

Cytomegalovirus Pneumonia Ten Years After Renal Transplant – A Manifestation Of Sustained Lymphocyte Depletion

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Citation

P Azevedo, C Freitas, P Aguiar, M Almeida, S Pedroso, L Martins, L Dias, A Marinho, J Pimentel, A C Henriques, A Cabrita. *Cytomegalovirus Pneumonia Ten Years After Renal Transplant – A Manifestation Of Sustained Lymphocyte Depletion*. The Internet Journal of Infectious Diseases. 2014 Volume 13 Number 1.

Abstract

Cytomegalovirus (CMV) remains a major cause of morbidity and mortality in renal transplant recipients. CMV pneumonia is a rare presentation, but it is associated with high mortality (up to 100%). Diagnosis of CMV pneumonia is a challenge, as it is often obscured by the presence of other respiratory pathogens causing overlapping symptoms and most of diagnostic techniques are insensitive. The polymerase chain reaction may be a useful test when other diagnostic methods are negative and biopsy cannot be done. Immunophenotyping of peripheral blood may provide an opportunity to realize the pathophysiology of CMV infection and contribute to differential diagnosis.

The authors present two cases of CMV pneumonia diagnosed ten years after renal transplantation and discuss its risk factors and pathophysiology, focusing on the immunologic alterations caused by prolonged immunosuppression. A review of literature on clinical manifestations, diagnostic approach and treatment options was also performed.

INTRODUCTION

Cytomegalovirus (CMV) is a common complication after renal transplantation and viremia can be found in up to 60% of all recipients [1].

Although most patients remain asymptomatic [1], CMV infection can be a major cause of morbidity due to direct multiorgan invasion and to indirect effects [2]. CMV has an immunomodulatory ability and causes suppression of host defenses, predisposing to secondary bacterial infections and invasion by pathogens, as *Pneumocystis jirovecii*, *Candida*, and *Aspergillus* [3]. CMV also contributes to the risk of graft rejection, Epstein Barr virus-mediated post-transplant lymphoproliferative disorder, Herpes virus (HHV)-6 and HHV-7 infections [3].

The direct effects of disease tend to appear during the first year of transplantation [4,5] when immunosuppression is more intense and CMV disease related-mortality has been reported in up to 85% of transplant recipients [6].

In renal-transplant patients, neutropenia syndrome, hepatitis and gastrointestinal invasion are the most frequent

manifestations of CMV infection [2]. Viral pneumonia is a rare manifestation (incidence of 2-10%) [7], with earlier onset [8] and higher mortality rate (up to 100%) [5, 9].

The authors present two cases of CMV pneumonia diagnosed ten years after renal transplantation and make a brief review of its pathophysiology, clinical presentation, diagnostic approach and treatment options.

CASE 1

A 67-year-old man with end-stage renal disease (ESRD) due to chronic glomerulonephritis, on hemodialysis since 1982, was submitted to a first renal transplantation (RT) in 1988. Immunosuppression included cyclosporine (CyA) and steroids. There were no surgical nor infectious complications. In 1990 he resumed hemodialysis because of progressive chronic graft failure due to chronic graft rejection.

A second renal transplant was performed in 1997 from a 47-year-old donor with 5 HLA mismatches. Both donors and receptor were CMV positive. Immunosuppression consisted of antithymocyte globulin (ATG), azathioprine, CyA and

steroids. Delayed graft function was documented due to acute tubular necrosis, without rejection.

One week after RT the patient presented fever and a positive serology for IgM CMV (negative CMV antigenemia). Anti-CMV immunoglobulin and ganciclovir were started, with clinical remission, and the patient was discharged with a 3 week course of ganciclovir therapy. Additional testing for other causes of acquired immunodeficiencies was negative. During late post-transplant period he experienced several episodes of gout flares treated with transitory increase of steroids. One year after RT (April 1998) another CMV infection episode occurred and he was successfully treated with ganciclovir.

Thirteen years after RT, in July 2012, anemia refractory to erythropoiesis stimulating agents appeared and the patient required multiple red blood cell transfusions. A diagnosis of bone marrow aplasia was established after a thorough investigation and he was started on danazol and increased doses of steroids (Prednisolone 1mg/Kg/day), with improved erythrocyte count. Azathioprine was suspended at the same time.

