transplant (SOT) recipients; adenovirus infection in such patients will be the primary focus of this chapter.

### HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS

In the stem-cell transplant (SCT) population, the incidence of disease due to adenovirus ranges from 3% to 47% (2, 12–21). Available data suggest that adenoviral infections are more frequent in allogeneic SCT recipients compared to those receiving autologous grafts (8.5% to 30% vs 2% to 12%); children compared to adults (20% to 47% vs 9% to 13.6%); patients who receive T-cell-depleted grafts (45% vs 11%); use of alemtuzumab, cord-blood donors, and patients with acute graft-versus-host disease (GVHD) (1, 12–15, 18, 19, 21–27). Most retrospective studies have documented the onset of adenovirus disease primarily during the first 100 days following HSCT (median 36 to 90 days), (1, 14, 16, 18, 19, 23, 28–30) although adenovirus disease after 100 days has also been clearly documented, especially in adults (14, 15).

In HSCT recipients, adenovirus can cause a wide range of disease including upper and/or lower respiratory-tract infection, GI disease, hepatitis, and cystitis; certain serotypes are more commonly associated with certain disease manifestations (See Table 1) (1, 2). Adenoviral disease can result from primary infection or reactivation of latent infection with higher rates of infection in pediatric age groups because of a greater incidence of de novo infections (1, 2). Respiratory-tract disease ranges from mild upper-tract involvement, typically with nonspecific cold-like symptoms, to severe pneumonia (15, 21, 31, 32). GI disease ranges from mild diarrhea to hemorrhagic colitis; severe hepatitis has also been described (15, 21, 31). Diarrhea can be caused by viruses that are limited to the GI tract (i.e., adenovirus [AdV] 40, 41, and 52) or a manifestation of GI involvement of disseminated disease (i.e., AdV 11, 34, and 35) (see Table 1). Adenovirus can cause an interstitial nephritis rarely, but more commonly is associated with hemorrhagic cystitis. Detection of adenovirus in the urine of a patient with hemorrhagic cystitis is strongly suggestive of adenovirus-induced hemorrhagic cystitis, although other causes should be considered and tested for (31–35). Unlike other forms of adenoviral disease, hemorrhagic cystitis can typically be treated with local therapy and rarely progresses to disseminated infection (10% to 20%) (1). Although disseminated disease only effects 1% to 7% of HSCT recipients, it is associated with a significant risk of mortality (8% to 26%) (1, 24, 25, 36).

Outcome of infections can be severe. Adenovirus infections may be associated with graft failure or delayed engraftment. Additionally, co-infection with cytomegalovirus (CMV), Aspergillus, or bacteria frequently occurs and may contribute to the poor outcomes associated with adenovirus disease (1). Untreated, the mortality for HSCT patients approaches 26% for all symptomatic patients, while pneumonia and disseminated disease portend more ominous outcomes (50% and 80% mortality, respectively) (1, 2, 37).

### SOLID ORGAN TRANSPLANT RECIPIENTS

Adenovirus infection has been reported in a wide variety of SOT populations, including those receiving heart, lung, liver, intestinal, and renal transplants (38). Among SOT recipients, risk factors for adenovirus infection include small bowel and liver-transplant recipients, pediatric-transplant recipients, patients who receive antilymphocyte antibodies, and patients with donor-positive/recipient-negative adenovirus status (39). Asymptomatic adenovirus DNA-emia is common among SOT recipients and generally is not associated with

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Infections associated with adenovirus species and serotype</th>
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<tr>
<td>Species</td>
<td>Serotype</td>
</tr>
<tr>
<td>A</td>
<td>12, 18, 51, 61</td>
</tr>
<tr>
<td>B</td>
<td>3, 7, 11, 14, 16, 21, 34–35, 50, 55, 66</td>
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<tr>
<td>C</td>
<td>1, 2, 5, 6, 57</td>
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<td>E</td>
<td>4</td>
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<tr>
<td>F</td>
<td>40, 41</td>
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<td>G</td>
<td>52</td>
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*from reference 136.*
progression to symptomatic disease; 6.5%, 6.7%, 8.3%, and 22.5% for adult kidney, heart, liver, and lung recipients, respectively, were DNA-emic in the first year post-transplant, generally without clinical evidence of disease (40, 41). Invasive disease occurs in up to 10% of patients (42, 43).

