What do we know about adenovirus in renal transplantation?

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ABSTRACT

Adenoviruses are common pathogens that have the potential to cause opportunistic infections with significant morbidity and mortality in immunocompromised hosts. The significance of adenoviral infection and disease is incompletely known in the setting of kidney transplantation. Reported adenovirus infections in renal transplant recipients have typically manifested as hemorrhagic cystitis and tubulointerstitial nephritis, less severe diseases than often seen in other solid organ transplant recipients (i.e. pneumonia, hepatitis and enteritis). The prevalent adenovirus subgroups associated with cystitis and nephritis are B1 and B2 with the serotypes 7, 11, 34, 35. However, disseminated or severe adenovirus infections, including fatal cases, have been described in renal transplant recipients. There is uncertainty regarding monitoring of and treatment for this virus. Although not supported by randomized clinical trials, cidofovir is used for the treatment of adenovirus disease not responding to reduction of immunosuppression.

INTRODUCTION

Adenoviruses are non-enveloped, lytic, double-stranded DNA viruses [1]. In immunocompetent patients, they are considered to be the cause of self-limited respiratory, gastrointestinal and conjunctival disease without seasonal predilection [1, 2]. Over the last decade, adenoviruses have been described as pathogens in solid organ transplant recipients, and have been associated with severe, prolonged and even life-threatening infections, with a significant impact on morbidity, mortality and graft survival [2, 3]. Information regarding the role of adenovirus infections in solid organ transplantation, including renal transplantation, is emerging.

Based on the hemagglutination properties, DNA homology and oncogenic potential in rodents, adenoviruses are classified into seven subgroups (A–G) [2]. On the basis of neutralization by specific animal antisera, these subgroups can be further divided into 52 serotypes [2]. There are different genotypes that can be further distinguished within the same serotype [2]. The serotypes have different organ tropisms that are reflected in the clinical manifestations of disease [2]. For example, serogroups B1 and B2, serotypes 7, 11, 34 and 35 have been mainly associated with hemorrhagic cystitis and tubulointerstitial nephritis, while serotypes 11, 35 and 37 have been described to cause allograft infections [5–7]. Respiratory tract disease is usually associated with serotypes 3, 4, 7, 11, 14, 16, 21, 34, 35 and 50, gastroenteritis with serotypes 40, 41 and 52, while hepatitis is associated with serotypes 1, 2, 3, 5 and 7 [2, 4]. Disseminated adenovirus disease is caused mainly by serotypes 11, 33, 34, 35 and 40 [2, 4]. Several serotypes from subgroup C are capable of inducing a latent infection in T lymphocytes [2, 8]. Adenoviruses can evade the host immune response by inhibiting the effects of interferon, and by preventing apoptosis of infected cells. They can also prevent major human leukocyte antigen class I expression on the cell surface [2, 9].

For the purpose of this manuscript, we will use the most common definitions for asymptomatic adenovirus infection and adenovirus disease. Asymptomatic adenovirus infection is defined by the detection of the virus in urine, blood, stool or upper airway specimens by viral culture, antigen tests or polymerase chain reaction (PCR) in the absence of signs and symptoms [10]. Adenovirus disease is characterized by the presence of organ-specific signs and symptoms with concomitant detection of the virus in biopsy specimens by immunohistochemical stain, or from bronchoalveolar lavage or cerebrospinal fluid by culture, antigen detection or PCR, in the absence of adenovirus in urine, blood, stool or upper airway specimens.
of another diagnosis [2, 10]. If two or more organs are involved, not including viremia, adenovirus disease is disseminated [2, 11].

**Epidemiology**

Due to the lack of consistency of definitions used in different studies, it is difficult to assess the true incidence of adenovirus infections in solid organ transplantation. Also, most of the data originate from studies of clinically manifested disease, with only few prospective epidemiological studies being performed. For unclear reasons, in kidney transplant recipients, adenovirus infections have, to date, more commonly been reported in adults, with incidence of up to 4.1% [12]. Data in renal transplantation are limited, arising from case reports and case series. However, the incidence seems to be comparable with the 5.8% incidence in adult liver transplant recipients [13], and much lower than the 22.5% incidence in adult lung transplant recipients [14]. The rate of adenovirus infection after transplantation thus depends on the organ transplanted, perhaps related to the content of lymphoid tissue associated with the allograft, as well as the risk of rejection and requirements for immunosuppression [3].