Two months later, he was admitted for arterio-venous fistula (AVF) surgery due to progressive deterioration of renal graft function, with no surgical complications. In post-operative period, the patient presented asthenia and dyspnoea, accompanied by febrile neutropenia. Thoracic computed tomography (CT) revealed bilateral pleural effusion (not suggestive of empyema), extensive peri-broncho-vascular opacities associated with interlobular septal thickening and "crazy-paving" appearance. There were cylindrical bronchiectasis in the upper lobes, middle lobe and right lower lobe (Fig.1). A diagnosis of acute interstitial pneumonia/organizing pneumonia was hypothesized and piperacillin-tazobactam was started. Two days later, respiratory complaints worsened and respiratory failure emerged. He was admitted to an Intensive Care Unit for mechanical ventilator support. Bronchoscopy was performed and CMV was isolated by polymerase chain reaction (PCR) analysis in bronchoalveolar lavage (BAL) fluid. Microbiological analysis were negative for other agents.

Figure 1

Thoracic CT showing pleural effusion, extensive peri-broncho-vascular opacities associated with interlobular septal thickening and "crazy-paving" appearance



Immunophenotyping of peripheral blood revealed reduction of natural killer (NK) cells, B-lymphocytes, CD4+ T-cells and inversion of CD4/CD8 ratio (Table 1).

Table 1

Immunophenotyping of bronchoalveolar lavage

BAL		PATIENT 1	PATIENT 2	REFERENCE VALUES
T-LYMPHOCYTES	CD3 (%)	99	95	63-83
	CD4 (%)	31	43	40-45
	CD8 (%)	59	47	30-35
CD4/CD8 RATIO		0.525	0.915	
B-LYMPHOCYTES	CD19 (%)	<1	1	0-4
NK (%)		<1	4	2-14

The patient was started on anti-CMV immunoglobulin (200 mg/Kg on alternate days) and ganciclovir; sustained low-efficiency daily dialysis (SLEDD) was initiated, due to progressive renal dysfunction. Despite all measures instituted, clinical condition gradually deteriorated and he died with multi-organ dysfunction.

CASE 2

A 39-year-old man, active smoker, with hypertension, dyslipidemia and diabetes mellitus (diagnosed at the age of six, with poor metabolic control), started HD in 1998, due to ESRD secondary to diabetic nephropathy. In 2001 he was submitted to reno-pancreatic transplant. Both donor and receptor were CMV positive. Immunosuppression included ATG, tacrolimus (TAC), mycophenolate mofetil (MMF) and steroids. There was an immediate function of both grafts but

the postoperative period was complicated by gastrointestinal bleeding, requiring right colectomy. No infectious complications nor acute rejection episodes were detected and additional testing for other causes of acquired immunodeficiencies was negative. Pancreatic function remained normal, but chronic renal allograft dysfunction and nephrotic proteinuria progressively developed and a femoro-femoral arterio-venous fistula was constructed in June 2012.

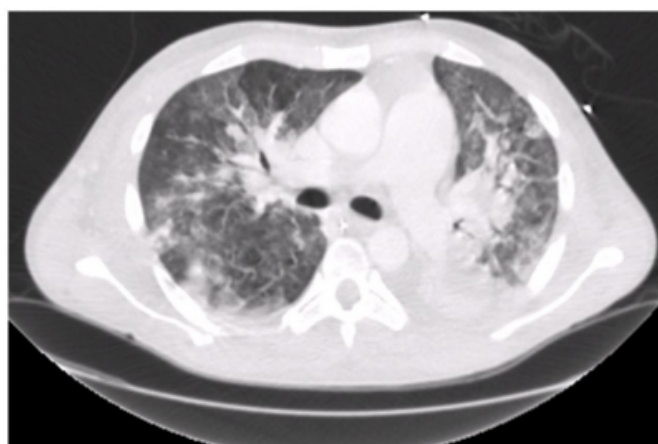
On August 2012, he was readmitted because of sepsis with abdominal origin. A laparotomy was performed with identification of peritonitis and perforation of an ileal ulcer. Histological analysis revealed perforated erosion with serositis; microbiological, CMV and tuberculosis PCR analysis were negative. Two weeks of antibiotic therapy with piperacillin-tazobactam was administered, with clinical improvement.

One month later, he was admitted with gastrointestinal bleeding with no colonoscopic and endoscopic pathologic alterations. One week after discharge, he was readmitted with profuse hematochezias and anemia (Hb 5.7 g/dL). Endoscopic studies revealed antrum mucosal erosions and histological study confirmed chronic gastritis without *Helicobacter pylori* or other infectious agents. Clinical condition rapidly worsened, with peritonitis and severe respiratory failure.