Among SOT recipients, hemorrhagic cystitis, nephritis, pneumonia, hepatitis, enterocolitis, and disseminated disease have been described (2, 39, 42–48). With the exception of hemorrhagic cystitis (the most common form of symptomatic disease in renal-transplant recipients), the transplanted organ is typically the site of infection: pneumonia is most frequent in lung-transplant recipients (49–51), hepatitis is most frequent in liver-transplant recipients (42, 43), and enterocolitis, which may mimic rejection, is most frequent in small-bowel recipients (52, 53). Among kidney-transplant recipients, hemorrhagic cystitis, which typically presents as fever and visible blood in the urine, may progress to involve the kidney; in this case, granulomatous-interstitial nephritis is typically seen on biopsy (54, 55). Management in transplant patients is generally focused on reduction of immunosuppression. In most cases, this alone is all that is needed to resolve the infections over time. In patients with pneumonia, severe enterocolitis, or progressive disease despite reduction of immunosuppression, cidofovir or brincidofovir are generally utilized and have the greatest body of evidence to support their use (40, 54, 56).

**THERAPEUTIC ALTERNATIVES**

There are limited options for therapy of adenovirus infection. There have been few prospective studies to-date, and the optimal timing for therapeutic intervention during the course of illness is unclear (1, 2). Of the available agents, most studies have focused on ribavirin and cidofovir; existing data suggests that cidofovir and its lipid ester analogue, brincidofovir, may provide the highest likelihood of antiviral efficacy (1, 25, 37, 57). Despite documented *in vitro* and *in vivo* efficacy, significant toxicity has so far limited the use of cidofovir more broadly (58). Brincidofovir appear to have increased *in vitro* efficacy against adenovirus and has less renal and bone-marrow toxicity as compared to cidofovir (1, 57, 59–62). Further, brincidofovir has excellent oral bioavailability allowing dosing once to twice weekly. Data from a recent small study of brincidofovir demonstrated a virologic response in nine of 13 patients with improved outcomes in those with response (57). Brincidofovir is currently available in an open-label safety and efficacy study (ClinicalTrials.gov Identifier #: NCT02087306). Ribavirin does not appear to have significant anti-adenovirus activity in humans and is generally not recommended to treat serious adenoviral infections (63). Other approved agents, including vidarabine, dideoxycytidine, and ganciclovir may have activity, but their efficacy in managing adenoviral infections remains uncertain (1). Adenovirus- and multi-virus-specific T cells can be produced reliably and can be safely used in patients with serious adenovirus infections, with a low frequency of de novo GVHD (64, 65). Most patients who received the infusions had a complete or partial response in their adenovirus infection. While these exogenous T cells remain experimental, they show promise and are currently being studied in prospective clinical trials.

**DIAGNOSTIC APPROACHES**

The approach to patients with adenovirus disease is complex. Optimal diagnostic strategy depends on the specific indication—screening asymptomatic patients versus diagnosing end-organ disease. Given the wide array of clinical presentations of adenovirus, it is important to consider the virus in any immunocompromised patient with a compatible syndrome (1, 2). Although there are limited treatment options, outcomes appear to be maximized when antiviral therapy is started before extensive disseminated disease is recognized (1, 2). Throat swabs, nasal washes, conjunctival swabs or scrapings, anal swabs, stool, urine, and blood may be collected for testing (7). Sample types collected and tested should correspond to clinically evident sites of infection (stool for GI disease, urine for genitourinary (GU) disease, for example). As will be discussed in greater detail below, infection may not directly affect the respiratory tract, so respiratory samples may be negative despite infections in other locations. Serial monitoring of peripheral blood by quantitative polymerase-chain reaction (PCR) should be considered for all clinical syndromes in immunocompromised patients (1, 2). Further, given the ability of adenovirus to remain latent in various tissues, biopsy is often required to document lytic infection in the tissue of interest.

Various diagnostic techniques, including detection of antibodies, antigen-detection methods, culture, electron microscopy, nucleic acid-amplification methods led by PCR, *in situ* hybridization, immunohistochemistry, and histopathology, have been described. Some of these are not widely used on a routine basis. Serology, for example, has limited practical value due both to a poor
humoral-immune response in many immunocompromised patients and to a high population seroprevalence for adenovirus, seen in the absence of active infection. Viral subtyping and serotyping can be accomplished through immunologic or nucleic acid sequence-based methods (66–68). These techniques are currently in use predominantly for epidemiologic studies and other research applications. The review below will focus primarily on culture, antigen detection, and molecular diagnostic methods.

