Cytomegalovirus Infection in Paediatric Haemopoietic Stem Cell Transplantation

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Abstract
A retrospective audit of CMV infection was undertaken to determine prevalence and outcome in the national paediatric Haemopoietic Stem Cell Transplant (HSCT) unit, with particular reference to surveillance and treatment. All patients undergoing HSCT (125 allogeneic, 50 autologous) from January 1994 to December 2004 were included. Nine underwent a second transplant for graft failure or disease recurrence. Of 134 allogeneic transplants performed, 52 were unrelated. Shell vial cultures of throat swabs and urine, and blood samples for pp65 antigenemia +/- PCR were tested weekly for a mean of 147 days post transplant. CMV negative blood products and filters were used in all. 11 rec+/donor-, 12 rec-/donor+ and 10 rec+/donor+ transplants were performed. All donors were chosen for CMV positive recipients. All recipients of unrelated grafts were prescribed intravenous immunoglobulin (IVIG) weekly to D+35, then three weekly for 3 months. Prophylactic CMV negative blood products and filters in all. Recipients of unrelated grafts were given CMV negative blood products and filters and pre-emptive therapy appears to be effective in controlling CMV disease/infection in the peri-transplant period.

Introduction
Cytomegalovirus infection (CMV) remains one of the most serious infectious complications after allogeneic haemopoietic stem cell transplantation (HSCT); despite prophylactic and pre-emptive strategies, it still remains a major cause of morbidity and mortality post transplant especially in those who are CMV antibody positive. The most common targets of CMV infection include lung, gastrointestinal tract and the eye with CMV pneumonia a major manifestation of late disease.

The aims of this audit were a) to determine the prevalence of CMV infection in a paediatric haemopoietic stem cell transplant (HSCT) population, b) to review the effectiveness of prophylactic and therapeutic strategies and c) to assess the surveillance policies with particular reference to clinical outcome.

Methods
Patients
All patients who underwent HSCT at Our Lady's Children's Hospital, Dublin between January 1994 and December 2004 were eligible for inclusion. The cohort was subdivided into three categories dependent on the expected risk of CMV infection and also type of transplant performed. A retrospective chart review was undertaken as well as collation of laboratory results. Donors and recipients had molecular typing of HLA Class II antigens and serotyping of Class I antigens, the latter being replaced by molecular typing in 2000. Donors were selected on the basis of HLA compatibility; from 1999, whenever possible, CMV positive donors were chosen for CMV positive recipients.

Conditioning regimens
Included a) cyclophosphamide and total body irradiation, b) busulphan and cyclophosphamide, c) BEAM and d) high dose melphalan. The pan T-cell antibody Campath was incorporated into conditioning regimens for patients undergoing unrelated transplants. Three patients had ex vivo T-cell depletion of unrelated grafts while a further two patients had T-cell addback. All patients received CMV negative, irradiated and filtered blood products.

GVHD prophylaxis
All patients were commenced on cyclosporin; short course methotrexate was added for patients over five years receiving grafts from HLA identical relatives and unrelated donor transplants.

Antimicrobial prophylaxis
Patients received cotrimoxazole for Pneumocystis prophylaxis and itraconazole as antifungal prophylaxis from start of conditioning. All recipients of unrelated grafts were prescribed intravenous immunoglobulin (IVIG) weekly to D+35, then three weekly for three months.

Risk adapted CMV preventive strategies
Patients were stratified into three groups based on CMV status of
donor and recipient: 1) Minimal risk (recipient and donor CMV negative), 2) Intermediate risk (recipient negative and donor CMV positive) or 3) High risk (CMV positive recipient and donor negative or both recipient and donor CMV positive). Intermediate and high-risk patients were prescribed Acyclovir (500mg/m² tds IV weekly for 3-4 weeks and then 400-800mg qds PO for 3 months) in conjunction with IVIG (weekly till D+35 and then q3 weekly for 3 months). Minimal risk patients received acyclovir (10mg/kg tds IV for 3 weeks and then 200-400mg qds PO up to 3 months).

CMV screening
Screening was performed at least once weekly post transplant for up to 6 months. Shell vial cultures were performed on urine and throat swabs in all patients; following engraftment, peripheral blood leukocytes were screened for pp65 antigenemia. From 1999, CMV-DNA screening by PCR (Roche Diagnostics, UK) was undertaken, in addition to pp65 antigenemia. Bronchoalveolar lavage (BAL) was undertaken only in those patients who developed severe respiratory symptoms.