**Risk Factors for Adenovirus Infections**

Overall, adenovirus infections are more common in children, particularly under the age of 5 years, because they are immunologically naïve and are more frequently exposed to the virus [3, 13, 15]. Although in small bowel and liver transplant recipients, most of the infections are reported in pediatric patients (age being considered an independent risk factor for adenovirus infection) [16], in renal transplant recipients the majority of the infections have been reported in adult patients. Adenovirus sero-mismatch at the time of transplantation might be a risk factor for infection [2]. However, adenovirus serology screening is not currently a routine test performed in transplant centers, and hence limits the data available to assess this. The role of immunosuppression, and in particular induction therapy, as a risk factor for adenovirus infection is supported by the high rate of adenovirus infections in the first months after transplantation, a period in which patients typically receive more potent immune suppression [12, 16, 17]. Additionally, development of adenovirus disease has been associated with treatment for acute rejection [12, 18]. Meanwhile, reduction of immunosuppression can lead to resolution of the infection, as demonstrated in multiple case reports [19]. Watcharananan et al. [12] showed that a low absolute lymphocyte count at the time of adenovirus viremia and recovery of the absolute lymphocyte count might be predictors of adenovirus disease and patient outcome. Isolation of the virus in the first months after transplantation, isolation of the virus from multiple sites, persistent asymptomatic infection, high initial viral load in blood and intensification of immunosuppression appear to be risk factors for progression to adenovirus disease, or even disseminated disease [12, 20, 21]. In adult solid organ transplant recipients, including kidney recipients, asymptomatic viremia is common (6.5–22.5%), but the risk of progression to adenoviral disease remains unknown [14, 22]. Consequently, at this time routine screening for adenovirus viremia is not recommended [3, 4].

**Clinical Presentation**

The incubation period for de novo adenovirus infections is reported to range from 2 days to 2 weeks [1]. Most infections are diagnosed in the first year after transplantation [18], including de novo infections and viral reactivation. More specifically, Watcharananan et al. [12] reported that the median time to diagnosis of infection after transplantation was 1.25 months (range 0.5–75 months), with 76.5% of the infections being diagnosed within 3 months of transplantation.

In general, clinical manifestations vary with the transplanted organ, with the allograft itself being the most frequent organ involved [2]. Renal transplant recipients frequently present with hemorrhagic cystitis, heralded by fever, dysuria, urgency, frequency and gross hematuria [18]. Hemorrhagic cystitis is usually accompanied by allograft dysfunction [12, 18], although this has been more frequently described in adult renal transplant recipients than in pediatric recipients [12, 23]. The majority of patients presenting with hemorrhagic cystitis have an associated acute graft dysfunction, 17 of 19 cases in the series reviewed by Hofland et al. [18] and 11 of 17 in the series presented by Watcharananan et al. [12]. In the large majority of cases, the renal function returned to baseline after resolution of the adenovirus disease.

The relationship between adenovirus infection and allograft rejection remains controversial. It is noteworthy that many patients in the case reports with hemorrhagic cystitis and decreased renal function were empirically treated for rejection with high-dose steroids [24]. When renal allograft biopsies were performed, rejection was present in a minority of cases, with the majority of the biopsy reports demonstrating changes consistent with viral infection [23, 24]. It is possible that the two processes are related, although clear evidence of such does not exist. A BK virus has been associated with acute renal allograft rejection [25], but it remains unclear whether the viral infection is causing the rejection through a direct effect or is the result of the change in immunosuppression level as a consequence of the infection. As with the BK virus, an adenovirus might be able to trigger rejection through activation of the innate immune system [26]. Adenovirus infection may also lead to acute allograft rejection following a decrease in the immunosuppressive regimen to allow clearance of the viral infection. Conversely, increases in immunosuppression employed to treat acute rejection can activate a latent adenovirus infection.

Watcharananan et al. reported that in patients with adenovirus viremia, involvement of other organs was common. These patients presented with testicular pain (orchitis), diarrhea (enteritis) or rhinorrhea, sore throat, cough and
shortness of breath (pneumonitis) [12]. In contrast, a study published by Humar et al. showed that 7.2% of the adult liver, heart, kidney and kidney–pancreas transplant recipients developed transient self-limiting adenovirus viremia in the first year post-transplantation [22]. The majority of these patients (79%) were asymptomatic, while a few had associated gastrointestinal (10.5%) and respiratory symptoms (10.5%). None required treatment [22]. However, these two studies are quite different [12, 22]. Watcharananan et al. [12] enrolled only kidney transplant recipients for which an adenovirus PCR was performed when the patients presented with clinical symptoms compatible with viral syndrome or adenovirus disease; the aim of the study was to evaluate the course of the adenovirus infection and patients’ outcome in relation to immune recovery and adenovirus viral load in blood. In this study, the clinical evolution and outcome data are only based on information from symptomatic patients, since monitoring of asymptomatic renal transplant recipients was not performed [12]. Humar et al. [22] sub-study was part of a randomized, double-blind, double-dummy clinical trial comparing 3 months of valganciclovir with oral ganciclovir as primary prophylaxis for cytomegalovirus in D+/R− SOT recipients; the aim of the sub-study was to assess the incidence and clinical significance of adenovirus infections in adult SOT recipients using molecular surveillance at Days 7, 28, 56 and 100, then Months 6 and 12 post-transplantation. This was mainly an observational study, not designed primarily to assess adenovirus infections; therefore, testing was not done specifically when adenoviral infection may have been present [22].

