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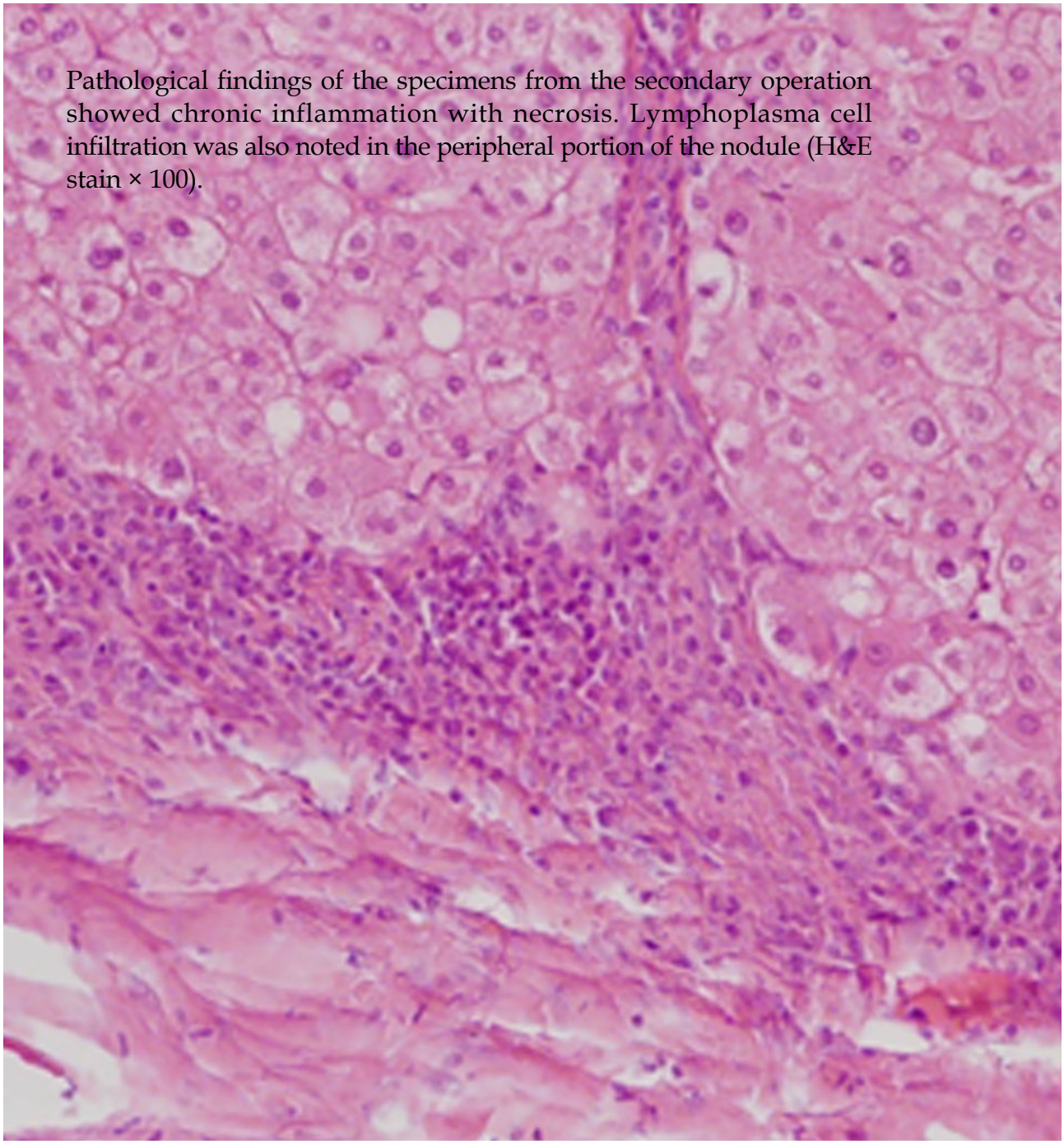

Pathological findings of the specimens from the secondary operation showed chronic inflammation with necrosis. Lymphoplasma cell infiltration was also noted in the peripheral portion of the nodule (H&E stain × 100).

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EDITORIAL

Current concepts on cytomegalovirus infection after liver transplantation

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Abstract

Cytomegalovirus (CMV) is the most common viral pathogen that negatively impacts on the outcome of liver transplantation. CMV cause febrile illness often accompanied by bone marrow suppression, and in some cases, invades tissues including the transplanted allograft. In addition, CMV has been significantly associated with an increased predisposition to allograft rejection, accelerated hepatitis C recurrence, and other opportunistic infections, as well as reduced overall patient and allograft survival. To negate the adverse effects of CMV on outcome, its prevention, whether through antiviral prophylaxis or preemptive therapy, is regarded as an essential component to the medical management of liver transplant patients. Two recent guidelines have suggested that antiviral prophylaxis or preemptive therapy are similarly effective in preventing CMV disease in modest-risk CMV-seropositive liver transplant recipients, while antiviral prophylaxis is the preferred strategy over preemptive therapy for the prevention of CMV disease in high-risk recipients [CMV-seronegative recipients of liver allografts from CMV-seropositive donors (D+/R-)]. However, antiviral prophylaxis has only delayed the onset of CMV disease in many CMV D+/R- liver transplant recipients, and at least in one study, such occurrence of late-onset primary CMV disease was significantly associated with increased mortality after liver transplantation. Therefore, optimized strategies for prevention are needed, and aggressive treatment of CMV infection and disease should be pursued. The standard treatment of CMV disease consists of intravenous ganciclovir or oral valganciclovir, and if feasible, one should also reduce the degree of immunosuppression. In one recent controlled clinical trial, valganciclovir was found to be as effective and safe as intravenous ganciclovir for the treatment of mild to moderate CMV disease in solid organ (including liver) transplant recipients. In this article, the authors review the current state and the future perspectives of prevention and treatment of CMV disease after liver transplantation.

Key words: Cytomegalovirus; Outcome; Hepatitis; Transplantation; Valganciclovir; Prophylaxis; Treatment

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INTRODUCTION

Cytomegalovirus (CMV) is the single most common viral pathogen that influences the outcome of liver transplantation[1,2]. CMV is a ubiquitous herpes virus that,

depending on the population studied, infects 60%-100% of humans[1,2]. Primary CMV infection in immune competent individuals presents most commonly as an asymptomatic illness or a benign febrile infectious mononucleosis-like syndrome. When CMV infection occurs in individuals with compromised immunity, such as liver transplant recipients, clinical disease with high morbidity may develop and, in some cases, this may lead to death[1,2].

The outcome of primary CMV infection is latency in various cells, which ensures persistence throughout the life of the host[1,2]. This characteristic of the virus plays a very important role in how liver transplant recipients develop CMV infection. Firstly, cellular sites of viral latency become reservoirs for reactivation during periods of stress and cytokine release (such as during allograft rejection, allogeneic stimulation, and critical illness). Secondly, cellular sites of viral latency (which is widespread in the human host) serve as vehicles for transmission to susceptible hosts (i.e. during blood transfusions and transplantation of liver allografts latently infected with virus). Moreover, the pharmacologically-induced impairment of immunity in liver transplant recipients markedly limits the ability of the patients to effectively control "endogenously-reactivated" or "allograft-transmitted" CMV, leading in the short-term to febrile and tissue-invasive diseases, and in the long-term to poor allograft and patient survival[1-5].

CLINICAL IMPACT OF CMV ON LIVER TRANSPLANTATION

Direct CMV effects

The classic illness caused by CMV after transplantation is manifested as fever, bone marrow suppression, and organ-invasive diseases (Table 1)[1]. These have been traditionally categorized either as CMV syndrome (fever with bone marrow suppression) and tissue-invasive CMV disease (which may involve virtually any organ system)[6]. The most common organ system involved during CMV disease is the gastrointestinal tract (in the form of CMV gastritis, esophagitis, enteritis, and colitis), accounting for over 70% of tissue-invasive CMV disease cases in solid organ transplant recipients[7]. The transplanted liver also seems to be more predisposed to develop tissue-invasion by CMV such that CMV hepatitis occurs more frequently in liver transplant recipients than in other solid organ transplant recipients. The involvement of the transplanted allograft is often manifested by symptoms that may be clinically indistinguishable from acute allograft rejection[8]. The availability of sensitive tests for the rapid detection of CMV in the blood may obviate the need for liver biopsy to differentiate CMV disease from organ rejection. However, in many cases, a liver biopsy is needed to differentiate or to demonstrate the co-existence of CMV disease and allograft rejection.

Among liver transplant recipients who are not receiving effective antiviral prophylaxis, the direct effects of CMV are observed most commonly during the first 3 mo after liver transplantation[6]. Overall, it is estimated that 18%-29% of all liver transplant recipients will develop

Table 1 Direct and indirect clinical effects of cytomegalovirus after solid organ transplantation

Direct effects	Indirect effects
CMV syndrome	Acute allograft rejection
Fever	
Myelosuppression	
Malaise	
Tissue-invasive CMV disease[1]	Chronic allograft rejection
Gastrointestinal disease (colitis, esophagitis, gastritis, enteritis)	Vanishing bile duct syndrome
	Chronic ductopenic rejection
Hepatitis	Hepatitis C virus recurrence
Pneumonitis	Allograft hepatitis, fibrosis and allograft failure
CNS disease	
Retinitis	
Mortality	Opportunistic and other infections
	Fungal superinfection
	Nocardiosis
	Bacterial superinfection
	Epstein-Barr virus and PTLD
	HHV-6 and HHV-7 infections
	Vascular thrombosis
	Mortality

PTLD: post-transplant lymphoproliferative disease; HHV: human herpes virus; CMV: cytomegalovirus. [1]Any organ system may be affected by CMV.

Table 2 Estimated incidence of cytomegalovirus disease during the first 12 mo after liver transplantation

	Use of anti-cytomegalovirus prophylaxis	
	Yes[1] (%)	No (%)
CMV D+/R-	12-30	44-65
CMV D+/R+	2.70	18.20
CMV D-/R+	3.90	7.90
CMV D-/R-	0.00	0.00
All patients	4.80	18-29

D: donor; R: recipient; CMV: cytomegalovirus. [1]Most cases occur as delayed-onset CMV disease. CMV disease occurs rarely during prophylaxis with oral valganciclovir. Data adapted from references [4,5,77].

CMV disease (Table 2)[4,5,9-11]. However, this incidence varies depending upon donor and recipient CMV serologic status; it may be as high as 44%-65% in CMV D+/R-, or as low as 8%-19% in CMV-seropositive (CMV R+) liver transplant recipients[4,9,11]. The incidence is markedly reduced in liver transplant recipients who received 3 mo of prophylaxis with valganciclovir or oral ganciclovir. Recent studies have reported CMV disease incidence rates of 12%-30% in CMV D+/R-, and < 10% in CMV R+ liver transplant recipients who received 3 mo of antiviral prophylaxis[3,4,9,11-13]. The onset of disease in these patients occurs most commonly during first 3 mo after completing antiviral prophylaxis; hence, the term "delayed-onset (also termed late-onset) CMV disease" to indicate that the onset has been delayed by antiviral prophylaxis (Figure 1)[3].

Indirect CMV effects

The clinical impact of CMV after liver transplantation extends beyond its direct effects to numerous indirect out-

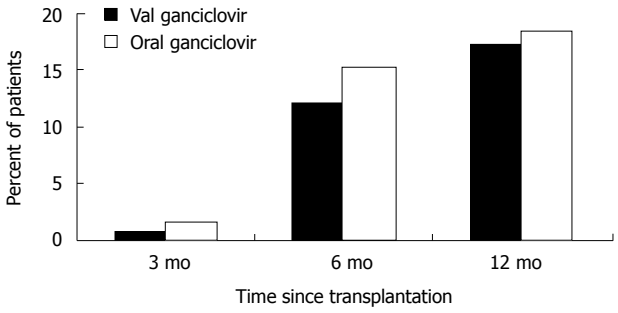

Figure 1 Time to the onset of cytomegalovirus disease in solid organ transplant recipients who received three mo of oral ganciclovir or valganciclovir prophylaxis. Data obtained from the study by Paya and colleagues[5].

comes that are believed to be mediated by the ability of CMV to modulate the immune system (Table 1)[1,2]. CMV is known to be a potent up-regulator of alloantigens, which increases the risk of acute rejection and chronic allograft dysfunction[14]. CMV has been associated with vanishing bile duct syndrome and ductopenic rejection that leads to chronic cholestasis and eventually to allograft failure[15-17]. A higher incidence of vascular and hepatic artery thrombosis has also been reported in a few studies of liver transplant recipients with CMV disease, and this effect is postulated to result from infection of the vascular endothelial cells[18,19]. The immunomodulatory effects of CMV have been suggested to account for a higher predisposition to develop infections due to other opportunistic infections including fungi, other viruses, and bacteria such as *Nocardia* sp[20,21]. CMV-infected transplant recipients are also more likely to develop Epstein-Barr virus associated post-transplant lymphoproliferative disorders (PTLD), or develop co-infections with other viruses such as the human herpesviruses HHV-6 and HHV-7[20,22]. There is a well-described interaction between members of the beta-herpes group of viruses, known as β-herpesvirus syndrome, as exemplified by observations that reactivations of HHV-6 and HHV-7 are significantly associated with an increased predisposition to CMV disease after liver transplantation[23-25]. Similarly, a significant association between CMV and hepatitis C virus (HCV) is also described after liver transplantation[26-31]. This is clinically manifested as an accelerated clinical course of HCV recurrence among patients who have developed CMV infection and disease. In one study of 92 HCV-infected liver transplant recipients, there was a 4-fold higher risk of allograft failure and mortality among those who developed CMV infection and disease. Three years after liver transplantation, 48% of patients who developed CMV disease had allograft loss or had died, compared to 35% of patients with asymptomatic CMV infection, and 17% of patients who did not develop CMV infection[29,31].

Impact on mortality

Through direct, indirect and possibly immunomodulatory mechanisms, CMV is an important predictor of mortality after transplantation[20,32,33]. Prior to the availability of intravenous (IV) and oral ganciclovir, CMV was a major cause of mortality after liver transplantation. With the use of these effective antiviral drugs for prevention and treatment, death due to CMV disease has been remarkably reduced. Indeed, several meta-analyses have demonstrated that the use of anti-CMV drugs, either through antiviral prophylaxis or preemptive therapy, is associated with significant reductions in mortality after transplantation[20,34-36].

