Antiviral therapy for polyomavirus-associated nephropathy after renal transplantation

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Polyomavirus-associated nephropathy (PVAN) has recently emerged as an important cause of allograft failure following renal transplantation. The BK virus is the most important polyomavirus associated with this condition. The mainstay of therapy for PVAN is a prompt immunosuppressive dose reduction in conjunction with careful monitoring for BK viraemia. A number of antiviral agents have been tried to help to reduce BK viral replication. So far, there has been only a single randomized controlled study on the use of one of these agents. Pooled data from various small case series did not show significant differences in outcome. Prospective randomized studies with a standardized protocol are urgently required.

Keywords: BK virus, renal transplantation, cidofovir, leflunomide, fluoroquinolones, intravenous immunoglobulin

Polyomaviruses are widespread among vertebrates and tend to be species specific. It is likely that human polyomaviruses co-evolved with their hosts, which accounts for their high prevalence, low morbidity, long latency and symptomless reactivation. Only two polyomavirus strains are thought to be pathogenic in humans, polyomavirus hominis 1 (BK virus) and polyomavirus hominis 2 (JC virus), named with the initials of the patients from whom they were first isolated. These viruses cause disease only in immunocompromised patients, the BK virus manifesting as a viral nephritis or cystitis and the JC virus as a viral encephalopathy. The BK virus is a double-stranded DNA virus with an ~5000 bp genome encoding the early regulatory large tumour (T) and small tumour (t) antigens and the late structural viral capsid proteins VP1, 2 and 3. VP1 on the outer shell of the virion interacts with cellular receptors to permit endocytic uptake into cells. SV40 is another polyomavirus that humans might encounter through receipt of contaminated vaccine made from primary simian cultures. Recently, two new polyomaviruses called KI and WU have been identified in humans with respiratory infections, though their clinical significance is as yet unknown.¹

Primary BK virus infection occurs during childhood, resulting in almost universal seropositivity worldwide. After infection, the virus persists in the genitourinary tract, in renal cortex and medulla, urothelial cells and the prostate.² Asymptomatic viral shedding into urine is relatively common, particularly in old age, pregnancy, diabetes mellitus and immunosuppression in association with transplantation or HIV infection.

During the last decade, polyomavirus-associated nephropathy (PVAN) has emerged as an important cause of allograft failure following renal transplantation. BK is the most important virus associated with this condition, though rare cases of the JC virus or SV40-associated nephropathy have been reported. The precise reason for the recent emergence of this condition is not clear, but may reflect the increasing use of newer and more potent immunosuppressive agents such as tacrolimus and mycophenolate mofetil. The reported prevalence of PVAN is between 1% and 10%, with a step-wise increase since the mid-1990s.³ The increase in prevalence may in part be due to heightened awareness and improved diagnostic techniques. PVAN is associated with a high risk (14% to >80%) of irreversible graft loss.³

The ‘gold standard’ for the diagnosis of PVAN is renal biopsy, with the demonstration of polyomavirus cytopathic changes, and the use of immunohistochemical staining to confirm the presence of the polyomavirus antigen. However, the histopathological changes are focal and may be missed in the biopsy. Increasingly therefore, molecular methods are being used in the diagnosis of PVAN.⁴ Quantitative viral load studies have shown that a plasma BK viral load of over 10⁴ copies/mL is a sensitive and specific surrogate marker for PVAN with a positive predictive value of between 50% and 60%.³ Serial
determinations of the level of viraemia have been shown to be effective in monitoring the resolution of disease.\textsuperscript{5}

The mainstay of therapy for PVAN is a prompt immunosuppressive dose reduction, which, in conjunction with careful monitoring for BK viraemia, can forestall progression to overt PVAN and graft loss. There are very few systematic studies on the outcome of immunosuppressive dose reduction. It is not clear whether the reduction or elimination of antimetabolites is more effective than the reduction of calcineurin inhibitors. One study achieved 95\% resolution of viraemia through discontinuation of mycophenolate mofetil, with additional dosage reduction in the calcineurin inhibitor if viraemia persisted.\textsuperscript{5} Another study showed that reduction in dosages of both mycophenolate mofetil and calcineurin inhibitors was effective in controlling viraemia.\textsuperscript{6} While early reduction of immunosuppression may be very effective, established disease is often more refractory.\textsuperscript{7} In these cases, reduction of immunosuppression may prove insufficient to control viral replication, and in all cases, clinicians must balance the risk of graft injury due to recurrent rejection against that due to PVAN.\textsuperscript{8} As yet, no antiviral drug with proven efficacy against the BK virus has been licensed, but owing to strong clinical demand, a number of drugs have been explored in small case series.