Thoraco-abdominal CT showed diffuse and bilateral pulmonary opacities, moderate ascitis and diffuse edema of the bowel loops without evidence of pneumatosis (Fig. 2).

Figure 2

Thoracic CT showing difuse and bilateral pulmonary opacities



Analytic study revealed negative CMV antigenemia with

positive CMV serology and positive PCR analysis for CMV in BAL fluid. There were no other agents identified in microbiological studies. Immunophenotyping of peripheral blood revealed reduction of NK and T-cells, with CD4+/CD8+ ratio inversion (Table 2).

Table 2

Immunophenotyping of peripheral blood

PERIPHERAL BLOOD		PATIENT 1	PATIENT 2	REFERENCE VALUES
T-LYMPHOCYTES	CD3/mm ³ (%)	124 ⁺ (98.3%)	966 (62%)	1100-1700 (67-76%)
	CD4 mm ³ (%)	112 (8.86%)	455 (29.2%)	700-1100 (38-46%)
	CD8 mm ³ (%)	915 (72.14%)	494 (31.7%)	500-900 (31-40%)
CD4/CD8		0.123	0.921	
B-LYMPHOCYTES	CD19 mm ³ (%)	7 (0.59%)	494 (31.7%)	200-400 (11-16%)
NK mm ³ (%)		0.57/7	3.30/51	200-400 (10-19%)

Tacrolimus and MMF were suspended and the patient was started on anti-CMV immunoglobulin (200 mg/Kg on alternate days) and ganciclovir. He was admitted in Intensive Care Unit for mechanical ventilation. The patient maintained gastrointestinal bleeding with hemodynamic instability and started SLEDD due to progressive renal dysfunction. His clinical condition progressively worsened and he died on November 2012.

DISCUSSION

Cytomegalovirus infection is defined as evidence of CMV replication regardless of symptoms and CMV disease requires both evidence of infection as well as symptoms or evidence of tissue invasion [10,11]. Cytomegalovirus infection is highly prevalent in general and transplant population [12]. In renal transplant (RT) recipients, in the absence of any preventive therapy, 20 to 60% develop CMV infection [1,13] and the incidence of CMV disease is 10-25% [14].

Although the majority of patients remain asymptomatic [1], CMV infection can be a major cause of morbidity and mortality. CMV infection related-mortality has been reported in 44% of immunocompetent patients and in up to 85% of transplant recipients [6,12].

Initial infection usually occurs in infancy and it can range from asymptomatic disease to mononucleosis-like syndrome [2]. As a β -herpesvirus, after primary infection, the virus remains in a persistent state within CD34+ myeloid progenitor cells, CD14+ monocytes, dendritic cells and megakaryocytes, with the risk of reactivation with

immunosuppression. This defines latent infection [15].

The host response to CMV comprises a specific (mediated by CD4+ and CD8+ T-cells) and nonspecific (mediated by NK and gamma delta T-cells) cellular and humoral immune responses, which vary in importance during the course of the infection. Earlier, during primary CMV infection, the mechanisms associated with innate immunity are essential for limiting viral replication, but in recurrent infection the critical role is performed by adaptive immunity with the intervention of B and T-cells [16]. In solid organ recipients, infective episodes have been preceded by depressed lymphocyte (CD3+, CD4+ and B-cells) and monocyte/macrophage counts [17]. In RT patients with CMV infection CD4+ T cell reduction is predominant and the consecutive CD4/CD8 inversion has been pointed as the most reliable and earliest indicator of infection [17].

Risk factors for CMV disease include older donor (> 60 years), donor seropositivity (especially if the recipient is seronegative), simultaneous kidney-pancreas transplantation, the presence of allograft rejection and concurrent infections with other viruses [11,18]. Other conditions, as metabolic derangements related to ESRD, longer time on dialysis [19], diabetes, malnutrition [11,20] and frequent blood transfusions [21,22] have also been pointed as predisposing factors for CMV infection. Immunosuppressive therapy is, by far, the leading risk factor for CMV primary or recurrent infection [23].

T-cells depleting agents (such as ATG and anti-CD3 antibodies) provide dose-dependent depletion of T cells [24,25]. ATG obtained from rabbit (rATG), used in our Center for induction therapy, has a half-life of 30 days [25] and is administered for 5-7 days, in the dose of 3-3,5 mg/kg/day.