Despite these improvements in outcome with the widespread use of antiviral drugs, CMV disease occurring at a delayed onset after prophylaxis remains a common problem, and notably, remains significantly associated with increased risk of mortality after liver transplantation[33]. In an analysis of 437 liver transplant recipients, CMV disease occurred in 37 patients (8.5%) and its occurrence was independently associated with a 5-fold increased risk of all-cause mortality, and an 11-fold increased risk of infection-related mortality[33].

Table 3 Selected traditional and novel factors associated with increased risk of cytomegalovirus disease after liver transplantation

Traditional factors	Recently identified factors
CMV D+/R- > CMV R+	Toll-like receptor gene polymorphism
Allograft rejection	Mannose binding lectin deficiency
High viral replication	Chemokine and cytokine defects (IL-10, MCP-1, CCR5)
Mycophenolate mofetil	Deficiency in CMV-specific CD4+ T cells
Muromonab-CD3	Deficiency in CMV-specific CD8+ T cells
Anti-thymocyte globulin	Expression of immune evasion genes
Alemtuzumab	Programmed cell death 1 expression
Basiliximab	
Human herpesvirus-6	
Human herpesvirus-7	
Renal insufficiency	
Others[1]	

D: donor; R: recipient; IL-10: interleukin-10; MCP-1: monocyte chemotactic protein-1; CCR5: chemokine (C-C motif) receptor 5; CMV: cytomegalovirus. [1]Others include re-transplantation, volume of blood transfusion, sepsis and other factors associated with high tumor necrosis factor-α secretion.

RISK FACTORS FOR CMV DISEASE AFTER LIVER TRANSPLANTATION

Lack of pre-existing CMV-specific immunity

The most important risk factor for the occurrence of CMV disease after liver transplantation is a lack of effective CMV-specific immunity. Specifically, CMV D+/R- patients are at highest risk of CMV disease[4,20], while CMV R+ patients have modest risk and CMV D-/R- have the lowest risk of CMV disease after liver transplantation (Table 3).

Drug-induced immunodeficiency

Severe pharmacologic immunosuppression impairs the ability of liver transplant recipients to mount an effective immune response against CMV, thereby predisposing to higher risk of CMV disease[4,20]. The severity of im-

mune dysfunction is particularly intense with the use of lymphocyte-depleting drugs, as either induction or re jection therapy, such as muromonab-CD3 (OKT3) and anti-thymocyte globulin[37,38]. When alemtuzumab, an anti-CD52 lymphocytic antibody, is used for short-course induction therapy only, the risk of developing CMV disease is low[39,40]. However, when patients receive alem tuzumab as rejection therapy, the risk of developing CMV disease is higher, suggesting that rejection per se also increases the risk[40]. Basiliximab and daclizumab are also used for induction therapy and they act as anti-CD25 directed non-depleting antibodies (interleukin-2 receptor antagonist). Transplant recipients receiving basiliximab induction therapy have a lower incidence of infection but a greater incidence of CMV disease than those receiving anti-thymocyte globulin[41].

Drugs used for maintenance immunosuppression have also been associated with CMV disease, particularly with high-doses of mycophenolate mofetil[31,42]. More recently, the use of newer maintenance immunosuppressive drugs such as sirolimus and everolimus [mammalian target of rapamycin (mTOR) inhibitor] has been found to be associated with lower risk of CMV disease[43,44]. These observations have generated special interest in the use of the mTOR agents for patients at high risk of CMV disease. However, it is very likely that not only do the specific immunosuppressive drugs predispose to CMV disease, but that the net state of combined pharmacologic immunosuppression increases the risk of CMV disease after liver transplantation[1,2,20].

Defects in innate and CMV-specific cell-mediated immunity

Inherent defects in innate immunity, such as mutations in innate immunity-associated genes, increase the risk of CMV disease after liver transplantation (Table 3). In our study of 92 liver transplant recipients, a specific genetic polymorphism in the Toll-like receptor (TLR)-2 gene, which resulted in the substitution of arginine to glutamine at position 753 in the protein-receptor, was significantly associated with a higher degree of CMV replication and a higher incidence of CMV disease. TLR2 is a pattern recognition receptor expressed in innate immune cells, and it functions to sense the glycoprotein B of CMV, thereby signaling the immune cells to produce antiviral peptides and other cytokines. Our *in vitro* data suggests that this specific genetic polymorphism causes in impairment of cellular recognition of CMV by TLR2-expressing cells[45].

CMV-specific T cells are necessary for the adequate control of CMV after liver transplantation[46], and the detection of these pathogen-specific cells after transplan tation appear to confer protection against the develop ment of CMV disease. In one study, secretion of interferon-γ by CD8+ T cells during in vitro stimulation with a pool of CMV peptides was significantly associated with a lower incidence of late onset CMV disease in a cohort of solid organ transplant recipients who received valganciclovir prophylaxis[47]. However, data from other studies do not yet support the potential clinical utility of CMV-specific T cells as prognostic markers of CMV disease predisposition[46]. There are ongoing studies in this field that will further clarify the prognostic role of CMV-specific T cell assays in stratifying CMV disease risk after liver transplantation.

Other immune measures, such as programmed death-1 expression[48], mannose binding lectin levels or gene mutation, and immune evasion genes[49] have also been assessed as prognostic indicators of CMV disease after transplantation. In one study, programmed death-1 receptor up-regulation was significantly associated with incipient and overt CMV disease and with CMV viremia[48].

Allograft rejection

Allograft rejection is often associated with CMV reactivation, and thus it is considered as a significant risk factor for CMV disease after liver transplantation[13]. It is hypothesized that cytokines that are released during episodes of acute rejection, particularly tumor necrosis factor-α[50], could transactivate CMV from its state of latency[51,52]. Subsequent therapy for allograft rejection with intensified immunosuppression further enhances the risk of CMV disease by enhancing viral replication and by impairing the generation of an effective CMV-specific cell-mediated immunity[53]. Conversely, CMV induces allogeneic stimulation thereby increasing the risk of allograft rejection, and creating a bidirectional relationship between CMV and allograft rejection[14].

Virus-to-virus interactions

Virus-virus interactions have been proposed to enhance the risk of CMV disease after liver transplantation[22,23,27-31]. HHV-6 has been associated with an increased predisposition to develop CMV disease after liver transp lantation[22,23,25]. Likewise, HCV-infected liver transplant patients have a higher incidence of CMV disease[54], although the advent of valganciclovir prophylaxis has allowed mitigation of this phenomenon[26].

Other factors

The risk of CMV disease after liver transplantation is associated, in direct proportion, with the degree of CMV replication, which is partly a function of over-immunosuppression[9,24,55,56]. Other factors associated with CMV disease after liver transplantation include cold ischemia time, bacterial and fungal infections and sepsis, the amount of blood loss, fulminant hepatic failure as the indication for liver transplantation, age, female gender, Hispanic race, and renal insufficiency[2,3,20,57].

PREVENTION OF CMV DISEASE AFTER LIVER TRANSPLANTATION

There are two major strategies for CMV disease prevention after liver transplantation: (1) preemptive therapy (wherein patients are monitored for CMV replication by sensitive assays such as PCR and pp65 antigenemia, and

upon the detection of asymptomatic CMV replication, antiviral therapy is administered preemptively to prevent progression to symptomatic clinical disease); and (2) antiviral prophylaxis (wherein antiviral drugs such as valganciclovir are administered to all patients at risk of CMV disease after liver transplantation)[20]. Both of these prevention strategies are considered similarly effective in preventing CMV disease after liver transplantation[4,5,58-61]. According to the current American Society of Transplantation (AST) and The Transplantation Society (TTS) guidelines, preemptive therapy may be an option in CMV D+/R- liver transplant recipients, although many authorities prefer to use antiviral prophylaxis and reserve preemptive therapy for lower-risk populations[62,63]. The main reason for this is the rapidity of CMV replication in CMV-naïve CMV D+/R- liver recipients, which may escape detection with once weekly CMV surveillance. Indeed, antiviral prophylaxis is used by the majority of American and European transplant centers in preventing primary CMV disease in high-risk CMV D+/R- liver transplant recipients[64,65]. Moreover, primary antiviral prophylaxis has the added benefit of reduction in bacterial and fungal opportunistic infections and mortality[34,35].

Preemptive therapy

The basic principle of preemptive therapy is to detect the presence of CMV replication prior to the onset of clinical symptoms, so that antiviral therapy is administered early in order to prevent the progression of asymptomatic infection to full-blown clinical disease[56,58,59,61,66]. Preemptive therapy has the potential advantage of targeting therapy to the highest risk patients and thereby decreasing drug costs and toxicity. The success of this approach relies on several aspects including: (1) the optimal laboratory test and frequency and duration of monitoring; (2) selection of the appropriate population for preemptive therapy; and (3) choosing the type, dose and duration of an antiviral drug.

Either the CMV pp65 antigenemia assay or quantitative PCR may be used for the preemptive approach. The pp65 antigenemia assay is a semi-quantitative fluorescence assay based on detection of CMV pp65 antigen present in infected cells in the peripheral blood. This assay is comparable in sensitivity to CMV PCR[67] and remains a valuable test for centers managing small numbers of specimens. However, because the pp65 antigenemia assay needs processing of samples within 6-8 h of blood collection, a large sample volume, subjective interpretation that leads to poor standardization, and is labor-intensive, many laboratories are moving towards quantitative PCR. PCR has significant potential power for patient surveillance in the preemptive approach. However, there is lack of CMV PCR standardization across different laboratories and each center needs to validate their own threshold values for preemptive therapy[62,63]. The optimal interval and duration of monitoring is unknown, but testing approximately once weekly for 12 wk after transplant is suggested. If a patient shows viremia above an institutionally-derived threshold during the surveillance, therapy should be initiated and continued until CMV viremia is no longer detectable[62,63].

Several studies have reported the success of IV or oral ganciclovir or valganciclovir in the preemptive treatment of CMV reactivation in liver transplant recipients, including high-risk CMV D+/R- patients[60,66]. However, some studies have indicated that preemptive therapy may not be completely effective in CMV D+/R- liver transplant recipients since the replication kinetics of CMV in immune-deficient individuals is so rapid[55] that it may escape detection with once weekly PCR or antigenemia assay, and this could result in clinical illness prior to its laboratory detection[9,58]. Indeed, in our clinical experience, nearly 25% of CMV D+/R- liver transplant recipients who developed CMV disease were not identified early by a protocol-based weekly CMV PCR assay[9,58]. Accordingly, the current AST and TTS guidelines prefer antiviral prophylaxis in CMV D+/R- liver transplant recipients[62,63]. In contrast, preemptive therapy is recommended and highly effective in CMV-seropositive liver transplant recipients.

Reassuringly, clinical trials have demonstrated the efficacy of preemptive therapy in CMV disease prevention[58-60,66]. Three meta-analyses that collectively analyzed data from prospective clinical trials demonstrated the benefits of preemptive therapy in preventing CMV disease[35,36,68]. When conducted properly, preemptive therapy, with the use of oral ganciclovir, IV ganciclovir, or valganciclovir resulted in the reduction of CMV disease by about 70%[35,36,68]. Moreover, preemptive therapy is much less likely associated with late onset CMV disease (unlike in antiviral prophylaxis, as discussed below)[59,66]. Currently, valganciclovir is the most commonly used drug for preemptive therapy[64], and in one non-controlled study, it was demonstrated to be as effective in terms of clinical and virologic response, as IV ganciclovir[59,66]. In addition, preemptive therapy may be beneficial in reducing the indirect effects of CMV, although to a much lesser degree than antiviral prophylaxis. In one study, the incidence of major opportunistic infections, bacteremia, bacterial infection, HCV recurrence, and rejection were not significantly different between liver transplant patients who received preemptive therapy and those who did not have CMV reactivation[69]. An example of a preemptive algorithm is shown in Figure 2.

Antiviral prophylaxis

Antiviral prophylaxis is highly effective in preventing the direct, as well as the indirect effects of CMV after liver transplantation[4,5,35,36,68]. Compared to placebo or no treatment, patients who received antiviral prophylaxis had lower incidence of CMV disease (58%-80% reduction) and CMV infection (about 40% reduction)[68]. In one meta-analysis, a 25% reduction in the incidence of acute allograft rejection was observed[35]. In two studies, a reduction in all-cause mortality was observed[35,68], mainly due to a decline in CMV-related death[68]. A reduction in the incidence of other herpes viruses, bacterial, and proto-

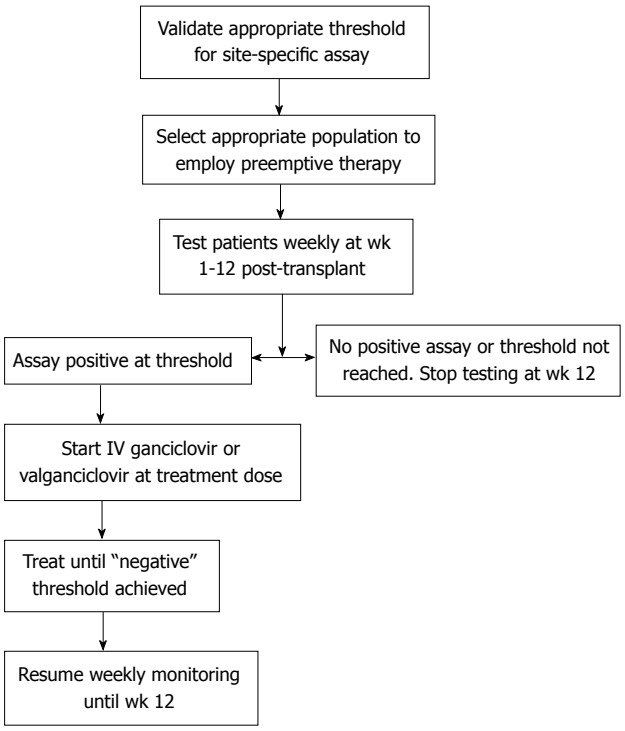

Figure 2 Suggested algorithm for preemptive therapy. Figure adapted from reference [62].

zoan infections were also observed[68]. Because of these additional benefits, liver transplant centers prefer the use of antiviral prophylaxis over preemptive therapy in the prevention of CMV disease, particularly in CMV D+/R- liver transplant recipients[64]. Table 4 shows the currently available antiviral drugs for CMV prophylaxis and treatment in liver transplant recipients.