### Cidofovir

Cidofovir is a nucleotide analogue of cytosine active against a wide array of DNA viruses. It is licensed in Europe and the USA for the treatment of cytomegalovirus (CMV) retinitis in AIDS patients and is also used as a second-line agent for the treatment of ganciclovir-resistant CMV infection. Its therapeutic use is limited by nephrotoxicity as it accumulates in renal tubular cells causing apoptosis and acute renal failure.\textsuperscript{9} Case reports of the successful use of cytosine arabinoside in the treatment of progressive multifocal leukoencephalopathy prompted an evaluation of the effect of several other nucleotide analogues on \textit{in vitro} replication of mouse polyomavirus and the primate virus SV40. Cidofovir emerged as the most selective antipolyomavirus agent.\textsuperscript{10} However, the mechanism by which cidofovir mediates antipolyomavirus activity is not clear as, unlike herpesviruses, polyomaviruses do not have viral-encoded DNA polymerase. A recent study suggested that the \textit{in vitro}-observed effect of cidofovir on polyomavirus infection may be mediated through the late stage of T-antigen expression and involves significant host toxicity.\textsuperscript{11}

### Leflunomide and derivatives

Leflunomide is an isoxazole derivative rapidly metabolized to its active metabolite A77 1726. Its mechanism of action is through inhibition of dihydroorotic acid dehydrogenase, an enzyme necessary for \textit{de novo} pyrimidine synthesis, and inhibition of tyrosine kinases involved in T cell, B cell, vascular smooth muscle cell and fibroblast signalling cascades.\textsuperscript{12} As an immunosuppressive agent, leflunomide is licensed for the treatment of rheumatoid and psoriatic arthritis. It has \textit{in vitro} anti-CMV and antipolyomavirus activity. A possible mechanism for the anti-CMV effect of leflunomide is through its effect on tegumentation and assembly of CMV. Again, the mechanism for antipolyomavirus activity is not clear.

### Fluoroquinolones

Fluoroquinolones inhibit bacterial DNA replication by targeting the essential bacterial enzymes gyrase and topoisomerase IV. Inhibition of gyrase activity involves interaction with the helicase component of bacterial gyrase, which may be of relevance, as polyomavirus T-antigen also has the helicase function essential for replication. Fluoroquinolones have been shown to inhibit SV40 plaque formation and to inhibit the helicase activity of SV40 T-antigen \textit{in vitro}.\textsuperscript{11} In a single-centre observational study, ciprofloxacin was shown to decrease BK viral load after haematopoietic stem cell transplantation.\textsuperscript{14}

### Intravenous immunoglobulin (IVIg)

IVIg has immunomodulatory properties and has been shown to contain polyomavirus-reactive antibodies.\textsuperscript{15} However, the role of antibody-mediated immunity in polyomavirus control remains unclear, as many patients with active polyomavirus infection have high specific antibody titres. IVIg is expensive and is in limited supply. It is therefore difficult to recommend its use in the absence of supporting data from adequate clinical trials.

### Published data on the use of these agents

Although most major transplant centres have reported cases of PVAN, there are relatively few small observational case series reporting the outcome of treatment with antiviral agents. So far, only one randomized controlled trial, which used a derivative of leflunomide, has been reported.\textsuperscript{16} Altogether, we have identified 44 reports (Table 1) from published data and meeting abstracts since 2002 describing the use of these four agents, and these can be compared to allow a measure of success in terms of clearance of viraemia and graft survival. Because polyomavirus replication in the allograft correlates with the detection of polyomavirus DNA in plasma by PCR assay, viraemia serves as a quantifiable surrogate marker of the course of the infection.\textsuperscript{59} There were 184 patients from 27 centres who were treated with cidofovir, the majority of whom received a low-dose treatment strategy, the commonest dosing schedule being 0.25 mg/kg intravenously once every 2 weeks with or without probenecid. The use of leflunomide was more recent (2003–08), with 189 patients from 18 centres. The most commonly used dose of leflunomide was 20–40 mg/day. Some centres were able to monitor levels of the active metabolite A77 1726 to achieve the target level. In three centres, a total of 25 patients received combination therapy with both cidofovir and leflunomide. The use of fluoroquinolones and IVIg has been less extensively evaluated and reported in only 14 and 29 patients, respectively.