After bone marrow transplantation, ATG is associated with late recovery of most leukocyte populations (including naive T-cells and CD4 T-cells), suggesting that ATG impairs thymopoiesis [18] with a sustained effect, as it often persists beyond 1 year post-transplantation [18,25]. In solid organ recipients, r-ATG (9mg/kg/day, for 5 doses) also induces a rapid T-cell depletion that only recovers to pretransplantation levels by 730 post-transplantation days [25]. In addition to T-cell depletion, rATG suppresses cytotoxic cells-dependent effect, induces apoptosis of B-cell lineages and interferes with dendritic cells, granulocytes, monocytes / macrophages, regulatory T-cells and NK cell

functions. ATG also induces TNF- α release, stimulating the binding of cellular nuclear factor κ B to the promoter region of the CMV immediate-early antigen gene and enhancing CMV replication [25-27]. Indeed, ATG is associated with a two- to seven-fold increase in rate of CMV [18,24,28]. Anti-CD25 agents (basiliximab and daclizumab) do not seem to increase this incidence [24,28].

The calcineurin inhibitors (CyA and TAC), commonly used with low dose of steroids for maintenance therapy, promote lymphocyte suppression [3]. Higher percentage of CMV infections (65% vs. 30%) have been described in patients treated with TAC compared to CyA [8]. Mycophenolate mofetil induces a change in primary immune response and has been pointed as an increasing risk factor for late CMV infection [3,29]. When compared to azathioprine, MMF increased by 3 times the incidence of CMV disease after RT [37] and the addition of MMF to CyA and steroids increased the likelihood of CMV infection, with longer infectious period and higher viral load [23].

mTOR inhibitors, by contrast, may impair intracellular CMV replication [31]. In RT recipients, sirolimus [32] and everolimus [33] have been associated with lower rates of CMV infection, when compared to MMF.

Our patients had risk factors for CMV disease, received ATG as induction therapy and were under maintenance therapy with calcineurin inhibitors and antimetabolites (MMF or azathioprine). Moreover, in case 1, bone marrow aplasia and increased dose of steroids contributed to extra immunosuppression. Even 10 years after RT, both patients showed reduced NK and CD4+ T-cell counts, reflecting both nonspecific and specific immunity deficiency. The reduction of NK-cell counts may be of particular interest. CMV, by the inhibition of proteasomal activity and by blocking MHC synthesis, tends to escape from CD8+-T cells' activity. NK-cells are activated by infected cells even in the absence of HLA class I molecules and, in association with macrophages, they produce IFN- γ , which is essential in the elimination of infected cells and inhibition of viral replication.

ATG therapy was associated to thymopoiesis impairment, T-cell depletion, B-cell apoptosis and disturbance of NK-cell function in up to three years after RT [25], but no studies described these effects beyond that time.

The global immunodeficiency found in our patients could explain their inability to limit viral replication and to control

infection, the severity of disease presentation and the poor response to treatment.

In RT recipients, inflammatory processes (either related to rejection, infection or healing) have been associated with CMV reactivation [25] and a strong correlation between elevated TNF- α plasma levels and CMV antigenemia was reported. Some authors described an increase in TNF- α production [34] in earlier stages of wound healing. They postulate that, as wound healing progresses, granulation tissues develop with many macrophage infiltrates and hypervascularity. During that process, CD34+ bone marrow progenitor cells (with latent CMV) differentiate into macrophages and endothelial progenitor cells that are incorporated into the foci of neovascularization. In the TNF- α -rich environment, proliferation of cells with latent CMV is stimulated, and it is possible that latent CMV could be reactivated, causing systemic infection and invasive disease after hematogenous dissemination [34]. This mechanism could explain the initial trigger to CMV reactivation in our patients, as both had inflammatory processes secondary to AVF healing and intestinal ulcer repair.

Clinical manifestations of CMV disease are nonspecific. Fever and Neutropenia Syndrome (leukopenia, myalgia and fatigue), thrombocytopenia, hepatitis, pancreatitis, chorioretinitis, encephalitis, myocarditis, pneumonitis and gastrointestinal invasion with ulcers, perforation and bleeding are typical of CMV infection [2].

CMV (both reactivation and acquired) disease tends to appear in 1-4 post-transplant months or after the cessation of antiviral prophylaxis. CMV retinitis and colitis occur later, after 6 to 12 months [3]. CMV pneumonia has an incidence of 2-10% in renal transplantation [7] and it often develops at a median of 5 months (1 month to 11 years) post-transplant [8]. The increased doses of immunosuppression during the first year of transplantation [4,5] may justify the higher incidence of CMV during this period.