Ganciclovir prophylaxis

Ganciclovir-based regimen is more effective than acyclovir or immunoglobulins in reducing the incidence of CMV after liver transplantation. In one study, the administration of IV ganciclovir for 90-100 d reduced the incidence of CMV disease in CMV D+/R- liver transplant recipients to 5.4% (compared to 40% in patients who received less than 7 weeks of prophylaxis)[70]. The major drawback to IV ganciclovir was the need for long-term vascular access and its risks of thrombosis, phlebitis, and line-associated bacterial and fungal infections[38,71]. Oral ganciclovir, administered at 1000 mg PO three times daily, circumvents these limitations of intravenous therapy. In a randomized trial that compared it to placebo, oral ganciclovir for 98 days reduced the 6-mo incidence of CMV infection (51.5% *vs* 24.5%; $P < 0.001$), and CMV disease (19% *vs* 5%; $P < 0.001$) in liver transplant recipients[4], including CMV D+/R- patients (44% *vs* 15%; $P = 0.02$) and patients who received antilymphocyte antibodies (33% *vs* 5%; $P = 0.002$)[4]. Among CMV R+ liver transplant recipients, oral ganciclovir for 12 wk reduced the incidence of CMV disease to 1% (compared to 7% in patients who received acyclovir)[72]. Oral ganciclovir, however, is poorly absorbed, and its oral administration results in low systemic ganciclovir levels[73]. This factor has been implicated in the emergence of ganciclovir-resistant CMV in certain clinical settings[74,75], such as high-risk CMV D+/R- patients, and those receiving potent immunosuppressive regimens.

Table 4 Currently available antiviral drugs for cytomegalovirus prophylaxis and treatment in liver transplant recipients

Drug	Route	Usual adult prophylaxis dose	Usual adult treatment dose	Comments on use and major toxicity
Ganciclovir	Intravenous	5 mg/kg once daily	5 mg/kg twice daily	Intravenous access; leukopenia
Ganciclovir	Oral	1 g three times daily	Not applicable	Low oral bioavailability; high pill burden
Valganciclovir	Oral	900 mg once daily	900 mg twice daily	Ease of administration; leukopenia

Valganciclovir prophylaxis

Valganciclovir, a valine ester of ganciclovir, provides systemic ganciclovir levels that are comparable to IV ganciclovir[73,76]. Pharmacokinetic studies indicate that a 900 mg dose of valganciclovir achieves a similar daily area under the concentration time curve (AUC_{24}) as an IV dose of 5 mg/kg of ganciclovir[73]. Hence, valganciclovir (900 mg once daily) has the advantage of avoiding the cost and risks of IV ganciclovir and the pill burden and poor absorption of oral ganciclovir. The role of valganciclovir in the prevention of CMV disease after liver transplantation was evaluated in a multicenter randomized non-inferiority clinical trial that compared it with oral ganciclovir in a cohort of 364 CMV D+/R- solid organ (including liver) transplant recipients (Figure 1). The 6-mo incidence of CMV disease was 12% and 15% in the valganciclovir and oral ganciclovir groups, respectively. Follow-up at one year, demonstrated that the incidence of protocol-defined CMV disease in all patients was 17% and 18% with valganciclovir and oral ganciclovir, respectively[5]. Overall, valganciclovir was as clinically effective and well-tolerated (except for a higher incidence of neutropenia; 8% and 3%, respectively) as oral ganciclovir for CMV prevention in high-risk solid organ transplant recipients.

However, in a subgroup analysis of the 177 liver transplant recipients who participated in the clinical trial, the incidence of CMV disease was 19% in the valganciclovir group as opposed to only 12% in the ganciclovir group. There was also a higher incidence of tissue-invasive CMV disease in the valganciclovir group[5]. As a result of these findings, valganciclovir did not gain approval from the US-FDA for prophylaxis against CMV disease after liver transplantation. Although not FDA-approved for prophylaxis in liver transplant recipients, valganciclovir is the most widely used drug for the prevention of CMV disease after liver transplantation[64].

Delayed- and late- onset CMV disease

With the success of a 3-mo anti-CMV prophylaxis program (in terms of the almost complete elimination of

CMV disease among individuals who are actively taking the antiviral drugs), the challenge of delayed- and late-onset CMV disease has emerged. In many high-risk CMV D+/R- individuals, the use of antiviral prophylaxis has only delayed the onset of CMV disease to 3-6 mo after liver transplantation[3-5,13]. In a retrospective study, CMV disease occurred in 14 of 54 (26%) CMV D+/R- liver transplant recipients who completed at least 3 mo of valganciclovir prophylaxis (Figure 1)[77]. In another retrospective study on 203 liver transplant recipients who received valganciclovir 900 mg daily for 3 to 6 mo, the overall incidence of CMV disease was 14%. The incidence varied among the different CMV serogroups (16% in D+/R+ group; 7% in D-/R+ group; and 26% in D+/R- group)[77]. These findings illustrate that the burden of delayed-onset CMV disease remains high, particularly in the CMV D+/R- group[5]. In our analysis of 67 CMV D+/R- liver transplant recipients who received 3 mo of oral ganciclovir and valganciclovir prophylaxis, the two year incidence of CMV disease was 29%. The incidence of delayed-onset CMV disease was not significantly different between patients who received oral ganciclovir or valganciclovir (22% *vs* 28%; $P = 0.63$)[3]. Thus, one out of every four CMV D+/R- liver transplant recipients will develop CMV disease after cessation of antiviral prophylaxis. Delayed-onset CMV disease most commonly presented as CMV syndrome, with fever and bone marrow suppression[3]. In less than half of the patients, CMV manifested as tissue-invasive disease, and frequently effected the gastrointestinal tract[3]. Factors such as age[3], female gender[3,78], renal dysfunction[78], and allograft rejection[13] predisposed to the development of delayed-onset primary CMV disease. Delayed-onset CMV disease appears to be clinically less severe, although it is associated with significant mortality after liver transplantation[33].

Because of the negative effect of late onset CMV disease on overall outcome, a better method for CMV prevention is needed among CMV D+/R- liver transplant recipients. The current AST and TTS guidelines suggest that the duration of antiviral prophylaxis may be prolonged from the standard 3 mo to 6 mo in CMV D+/R- liver transplant recipients[62,63]. This recommendation is based on a trial that investigated this approach in CMV D+/R- kidney transplant recipients. It is emphasized that this duration has not yet been studied in the liver transplant recipients, and that valganciclovir is not FDA-approved for the prevention of CMV disease after liver transplantation. Nonetheless, in this study of kidney transplant recipients, the incidence of CMV disease was reduced from 36.8% in patients who received 3 mo of valganciclovir prophylaxis to 16.1% in those who received the drug for 6 mo. While this represents a significant reduction in the incidence of CMV disease, the data also highlights the continued risk in some patients despite the prolonged prophylaxis (in this case, 16% still developed CMV disease despite 6 months of valganciclovir prophylaxis). In addition, there are theoretical concerns about ganciclovir resistance and drug toxicity particularly with leukopenia during prolonged prophylaxis, although these were not demonstrated in the clinical trial. The cost of the use of prolonged prophylaxis will need to be evaluated. Another strategy that is gaining interest is an aggressive minimization of immunosuppression, including the use of prednisone-free regimens. Many liver transplant programs (including ours) have adapted this approach, and have minimized immunosuppression gradually so that patients are maintained on tacrolimus monotherapy beyond the 4th mo after liver transplantation. In a retrospective analysis, we observed a higher incidence of CMV disease among liver transplant recipients who were still receiving mycophenolate mofetil and prednisone at the time they discontinue antiviral prophylaxis[14].

TREATMENT OF CMV DISEASE AFTER LIVER TRANSPLANTATION

The first line treatment of CMV disease after liver transplantation is IV ganciclovir or valganciclovir[62,71,79]. In contrast, oral ganciclovir should not be used for the treatment of CMV disease because of its poor bioavailability[20]. In addition, the degree of pharmacologic immunosuppression should be reduced if possible[20]. In a multi-center non-inferiority trial, 321 solid organ (including liver) transplant recipients with non-severe CMV disease were randomized to valganciclovir (900 mg twice daily) or IV ganciclovir (5 mg/kg twice daily) for a fixed 21-d course, followed by valganciclovir (900 mg once daily) maintenance treatment for 4 wk. The proportion of patients with viral eradication at 21 and 49 d were comparable in the IV ganciclovir and valganciclovir groups (Figure 3)[79]. The overall time to viral eradication was 21 d with valganciclovir and 19 d with IV ganciclovir. The calculated viral decay was 11.5 d with valganciclovir and 10.4 d with IV ganciclovir. Likewise, clinical resolution was not different between the two groups. It was noted that patients enrolled into this trial were mostly CMV-seropositive, the majority were kidney recipients (although there were good number of liver transplant recipients), and patients with severe CMV disease were excluded. Despite these limitations, this pivotal trial now supports the use of valganciclovir for oral treatment of CMV disease, at least in selected transplant patients[79]. IV ganciclovir is preferable to valganciclovir in patients with severe or life-threatening disease, or in patients who may have a problem with gastrointestinal absorption of oral drugs such as those with gastrointestinal CMV disease. In many instances, valganciclovir is used as a step-down treatment when the clinical symptoms have resolved after an initial induction treatment with IV ganciclovir.

The duration of treatment of CMV disease should be individualized[62,80]. The persistence of the virus at the end of therapy (as indicated by PCR or pp65 antigenemia) is associated with a higher risk of clinical relapse[81]. It is now generally accepted that multiple (at least two) weekly negative CMV PCR results should be obtained before antiviral therapy is discontinued. Although this may be correct for non-tissue invasive CMV syndromes, the utility of such an approach may not necessarily apply

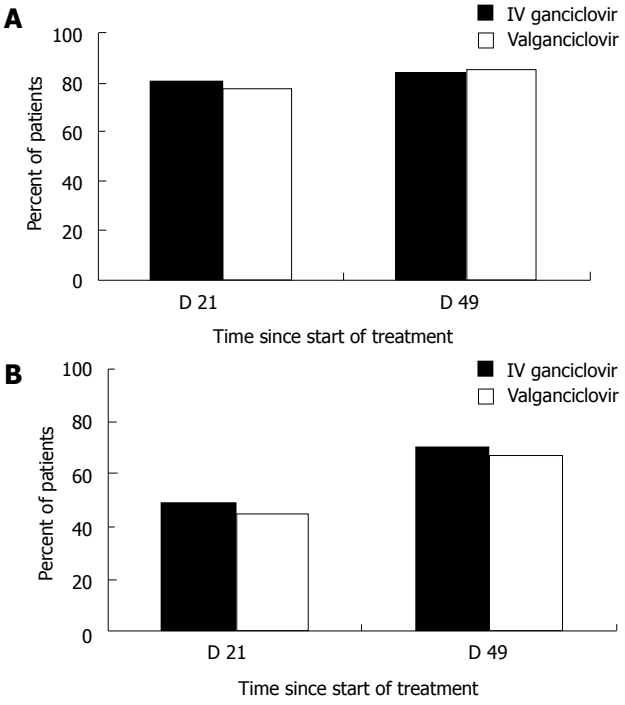

Figure 3 Proportion of solid organ transplant patients with resolution of clinical symptoms (panel A) and viremia eradication (panel B) at day 21 and 49 following the start of valganciclovir or intravenous ganciclovir treatment of CMV disease. Data obtained from the study by Asberg and colleagues[79].

to some tissue-invasive disease, which may manifest as "compartmentalized disease"[20].

Treatment of compartmentalized CMV disease

Compartmentalized CMV disease refers to clinical syndromes wherein the virus is detected in the effected tissues but is minimally detectable or undetectable in the blood[20]. In the current era, gastrointestinal CMV disease constitutes the vast majority of tissue-invasive cases[3,7,20], and in a number of cases, especially in CMV R+ patients, this type of CMV disease is "compartmentalized." In a retrospective study, the sensitivity of pp65 antigenemia assay (defined as detection of $\geqslant$ 1 positive cells/2 $\times 10^5$ leukocytes) for diagnosis of CMV gastrointestinal disease was only 54%[82]. Such a clinical presentation is reminiscent of CMV retinitis, a very rare manifestation of tissue-invasive CMV disease after transplantation, that is often not accompanied by viremia[83,84]. This dilemma brings to the forefront the limitation of viral load monitoring in assessing duration of treatment. In our clinical practice, it is not uncommon to have negative blood PCR assays even when there remains histologic evidence of tissue invasion. Accordingly, it has been suggested that colonoscopy or upper endoscopy should be performed to document clearance of gastrointestinal CMV disease prior to discontinuation of therapy. However, our retrospective review of this practice suggests that this should not be generalized to all patients with gastrointestinal CMV disease. We observed that relapse of gastrointestinal CMV disease was significantly associated with extensive involvement of gastrointestinal tract at the time of diagnosis[85]. In contrast, CMV serologic conversion, degree of viral load, treatment duration, maintenance therapy, and endoscopic findings at the end of therapy were not significantly predictive of CMV relapse. Our experience indicates that endoscopic evidence of resolution of gastrointestinal disease may not be necessary in mild to moderate disease as long as sufficient therapy is provided[85].

Treatment of ganciclovir-resistant CMV disease

Ganciclovir-resistant CMV is now emerging as an important complication of prolonged antiviral drug use after transplantation[2,20,75]. Currently, ganciclovir-resistant CMV is very rarely seen in liver transplant recipients (while it is relatively more common after kidney-pancreas and lung transplantation). The estimated incidence of ganciclovir-resistant CMV after liver transplantation is < 0.5%[75,86]. Several studies have identified risk factors for ganciclovir resistance CMV[2,20,75], including CMV D+/R- status, high levels of viral replication, potent immunosuppressive therapy, and suboptimal ganciclovir levels. The vast majority of drug-resistant cases involve the selection of viral strains with UL97 (kinase) mutation[2,20,75,87,88]. UL97 mutation generally confers resistance to ganciclovir, although in some cases, a concomitant UL54 mutation (CMV DNA polymerase) is also observed, in which case, cross-resistance to cidofovir and/or foscarnet is likely.