Pre-emptive treatment
Criteria for pre-emptive therapy included CMV pp65 antigenemia on peripheral blood sample or 2 consecutive positive CMV-DNA PCR tests. These patients were commenced on IV Ganciclovir at a dose of 5mg/kg bd for 2-3 weeks, then 5mg/kg/day for 5 days/week for 6 weeks. The course was continued if screening remained positive or if patient redeveloped antigenemia.

CMV treatment
Ganciclovir was given as per the pre-emptive protocol, with the addition of Foscarnet (60mg/kg tds IV and IVIG IV alternate days) for 10 doses.

Results
A total of 175 patients (95M:80F), age range 3 weeks to 18.5 yrs (mean 7yrs, median 6.6) who underwent HSCT between January 1994 and December 2004 were included in the study. Allogeneic transplants were performed on 125 patients while 50 patients underwent autologous transplantation. Nine CMV negative patients underwent a second transplant from CMV negative recipients for recurrent disease or graft failure. Indications for HSCT included ALL, AML & CML (n=79), Aplastic anaemia and Fanconi anaemia (n=23), solid tumours (n=45) and Inborn errors/Immunodeficiencies (n=37). 133 patients had stem cells from bone marrow, 36 from peripheral blood (auto 33, allo 5) and six patients received cord blood transplants.

Of the 138 patients, in which both the recipient and donor were CMV negative there were no cases of post transplant acquired CMV disease. Thirty-nine of 175 (22%) patients were deemed at risk of CMV reactivation (on the basis of recipient and/or donor CMV positivity), 33 allogeneic and 6 autologous. The 3 at risk group, will be the focus of this review and information pertaining to BMT type, screening results, treatment and outcome is summarised in Table 1.

Recipient positive/donor negative
Of eleven patients in this high-risk category, four patients had positive screening tests, three of whom were treated. One patient was treated pre-emptively, based on antigenemia. One patient developed early respiratory signs, coinciding with a positive BAL, was treated with ganciclovir and made a complete recovery. One patient received an unrelated, unmanipulated graft and developed grade 3 GVHD on D+12. On D+33, he had severe haematuria and BK virus was isolated from urine. Having had 7 negative urine tests, he developed CMV interstitial pneumonitis on D+45, coinciding with CMV antigenaemia, which failed to respond to ganciclovir and foscarnet and died on D+56.

Recipient negative/donor positive
One out of twelve patients in this intermediate-risk category developed CMV antigenaemia and CMV pneumonitis. She had engrafted within 15 days and was discharged home on D+29. Early screening was negative. While on prophylactic acyclovir, she developed antigenaemia on D+75 coinciding with respiratory signs, and was treated pre-emptively, based on antigenemia. One patient received an unrelated, unmanipulated graft and developed grade 4 GVHD on D+12. On D+33, he had severe haematuria and BK virus was isolated from urine. Having had 7 negative urine tests, he developed CMV interstitial pneumonitis on D+45, coinciding with CMV antigenaemia, which failed to respond to ganciclovir and foscarnet and died on D+56.

Table 1.