The manifestations of CMV pneumonitis may range from mild dyspnoea to severe signs and symptoms such as cough, fatigue, malaise, anorexia, myalgias, arthralgias, fever, profuse sweating and tachypnoea [1,5]. Respiratory insufficiency may develop and progress to acute respiratory distress syndrome (ARDS) requiring invasive mechanical ventilation [1]. ARDS is the most direct cause of death [5,35] and CMV pneumonia has an overall mortality rate of 48% increasing to 90-100% in ventilator assisted patients

[4,5,9,35].

Diagnosis is not always easy, as it is often obscured by the presence of other concomitant respiratory pathogens causing overlapping symptoms and most of diagnostic techniques are insensitive [5].

Imaging findings include diffuse ground-glass opacities, dense areas of consolidation, small poorly defined nodules, and, less commonly, irregular linear opacities [36,37]. Ground-glass opacities represent early changes of diffuse alveolar damage, consolidation correlates with interstitial pneumonia with associated edema and fibrinous exudates and micronodules correspond to inflammatory and hemorrhagic nodules and focal areas of organizing pneumonia [37]. CT pattern of bilateral, diffuse or patchy ground glass opacities followed by progressive consolidation, described in our patients, has been associated with an increased mortality [38].

Serological methods for determining antibody responses to CMV have commonly been used. Before transplantation, serological markers (IgM and IgG) against CMV are reasonable as a baseline and can be used in order to adjust for the risk of CMV disease [1,39]. However, after transplantation, antibodies may not develop due to immunosuppression or they may appear after the disease is already cured and persist for years [40]. The sensitivity and specificity reported of CMV IgM is 73% and 62%, respectively, and it does not differentiate between latent and active infection [39]. The use of serological markers in bronchoalveolar lavage has also been disappointing. Detection of IgG CMV in BAL is nonspecific, as IgG CMV has been found in BAL in RT patients with pneumonitis without CMV infection [41].

The antigenemia assay detects in peripheral blood leukocytes a 65-kD CMV protein. In most cases, higher levels of antigenemia correlate well with CMV disease and lower levels with asymptomatic infection [2,42]. This test has a sensitivity of 64-89%, a specificity of 81-100% [43]. It is useful in diagnosis and monitoring CMV disease activity. Although, antigenemia has been often negative in neurologic and gastrointestinal disease (including invasive colitis and gastritis) [3]. Moreover, like our patients, CMV antigenemia can be negative even with active disease and the diagnosis can be achieved by polymerase chain reaction (PCR) or biopsy.

The protein chain reaction of BAL is a useful method for the

diagnosis of CMV pneumonia. It allows an earlier detection of CMV infection with a sensitivity of 100% [39]. However, due to the high sensitivity of DNA amplification, it can detect CMV in patients with asymptomatic infection (who will never progress to active disease) and it remains positive, even after resolution of the infection [39,44]. Many authors point PCR specificity (45-72%) as a major disadvantage in the diagnosis of CMV pneumonia [44]. To overcome this issue, Ye et al. proposed that CMV serum viral load superior to 104 copies/mL (maintained for 3 weeks) could be used as a cutoff to predict CMV pneumonia in RT recipients [45].

The definitive diagnosis of CMV pneumonitis is made with histological confirmation, after transbronchial or open lung biopsy. Typical findings suggestive of CMV infection include epithelial and endothelial cells markedly enlarged with nuclear inclusions, surrounded by clear halo and eosinophilic cytoplasmatic inclusions [37]. Transbronchial biopsy is rarely performed due to hemorrhagic risk and even with multiple transbronchial biopsies the diagnosis may be missed [1,44].

Immunophenotyping of peripheral blood may provide an opportunity to realize the pathophysiology of CMV infection and contribute to differential diagnosis with acute rejection [17]. In RT recipients, an increase in CD4+ T-cells is found in allograft rejection and reduction of CD4+ T cells with CD4/CD8 ratio inversion, as reported in our patients, is suggestive of CMV infection [17]. In patients with CMV pneumonia and ARDS the persistence of the CD4+/CD8+ ratio inversion was also associated with poor outcomes [46]. Therefore, flow cytometric analysis of peripheral T lymphocytes may be useful in diagnosis and monitoring of viral infections [17].

Treatment of established CMV disease requires a multifactorial approach, including a reduction of immunosuppressive agents, antiviral agents and, in some cases, adjuvant therapy [18].