Drug-resistant CMV is associated with significant morbidity and mortality, and there is a very limited number of antiviral drugs (which are often toxic) available for treatment[86]. Drug-resistant CMV should be suspected when viral load or antigenemia rises or does not decline to undetectable levels despite IV ganciclovir treatment. In our retrospective study of 225 CMV D+/R- solid organ transplant recipients who received 3 mo of valganciclovir prophylaxis, CMV disease occurred in 65 patients (29%), including four (8%) caused by drug-resistant CMV, judged by the failure of the viral load to decline to undetectable levels while on IV ganciclovir treatment. This diagnosis was confirmed by genetic analysis to demonstrate mutational changes in UL97 and UL54 genes encoding for kinase and polymerase, respectively[75,86]. In patients where foscarnet or cidofovir was used, nephrotoxicity was a major and common adverse effect[89]. Other potential drugs for treatment of multi-drug resistant CMV include the off-label use of immunoglobulins, leflunomide (an immunosuppressive drug), and artesunate (anti-malaria drug), although data supporting their use are only anecdotal[20,90]. The potential clinical utility of maribavir in treatment of resistant CMV has also been suggested. However, the clinical development of this drug is currently halted in view of disappointing results from a phase III clinical trial in bone marrow transplant recipients, which also resulted in the premature termination of the randomized clinical trial in liver transplant recipients[87,88,91,92].

CONCLUSION

Remarkable advances in molecular diagnostics and therapeutics have led to marked reduction in the incidence and

severity of CMV disease after liver transplantation, and a parallel decline in associated morbidity and mortality. However, despite these improvements, CMV remains a common infectious complication and continues to negatively influence the outcome of liver transplantation. In addition to viral factors and pharmacologic immunosuppression, the role of innate and adaptive immune deficiencies is being recognized in the pathogenesis of CMV disease after liver transplantation. Such novel findings should provide additional avenues and opportunities for improving our management strategies. Prevention of CMV with antiviral prophylaxis and preemptive therapy is effective, although a well-controlled trial assessing these two strategies in a head-to-head comparison is yet to be conducted after liver transplantation. Currently, valganciclovir prophylaxis is the most common approach for the prevention of CMV disease in CMV D+/R- and R+ liver transplant recipients. The availability of predictive diagnostic tests has paved the way for the successful use of preemptive therapy in preventing the progression of CMV reactivation to clinical disease even among high-risk liver transplant patients. IV ganciclovir and oral valganciclovir are the standard drugs for treatment of established CMV disease, although valganciclovir should be limited to patients with mild to moderate CMV disease. Oral valganciclovir should be avoided as initial therapy for patients with severe CMV disease and those with questionable gastrointestinal absorption. The duration of treatment should be individualized, depending upon clinical and laboratory parameters such as the decline of CMV load in the blood as measured by rapid and sensitive molecular testing. In this context, it is generally recommended that treatment be continued until all evidence of active infection, such as positive CMV viral load, has resolved. Ganciclovir-resistant CMV and compartmentalized tissue-invasive disease (most commonly with gastrointestinal CMV disease) are emerging challenges to the management of CMV after liver transplantation. These, together with the common occurrence of late-onset CMV disease in high-risk patients, should serve as catalysts to the ongoing search for the optimal management strategy for CMV disease after liver transplantation.

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GUIDELINES FOR BASIC SCIENCE

Nitric oxide and cancer

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Abstract

Nitric oxide (NO) is a lipophilic, highly diffusible and short-lived physiological messenger which regulates a variety of important physiological responses including vasodilation, respiration, cell migration, immune response and apoptosis. NO is synthesized by three differentially gene-encoded NO synthase (NOS) in mammals: neuronal NOS (nNOS or NOS-1), inducible NOS (iNOS or NOS-2) and endothelial NOS (eNOS or NOS-3). All isoforms of NOS catalyze the reaction of L-arginine, NADPH and oxygen to NO, L-citrulline and NADP. NO may exert its cellular action by cGMP-dependent as well as by cGMP-independent pathways including postranslational modifications in cysteine (S-nitrosylation or S-nitrosation) and tyrosine (nitration) residues, mixed disulfide formation (S-nitrosoglutathione or GSNO) or promoting further oxidation protein stages which have been related to altered protein function and gene transcription, genotoxic lesions, alteration of cell-cycle check points, apoptosis and DNA repair. NO sensitizes tumor cells to chemotherapeutic compounds. The expression of NOS-2 and NOS-3 has been found to be increased in a variety of human cancers. The multiple actions of NO in the tumor environment is related to heterogeneous cell responses with particular attention in the regulation of the stress response mediated by the hypoxia inducible factor-1 and p53 generally leading to growth arrest, apoptosis or adaptation.

Key words: Nitric oxide; Cancer; Carcinogenesis

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INTRODUCTION

Discovery of nitric oxide

Nitric oxide (NO) is a lipophilic, highly diffusible and short-lived physiological messenger[1]. NO regulates a variety of important physiological responses including vasodilation, respiration, cell migration, immune response and apoptosis. Ignarro *et al*[2] and Moncada *et al*[3] identified simultaneously the endothelium-derived relaxing factor (EDRF) as NO. Hibbs *et al*[4] demonstrated that the reaction using L-arginine as substrate results in the formation of L-citrulline and the end products, NO_2^-/NO_3^-. The 1990s brought several landmarks to the field including the molecular characterization of the NO synthase (NOS) family of enzymes[5-8], the discovery of peroxyntrite ($ONOO^-$)[9], the importance of NO-mediated posttranslational protein modifications[10], the regulation of mitochondrial function by NO[11-14] and the chemistry of NO diffusion/reactivity[15].

Nitric oxide synthase isoenzymes

NO is synthesized by three differentially gene-encoded NOS in mammals: neuronal NOS (nNOS or NOS-1), inducible NOS (iNOS or NOS-2) and endothelial NOS (eNOS or NOS-3). All three isoforms share similar structures and catalytic modes, yet the mechanisms that control their activity in time and space are quite diverse. The expression of NOS-2 is induced by inflammatory stimuli while NOS-1 and NOS-3 are more or less constitutively expressed[16]. The full active NOS form requires two NOS monomers associated with two Ca^{2+}-binding protein calmodulin (CaM). NOS contains relatively tightly-bound cofactors such as (6R)-5,6,7,8-tetrahydrobiopterin (BH4), FAD, FMN and iron protoporphyrin Ⅸ (haem) and catalyze the reaction of L-arginine, NADPH and oxygen to NO, L-citrulline and NADP[16]. The reaction is composed of two sequential steps involving the hydroxylation of guanidino nitrogen of L-arginine generating the intermediate N^{ω}-hydroxy-L-arginine (NOHA) which is oxidized to NO and L-citrulline[17]. HB_4 acts as a redox cofactor in the second reduction step and prevents the uncoupling of NOS and generation of anion superoxide (O_2^{-}). All NOSs require similar amounts of L-arginine, BH4 and NADPH for activity. NOS isoforms are differentially regulated at transcriptional, translational and post-translational levels. However, the activity of NOS-1 and NOS-3 is highly dependent upon intracellular Ca^{2+} concentration whereas NOS-2 forms an active complex with CaM. NOS-2 is already maximally activated by Ca^{2+}/CaM even at basal levels of intracellular Ca^{2+}[16]. Several inhibitory and activator phosphorylated sites in NOS-1 and NOS-3 tightly regulate their NO production[18].

The intracellular localization is relevant for the activity of NOS. It appears that there are compartments that allow full activation of NOS with free access to substrates and cofactors as well as the presence of activators[19]. In this sense, accumulating evidence indicates that NOSs are subject to specific targeting to subcellular compartments (plasma membrane, Golgi, cytosol, nucleus and mitochondria) and that this trafficking is crucial for NO production and specific posttranslational modifications of target proteins[20,21].

NITRIC OXIDE CELL SIGNALING

The biological activity of NO is classified by cGMP-dependent and cGMP-independent pathways, both attributed to physiological and pathological conditions[22-24]. cGMP-dependent protein kinases, cyclic-nucleotide-gated ion channels and cGMP-regulated phosphodiesterases mediate several cellular effects. However, during the last decade, cGMP-independent reactions have gained considerable interest. A variety of effects are achieved through its interactions with targets *via* redox and additive chemistry that may promote covalent modifications of proteins as well as oxidation events that do not require attachment of the NO group. In fact, NO is the prototypic redox-signaling molecule more versatile than O_2^{-} or H_2O_2 and clearly better identified with redox-related modifications of intracellular proteins[25].

Nitric oxide cGMP-independent pathways

The most prominent and recognized NO reaction with thiols groups of cysteine residues is called S-nitrosylation or S-nitrosation which leads to the formation of more stable nitrosothiols[26]. However, other modifications such as disulfide, mixed disulfide formation with reduced glutathione (S-nitrosoglutathione or GSNO) or oxidation towards sulfenic acid are also important since they are reversible. Higher thiol oxidation states such as sulfinic or sulfonic acids are irreversible modifications with subsequent loss of functional control. Nitrosothiol formation can be the result of a direct reaction with NO or of an oxidative nitrosylation reaction involving the preformation of $ONOO^{-}$[27]. The pattern of nitrosylated proteins is specific, probably dependent of the presence of specific consensus motifs which influence the accessibility of the thiols groups to NO[28]. Different proteins such as NMDA and ryanodine receptors, ras, caspases, glyceraldehyde-3-phosphate dehydrogenase and DNA repair proteins are widely post-translationally modified by nitrosylation[29].

Oxidative and nitrosative stress is sensed and closely associated with transcriptional regulation of multiple target genes[30]. The net effect of NO on gene regulation is variable and ranges from activation to inhibition of transcription. S-nitrosation of specific cysteines in active zinc fingers sequences in SP-1, EGR-1 and glucocorticoid receptors, induces Zn^{2+} release, concomitant conformational changes and reduced DNA-binding[31]. The impact of NO in other transcription factors such as NF-*k*B may affect at different levels such as I*k*B expression and stability, NF-*k*B activation, nuclear translocation or cysteine residue modification involving alteration of DNA binding. The administration of NO donors reduces NF-*k*B activation and downstream expression of anti-apoptotic gene products[32] which is relevant for NO-dependent sensitization chemotherapy-resistant tumor cells[33,34]. It now seems more certain that reducing conditions are required in the nucleus for NF-*k*B DNA binding whereas oxidizing conditions in the cytoplasm promote NF-*k*B activation[30]. AP-1 is a transcription factor that belongs to the basic leucine zipper (bZip) family in which a single cysteine residue is present that confers redox sensitivity[35]. NO, mostly by S-nitrosylation[36] and glutathiolation[37] of cysteine, inhibits c-Jun and c-Fos DNA binding in a reversible manner. p53 also binds to its specific DNA sites in a reducing environment and mutations of cysteine residues in the p53 core binding domain (loop-sheet-helix motif linked to a loop-helix motif) prevents DNA binding and p53-induced transcription[38]. HIF-1α has a single cysteine in the basic-helix-loop-helix of the carboxyl-terminal trans-activating domain which participates in protein–protein interactions that activate transcription[39]. Other transcription factors whose binding to DNA is facilitated under reducing conditions include c-Myb, USF, NFI, NF-Y, HLF, PEBP2, GABPa, TTF-1 and Pax-8[30].

The generation of $O_2^{\cdot-}$ and NO may lead the production of the harmful molecule $ONOO^{-}$[40]. $ONOO^{-}$ may result in S-nitrosylation and tyrosine nitration of proteins with a concomitant change in their function[41]. The generation of $ONOO^{-}$ may exert a negative feedback regulation on the NO production. In this sense, the reaction of $ONOO^{-}$ with Akt and BH4 altered NO production generated by NOS[42,43]. Proteins that can be nitrated on tyrosine residues include actin, histone proteins, protein kinase C, prostacyclin synthase, manganese superoxide dismutase, tyrosine hydroxylase, cytochrome P450B1, transcription factor STAT1 and p53[44]. Also, different proteins appeared to be nitrated in cultured human hepatocytes[45]. Alternatively, NO may indirectly induce gene transcription *via* activation/modulation of signaling pathways such as mitogen-activated protein kinases (MAPK), G-proteins, Ras pathway or phosphatidylinositol-3 kinase (PI3K) pathways[46].

NITRIC OXIDE, CELL PROLIFERATION AND CANCER

Nitrogen oxide chemistry is critical in the nitrogen cycle, converting nitrate(NO_3^-) and nitrite (NO_2^-) to ammonia (NH_4^+), an essential component of protein synthesis as well as in the vascular tone and cell signaling regulation. However, it has also been associated to the deleterious/cytotoxic effects in air pollution, antibacterial in the preservation of food as well as the generation of carcinogenic nitrosamines[47]. In this sense, NO may participate in the induction of genotoxic lesions as well as promoting angiogenesis, tumor cell growth and invasion[48].

Participation of nitric oxide in carcinogenesis

The infectious and non-infectious generation of chronic injury and irritation initiates an inflammatory response[49]. A subsequent respiratory burst, an increased uptake of oxygen that leads to the release of free radicals from leukocytes, including activated macrophages, can damage surrounding cells. This process can drive carcinogenesis by altering targets and pathways that are crucial to normal tissue homeostasis. NO and NO-derived reactive nitrogen species induce oxidative and nitrosative stress which results in DNA damage (such as nitrosative deamination of nucleic acid bases, transition and/or transversion of nucleic acids, alkylation and DNA strand breakage) and inhibition of DNA repair enzymes (such as alkyltransferase and DNA ligase) through direct or indirect mechanisms[50]. However, the diversity of reactive species produced during chronic inflammation under different cellular microenvironments has impaired identification of a clear biomarker that identifies the involvement of a single reactive species in the carcinogenic process[51]. Chronic inflammation contributes to about one in four of all cancer cases worldwide[49]. The induction of mutations in cancer-related genes or post-translational modifications of proteins by nitration, nitrosation, phosphorylation, acetylation or polyADP-ribosylation are some key events that can increase the cancer risk. In particular, high levels of NO are genotoxic through formation of carcinogenic nitrosamines or by directly modifying DNA or DNA repair proteins. It was found that aerobic solutions of NO, NO_2 and N_2O_3 led to deamination of nucleic acids[52]. Unlike oxidation by $ONNO^{-}$ or reactive oxygen species (ROS) that preferentially results in transversions, nitrosative mixtures of NO_2/N_2O_3 mediate transitions[53]. However, NO may also influence the carcinogenesis process by alteration of cell-cycle checkpoints[54], apoptosis[55] and DNA repair[56]. NO donors sensitize tumor cells to chemotherapeutic compounds by nitrosilation of critical thiols in DNA repair enzymes in hepatoma cell line[57]. Other studies have demonstrated increased susceptibility to chemotherapy to cisplatin[58] and melphalan[59] by NO donors in different cell lines. These results implied substantial modification of key biological target(s) including DNA repair proteins and transcription factor known to be inhibited by NO.