In the study by Watcharananan et al., the infections occurred earlier after renal transplantation, correlating with low absolute lymphocyte counts and induction therapy; these patients had more complications and progressive disease [12]. The number of patients included in this study was too small to warrant drawing definite conclusions. Finally, there are several reports of renal transplant recipients with disseminated adenovirus infections who died and the diagnosis was established at autopsy [27–29].

**DIAGNOSTIC METHODS**

Several methods are available for the confirmation of adenovirus infection. The methods chosen in clinical practice depend on the site of infection and the samples that can be collected, with the goal being an accurate and rapid result. Viral culture, direct antigen detection, molecular methods and histopathology are the most used diagnostic tests. Serologic testing, although available, is not frequently used in clinical practice due to low sensitivity and unclear significance in immunocompromised patients who may be unable to mount an immune response [2].

With the exception of serotypes 40 and 41, all the adenovirus serotypes grow well in human epithelial cell lines, producing a characteristic cytopathic effect. However, viral culture has limited clinical application because the results are available only after a considerable delay (2–28 days) [1, 2]. Furthermore, isolation of adenovirus by culture from a site (urine, respiratory secretion or stool) does not necessarily correlate with the clinical diagnosis, since adenovirus can be asymptomatically shed for prolonged periods of time [11, 16]. For these reasons, clinical signs and symptoms, and histopathological findings consistent with adenovirus disease should be correlated with the culture results.

Several rapid antigen detection tests are commercially available with unclear sensitivity and specificity in the solid organ transplant population. Immunofluorescence assays are used for respiratory specimens [2]. Enzyme immunoassays, immunochromatography and latex agglutination tests are used for stool samples [2].

The most common diagnostic tool used in clinical practice remains the amplification and detection of the viral genome by a PCR, by either a qualitative or quantitative assay. It is a highly sensitive diagnostic test that provides relatively rapid results, especially if the test is available on site. There are several commercial and ‘home brewed’ PCR assays available. Since the correlation of the results between these different assays was performed in a limited number of studies [30], the patients should be monitored using the same assay. PCRs have the advantage of picking up all adenovirus serotype, but different PCR assays can give a difference in viral load degree and the tests are not interchangeable for monitoring response to treatment or disease progression. As with viral culture, the PCR results should be correlated with the clinical presentation and histopathology results to differentiate disease from asymptomatic shedding of the virus. In clinical practice, a serial quantitative PCR is probably useful for informing decisions to initiate therapy, as well as to assess the response to therapy [12, 31–33]. Serial quantitative measurement of adenovirus DNA-emia should be used to monitor response to therapy.

Cystoscopy and renal biopsy are very useful tools in the setting of renal transplantation to provide samples that can show histological changes consistent with viral infection, rejection or other concomitant pathology. Pathological findings in the renal allograft associated with adenovirus infection consist of tubular cell necrosis with viral cytopathic effects: nuclear enlargement, peripheral condensed chromatin and basophilic nuclear inclusions representing viral particles (Figures 1 and 2) [34–36]. The tubular cell abnormalities are associated with severe interstitial inflammation with lymphocytes, plasma cells and neutrophils. Granuloma formation has been described in selected cases [34, 35]. The glomerulitis and the blood vessels are free of inflammation in viral infection. The presence of adenovirus within tissue can be confirmed by using immunoperoxidase staining and/or in situ hybridization (Figure 3). Electron microscopy shows the typical 70–80 nm diameter adenoviral particles within the nuclei and cytoplasm of tubular epithelial cells [35, 37, 38] (Figure 4). The similarities between the pathological findings of adenovirus-associated interstitial nephritis and acute rejection further complicate the diagnosis. The frequently granulomatous character of the interstitial inflammation associated with adenovirus infection, as well as the viral cytopathic effects, is an important clue in differentiating the two conditions. The
The presence of intimal arteritis (vascular rejection) or C4D-positive staining of the peritubular capillaries (humoral rejection) would support the diagnosis of rejection.