<table>
<thead>
<tr>
<th>Rec/Donor</th>
<th>Type</th>
<th>BMT</th>
<th>In vivo Tcell Depletion</th>
<th>Urine</th>
<th>Pp65/P CR</th>
<th>Throat Swab BAL Treatment</th>
<th>Outcome</th>
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<tr>
<td>R+/D-</td>
<td>ALL</td>
<td>UD</td>
<td>Yes</td>
<td>D+103</td>
<td>D+117</td>
<td>No ND</td>
<td>Gan (D+127) A+W</td>
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<td>UD</td>
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<td>No</td>
<td>No</td>
<td>D+49</td>
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<tr>
<td>AML</td>
<td>Sib</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>D+29</td>
<td>ND Did not progress further</td>
<td>A+W</td>
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<tr>
<td>AML</td>
<td>Sib</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>D+27</td>
<td>ND Did not progress further</td>
<td>A+W</td>
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<tr>
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<td>UD</td>
<td>Yes</td>
<td>D+67</td>
<td>D+57</td>
<td>No Neg x 2</td>
<td>Gan (D+77), Fos (D+97) Died D+119</td>
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<tr>
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<td>UD</td>
<td>Yes- add back</td>
<td>No</td>
<td>D+32</td>
<td>D+17 ND</td>
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<td>HS</td>
<td>Sib</td>
<td>No</td>
<td>D+54</td>
<td>D+49</td>
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<td>Gan x 2* (D+42/109) DOD</td>
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<td>UD</td>
<td>Yes</td>
<td>D+156</td>
<td>D+44</td>
<td>D+40</td>
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<td>A+W</td>
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<tr>
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<td>Sib</td>
<td>No</td>
<td>D+33</td>
<td>D+51</td>
<td>D+40</td>
<td>ND Did not treat</td>
<td>A+W</td>
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<tr>
<td>ALL</td>
<td>Sib</td>
<td>No</td>
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<td>A+W</td>
</tr>
<tr>
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<td>No</td>
<td>D+36</td>
<td>D+36</td>
<td>No ND</td>
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<td>A+W</td>
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</table>

R+/D- Recipient CMV positive/Donor CMV negative, R-/D+ Recipient CMV negative/Donor CMV positive, R+/D+ Recipient CMV positive/Donor CMV negative
ALL Acute lymphoblastic leukaemia, AML Acute myeloid leukaemia, FA Fanconia anaemia, HS Hurlers Syndrome
UD Unrelated donor, Sib Sibling donor
ND Not done
Gan Ganciclovir
A+W Alive and well, DOD Died of primary disease
*2 courses Ganciclovir

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deteriorated despite ganciclovir and foscarnet and died on D+118 of CMV pneumonitis. Screening of all other patients was negative.

Recipient and donor positive
Seven of ten patients in this high-risk group, developed antigenemia, six of whom met the criteria (>5x10^12 neutrophils or two positive PCR) for pre-emptive treatment. Three received grafts from unrelated donors and two patients had T-cell addback. Two patients required repeated courses of ganciclovir. No patient in this category developed CMV disease.

PCR testing only became available in recent years, initially exclusively for high-risk patients. While there was insufficient data to make meaningful comparison, positivity coincided with pp65 antigenemia. The initial site of detection of CMV was most commonly in urine, which was noted in five patients, four of whom developed antigenemia.

Discussion
While management of CMV infection in the pre-transplant period has improved in recent years, this herpes virus remains a significant cause of morbidity and mortality in both children and adults. Current strategies aimed at reducing CMV disease in transplanted patients are focused on a) identifying the most appropriate donor b) universal antiviral prophylaxis c) improved surveillance accompanied by pre-emptive therapy and d) therapy which encompasses both pharmacological and immune modulated therapy.

Controversy is ongoing in relation to choice of donor. In 2000, the Center for Disease Control and Prevention issued guidelines for preventing opportunistic infections in patients undergoing HSCT, using an evidence based system to guide practice. While the choice of a CMV negative donor is strongly recommended for a CMV positive recipient, Ljungman et al for European Bone Marrow Transplant Group reported improved survival in CMV positive patients undergoing unrelated non T-depleted transplant from CMV positive donors, although this was not supported in a National Marrow Donor Program Study. Two recent paediatric reports have noted reduced incidence of CMV reactivation and disease in double positive transplanted patients. The only variable which appears to significantly influence incidence of CMV disease and mortality in CMV positive recipients is donor status. Worth noting, Ljungman et al demonstrated improved disease free survival in CMV negative recipients who received grafts from unrelated CMV positive donors, commenting that the development of HLA-restricted cytotoxic T-lymphocyte (CTL) function was the most important immune response for protection from severe disease and mortality after BMT. In that study, CMV status was not relevant in HLA identical related transplants.