Cell signaling of NO in carcinogenesis

It is difficult to identify the specific role of NO in carcinogenesis because it is dependent on its concentration, interaction with other free radicals, metal ions and proteins, and the cell type and the genetic background that it targets. The expression of NOS-2 has been found to be increased in a variety of human cancers[60-62]. However, NOS-3 has also been suggested to modulate different cancer-related events (angiogenesis, apoptosis, cell cycle, invasion and metastasis)[63]. NO can both cause DNA damage and protect from cytotoxicity, can inhibit and stimulate cell proliferation and can be both pro- and anti-apoptotic[64-67]. Lancaster and Xie[68] suggest that the multiple actions of NO in the tumor environment are related to its chemical (post-translational-related modifications) and biological heterogeneity (cellular production, consumption and responses). However, one of the critical insights into this dichotomy may be the regulation by NO of the stress response mediated by the hypoxia inducible factor-1 (HIF-1)[69] and p53[70] generally leading to growth arrest, apoptosis or adaptation[71].

Nitric oxide and p53

The biological outcomes of p53 activity include apoptosis, inhibition of cell cycle progression, senescence, differentiation and accelerated DNA repair. The types of stress that promote p53 activation include many conditions associated with cancer initiation and progression such as direct DNA damage, chromosomal aberrations, illegitimate activation of oncogenes, hypoxia, telomere shortening *etc*. p53 is found at very low steady state in non stress conditions by the mouse double minute (Mdm2, the human orthologue form is named Hdm2) protein[72]. Mdm2 displays an E3 ubiquitin ligase activity towards p53 for ubiquitin-dependent proteasomal degradation, although non-proteasomal mechanisms for p53 degradation may also play a significant role under certain

circumstances[73]. The induction of cell death or anti-tumoral properties of NO has been extensively related to nuclear p53 accumulation[74-77]. NO donors induce p53 accumulation and apoptosis through JNK-1/2, but not ERK1/2 or p38, activation in RAW 264.7 macrophages[78]. A peptide corresponding to the JNK binding site on p53 protein efficiently blocks its ubiquitination and consequently increases p53 half-life[79]. The nuclear accumulation of p53 is mainly regulated by posttranslational modifications by phosphorylation[80] or tyrosine nitration[81]. S-nitrosoglutathione and NO donors prevent poly-ubiquitinated-dependent p53 degradation by proteasome which it is antagonized by reducing agents[82]. Ubiquitin-activating enzyme (E1), a key component of ubiquitinization process, has a cysteine residue that is S-nitrosylated by NO donors[83].

The overexpression of NOS-2 reduced *in vitro* cell proliferation[84-86] and *in vivo* tumor progression in xenograft experimental models[84,86] using different carcinoma cell lines. Le *et al*[87] have recently shown that NOS-2 overexpression exerts antitumor activity *in vitro* and *in vivo* dose dependently, regardless of its up-regulation of protumor factors. Also, NOS-2-derived NO suppresses lymphomagenesis even in a *p53*$^{-/-}$ back-ground by promoting apoptosis and decreasing tumor cell proliferation[88]. NOS-2 overexpression has also been shown to induce radiosensitization through p53 accumulation in *in vitro* and *in vivo* xenograft models[89,90]. Furthermore, wild-type p53-induced transrepression of NOS-2 provides a protective mechanism against prolonged exposure to pathological conditions of NO[75,91]. The frequency of p53 mutations occurs in about 50% of all human tumors suggesting that can be an early event in the process of hepatocellular carcinogenesis[92,93]. The expression of NOS-2 has been associated with increased expression of p53 mutated isoforms in liver sections from patients with hemochromatosis and Wilson diseases[94], ulcerative colitis[95], colon cancer[96] and stomach, brain and breast cancers[60,62,97]. Ambs *et al*[84] have observed that NOS-2 overexpression in cells with mutated p53 accelerated tumor growth, increased vascular endothelial growth factor (VEGF) expression and neovascularization. These studies indicate that exposure of cells to high levels of NO and its derivatives during chronic inflammation in the absence of wild-type p53, and therefore the negative NOS-2 regulation, may increase the susceptibility to cancer. Therefore, loss of functional p53 may lead to a reduction of NO sensitivity and transfer to other stress survival response such as HIF-1 that may promote a selective tumoral growth advantage. However, recent study has shown that NOS-2 overexpression abrogated the growth of various human tumor cells with different p53 functional status (wild-type, mutated and gene loss)[87]. The contradictory results among studies showing the potential role of p53 in high NO production may be explained by the different overall genetic background of tumoral cell lines as well as the stromal cell-derived NO in xenograft models that may modulate the response of surrounding tumor cells.

Nitric oxide and HIF-1

NO has also revealed an impact on the redox-sensitive target HIF-1. HIF-1 is predominantly active under hypoxic conditions because the HIF-1α subunit is rapidly degraded in normoxic conditions by proteasomal degradation[98]. Different genes involved in erythropoiesis and iron metabolism (erythropoietin or transferrin), glucose/energy metabolism (glucose transporters), cell pro liferation/viability decisions (transforming growth factor-β), vascular development/remodeling and/or vasomotor tone (VEGF or NOS-2) contain HRE (hypoxia responsive element)[99]. HIF-1α is overexpressed as a re sult of intratumoral hypoxia and/or genetic alterations affecting key oncogenes and tumor suppressor genes in human cancer[100]. Different signals other than hypoxia such as growth factors, reactive oxygen species, cytokines, NO and/or NO-derived species participate in hypoxic signaling[101]. NO, through cGMP-dependent pathways, regulates different modifications during drosophila development in oxygen deprivation conditions[102]. However, thiol groups in HIF-1 or the proteins that are involved in the regulation of HIF-1 are also potential targets for post-translational modifications by NO. GSNO or selected NO donors enhance S-nitrosylation of propyl hydroxylase which lead to HIF-1α accumulation[103,104] and HIF-1 DNA-binding activity in cell systems[105]. However, small NO concentrations induce a faster but transient HIF-1α accumulation than higher doses of the same donor[106]. NO-related HIF-1 activation mediates up-regulation of VEGF expression in normoxic human glioblastoma and hepatoma cells[107]. Different studies have also shown that phosphorylation mechanism by PI3K/Akt pathway is also involved in GSNO-induced HIF-1 accumulation[108].

As described above, HIF-1 is predominantly active under hypoxic conditions in which the generation of oxygen species, specifically H_2O_2, is supposed to attenuate HIF-1 activation. Similarly, the redox cycler DMNQ (2,3-dimethoxy-1,4-naphthoquinone) generating $O_2^{\cdot-}$ and/or H_2O_2 (derived from superoxide dismutase-triggered conversion of $O_2^{\cdot-}$ to H_2O_2) attenuated NO-derived reactive nitrogen species-elicited HIF-1α accumulation[108]. The attenuation by NO of hypoxia-evoked reporter gene activation has been extended to several genes such as insulin-like growth factor binding protein (IGFBP-1), endothelin-1 and VEGF[101]. In this condition, NO has been shown to prevent HIF-1 accumulation in Hep3B and PC-12 cells which it reduced by addition of a lipophilic glutathione analog or $ONOO^-$ scavenger[109]. If indeed the steady state of $O_2^{\cdot-}$ increases under hypoxia, it may be hypothesized that hypoxia in the presence of NO-derived reactive nitrogen species delivery promotes formation to the strong oxidant $ONOO^-$. $ONOO^-$ in turn may not only oxidize reduced glutathione but also damage mitochondria. The differential behavior of NO in normoxic and hypoxic conditions may also be related to its capacity to regulated mitochondrial oxidative phosphorylation which may limit ROS generation and HIF-1 accumulation in hypoxic conditions[110]. In addition, the

interference of NO signaling by mitochondrial $O_2^{\cdot-}$ generation can be rationalized by the diffusion-controlled radical interaction which may redirect signaling qualities of NO towards other species, i.e. $ONOO^{-}$ that may not share the ability to stabilize HIF-1α. Hypoxic intracellular environment is characterized by a complex network of radical pattern generation that in conjunction with variable amounts of defense-systems may reveal a variable HIF-response to NO.

CONCLUSION

Different studies have shown that increased and continuous NO production plays a pivotal role in the regulation of carcinogenic process. The alteration of redox status and transcriptional pattern modifications induced by NO in tumoral cells increase cell death and exerts antineoplastic properties. In this sense, more studies should be done in order to identify the temporal, spatial and concentration-dependent intra- and extra-cellular NO generation that exerts its maximum antitumoral activity either as monotherapy or combined treatment with chemotherapy.

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REVIEW

Impact of human herpes virus 6 in liver transplantation

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Abstract

Human herpes virus 6 (HHV-6) infects > 95% of humans. Primary infection which occurs mostly during the first 2 years of life in the form of roseola infantum, non-specific febrile illness, or an asymptomatic illness, results in latency. Reactivation of latent HHV-6 is common after liver transplantation. Since the majority of human beings harbor the latent virus, HHV-6 infections after liver transplantation are most probably caused by endogenous reactivation or superinfection. In a minority of cases, primary HHV-6 infection may occur when an HHV-6-seronegative individual receives a liver allograft from an HHV-6-seropositive donor. The vast majority of HHV-6 infections after liver transplantation are asymptomatic. Only in a minority of cases, when HHV-6 causes a febrile illness associated with rash and myelosuppression, hepatitis, gastroenteritis, pneumonitis, and encephalitis after liver transplantation. In addition, HHV-6 has been implicated in a variety of indirect effects, such as allograft rejection and increased predisposition to and severity of other infections, including cytomegalovirus, hepatitis C virus, and opportunistic fungi. Because of the uncommon nature of the clinical illnesses directly attributed to HHV-6, there is currently no recommended HHV-6-specific approach prevention after liver transplantation. Asymptomatic HHV-6 infection does not require antiviral treatment, while treatment of established HHV-6 disease is treated with intravenous ganciclovir, foscarnet, or cidofovir and this should be complemented by a reduction in immunosuppression.

Key words: Human herpes virus 6; Opportunistic infections; Liver transplantation; Antivirals

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INTRODUCTION

Human herpes virus 6 (HHV-6), a member of the β-Herpesviridae subfamily, was first isolated from human peripheral blood lymphocytes in 1986[1]. It was initially considered an orphan virus, because it was not associated with any human illness until two years after its discovery, when HHV-6 was implicated as the etiologic agent of a common childhood febrile illness known as roseola infantum (also termed exanthem subitum or sixth disease)[2].

Primary infection with HHV-6 occurs most commonly during the first two years of life, and the peak incidence

occurs between 6 and 12 mo after birth[3]. By two years, more than 90% of humans have been infected with the virus, as indicated by the presence of a positive HHV-6 IgG antibody[3]. Primary HHV-6 infections may present as an asymptomatic illness or as a febrile syndrome, later accompanied by a maculopapular rash (exanthem subitum)[3]. Primary HHV-6 infection has also been associated with otitis, gastrointestinal symptoms, respiratory distress, seizures, and, more rarely, with encephalitis or hepatitis in children[3-5].

There are two variants of HHV-6, designated variant A and variant B (HHV-6A and HHV-6B), respectively[6,7]. The two subtypes share certain biological properties and a high level of sequence homology, but are clearly two distinct viruses, both virologically and epidemiologically[8,9]. HHV-6B is implicated in the majority of primary HHV-6 infections during the first two years of life. Since HHV-6B replicates in the salivary glands, the mechanism of transmission between humans is thought to be via salivary secretions[10]. On the other hand, HHV-6A seems to be more neurotropic and it has been implicated mainly in neurologic diseases, especially in patients with HIV infection[11-13]. The age of acquisition of HHV-6A remains undetermined, and, unlike HHV-6B, it does not seem to replicate in salivary glands, and its primary mode of transmission is currently not known.

LATENCY AND CHROMOSOMAL INTEGRATION

HHV-6 is a lymphotropic virus that replicates in CD4+ T-lymphocytes[14], and by using the CD46 molecule as its cellular receptor[15], it may also infect other cell types, such as monocytes and macrophages, astrocytes, fibroblasts, and cells of endothelial or epithelial origin[16-20]. HHV-6 infects various organs, including the brain, salivary glands, tonsils, lungs, kidneys and liver[14,21-29]. Primary HHV-6 infection results in the establishment of latent infection, with the virus being located primarily located in mononuclear cells. During latency, HHV-6 genome is harbored mainly as a separate circular DNA inside various cells, such as lymphocytes and monocytes[16]. Indeed, in one study, HHV-6 DNA sequences were detected in peripheral blood mononuclear cells in as many as 90% of the subjects being studied, thus implying the ubiquitous nature of the virus[30].

In the minority of HHV-6 infected individuals, instead of existing as a separate circular DNA, the virus becomes integrated into the host chromosome[31,32]. This is known as a chromosomally-integrated HHV-6 infection. Both HHV-6A and HHV-6B variants have the ability to be integrated into the chromosome. The incidence of chromosomally-integrated HHV-6 infection is not exactly known, although a recent study of blood donors from the United Kingdom estimated an incidence between 0.2%-1%[31]. Because the virus can be found in germ lines, it has also been suggested that chromosomally-integrated HHV-6 may be transmitted from mother to child (i.e. vertical transmission)[32,33]. However, this has not been confirmed by other investigators[34]. Individuals with chromosomally-integrated HHV-6 infection have a characteristic persistently high level of HHV-6 DNA in the blood, serum, and hair follicles, usually millions of genomic copies, but without causing clinical illness[35]. The high level of HHV-6 DNA in the blood and other body fluids in individuals with chromosomally-integrated HHV-6 infection is thought to be due to cellular proliferation and lysis, and not as a result of viral replication. However, in a few individuals with chromosomally-integrated HHV-6, some degree of viral replication may also occur[33]. Many believe that chromosomally-integrated HHV-6 infection is not clinically significant, although there are a few studies reporting on its association with an increased risk of lymphoproliferative diseases[36,37].

MECHANISMS OF HHV-6 INFECTION AFTER LIVER TRANSPLANT

Latent HHV-6 serves as the reservoir for endogenous viral reactivation after transplantation, or as a potential vector of transmission to susceptible individuals via the transplanted organ itself. Given the high seroprevalence of HHV-6 in adults, which is estimated at over 90%-95%, most active infections after liver transplantation are thought to originate from reactivation of the endogenous latent virus. However, primary HHV-6 infection may occur in previously non-infected individuals, and this scenario is more commonly observed in pediatric transplant recipients who are under 2 years of age, who may not have been infected with the virus, and who may in turn have received an organ transplant from an HHV-6 positive donor[38]. Some of the primary HHV-6 infections, which were presumably of donor origin, have resulted in fatal primary HHV-6 diseases[39,40]. Viral reactivation may also occur in the transplanted allograft to cause HHV-6 superinfection in a previously-infected individual[41]. In a minority of cases, primary HHV-6 infection may occur when a transplant patient acquires the virus through blood products, or through natural transmission (e.g. exposure to oropharyngeal secretions).