TREATMENT

T-cell mediated immunity plays a key role in recovery from adenovirus infection. In many cases, reduction of immunosuppression leads to resolution of disease manifestations [12, 39]. It has been noted in some cases that even after reduction of immunosuppression, viral load remains stable or increases, changes that may not necessarily correlate with disease progression. In the cases previously published, the majority of the patients were maintained on an immunosuppressive regimen of mycophenolate mofetil (MMF), tacrolimus or cyclosporine and low-dose steroid. The immunosuppression reduction strategy varied widely among cases (perhaps relating to the severity of disease) ranging from only decreasing the MMF dose to discontinuing all immunosuppression [18, 34, 40, 41]. Currently, there are no data to suggest which immunosuppressive regimen changes are more effective in allowing viral clearance, while still preventing rejection.

The need for antiviral medication to treat adenovirus disease in renal transplant recipients depends on the site and severity of involvement. In reported cases, it remains unclear how much of the recovery can be attributed to additional antiviral therapy, reduction of immunosuppression, or to a combination of these interventions [16, 20]. It also remains unclear whether antiviral therapy would have hastened resolution in cases where the adenovirus disease resolved following immunosuppression reduction alone. Hemorrhagic cystitis and nephritis probably can generally be treated only with reduction in immunosuppression without specific...
antiviral therapy. The optimal role of antivirals in kidney transplantation is not well studied. However, in patients with early adenovirus disease (in the first months after transplantation), and especially for patients diagnosed with adenoviral pneumonia, hepatitis or enteritis, antiviral treatment may be beneficial. It is important to note that no prospective randomized clinical trials have been performed to date to support the use of any antiviral agent for adenovirus infection, and that none of the antiviral agents used to treat adenoviral infections has been approved by the US Food and Drug Administration for such.

Ribavirin appears to have in vitro anti-adenovirus activity [2], against only subtype C viruses (serotypes 1, 2, 5, and 6) [42]. The main associated side effect is anemia [2, 43]. Clinical efficacy is highly conflicting and the best study that assessed serial quantitative virology, suggested lack of clinical efficacy [32, 42, 43]. Thus, based on the limited existing evidence, ribavirin should not be routinely used in the treatment of adenovirus infections.

Of all antiviral agents used in clinical practice to treat adenovirus infections, cidofovir has the most data to support its use [19, 43–49]. It has activity against all adenovirus serotypes [2]. In many centers, cidofovir is considered the standard treatment of adenovirus disease, although its use is not supported by any prospective randomized clinical trials. At the current time, it remains unclear when therapy with cidofovir should be initiated and which dosing regimen is most effective. Data published by Leruez-Ville et al. suggest that high adenovirus viral load prior to initiation of treatment and long interval between the onset of symptoms and administration of treatment might be risk factors for poor response to cidofovir [31]. In the same study, it was shown that failure to decrease the adenovirus viral load by at least one log in the first 2 weeks of therapy was associated with progression of clinical symptoms, and death due to symptomatic disease [31]. Conversely, virologic response correlated with clinical improvement and survival [31, 33]. Most of the data regarding the use of cidofovir for treatment of adenovirus comes from the bone marrow transplantation setting, where the infections tend to be more severe than in kidney transplantation and require more aggressive intervention. Two regimens of cidofovir have been widely used and are supported by the current literature: 1 mg/kg intravenous i.v. thrice weekly or 5 mg/kg i.v. weekly for 2 weeks followed by 5 mg/kg i.v. every other week until complete resolution of the symptoms and documentation of at least 3 negative adenovirus samples, one week apart, from the sites that were originally positive [2, 16, 31, 50, 51]. Cidofovir dosing needs to be adjusted for renal function, and should be decreased to 0.5 mg/kg i.v. thrice weekly in adult patients with creatinine clearance <50 mL/min or in the pediatric population with creatinine clearance <0.3 mL/min/kg [16]. For patients on hemodialysis, hemodialysis should be avoided for 1 h before and 4 h after cidofovir administration to allow intracellular distribution of the drug [16]. Cidofovir use is associated with significant side effects, including nephrotoxicity (up to 50% of patients), neutropenia (up to 20% of patients) and uveitis [52, 53]. Probenecid may protect against nephrotoxicity by blocking uptake into the proximal tubular epithelial cells. In most reports, probenecid 0.5–1.25 g/m² i.v. was administered 3 h before, 2–3 h after and 8 h after cidofovir [16, 31, 50]. Hydration is an important adjunct to probenecid to help reduce the risk of renal toxicity [2, 16]. While the thrice weekly regimen might be less nephrotoxic [19], it may also be associated with breakthrough cytomegalovirus and herpes simplex infections and the emergence of adenovirus resistance [53, 54].