From a survey by the Irish Blood Transfusion Service, 24% of the general population have had previous exposure, representing one of the lowest reported CMV sero-prevalence rates. This might at least partially explain the lower incidence of CMV (9%) noted in this study, in comparison with other paediatric groups (30-38%) although this figure is likely to change with the increasing ethnic diversity. Twelve of 125 patients in one series developed CMV disease, nine of whom died. In this study, only two patients developed CMV disease, both of whom, died. While GVHD may well have been a precipitating factor in one patient, the development of CMV pneumonitis in a CMV negative, donor positive patient was unexpected. CMV pneumonitis, once established, carries a grave prognosis, in spite of the considerable improvement in intensive care, mortality rates are high. Onset is approximately 7 to 10 weeks after BMT and most episodes occur in the first 100 days. Late onset CMV pneumonitis has also been increasingly reported, at least partially attributable to prophylactic and pre-emptive therapy delaying recovery of CMV-specific T cell immunity and chronic GVHD. Mortality in this situation was 46% in one study and, with concurrent infections, is usually associated with 100% mortality.

Four of the seven CMV+ve/+ve patients who developed antigenemia had sibling transplants and had neither in vivo or in vitro T cell depletion. None of these patients developed CMV disease. All but one patient in the entire group reactivated within 100 days of transplant. Beoch & Ljungman have reported that almost 80% of CMV positive patients reactivated within 100 days of transplant, while only 28% of seronegative patients transplanted using a seropositive donor did so. Donor source was also relevant in that study; the risk of CMV reactivation was 2.4% in recipients of a related donor and 4.4% using matched unrelated donor, compared with autologous transplants. None of the six CMV positive patients surviving autologous transplants in this study reactivated; none of these patients had a haematological malignancy although all were heavily pretreated. Stem cells were unmanipulated in all. Numbers in this study are too small to make meaningful comparisons.

All intermediate and high risk patients in this group received intravenous immunoglobulin. This is no longer recommended as effective prophylaxis (DII) although, a modest beneficial effect has been demonstrated by others. The same can be stated for more specific passive immunity in the form of CMV specific monoclonal antibody. The incorporation of pre-emptive therapy has now become established practice in HSCT; it is recommended that any level of antigenemia or two positive qualitative PCR CMV assays within one week, occurring within 3 months of transplant should warrant antiviral therapy. Ganciclovir and foscarnet are the drugs of choice. Foscarnet is associated with nephrotoxicity and close electrolyte monitoring is strongly recommended. Ganciclovir, on the other hand is not without side effects, most notable of which is the association with myelosuppression, occurring at a time when engraftment is at a precarious stage. An induction period of 1-2 weeks is recommended or until CMV load begins to decrease. Maintenance period varies between centres, ranging from 2 weeks to treatment until CMV load is negative. After maintenance therapy, 30-50% of patients are likely to relapse, particularly after short-term treatment. While the standard pre-emptive strategies have generally proved successful in matched sibling and matched unrelated transplants, some authors recommend that patients whose grafts are T cell depleted and are in vivo T cell depleted prior to transplant as well as patients at high risk for development of GVHD should be considered for prophylactic, rather than pre-emptive treatment. Reported in an unrelated donor transplant population who were T cell depleted pre transplant, mortality in CMV seropositive patients was three times higher than seronegative patients; this was despite monitoring for CMV antigenemia and pre-emptive therapy with ganciclovir. They noted that transplant related mortality was identical in seropositive patients with or without reactivation. Late reactivation of CMV has been well-documented and is attributable to factors including chronic GVHD and delayed immune reconstitution raising questions in relation to duration of prophylaxis.

Given that the risk of CMV reactivation is greatest after engraftment, when pp65 antigenemia and CMV PCR can be employed, the role of serial viral culture assay of urine, which comprised a major component of surveillance in this study, would appear to be in doubt. An advantage of the latter however might be an awareness of imminent antigenemia and an indication for greater vigilance with surveillance. Throat swabs, on the other hand, were unreliable and negative in both patients with CMV pneumonitis.

The role of acyclovir in reducing reactivation of CMV is not completely understood. It is postulated that its pan herpes effect improves the overall survival in patients with CMV disease, rather
than a specific CMV effect. This has not been observed however in patients undergoing autologous transplant or in allogeneic transplant recipients who developed antirejection or were treated with ganciclovir at engraftment. Valyclovir, an improved oral formulation of acyclovir, has been demonstrated to be superior for maintaining CMV suppression and may have a role in CMV prevention. Recent discussion has focused on universal prophylaxis with valyclovir, an oral prodrug, which is rapidly converted to ganciclovir by intestinal and hepatic esterases. Data has indicated comparable activity with IV ganciclovir when given pre-emptively in HSCT patients. While the efficacy of this agent does not appear to be in doubt, current debate is focused on the most appropriate scheduling, whether as prophylaxis in high-risk patients or as an alternative to IV ganciclovir as pre-emptive therapy. While there are advantages and disadvantages to both approaches, it would appear that this agent might well replace conventional treatment in the foreseeable future. To date, however, there has been no guideline re appropriate scheduling for paediatric patients.