EPIDEMIOLOGY OF HHV-6 AFTER LIVER TRANSPLANTATION

The reported incidence of HHV-6 infection after liver transplantation ranges widely, between 14% to 82%[42-44]. These estimates vary depending on the population being studied (adults versus children), the use of anti-CMV prophylaxis (ganciclovir products are active against HHV-6 and may suppress its reactivation)[45], the severity of pharmacologic immunosuppression, and the sensitivity of the laboratory methods used for the diagnosis[46]. HHV-6 infections typically occur during the first 2-8 wk after liver transplantation, when the level of immunosuppression is at its most intense. However, HHV-6

Table 1 Clinical syndromes attributed to human herpes virus 6 after liver transplantation

Direct effects	Indirect effects
Fever	Increased incidence and severity of cytomegalovirus disease
Rash	Earlier and more severe recurrence of hepatitis C virus
Myelosuppression	Higher incidence of fungal infections
Pneumonitis	Higher incidence of other opportunistic infections
Hepatitis	Higher incidence of allograft rejection
Gastritis and colitis	Higher incidence of allograft failure
Neurologic illness	Higher all-cause mortality

infections occurring as early as 10 d, and as late as 5 years, after liver transplantation have been reported[47].

While subclinical HHV-6 infection may be common, the reported incidence of HHV-6-associated clinical disease is rare. HHV-6B accounts for the majority of documented infections and disease in transplant recipients. The epidemiology of HHV-6A after liver transplantation is less well-defined, although it has certainly been reported to cause clinical disease, including those that have resulted in fatal outcomes[39,40].

The risk factors for HHV-6 infections and disease after liver transplantation are not completely defined. Since most infections after liver transplantation probably represent reactivation of latent viruses, it is reasonable to assume that the intensity of pharmacologic immunosuppression may be a risk factor, potentially through prolonged suppression of HHV-6 specific memory responses. Indeed, factors that have been associated with HHV-6 reactivation after liver transplantation are acute allograft rejection and the result of receiving high doses of corticosteroids[47,48]. Certain immunosuppressive agents, including muromunab-CD3 (OKT3), an investigational anti-CD3 monoclonal antibody (BC3), and alemtuzumab have been associated with active HHV-6 infection after transplantation[49,50]. The presence of active HHV-6 infection during acute liver failure has also been reported as a risk factor for the development of hepatic allograft infection after liver transplantation[51].

CLINICAL SYNDROMES ASSOCIATED WITH HHV-6 INFECTION AFTER LIVER TRANSPLANTATION

The vast majority of HHV-6 reactivations and infections after liver transplantation are asymptomatic[47,52-54]. Only in a minority of cases do they cause clinical disease. Nonetheless, there have been a myriad of clinical syndromes associated with HHV-6 infection after liver transplantation, and these have been classified either as direct or indirect effects. The direct clinical manifestations due to HHV-6 include a febrile illness with or without rash, myelosuppression, hepatitis, pneumonitis, gastrointestinal disease, and neurological diseases[47,54-56]. These overt clinical diseases that have been directly attributed to HHV-6 have been estimated to occur in less than 1% of liver transplant patients[57,58]. The indirect effects attributed to HHV-6 are presumed to be consequences of virus-induced immunomodulation and include the exacerbation of cytomegalovirus (CMV) disease, an increased severity of hepatitis C virus (HCV) recurrence, an increased risk of other opportunistic infections, allograft dysfunction, and acute cellular rejection (Table 1)[45,47,53,54,58-62].

Direct HHV-6 effects

Fever and rash: The most frequently reported clinical presentation of HHV-6 infection after liver transplantation is a febrile illness, frequently associated with myelosuppression and rash[48,54,63]. This clinical presentation of HHV-6 infection mimics the syndrome classically attributed to CMV, and it could thus possibly be misdiagnosed as a CMV syndrome[64]. In a retrospective study of 200 liver transplant recipients, two patients (1%) presented with a febrile illness and, after excluding all other pathogens and etiologies for the fever, HHV-6 was implicated as the causative agent[54]. In a second study involving 67 living-donor liver transplant recipients, five patients with HHV-6 viremia or DNAemia had unexplained fever at the time of infection, and this was accompanied by elevations in serum aminotransferase levels[65]. In a prospective study on 51 adult liver transplant recipients who were frequently monitored for HHV-6, eleven patients developed HHV-6B antigenemia at 7-280 d after transplantation[66], including four patients with HHV-6 associated fever or abdominal pain[66]. Skin rash or myelosuppression were not recorded in any of these patients. In some cases, co-infections between HHV-6 and CMV have been observed[64,67]. However, the contribution of HHV-6 to the clinical illness when CMV is present is debated. In a large study of liver and other solid organ transplant recipients with CMV disease, the presence of concomitant HHV-6 was not significantly associated with more severe clinical symptoms or higher CMV viral load[45,68].

Hepatitis: HHV-6 may infect the liver and cause allograft dysfunction in liver transplant recipients[47]. Clinically, HHV-6 infection of the liver has presented with elevated aminotransferases, allograft dysfunction, acute rejection, and lymphocytic infiltration of the graft (Figure 1A)[47,54]. In one study of 51 liver transplant recipients, there was significant graft dysfunction observed in eight out of 11 patients with HHV-6 antigenemia, and in three of these patients, HHV-6 antigens were detected in their liver biopsy specimens (5.9%)[66,67]. In another study, which was a retrospective review of 121 patients, HHV-6 infection was thought to be the etiologic agent of liver allograft infection after liver transplantation in eight (6.7%) cases[47], shown by serology and immunostaining of HHV-6 antigens in liver biopsy specimens (Figure 1B). HHV-6 antigens were detected in all six available liver biopsy specimens. Histologic examination of biopsy specimens demonstrated acute rejection in 5 of the 8 patients, and 3 patients had portal lymphocyte infiltration[47]. In a study

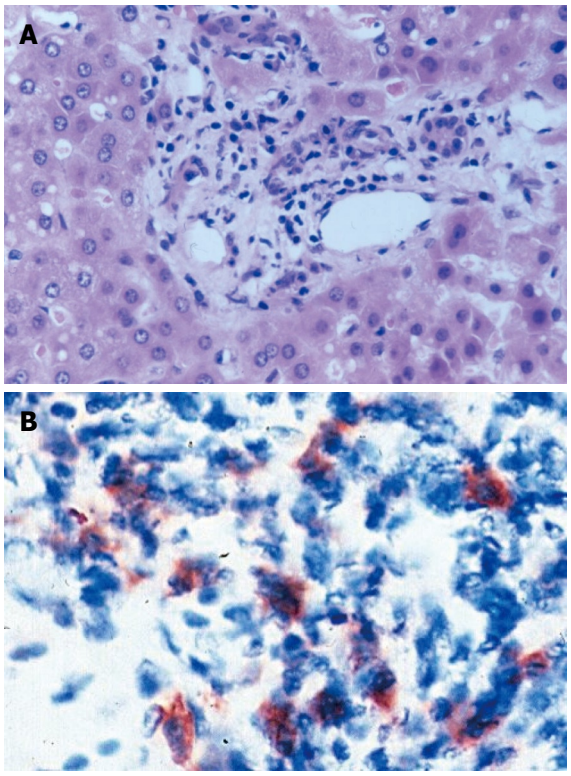

Figure 1 The histological findings associated with intragraft human herpes virus 6 infection. A: Portal area with mild lymphocyte dominated inflammatory infiltrate (H&E staining, original magnification × 400); B: Human herpes virus 6 positive cells in the portal area demonstrated by immunohistochemistry (original magnification × 1000). From Härmä *et al*. *Transplantation* 2006; 81: 367-372 with permission[52].

involving 67 living-donor liver transplant patients, the five patients with HHV-6 viremia or HHV-6 DNAemia had elevated aminotransferase levels that accompanied the febrile illness during the time of HHV-6 infection[65]. Another study showed that nine of 18 patients who had pre-transplant hepatic HHV-6B infection developed intra-allograft HHV-6B infection after liver transplantation[51], while another report documented donor-transmitted HHV-6A superinfection in an HHV-6B infected liver transplant recipient, which manifested itself as syncytial giant cell hepatitis[41].

Gastrointestinal disease: HHV-6 infection may manifest with symptoms of gastroenteritis and colitis, and this may or may not be accompanied by detectable HHV-6 viremia[69]. One study evaluated the presence of HHV-6 in the gastroduodenal mucosa of 90 liver transplant recipients who were undergoing gastroscopic examination for dyspeptic symptoms. HHV-6-positive cells were found in the biopsy specimens from 21 (23%) of the liver transplant recipients (Figure 2)[70]. Fifteen of the transplant recipients with positive HHV-6 findings in the gastroduodenal mucosa were also found to have HHV-6 antigenemia. The histopathological findings were, however, nonspecific, and included very mild inflammatory changes[70].

Bone marrow suppression: Suppression of the blood

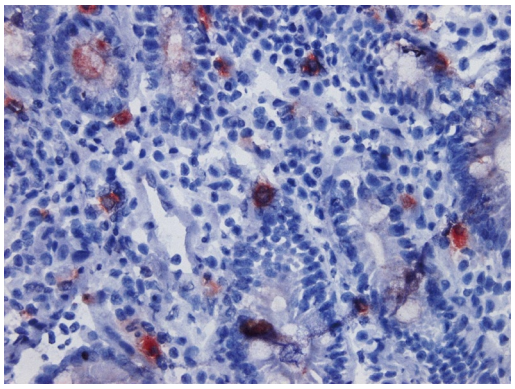

Figure 2 Human herpesvirus-6 positive cells in the gastroduodenal mucosa demonstrated by immunohistochemistry (original magnification × 400). (Courtesy of Dr. Johanna Arola, University of Helsinki, Finland).

cell lines is one of the more common manifestations of HHV-6 infection. In one report, four liver transplant recipients developed HHV-6 associated myelosuppression at a median of 50 d (range 17-90 d) after transplantation. While all the cell lineages were affected, leukopenia was the most common presentation[55].

Pneumonia: HHV-6 may infect the lungs to cause pneumonitis, often in association with other abnormalities such as bone marrow suppression. In one of the four patients with myelosuppression, concomitant interstitial HHV-6 pneumonitis was observed, as documented by positive HHV-6 immunostaining of the lung biopsy specimen[55].

Encephalitis: The vast majority of clinical reports of HHV-6 encephalitis have described its occurrence in allogeneic bone marrow transplant recipients, although HHV-6 has been reported to cause encephalitis in liver transplant recipients[56,71]. Encephalitis typically occurs within 4-6 wk after transplantation and is characterized by confusion, loss of short-term memory, and seizures. Patients will often have normal cerebrospinal fluid profiles, although elevated cerebrospinal fluid protein may be observed. Brain imaging may be abnormal, with hallmark abnormalities found in the medial temporal lobes. In one report, central nervous system complications such as mental status changes of unidentified etiology were more likely to occur in liver transplant recipients who had developed HHV-6 infection[62]. However, another report found no significant association between HHV-6 infection and neurological illnesses[54]. These contradictory results may be due to the differences in neurotropism between HHV-6 variants, with HHV-6A as the neurotropic variant.

Indirect HHV-6 effects

CMV disease: Many studies have described the association among the β-herpes viruses that results in a predisposition to develop CMV disease after liver transplantation. In one study, concurrent HHV-6 antigenemia was detected in 50% of 42 liver transplant patients with active CMV infection[67]. In another study, concurrent CMV

and HHV-6 infections were detected in 16 of 19 liver transplant patients at a mean of 11 d (range 6 to 24 d) after transplantation[60]. Since HHV-6 had been detected prior to CMV in most cases, there was a suggestion that HHV-6, through its immunomodulating property, enhanced the reactivation of CMV[60]. Indeed, another study demonstrated that liver transplant recipients with primary HHV-6 seroconversion were more likely to develop symptomatic CMV disease than those who did not have HHV-6 seroconversion[59]. This finding was again demonstrated in a prospective study which revealed that liver transplant recipients who developed CMV disease also had detectable HHV6 DNA in the blood[43]. However, this association between HHV-6 and CMV was not observed in a large cohort of solid organ transplant recipients who received oral ganciclovir and valganciclovir prophylaxis, and which revealed that the incidence of CMV disease was not significantly different between those who did and those who did not develop HHV-6 DNAemia[45]. Alternatively, the presence of HHV-6 may serve as a marker of an over-immunosuppressed state and hence the predisposition to develop other infections, such as CMV.

HCV disease progression: Liver transplant patients with HCV-induced liver cirrhosis were more likely to have HHV-6 infection[65]. Conversely, HHV-6 may be associated with early fibrosis due to HCV recurrence after liver transplantation[58,61], thereby creating a bidirectional relationship. A prospective study reported that HCV-positive patients who developed HHV-6 viremia after liver transplantation had an earlier recurrence and a higher fibrosis score upon hepatitis C recurrence when compared to patients without HHV6 viremia[61]. In another analysis of 60 liver transplant recipients with chronic hepatitis C, HHV-6 infection was associated with more severe hepatitis and higher fibrosis scores[58]. In contrast, a study of 93 HCV-infected liver transplant recipients showed no association between HHV-6 and the incidence and severity of hepatitis C recurrence after transplantation[72].

Fungal and other opportunistic infections: Possibly due to its immune modulating properties, HHV-6 may influence other opportunistic infections after liver transplantation. One study of 200 liver transplant recipients demonstrated the impact of HHV-6 infection on opportunistic infections, including CMV, Epstein Barr virus-related post transplant lymphoproliferative disease, varicella zoster virus, invasive fungal infections, and mycobacterial disease. In a multivariate analysis, HHV-6 was found to be a significant risk factor for the occurrence of these opportunistic infections[54]. In another study, HHV-6 infection was independently associated with the occurrence of invasive fungal infections in a cohort of 80 liver transplant recipients[62]. Likewise, in a study of 247 patients, the incidence of invasive fungal infection was twice as high in patients with HHV-6 seroconversion compared to those without HHV-6 seroconversion[54]. It was further demonstrated that HHV-6 infection was an independent predictor of invasive fungal infections during the first 90 d after liver transplantation[54].