Antigen Detection
Monoclonal and polyclonal antibodies directed against the group-specific hexon antigen can be used for the direct detection of all adenoviruses and are commercially available. Signal may be detected for up to 3 weeks post-infection; greatest sensitivity is achieved within the first 5 days of illness. Sensitivity for immunofluorescence assays performed on primary specimens has ranged from 28% to 75% while other antigen-detection methods have had sensitivities that ranged from 43% to 89% (69–75).

Sensitivity is greatest from children and is markedly lower in adults (7). In general, commercially available assays are not widely used clinically and their true analytical and clinical sensitivities are poorly studied, particularly among immunocompromised patients; available data suggests that antigen detection is not sufficiently sensitive for clinical care in the immunosuppressed (76).

Fluorescence antibody (FA) testing and immunohistochemistry (see section below on morphologic methods) are used clinically to diagnose respiratory and tissue-invasive adenoviral disease, respectively (49, 77, 78). FA is widely used, both for primary, direct detection (usually from respiratory specimens), and for confirmatory identification of viral isolates grown using both conventional and shell-vial methods. Although FA testing may be limited to adenovirus, the virus is typically one of several viruses tested as part of either a screening pool or a panel consisting of antibodies targeting several of the most common viral respiratory pathogens. FA offers rapid turnaround time for the detection of respiratory viruses, but may have limited sensitivity (49), particularly in the detection of dual infections (49, 77, 78).

Culture
Traditional culture methods have been described elsewhere in detail (5, 7, 79). Most adenoviruses can best be grown efficiently on human embryonic-kidney (HEK) cells, although continuous cell lines such as A549, KB, HeLa, HEP-2, and MRC-5 are more commonly used (80). Notably, AdV 40 and AdV 41 grow best on Graham 293 HEK cells, and therefore, maybe missed in traditional cell cultures (81, 82). For maximum yield, initial culture of primary clinical specimens should be set up on at least 2 optimal cell lines (7). A number of mixed cell lines are available for screening for viruses with the goal of allowing recovery of a broader spectrum of viruses than any single cell line; often these are combined with immunofluorescence to confirm the presence of adenovirus (71–73). Caution should be used in diagnosing adenovirus in these mixed cell lines as yield for adenovirus is typically the lowest of the respiratory viruses (as low as 25%) (83–85). Cytopathic effect (CPE) associated with adenovirus is classically described as a grape-like, with irregular aggregates of enlarged, rounded-up cells showing characteristic refractile intranuclear inclusions. CPE develops slowly and typically begins at the periphery of the cell monolayer (see Fig. 1) (79). Changes may be observed up to 28 days following inoculation, with most appearing in the first week of culture (86). The time to detection is dependent on multiple factors including viral load inoculated, subgroup or serotype of adenovirus, cell lines in use, and culture conditions (7). Once CPE is noted, identification can be confirmed by immunofluorescence, enzyme immunoassay, or latex agglutination (7, 87).

Shell-vial cultures can enhance the speed of adenovirus detection in clinical specimens (71). In general, a cell-line monolayer is grown on a coverslip and inoculated using centrifugation (88). The slides are incubated for one to two days and then stained with monoclonal antibodies to the adenovirus hexon protein (7, 71, 87).

FIGURE 1 Typical adenovirus cytopathic effect in hybrid cell lines composed of A549 and mink lung (Mv1Lu) cells. (Photograph courtesy of M. Bankowski)
Cell lines used are similar to those for conventional cell culture, with mixed cell lines used primarily to target multiple viruses simultaneously in respiratory-tract specimens and with limitations as described above (88). Shell-vial technology offers improved turnaround time with potential cost savings (89), but they typically have a lower sensitivity than conventional cell-culture methods and may be more susceptible to degradation with certain specimen types (90).

Electron Microscopy
Electron microscopy may be used to identify adenovirus, particularly in stool. In general, although enteric adenoviruses occur in stool in considerably larger quantities than do respiratory adenovirus isolates, presence of adenovirus in the stool is not, in and of itself, diagnostic of GI disease. Adenoviruses are non-enveloped icosahedral viruses (20 triangular surfaces and 12 vertices). They form typical diagnostic intranuclear inclusions that appear as “paracrystalline” arrays in infected cells. Individual mature virions are 65–80 nm in diameter and the entire virus is about 1.5 x 10^8 Da (Fig. 2) (7). Sensitivity of detection may be enhanced through the use of immunoelectron microscopy. While in the past such methods were a principle means of identifying adenovirus in clinical specimens (particularly in GI specimens), their routine use has diminished in recent years.