Other drugs include Cidofovir, which has been used as an alternative agent when first line therapy fails; while recommended for treatment of CMV retinitis, it has not been recommended for prophylaxis. Limited success has been reported in treatment of CMV disease. Newer agents e.g. Leflunomide, an immunosuppressant used in autoimmune disorders and treatment of solid organ rejection has been noted to have anti CMV properties but has yet to be investigated in clinical studies.

Other approaches including the concept of donor-derived, adoptive cellular immunotherapy are being currently investigated with the ex vivo expansion of CMV-specific CTLs. This approach would appear to offer realistic options, particularly in CMV resistant disease. Research is also ongoing in the area of vaccine development, applicability for which would extend far beyond the confines of organ transplantation.

In conclusion, the incidence of CMV antigenemia, reactivation and disease in this paediatric population has been lower than published series and may well be partially attributable to the low prevalence of CMV in the normal population. CMV surveillance with pp65 antigenemia +/-PCR was effective in monitoring patients at risk of reactivation, while routine use of CMV negative, filtered blood products may have contributed to the relatively low incidence of CMV disease. Pre-emptive therapy with ganciclovir was effective while the incorporation of shell vial cultures of urine immunoglobulin contributed to a reduction of infection related morbidity remains an unanswered question.

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A Role for Myelography in Assessing Paraparesis

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Abstract
Imaging of the spine is a fundamental part of assessment of paraparesis. Since the advent of MRI the indications for myelograms have diminished. However, a myelogram, although an invasive test, should still be considered a useful investigation for localising lesions in the spinal cord and for identifying rare causes of myelopathy. This case illustrates how a CT myelogram identified an arachnoid cyst, which is a potentially treatable cause of paraparesis.

Introduction
Spinocerebellar ataxia is a rare, but potentially treatable cause of paraparesis. Radiological investigations are essential for diagnosing and localising the lesion. We illustrate a case where a myelogram was the key diagnostic tool.

Case Report
A 58-year-old Caucasian woman was referred for assessment of spastic paraparesis. She had a 4-year history of bilateral lower limb paresis and decreased pain perception. She developed increasing difficulty walking upstairs and described a gradual deterioration in lower limb power. She also experienced constipation and urinary urgency. She is a non-smoker, with no history of travel or residence in tropical areas. She has no family history of neurological disease.

Clinical examination showed proximal weakness (4/5) bilaterally in the hip flexors, a scissor gait and gait with tandem gait. There was mild decreased pain sensation to T10. Hyperreflexia bilaterally at knees and ankles with upgoing plantar responses was noted.

Investigations
Nerve conduction studies were normal.
CSF analysis was normal, without oligoclonal bands.
Serology for poliovirus, syphilis and Lyme disease was negative.

MRI of spine demonstrated thinning of the spinal cord in the thoracic region. CSF signal intensity was seen surrounding the cord and there was no evidence of an extra-axial mass causing extrinsic cord compression (Figure 1).

A CT myelogram was performed following fluoroscopically-guided injection of 8ml of nonionic contrast medium into the subarachnoid space. This demonstrated a well-circumscribed, extra-meningeal, CSF-density collection posterior to the thoracic spinal cord, which did not fill with contrast. This collection extended from T4 to T8 levels, measuring 1.1cm in maximum anteroposterior diameter and 9.6cm from its superior to its inferior margin (Figure 2). The findings were consistent with a large arachnoid cyst.

Figure 1 Sagittal T2-weighted MRI of dorsal spine showing focal thinning of the thoracic spinal cord, surrounded by CSF signal intensity

An elective T6 laminectomy was performed. Following a midline dural opening a bulging arachnoid cyst was seen, which was punctured after introduction of a microscope. Histology showed sclerosed fibrovascular connective tissue partially lined by meningothelial cells.

At 2 months follow-up after surgery, our patient was independently mobile. Her power was grade 5 throughout the lower limbs, with normal reflexes and sensory examination.