CMX001 is a lipid conjugate of cidofovir that has been recently developed for treatment of dsDNA virus infections. CMX001 is being evaluated in a clinical trial for prevention of adenovirus infection in stem cell transplant patients. CMX001 has good oral bioavailability, achieves much higher intracellular levels of active drug compared with cidofovir and has not been associated with nephrotoxicity [56]. In a case series that included 13 immunocompromised patients with adenovirus disease refractory or intolerant to cidofovir, sustained virologic response to CMX001 was associated with better outcome [57]. Although promising, the ultimate utility of this drug will be determined by the outcome of ongoing investigations.

Immunoglobulin preparations have been used in a few cases of adenovirus disease [51]. Hypogammaglobulinemia (IgG levels <350 mg/dL) in transplant recipients appears to be a risk factor for severe opportunistic infections [58], risk that might be modified by administration of immunoglobulin at regular intervals and serial monitoring of levels [58]. Broebers et al. showed a high incidence of hypogammaglobulinemia during the first year post-transplantation, with 45% and 30% patients being hypogammaglobulinic at 3 and 12 months, respectively. Hypogammaglobulinemia seems to be clinically relevant, since these patients developed a higher number of infections within 3 months post-transplantation compared with patients with a normal concentration of immunoglobulins in their serum [59]. Previously published data showed that MMF-containing immunosuppressive regimens are more prone to induce hypogammaglobulinemia than regimens employing azathioprine-containing regimens [60, 61].

**OUTCOME**

Hofland et al. reported that with just reduction of immunosuppression, the adenovirus disease and all associated symptoms resolved within 30 days, and the serum creatinine returned to baseline or near baseline in all cases [18]. However, in severe, disseminated adenovirus infection, the creatinine might not return back to baseline, as in the case described by Sujeet et al. [34]. Watcharananan et al. reported clearance of adenovirus viremia at a median of 2 weeks (range 1–3 weeks) and viruria at 4.5 weeks (range 2–7 weeks) [12].

**FUTURE RESEARCH**

There remain numerous areas of uncertainty with respect to adenovirus in the setting of renal transplantation. First of
all, a better understanding of the natural history of adenovirus infection is necessary, including more accurate knowledge of the incidence and timing of infection and disease, as well as elucidation of specific risk factors. In particular, the effects of donor and recipient serostatus and those of various immune suppression regimens (including induction agents) need to be examined. Similar to cytomegalovirus prophylaxis, there might be a need for adenovirus prophylaxis after transplantation. The specific agents, target population and timing of regimens would all be areas of research. Finally, questions regarding the treatment of established infection still have no definite answer. Should monitoring of adenovirus viremia be used as a possible indicator of invasive disease? If so, at what viral load should therapy be considered? It is unknown whether monitoring of viral load is a reliable way to assess response to therapy, let alone how often it should be tested.

**AUTHORS’ CONTRIBUTIONS**

All authors contributed equally in the conception or design, or analysis and interpretation of data, or both; drafting the article or revising it; providing intellectual content of critical importance to the work described and final approval of the version to be published.

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**CONFLICT OF INTEREST STATEMENT**

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**REFERENCES**

As the rate of living kidney donor (LKD) transplantations increases, the selection of extended criteria donors such as old donors (>60–65 years) becomes more common. The pool of these old donors is probably wider than we think, especially if we tolerate a lower glomerular filtration rate (GFR) than the gold standard of 80 mL/min/1.73 m². Several important studies with large cohorts of living donors including old subjects have been published these last few years and give insights on the outcome in this subpopulation. The risk of death and end-stage renal disease (ESRD) is similar to that of matched controls from the general population. Post-donation GFR, as a result of glomerulopaenia, is lower in old than in younger donors but pre-donation as well as the rate of function loss is not different between young and old donors. Nearly 80% of donors over 60 have <60 mL/min GFR post-donation, the risk of cardiovascular mortality and progression to ESRD in the long term, as in the general population, is under question. Despite reduced renal function of the old kidney, the results of transplantation from an old living donor appeared to be equivalent to deceased transplantation from a younger donor. Finally, transplantation from an old living donor appeared to be a reasonably safe procedure for both the donor and the recipient and the age per se is certainly not a contraindication to donation.