Though the format and data from each report are not entirely comparable, it can be seen that the proportion of patients achieving viral clearance is very similar between cidofovir, leflunomide and IVIg (49\% to 52\% for each agent). The rate of graft loss is slightly lower with the use of leflunomide versus cidofovir (17\% versus 23\%). This difference is unlikely to be statistically significant. The reported percentage graft loss appears to be lower with IVIg (7\%) compared with either cidofovir or leflunomide, but the number of patients treated is small. However, such a comparison is crude, as the duration from the onset of treatment to the loss of viraemia was neither standardized nor adjusted between studies. Moreover, in
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Table 1. Summary of outcomes in small case series of treatment of polyomavirus-associated nephropathy (PVAN) with cidofovir, leflunomide, fluoroquinolones or intravenous immunoglobulin (IVIg) since 2002

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cidofovir</th>
<th>Leflunomide</th>
<th>Fluoroquinolones</th>
<th>IVIg</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of reports</td>
<td>27</td>
<td>18</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>No. of patients per report</td>
<td>1–26</td>
<td>1–30</td>
<td>4–10</td>
<td>1–11</td>
</tr>
<tr>
<td>Total no. of patients</td>
<td>184&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>189&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14</td>
<td>29&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Percentage of eventually cleared viraemia</td>
<td>82/168 (49%)</td>
<td>72/148 (49%)</td>
<td>0/10 (0%)</td>
<td>15/29 (52%)</td>
</tr>
<tr>
<td>Percentage of graft loss</td>
<td>42/184 (23%)</td>
<td>32/189 (17%)</td>
<td>0/14 (0%)</td>
<td>2/29 (7%)</td>
</tr>
<tr>
<td>References</td>
<td>12, 17–42</td>
<td>12, 16, 27, 32, 37, 40, 43–54</td>
<td>55, 56</td>
<td>32, 41, 48, 57, 58</td>
</tr>
</tbody>
</table>

All patients also had concomitant immunosuppression dose reduction.

<sup>a</sup>Three reports included a total of 25 patients on combined cidofovir and leflunomide.

<sup>b</sup>Two reports included 16 patients on combined leflunomide and IVIg.

<sup>c</sup>One report included one patient on combined cidofovir and IVIg.

many case series, more than one agent has been used in patients, either in combination or sequentially. Another confounding factor is that both leflunomide and IVIg have been used more recently, and the trend towards better outcomes may reflect better management of immunosuppressive dose reduction. However, an analysis of reported outcomes using cidofovir before and after 2006 shows an increase in percentage graft loss from 14% to 30%, which argues against a cohort effect.

Both cidofovir and leflunomide must be used with caution. Cidofovir is used in very low doses to treat PVAN because of its potential nephrotoxicity. Anterior uveitis in association with low-dose cidofovir therapy to treat PVAN has also been reported. There are also factors that limit enthusiasm for the use of leflunomide. A high dose (~40 mg/day) is generally required to achieve a therapeutic effect; the relationship between drug dose and level is unpredictable and few centres have access to assays of the active metabolite. There is also a view that, as leflunomide is a weak immunosuppressive agent, any beneficial effects that arise from its use may simply reflect a lower overall immunosuppressive burden. The use of higher doses of leflunomide has been associated with haemolysis. Relatively few patients have been treated with combination therapy and it is not clear whether this confers any advantage.

Conclusions

After more than a decade, PVAN remains a significant post-transplant challenge. There is a lack of adequate randomized controlled studies and no consensus view regarding appropriate antiviral therapy. Reduction of immunosuppressive therapy in conjunction with careful monitoring of viraemia remains the mainstay of management. Prospective randomized studies with standardized treatment protocols are urgently required in order to properly evaluate the risks and benefits of antiviral therapy.

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Transparency declarations

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References


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