Allograft rejection and function: HHV-6 may cause graft dysfunction and may be associated with rejection[47,53,54]. Local HHV-6 infection of the allograft was associated with increased expression of adhesion molecules on vascular endothelial cells and infiltrating leukocytes, and this could lead to local inflammation and graft damage, leading to dysfunction and rejection[73]. In liver biopsies, there was mild to moderate lymphocyte infiltration associated with HHV-6 infection. HHV-6 significantly increased the vascular expression of ICAM-1 and VCAM-1, and the number of graft-infiltrating lymphocytes positive for LFA-1, VLA-4 and class Ⅱ antigens[73]. In an analysis of liver transplant recipients who developed allograft rejection, HHV-6 infection was the only factor significantly associated with rejection beyond 30 d after liver transplantation[54]. Another study further supported the independent association between HHV-6 and biopsy-proven acute allograft rejection after liver transplantation[53].

Mortality: A higher all-cause mortality rate after liver transplantation has been reported in patients with HHV-6 infection[62]. This concurs with data from heart-lung[74] and bone marrow transplantation[75]. In another study, all-cause mortality at the last follow-up in liver transplant recipients with HHV-6 reactivation was significantly higher than for those patients without viral reactivation[65].

DIAGNOSIS OF HHV-6 INFECTION

Identification of clinically-relevant HHV-6 infection is hampered by the ubiquitous nature of latent infection[46]. The diagnostic tests that are available for the detection of HHV-6 infection include serology, culture, antigenemia, immunohistochemistry, and nucleic acid amplification assays[76]. In general, serology has inadequate sensitivity and specificity in identifying acute infection in immunocompromised transplant patients, who have impaired ability to mount an effective immune response[46,76]. Moreover, the high HHV-6 seroprevalence rates in adults further limits the potential utility of serology in detecting clinically relevant infections. Culture techniques, on the other hand, are very laborious and are not helpful in real-time management of patients[46,76].

Methods exploiting direct viral detection, such as the detection of nucleic acids by polymerase chain reaction (PCR) or antigenemia, are preferred for the detection of HHV-6 after liver transplantation[46,76]. HHV-6 antigenemia can be detected on mononuclear cells of a whole blood sample by using specific monoclonal antibodies and immunostaining[66]. This technique indicates the presence of an active infection and enables differentiation between HHV-6A and HHV-6B infections. However, it is labor-intensive, semi-quantitative, and it is not widely available for clinical use. This same technique and specific antibodies can be used to demonstrate HHV-6 infection in tissue specimens[47,66].

Molecular assays that detect and amplify HHV-6 nucleic acid are currently the most common laboratory methods for the detection of HHV-6 infection after transplantation. Both quantitative and qualitative methods have been developed to detect HHV-6 DNA in the blood and other clinical samples[77-80]. In addition to blood samples, HHV-6 detection by PCR can also be performed on biopsy and tissue specimens[81]. These assays, depending on the primers used, may differentiate between variants of HHV-6A and HHV-6B as a result of base-differences[77]. PCR testing has some limitations, especially the qualitative assays, which are mainly due to the inability of most assays to distinguish latent from replicating viruses. To address this, the use of serum samples has been suggested, since the virus is cell-associated, and the detection of free viral particles in cell-free serum would be more indicative of active HHV-6 infection[77]. However, this is not the case for whole blood specimens where latent HHV-6 may be present and amplified from leukocytes.

The use of quantitative PCR assays may be helpful in distinguishing replicating from latent HHV-6, with the premise that high HHV-6 levels or increasing viral levels over time would indicate true HHV-6 replication[78]. In the interpretation of HHV-6 viral loads, however, one should take into consideration the rare presence of chromosomally-integrated HHV-6 infections[35,37]. While chromosomally-integrated HHV-6 is rare[31,32], this possibility should be considered when interpreting HHV-6 results in the liver transplant population, in order to avoid unnecessary treatment[82]. Chromosomally-integrated HHV-6 infections are usually detected in high millions of copies of genomic DNA. Detecting HHV-6 by PCR of hair follicles samples also indicates chromosomally-integrated HHV-6[31,32]. The detection of HHV-6 RNA by real-time reverse transcriptase PCR assay, on the other hand, would indicate the presence of an actively replicating virus[83].

Because of the low rate of clinical HHV-6-associated disease and the relatively high rate of subclinical HHV-6 reactivations, it is generally not recommended to perform routine monitoring for HHV-6 after liver transplantation[46]. However, when clinically indicated, such as in certain clinical scenarios including encephalitis, hepatitis or liver allograft dysfunction, these molecular assays may be helpful in confirming a clinical suspicion of HHV-6 infection[11]. In addition, demonstration of HHV-6-specific antigens by immunostaining in the biopsy specimens may be useful in the diagnosis of HHV-6 hepatitis or gastrointestinal infection[47,66,70].

PREVENTION AND TREATMENT OF HHV-6 INFECTIONS

There are no randomized clinical trials that have been conducted of an antiviral drug for the prevention and treatment of HHV 6 disease in humans. As a result, there is currently no antiviral drug that is FDA-approved for clinical use in HHV-6 infection. In the absence of specific antiviral drug therapies, HHV-6-associated diseases have been managed clinically with broad-spectrum anti-herpes drugs such as foscarnet and ganciclovir, and less commonly cidofovir[84]. The clinical use of these drugs has been based mainly on *in vitro* data and several anecdotal case series and reports[84]. *In vitro*, HHV-6 is sensitive to achievable concentrations of ganciclovir, foscarnet, and cidofovir[85,86]. Generally, once the anti-herpetic drugs become activated after a series of phosphorylation, they act by inhibiting viral DNA polymerase. However, *in vitro* data suggests that the ability of HHV-6 pU69 protein kinase to phosphorylate ganciclovir is about 10 fold less when compared to its phosphorylation of CMV[87], suggesting potentially lower efficacy against HHV-6. In addition, the HHV-6A and HHV-6B variants have been demonstrated by different studies to have varying susceptibilities to ganciclovir (although this could potentially be due to the type of cell cultures and viral strains)[85,86]. The HHV-6 variants are resistant to acyclovir and penciclovir[85,86].

The majority of HHV-6 infections are subclinical and transient, and treatment of asymptomatic viral reactivation is not recommended[46]. However, treatment directed against HHV-6 should be initiated in the setting of HHV-6 encephalitis, and should also be considered for other clinical syndromes attributable to HHV-6[46]. The International Herpes Management Forum recommends that HHV-6 infection be considered in the differential diagnosis of encephalitis, particularly in immunocompromised patients[88]. The forum goes on to recommend ganciclovir and foscarnet either alone or in combination as first line therapy for treatment of HHV-6-related central nervous system illness[88]. While studies in order to define precise dosing and duration recommendations have not (yet) been performed, dosing typical for CMV disease is often used. In the setting of HHV-6 encephalitis, some clinicians base duration of therapy on a minimum course of 3-4 wk, and factor in the patient's clinical course and viral levels over time to define the ultimate course[88]. It should be recognized, however, that active viral replication in brain tissue may persist even after levels in blood and cerebrospinal fluid have resolved[89].

As in the treatment of most cases of opportunistic infections, strong consideration should be given to reducing the degree of pharmacologic immunosuppression when treating HHV-6 disease. This will allow the immune system to develop (among HHV-6-seronegative recipients) or recover (among seropositive recipients) sufficient HHV-6 specific immunity needed for adequate control of infection. Although there is no direct evidence to support this strategy, it is assumed that the degree of immunosuppression is a risk factor that could have led to HHV-6 reactivation and clinical disease.

Indirect evidence suggests that anti-CMV prophylaxis with ganciclovir-containing regimens has been associated with a lower rate and degree of HHV-6 detection[45,90,91]. However, these observations were contradicted by other studies that demonstrated that ganciclovir treatment of

CMV disease was not as effective against HHV-6 co-infections[68,92]; these differences in antiviral efficacy may be due to several factors, including viral strains (with HHV-6B considered as the less susceptible variant) and the degree of immunosuppression. Currently, there is insufficient evidence to recommend the routine use of antiviral prophylaxis or preemptive therapy for HHV-6 infection. And since the majority of HHV-6 infections after liver transplantation are subclinical, antiviral prophylaxis or preemptive therapy are currently of questionable benefit.

CONCLUSION

Subclinical HHV-6 infection in liver transplant recipients is common, while clinical HHV-6 disease is rare. Nonetheless, some of the reported HHV-6-associated diseases have led to serious complications and even mortality. The immunomodulatory effect of HHV-6, particularly its interaction with other viruses, and its effect on allograft survival in liver transplant recipients is very intriguing, and needs to be further elucidated. Hence, a better understanding of the impact of HHV-6 in liver transplant recipients is needed. However, this goal is hampered by the challenges in clinical diagnosis, due to the lack of standardized diagnostic methodologies. Although currently available antivirals have been used for treating severe cases of HHV-6 infections, well-controlled clinical studies that support their clinical use are still lacking.

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CASE REPORT

Isolated liver tuberculosis abscess in a patient without immunodeficiency: A case report

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Abstract

Although hepatic tuberculosis is not a rare disease entity, tubercular liver abscess (TLA) is extremely rare. It is usually associated with foci of infection either in the lung and/or gastrointestinal tract or with an immunocompromised state. An isolated or primary TLA with no evidence of tuberculosis elsewhere is even rarer. We report on a 28 year old man who developed an isolated tuberculous liver abscess not associated with lung involvement. Ultrasonography and computed tomography of the abdomen showed the abscess lesions in the liver but the diagnosis of tuberculosis was confirmed by histological examination of the wall of the abscess after surgical drainage. Although tuberculous liver abscess is very rare, it should be included in the differential diagnosis of abscess and unknown hepatic mass lesions.

Key words: Liver; Isolated abscess; Tuberculosis; Surgical drainage; Histological examination

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INTRODUCTION

Isolated tubercular abscess of liver is a very rare form of extrapulmonary tuberculosis, even in countries where tuberculosis is an alarming public health problem. The diagnosis is usually difficult and is frequently confused with pyogenic or amoebic liver abscess.

The histological examination of the liver and the specific treatment with anti-tuberculous agents led to the final diagnosis of tuberculous liver abscess. In this report, we describe a rare case of tuberculous liver abscess with no evidence of infection in the lung or gastrointestinal tract in a patient without immunodeficiency.

CASE REPORT

We report on a case of 28 year old man who presented to the emergency department with non-radiating pain in the right hypochondrium and epigastrium associated with vomiting, intermittent fever with chills and rigors, anorexia and weight loss for 17 d. There was no previous history of tuberculosis (TB) or contact with any patient with TB. At the time of admission, the physical examination revealed a conscious man with a temperature of

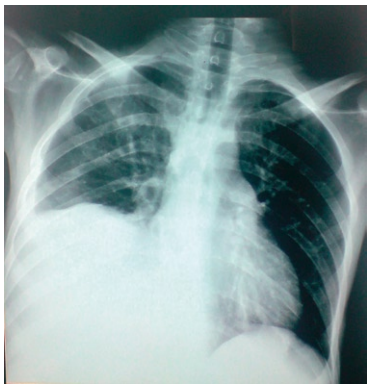

Figure 1 Chest X-ray showing a right-sided sub diaphragmatic pathology as the right hemi-diaphragm is raised and the costophrenic angle is blunted.

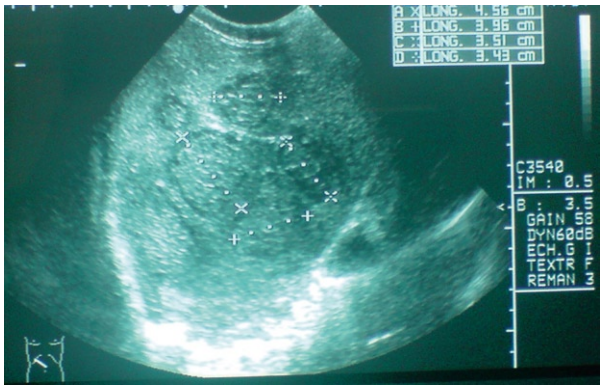

Figure 2 Ultrasonography of the abdomen revealing heterogeneous hypoechoic lesions in the right lobe of the liver suggestive of a multiseptate abscess.

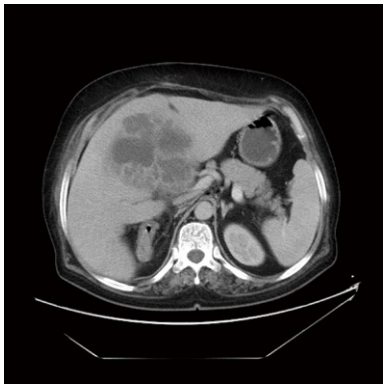

Figure 3 Computerized tomography of abdomen showing the multiseptate abscess in the right lobe of liver.

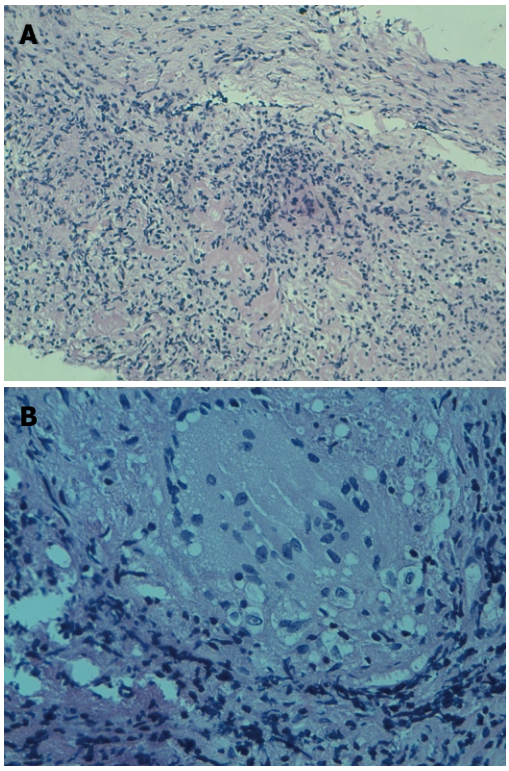

Figure 4 Histological examination of the abscess wall showing a Liver epithelioid granuloma with giant-cell and foci of caseous necrosis. A: HES × 10; B: HES × 30.

39℃, dehydration and who looked very ill with a pulse rate of 98/min, blood pressure 100/75 mmHg and respiratory rate of 19/min. There was no jaundice or lymphadenopathy. Abdominal examination revealed a painful hepatomegaly, the liver span was 16 cm and there was no splenomegaly, ascites or any other palpable mass in his abdomen. Respiratory and cardiovascular system (CVS) examination revealed no abnormality.