**FIGURE 2** Transmission electron microscopy of AdV 41 negatively stained with uranyl acetate. (Courtesy of Karin Boucke)

Histopathology
Although there is little call for such methods in the immunocompetent host, histopathology can be very useful in the detection of invasive adenovirus in those with impaired immunity. Routine examination of hematoxylin and eosin-stained slides, most commonly from sites such as lung, liver, or the GI tract, may show variable degrees of inflammatory, reactive, and regenerative changes in the presence of adenovirus infection. In addition, characteristic intranuclear inclusions are often seen, which can be very suggestive of the diagnosis (91). In patients with adenoviral nephritis, which is most commonly seen in renal-transplant recipients, enlarged tubular cells with amphophilic, glassy nuclear inclusions and granuloma formations are most commonly noted (55). Immunohistochemistry (IHC) (47, 53, 92) and in situ hybridization (ISH) techniques for detection of adenovirus have been described elsewhere (55, 93–95). When used as an adjunct to morphologic examination, either method may help definitively identify adenovirus infection in tissue sections, improving the sensitivity of detection, while also localizing disease and providing evidence for causality (55, 93). Since adenoviral enteritis may mimic rejection, the use of these methods is of particular value in situations such as small-bowel transplantation (52, 53, 96–104).

Nucleic Acid Detection
Adenovirus nucleic acid can be detected by a number of molecular methods. ISH techniques were briefly discussed above as adjuncts to morphologic detection in tissue (95). Outside of this limited application, direct-hybridization techniques have not gained widespread use. Since the transactivating regions of the E1A and the N-terminal region of the hexon gene are well conserved between serotypes, PCR assays with primers directed at these regions are commonly used (105, 106). Unfortunately, there is still significant genetic heterogeneity in the adenovirus genome (greater than 80% sequence dissimilarity between some species) (7). This presents challenges in designing a single robust assay to detect and quantify all serotypes. Numerous traditional and quantitative real-time PCR techniques have been described; often using nested primers or multiplex methods to detect many or all virus serotypes (58, 63, 72, 76, 93, 107–118). Although false negatives have been described (119), these PCR methods generally have the advantage of high sensitivity and, in some cases, the ability to provide quantitative data or differentiate specific sub-species (1, 58, 63, 72, 76, 93, 107–116). Recently, a series of serotype-specific, real-time quantitative PCR for
serotypes 3, 4, 7, 11, 14, 16, and 21 has been described with excellent sensitivity and specificity (68).

PCR applied to whole blood has emerged as a significant screening method with proven impact on pediatric and adult HSCT recipients (25, 31, 120). The specific blood compartment used for testing has varied widely, with different groups using whole blood, peripheral blood-mononuclear cells (PBMC), or plasma. There is little direct comparative data available, but a recent study showed similar sensitivity among the three sample types, with PBMC testing yielding reduced viral loads compared to the other two compartments (121). Even less has been published regarding other (non-blood) specimens, with the notable exception of PCR for detection of adenovirus in respiratory-tract specimens (49, 72, 114, 115, 118, 122).

One particular type of PCR assay that requires particular caution is the use of multiplex assays on respiratory samples (Table 2). Increasingly, clinical laboratories have turned to multiplex PCR-based assays to detect respiratory viruses from upper and lower respiratory-tract samples. These assays generally have excellent sensitivities and specificities. That being said, each assay has divergent pathogen-specific sensitivities, especially for different species and/or serotypes of adenoviruses (123). Further, some assays may not detect all subtypes of adenoviruses (123). Lastly, if the respiratory tract is not the primary site of infection, testing of respiratory specimens, particularly upper-tract specimens, may yield negative results despite ongoing replication elsewhere. In one recent study, for example, the sensitivity of detecting adenovirus by the FilmArray Respiratory Panel, the eSensor Respiratory Viral Panel, the xTAG Respiratory Viral Panel, and the xTAG Respiratory Viral Panel Fast was 57.1%, 100%, 74.3%, and 82.9%, respectively (123).