As immunosuppression is the major risk factor for infection, the reduction of immunosuppressive drugs is probably the most important treatment of CMV infection [23], but it carries the risk of allograft rejection. Once protective cytotoxic immunity to viruses is generally T cell-mediated, an initial reduction of antimetabolites and calcineurin inhibitors merits consideration. Steroid dose reduction is not recommended since it has the risk of developing adrenal insufficiency, during the acute phase of the infection [3].

Several antiviral agents are available for treatment of CMV infection, of which intravenous ganciclovir and the oral prodrug of ganciclovir, valganciclovir, are the most commonly prescribed. The standard of care for treating CMV disease is 2 to 3 weeks of antiviral therapy with demonstration of clinical and viral responses to therapy [18].

In mild to moderate disease intravenous ganciclovir (5 mg/kg twice daily, dosage adjustments for renal dysfunction) [18] or oral valganciclovir (900 mg twice daily) are recommended [47,48], but in patients with life-threatening CMV disease, high viral loads, leukopenia and impaired absorption, intravenous ganciclovir is preferable [18,48].

Recurrence rates after successful treatment of the initial episode range from 6% to 59% in transplant recipients [49]. Primary CMV infection, multiorgan disease and anti-rejection therapy are known predictors of recurrence [49]. In order to avoid disease recurrence, it is recommended to follow-up the treatment with secondary prophylaxis with valganciclovir for 3 to 6 months [3].

The use of CMV-specific hyperimmune globulin is not consensual [50]. Some authors advocate administration of standard immunoglobulin or CMV-specific hyperimmune globulin (100-150 mg/kg per dose) as adjuvant therapy in individuals with hypogammaglobulinemia [47], severe systemic infection [3,18], failure to respond to standard therapy or ganciclovir resistant CMV infection [3].

CMV resistance must be suspected if disease worsens or viral load increases despite 2 weeks of therapy [18]. Patients with several courses of ganciclovir with subtherapeutic levels are at higher risk of developing ganciclovir resistance. This is usually secondary to a mutation in the UL97 and UL54 CMV genes [18,47,51]. Resistant infection is treated with reduction of immunosuppression and adjustment of antiviral agents [18]. Alternative antiviral therapies include foscarnet, cidofovir, and leflunomide [3,44,52], but there are no controlled comparative trials using these therapeutic agents. The investigational drug maribavir, a benzimidazole antiviral agent, has a different mechanism of action and it has been proposed for treatment of ganciclovir-resistant CMV disease [53]. Maribavir efficacy has not yet been established and the combination of ganciclovir with maribavir does not seem to be advantageous [53,54].

Experimental studies are ongoing with the use of immunoenhancement therapy. Thymosin- α 1 has various immunomodulatory properties that lead to augmentation of

T-lymphocyte function, including modulation of IL-2, stimulation of IFN- γ production, induction of T-lymphocyte and NK-cells, and stimulation of thymopoiesis [22]. Ji et al included subcutaneous thymosin- α 1 in treatment of CMV disease accompanied with ARDS [4] and they showed, in the treated group, an increase in CD4+/CD8+ ratio, higher treatment success rate (78% vs. 50%) and lower mortality (22% vs. 50%) [4]. Gen-shu et al. used thymosin- α 1 in liver transplant recipients with CMV pneumonia and they reported a mortality rate of 16,7%, with no episodes of acute rejection [22].

Despite remarkable advances in the diagnostic and therapeutic modalities, CMV pneumonia persists as one of the most serious infections of RT recipients. The prognosis is poor, especially in patients requiring invasive ventilatory support.

Cytomegalovirus pneumonia with late presentation has been a growing concern and its diagnosis requires high clinical suspicion. These cases illustrate a very late presentation of this opportunistic infection and reflect a sustained state of intense immunosuppression, not expectable until recently. Several other questions, related to prophylaxis and treatment, remain also to be answered. The future may depend on the progressive development of immunobiology and the creation of an effective CMV vaccine.

CONCLUSION

A high index of suspicion is necessary for diagnosis of CMV pneumonitis, as symptoms can be mild and the disease may appear later than 3 months after renal transplant. The study of BAL with PCR can assist in identifying CMV infections that are negative by other diagnostic methods. Unsuspected lifelong lymphocyte depletion should be persistently researched. Determination of CD4+ and CD8+ T-cells reflects the status of cellular immunity and it may be useful in the diagnostic approach and therapeutic options. Individualized treatment of CMV pneumonia is essential to immunosuppression adjustment, antiviral and adjuvant therapy.

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