There are also a number of multiplex assays for detection of adenovirus from the stool of patients (124–126). Like the respiratory viral-multiplex panels, such assays allow for the screening of stool for multiple viral, parasitic, and sometimes bacterial, pathogens that cause diarrhea. There are few studies of these multiplex assays for the diagnosis of adenovirus as the cause of diarrhea in immunocompromised patients. Further, some of the available assays are optimized for the detection of the GI adenoviruses, such as AdV 40 and 41, and may fail to detect adenovirus resulting in diarrhea as a manifestation of disseminated disease.

In addition to the multiplex assays, there are commercially available adenovirus-specific assays (see Table 2). In general, these adenovirus-specific singleplex assays have improved diagnostic sensitivity as compared to the multiplex assays, but cannot require multiple assays to detect the range of respiratory viral pathogens (127, 128). The commercially available assays are qualitative and require the use of laboratory-developed assays for quantification. Quantitative PCR assays, typically locally developed laboratory assays, have been developed to monitor trends in viral shedding in the stool and replication in the blood as described in detail in the Results Interpretation section below.

The use of PCR and other molecular-amplification assays has increased markedly in recent years. As noted elsewhere in this chapter, the evaluation of PCR results must be interpreted with caution, and there is much yet to be achieved, in aspects of testing related to standardizing methods and quantitative controls. Since there is no international standard, there is the potential of significant variability in reported quantification by various assays and laboratories. It is therefore essential that serial-quantitative virology be assessed by the same assay in the same laboratory over time. In addition, some assays that claim to detect and quantify all serotypes of adenovirus may not, in fact, do so; further, there may be differences in quantitative linearity by serotypes in quantitative methods. It is important, particularly in the case of such a genetically heterogeneous group of viruses, that the

**Table 2** FDA-approved nucleic acid tests for adenovirus

<table>
<thead>
<tr>
<th>Test</th>
<th>Company</th>
<th>Serotype specificity</th>
<th>Sensitivity</th>
<th>Specimen types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus R-gene US</td>
<td>Argene SA</td>
<td>Pan-AdV</td>
<td>91.7% to 100% (95% CI: 80% to 100%)</td>
<td>NPS and NPA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ProAdeno+ Assay</td>
<td>Gen-Probe Prodesse, Inc</td>
<td>Pan-AdV</td>
<td>97.5% (95% CI: 87.1% to 94.3%)</td>
<td>NPS</td>
</tr>
<tr>
<td>FilmArray Respiratory Panel</td>
<td>BioFire</td>
<td>Multiplex</td>
<td>See text</td>
<td>NPS</td>
</tr>
<tr>
<td>xTAG Respiratory Viral Panel</td>
<td>Luminex Molecular Diagnostics</td>
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<td>Multiplex</td>
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<tr>
<td>eSensor Respiratory Viral Panel</td>
<td>GenMark Diagnostics</td>
<td>Multiplex</td>
<td>See text</td>
<td>NPS</td>
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<sup>a</sup>As listed in the package insert. See text for full details.

<sup>b</sup>95% CI = 95% confidence interval.

<sup>c</sup>NPA = Nasopharyngeal wash/aspirates; NPS = Nasopharyngeal swabs.
performance characteristics of each method are thoroughly verified against all expected targets (129). Other factors, including collection, preservation, and preparation of samples prior to testing may also significantly affect results. Adenovirus PCR has been used in case reports to detect adenovirus in the cerebrospinal fluid (CSF) from patients with encephalitis, ocular fluid in disseminated disease and conjunctivitis, and from tissue with tissue-invasive disease in case reports (130–132).

Typing

Although not currently in wide clinical use, specific subgroups and serotypes of adenovirus can be used to predict oncogenic potential, clinical disease, and susceptibility to antiviral agents (see Table 1). Species-F viruses, for example, almost exclusively cause GI disease, have specific growth characteristics, and are resistant to ribavirin (7, 133). Classification of the identified virus into individual species (A–G) and serotypes (1–67) can be accomplished by traditional serotyping, restriction-endonuclease digestion of viral DNA, sequencing, and serotype-specific PCR. Serotyping by neutralization or hemagglutination-inhibition assays using reference sera is losing favor (86, 136). Classical methods are labor- and time-intensive and require specialized reagents, which are in short supply; as a result, only a few state labs and the Centers for Disease Control and Prevention (CDC) commonly perform traditional serotyping. Type-specific monoclonal antibodies have been developed for a few adenovirus serotypes, but they are not widely available (135). Furthermore, several of the more newly recognized serotypes may have viral DNA from related viruses and may give inaccurate results if serologic type is used alone. Restriction fragment-length polymorphism analysis is also labor intensive and may be difficult to interpret for some viruses (136). Numerous PCR assays have been developed based on species- and type-specific primers targeting the hexon fiber, and virus-associated (VA) ribonucleic acids (RNA)-coding regions; unfortunately, reference sequences are not readily available for all recognized adenoviruses (7, 137). A more adaptable method focused on PCR and sequencing of hexon-gene hypervariable regions (HVR)1-6 and HVR7 has been developed as well (137). Using these more contemporary techniques allows for greater throughput and real-time information to inform clinical and epidemiologic studies. (67, 137–141).

Antiviral-Susceptibility Testing

Few labs are able to perform antiviral-susceptibility testing. There are no standard methods that have been universally agreed upon. An in vitro susceptibility assay has been developed, but its use in the context of clinical care has not been studied (70). Recently, a real-time PCR assay for rapid determination of adenovirus susceptibility from cell cultures has been published that promises to improve the availability and reliability of detection of reduction of susceptibility to selected agents from clinically derived specimens (142). In vitro resistance does not clearly correlate with clinical outcomes in the management of adenovirus (133, 143–145). Antiviral-resistance testing is available outside a research setting and is not recommended as part of routine clinical management of patients. A variety of point mutations in the adenovirus polymerase (V180I, A359E, L554F, F740I, L741S, and V817L) have been identified in individual viruses with reduced in vitro susceptibility to cidofovir (146). The impact of these mutations on treatment with brincidofovir is currently still being investigated.

Result Interpretation

Interpreting the results of adenovirus testing requires careful correlation of results with the clinical situation to rule out other potential pathogens or co-morbid conditions that may confound efforts to determine disease causality (see Fig. 3). As described above, shedding of virus from some sites may be prolonged and may represent either persistent active infection or asymptomatic latent virus. Despite a growing body of published work on the subject, interpretation of positive test results can be difficult, particularly when using highly sensitive nucleic acid-amplification techniques (1, 112). In general, while a positive test for adenovirus in the appropriate clinical setting may be considered diagnostic of adenovirus infection, pathologic evidence of invasive disease is still required to definitively prove causality. Unfortunately, patients with altered immune systems often have multiple concomitant problems, so co-infections must also be sought. Management of immunocompromised patients with adenovirus infections can be complex and should be done in consultation with an expert in the field (1, 31, 147), and communication among multiple laboratory sub-specialties. In summary, definitive diagnosis typically combines pathologic evidence of invasive adenoviral disease with positive culture or PCR from the site of infection. The interpretation of PCR-based data has become an important issue in the clinical care of immunocompromised patients with suspected systemic adenoviral infection and will constitute the balance of this discussion.

Quantitative viral-load measurements can contribute to the diagnosis of infection and act as surrogates that correlate with clinical response to therapy (58, 63).
Given the limitations of laboratory-developed assays and lack of a universal standard, screening and monitoring should utilize the same assay performed in the same laboratory over time. In part as a result of this lack of standardization, there are currently no well-defined diagnostic or treatment thresholds. In the appropriate clinical setting, such as fever and evidence of end-organ disease, a high viral load from appropriately collected samples is highly suggestive of invasive adenoviral infection. In general, larger viral loads have a stronger association with disease (148). However, it is critical to note that interpretation of actual values depends on the assay used and the blood compartment or other sample type tested. Perhaps more importantly, the trend of viral loads over time provides insight into the risk of disease—persistent or rising viral replication is suggestive of progressive or disseminated disease (31).

PCR, particularly of blood samples, may be an effective screening modality to identify asymptomatic patients at risk for progressive adenovirus-associated disease. However, the value of such surveillance has been demonstrated most clearly among pediatric HSCT recipients, with more limited data in adult HSCT patients (25, 31, 120, 149, 150). In pediatric HSCT patients, adenoviremia, but not detectable virus from urine, throat, and stool, has been associated with progression to invasive disease and with increased mortality (20, 148). In most patients, detectable adenoviremia preceded onset of clinical symptoms (20). Persistent adenoviremia in pediatric HSCT recipients has been linked to disease progression, especially in the presence of high viral load, lymphopenia, or continued immunosuppression (31). Routine screening of peripheral blood by PCR and of end-organ sites of infection (stool, throat, and urine) by culture or PCR has been suggested in pediatric HSCT patients (31). Patients would be treated with antiviral therapy in the setting of two consecutive PCR-positive blood samples or any positive end-organ result together with either severe lymphopenia or an inability to reduce immunosuppression (25). Patients with minimal or slow increases in viral titers in the stool or persistent levels below 10⁶ copies/gram stool appear to be at low risk of developing viremia and dissemination. On the other hand, patients with rapidly progressive viral loads or viral loads >10⁶ copies/gram stool are at higher risk of developing viremia and dissemination, with the highest risk in patients with >10¹¹ copies/gram stool (2).

Adenoviremia does not appear to be predictive of disease or complications among adult SOT recipients. In prospective studies, adenovirus viremia was detected in 6.5%, 6.7%, 8.3%, and 22.5% for adult kidney, heart, liver, and lung recipients, respectively (112, 151). Few of the patients had symptoms at the time that viremia was detected; viral loads were, in general, low, and none developed end-organ disease. There was no compromise of pulmonary function among lung-transplant recipients (112, 151).

An important use of PCR is the monitoring of therapeutic response (1, 57, 58, 63, 147, 152). Often, patients with severe adenovirus infections have co-morbidities,
complicating the determination of response to therapy based on clinical signs and symptoms. Dynamic trends in viral-load measurements can help overcome such obstacles. Three recent studies have provided insight regarding the optimal management of adenovirus infection among HSCT recipients and have demonstrated the clinical course of disease during treatment. In all three instances, serial samples of blood were collected for quantitative viral-load testing (57, 58, 63). In the first study, four patients with adenovirus were treated with ribavirin (63); 3 of the patients had C-species viruses, for which ribavirin has been shown to be active in vitro (133). None of the patients had a decline in viral load and all showed continued progressive clinical disease (63). In the second study, eight patients with adenovirus disease with adenoviremia were treated with cidofovir (58), which appears to have in vitro activity against all tested adenoviruses (133). In this case series, five of the eight patients had reductions in viral load, which was associated with clinical response; the remaining three had no improvement in viral load and had progressive clinical disease (58). Lack of reduction of viral load following the first two doses of cidofovir was predictive of a progressive clinical course (58). Most recently, nine of 13 patients had a virological response to brincidofovir a median of 7 days (range 3 to 35 days) after starting therapy. Patients with virological response had a longer survival than those without a response (median 196 days versus 54.5 days) (57). Based on these findings, most experts suggest that all HSCT patients with adenoviremia, who are on antiviral therapy, should have serial viral-load testing to monitor response to therapy (25, 39).

Most studies of quantitative PCR of stool or serum have utilized weekly surveillance for identifying patients who may benefit from reduction of immunosuppression or antiviral therapy. Likewise, in patients who are receiving therapy for adenovirus should be monitored weekly as outlined above (153).

**PROGNOSTICATION**

As described above, detection of adenovirus DNA in the peripheral blood of pediatric HSCT recipients may predict the onset of adenovirus disease (see Fig. 4) (1). Similarly, sequential-quantitative monitoring of adenoviremia may help indicate the likelihood of therapeutic response; most patients who will respond to therapy, will do so within the first two doses of antiviral agent (58). Viral load may also useful for determining prognosis as higher values (>1 x 10⁶ copies/ml, in one study)
and has been associated with a greater likelihood of death among pediatric-transplant recipients (109, 116).

Detection of adenoviral genome in myocardial biopsy specimens, post-transplant, may also be predictive of adverse clinical outcomes, including coronary vasculopathy and graft loss (odds ratio [OR] 4.7 compared to adenovirus-negative patients; 95% confidence interval 1.3 to 17.1) among cardiac transplant recipients (25, 39, 56, 154). A more recent study found that 11% of donor hearts had evidence of latent adenovirus by PCR, suggesting that donor-derived infections are common (156). Still lacking is a prospective study to determine whether adenovirus infection in the donor heart prior to transplantation is associated with graft loss or other preventable outcomes.

CONCLUSION

Adenovirus is associated with a wide spectrum of clinical disease in immunosuppressed patients, with significant attributable morbidity and mortality. Nucleic-acid testing has emerged as a primary diagnostic modality, primarily as a surveillance tool to enable preemptive antiviral therapy, and as a critical means of guiding patient management and treatment with antiviral agents. Management of patients with proven adenovirus disease has not been studied prospectively and the care of such individuals, where possible, should be done in consultation with an expert in infectious diseases of immunocompromised hosts.

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