Laboratory data revealed hematocrit 26%; hemoglobin 9.6 g/dL; white blood cells 13 300/mm³ (78% neutrophils); blood urea 0.25g/; and creatinine level 10 mg/L. Liver enzymes showed a total bilirubin of 13mg/L with a direct component of 6 mg/L; SGOT and SGPT were 77 U/L and 96 U/L respectively (normal range for SGOT is 17-59 U/L and for SGPT is 21-72 U/L). The patient was non-reactive in HIV serology. Chest X-ray showed no lesion suggestive of TB but revealed a right-sided subdiaphragmatic pathology as the right hemi-diaphragm was raised and the costophrenic angle was blunted (Figure 1). Pleural biopsy was performed and histological examination showed no signs of TB. Ultrasonography (US) of the abdomen revealed ill-defined, heterogeneous hypoechoic lesions reaching up to the liver surface with cystic areas in the right lobe of the liver suggestive of an abscess (Figure 2). Liver was enlarged with a span of 16.6 cm with no other focal lesion. No perihepatic effusion was seen. All other abdominal viscera appeared normal with no free fluid. A computerised tomographic (CT) scan of abdomen revealed a 14 cm/12 cm/7 cm multiseptate abscess in the right lobe of liver (Figure 3) for which a US guided aspiration of 250 cc of yellow colored pus was carried out. The aspiration material showed 10 200 leukocytes/L with 9200 neutrophils/L. Gram staining of the aspiration fluid and Ziehl-Neelsen staining for acid-fast bacteria were negative. Routine bacteriological cultures of the aspiration fluid were negative for bacterial infection and fungus. The patient was started on third generation cephalosporin and aminoglycoside with the provisional diagnosis of pyemic liver abscess. Despite this treatment, the patient's symptoms worsened. A repeat CT scan of the abdomen showed a persistence of the right lobe liver abscess. In view of the patient's condition and the refilling of the multiseptate abscess cavity, a decision to perform a laparotomy was taken. During the surgical exploration, a multiseptate intraparenchymal liver abscess was seen in

the right lobe with no other positive abdominal findings. 750 cc of thick yellow colored pus was drained and after breaking of all of the loculi, a biopsy of the hull of the abscess was performed and the liver cavity was closed over a tube drain, the output of which in the initial period was about 250 cc and decreased after 5 d. The drain was removed one week post operatively. Histological examination of the biopsy showed Tuberculosis follicle with central caseous necrosis surrounded by lymphocytes, multinucleate giant cells and epithelioid macrophages (Figure 4). The polymerase chain reaction (PCR) of the specimen for *Mycobacterium tuberculosis* was not performed because it was not available in the hospital and because the culture in Löwenstein-Jensen medium using the specimen revealed *Mycobacterium tuberculosis* and confirmed the diagnosis of tuberculosis.

Four drugs for antitubercular therapy (Rifampicin, Isoniazid, Ethambutol and Pyrazinamide) were started. The patient had an uneventful postoperative therapy. At 4 wk follow up he was asymptomatic and the hepatomegaly had regressed. The repeat sonography of abdomen at 2 mo showed a complete resolution of the abscess cavity.

DISCUSSION

In extra pulmonary TB, hepatic tuberculosis has been regarded as a rare but not exceptional form of TB[1]. Tuberculous liver abscess (TLA), however, is extremely rare. Most of cases usually occur in association with miliary lung tuberculosis, mainly through hematogenous dissemination[2]. In our patient there was no evidence of any pulmonary or gastrointestinal tuberculosis. The first description of TLA was given by Bestowe in 1858[3]. The prevalence of TLA was just 0.34% in patients with hepatic tuberculosis, as shown in a study where the patient age ranged from 6 mo to 72 years with an average age of 39.2 years[4]. TLA is frequently confused with hepatoma, amoebic liver abscess and pyogenic liver abscess, as in the case of our patient. Because of the non-specific clini cal presentation, the diagnosis of TLA is usually made at autopsy or occasionally after laparotomy has been performed[5]. The symptoms and signs of TLA are non-specific and include fever, vague abdominal pain, an orexia and weight loss[6]. Hepatomegaly is a common physical finding[7]. Jaundice is seldom encountered and may be caused by extra or intrahepatic obstruction[8]. No clear relationship exists between the degree of liver involvement and jaundice[9]. US and CT scan findings have a low specificity in TLA[10]. However, they are very helpful in delineating the site, the size and multiseptate nature of the abscess[11,12]. Their finding usually reflects different stages of disease varying from granulomatous tubercles with or without caseous necrosis to fibrosis and calcification in the healing stage[2]. Confirmation of the diagnosis depends on demonstration of acid-fast bacilli (AFB) in the aspirated pus, pus culture showing Mycobacterium Tuberculosis, positive ELISA and PCR for Mycobacterium Tuberculosis. AFB is most easily found in caseous necrotic material but even the absence of AFB should not detract from diagnosis, especially in a high TB prevalence country such as ours, as is evident from some other studies[1]. Recently, PCR assay was demonstrated to be useful in a diagnosis of hepatic tuberculosis[13,14]. Anti tubercular therapy alone or percutaneous aspiration along with antitubercular therapy are the preferred therapeutic options[5]. Quadruple therapy with antitubercular drugs is recommended for 1 year which was effectively advocated in our patient[7]. Gracey postulates that thick fibrous tissue around the abscesses and their large size may prevent antibiotics from reaching the target[15]. In some cases, TLA have been successfully treated by percutaneous drainage combined with transcatheter infusion of antitubercular drugs[6,16]. Surgery is reserved for cases in which percutaneous aspiration is not successful or not possible because of site and multiseptate nature of the abscess[17]. The diagnosis of LT is established by histological examination of the abscess wall which can show the characteristic aspect of a Tuberculosis follicle with central caseous necrosis surrounded by lymphocytes, multi-nucleate giant cells and epithelioid macrophages, as also seen in our patient. In our patient, the decision to operate was taken after CT scan because the percutaneous aspiration failed to drain the pus and the abscess was multiseptate and not accessible to percutaneous aspiration.

In conclusion, isolated hepatic tubercular liver abscess, although very rare, should always be considered in the differential diagnosis of multiloculated abscess and unknown hepatic mass lesions. Symptoms and radiological findings of the disease are commonly non-specific and the ultimate diagnosis depends upon the demonstration of AFB in pus, aspirate or biopsy specimen or the necrotic tissue. The prognosis with antitubercular treatment is good.

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CASE REPORT

Pericarditis and chronic inflammatory demyelinating polyneuropathy during therapy with pegylated interferon alfa-2a for chronic hepatitis C

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Abstract

We report a case of pericarditis and chronic inflammatory demyelinating polyneuropathy with biological signs of a lupus-like syndrome due to pegylated interferon alfa-2a therapy during treatment for chronic hepatitis C. The patient developed moderate weakness in the lower limbs and dyspnea. He was hospitalized for congestive heart failure. An electrocardiogram showed gradual ST-segment elevation in leads V_1 through V_6 without coronary artery disease. A transthoracic cardiac ultrasonographic study revealed moderate pericardial effusion with normal left ventricular function. Anti-DNA antibody and anti-ds DNA IgM were positive. Neurological examination revealed a symmetrical predominantly sensory polyneuropathy with impairment of light touch and pin prick in globe and stoking-like distribution. Treatment with prednisolone improved the pericarditis and motor nerve disturbance and the treatment with intravenous immunoglobulin improved the sensory nerve disturbance.

Key words: Chronic hepatitis C; Chronic inflammatory demyelinating polyneuropathy; Peginterferon alfa-2a; Pericarditis

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INTRODUCTION

Therapy with interferon alpha is widely accepted for the treatment of chronic hepatitis C. Secondary effects on the cardiovascular and neurological systems are rare. Acute pericarditis complicating interferon therapy has been described previously[1-5]. Some reported interferon-related peripheral neuropathy[6-9].

We report a case of acute pericarditis and chronic inflammatory demyelinating polyneuropathy (CIDP) with biological signs of a lupus-like syndrome during the treatment of hepatitis C infection in which standard dosage levels of pegylated interferon alfa-2a (PEG-IFN-2a) were used. We also review interferon alpha therapy and hepatitis C virus side effects on the cardiovascular and neurological systems.

CASE REPORT

In November 2009, a 67 year old man was referred to our hospital because of hepatitis C virus (HCV) infection.

Chronic active hepatitis C without signs of fibrosis was diagnosed. Chronic HCV serology was positive while HBsAg and human immunodeficiency virus (HIV) serology was negative. The patient received therapy with PEG-IFN-2a, 180 μg a wk from November 5th 2009 until November 19th 2009, because of high HCV infection. The treatment with PEG-IFN-2a was discontinued because of leg edema on December 3rd 2009. Chest radiographs showed mild cardiomegaly (CTR = 0.52). A transthoracic cardiac ultrasonographic (UCG) study showed that the size of the left ventricle and systolic function was normal. The estimated ejection was 56.1%. The early diastolic filling wave/atrial filling wave (E/A) was 1.27. Computed tomographic (CT) scanning of the chest showed no congestion, pleural effusion or pericardial effusion. Treatment with furosemide 10 mg per day and eplerenone 25 mg per day was started.

The patient was admitted to our hospital because of dyspnea, edema and paraesthesia on January 18th 2010. Blood pressure was 123/69 mmHg, pulse 101 beats/min temperature 36.9℃, respiratory rate 22/min and oxygen saturation 97% (room air). A portable chest radiograph revealed pulmonary vascular congestion without pleural effusion. An electrocardiogram (ECG) showed no ST segment elevation or T-wave abnormality. The UCG study revealed moderate pericardial effusion with normal left ventricular function. The CT scan of the chest revealed pulmonary vascular congestion, pleural effusion and pericardial effusion. An injection of furosemide 40 mg per day and carperitide 0.05 γ (μg/kg per min) were given for congestive heart failure. ECG showed gradual ST-segment elevation in leads V_1 through V_6 without elevated myocardial enzyme. After treatment of congestive heart failure, coronary angiography (CAG) was performed. The CAG showed that there was no significant coronary arterial stenosis. The left ventricular ejection fraction was 60.5% and the left ventricular end-diastolic pressure was 10 mm Hg. Right-sided cardiac catheterization revealed a right atrial pressure of 14 mm Hg, right ventricular pressure of systolic 46/diastolic 10/mean 44 mm Hg, pulmonary arterial pressure of systolic 40/diastolic 20/mean 40 mm Hg, pulmonary-capillary wedge pressure of 16 mm Hg, cardiac output of 6.9 liters per minute and cardiac index of 4.1 liters per minute per m^2. These data suggested diastolic heat failure.

The blood sample examination showed no minor inflammatory syndrome (CRP = 0.1 mg/dL, normal value 0-0.3 mg/dL). The hepatic enzymology was normal. The pair viral serologies (influenza, echo, coxsackie, polio and mumps) proved negative. The anti-nuclear antibody, LE test, anti-ds DNA IgG, anti-CCP antibody, anti-RNP antibody, anti-Sm antibody, anti-SS-A antibody, anti-SS-B antibody, anti-Scl-70 antibody, anti-Jo-1 antibody, anti-centromere antibody and lupus anticoagulant proved negative but anti-DNA antibody and anti-ds DNA IgM were positive. Cryoglobulin and M-protein were negative. The polymerase chain reaction (PCR) for Mycobacterium tuberculosis proved negative in the sample from stomach. A diagnosis of autoimmune pericarditis was made.

The patient became unable to walk and stand. Neurological examination revealed moderate weakness in lower limbs and a symmetrical predominantly sensory polyneuropathy with impairment of light touch and pin prick in globe and stoking-like distribution. Tendon reflexes were absent in all extremities. Romberg's test was positive. Electrophysiological studies revealed normal motor conduction velocities in the median nerves but absent in the tibial nerves and normal sensory nerve action potentials in the median nerves but absent in the tibial nerves. There was cytoalbuminologic dissociation in the cerebrospinal fluid (CSF). The protein content was elevated (68 mg/dL) although the cell count was normal (9 lymphocytes per 3 fields) in the CSF. The limbs weakness showed gradual progression after cessation of PEG-IFN-2a.

After discontinuation of interferon and initiation of prednisolone 10 mg per day, the pericardial effusion resolved within 16 d. The patient was treated with a course of intravenous immunoglobulin (IVIG) of at a dose of 0.4 g per kg per day for 5 d. The treatment with prednisolone improved the motor nerve disturbance and the treatment with IVIG improved the sensory nerve disturbance.

DISCUSSION

We report the case of a 67 year old man who developed congestive heart failure, acute pericardial effusion and gait disturbance during treatment with PEG-IFN-2a for chronic active hepatitis C viral infection.

The most common secondary effects with interferon alpha include influenza-like symptoms, headache, fatigue, fever, rigors, myalgia, thrombocytopenia and induction of autoantibodies, reported in over 30% of cases. Reported with rarer frequency are polyneuropathy, paranoia and suicidal thoughts, diabetes mellitus, retinopathy, optical neuritis, diminution of hearing, seizures, loss of libido and cardiotoxicity[10].

The cardiac toxicity of interferon alpha is well known and uncommon. Most frequently, cardiac adverse effects of interferon alpha for HCV hepatitis are arrhythmia (atrial fibrillation, sinus bradycardia, atrioventricular block and ventricular fibrillation), ischemic cardiomyopathy, cardiomyopathy, myocardial infarction and pericarditis[11,12]. Interferon alpha is the most cardiotoxic of the three interferons, followed by interferon beta and interferon gamma. Toxicity does not depend on the daily dose, the total amount or the duration of treatment. There are no established predisposing factors for interferon cardiotoxicity. The mechanism of interferon cardiotoxicity is unclear and probably multifactorial. Interferon evokes the release of several cytokines including tumor necrosis factor alpha and interleukin 1, 2 and 6. There is a high degree of individual variation in toxicity but most adverse events are reversible upon cessation of interferon.

A high prevalence of HCV infection has recently been noted in patients with hypertrophic cardiomyopathy, dilated cardiomyopathy and myocarditis[13]. Chronic infection with HCV is sometimes associated with clinical

and biological manifestation of auto-immune pathologies such as cryoglobulinemia, membranoproliferative glomerulonephritis, Sjögren syndrome, rheumatoid arthritis and systemic lupus erythematosus (SLE)[14]. Okanoue *et al* reported autoimmune phenomena in 987 patients treated with interferon alpha for HCV hepatitis[15]. 12 patients developed hyperthyroidism, 6 hypothyroidism, 3 interstitial pneumonia, 2 rheumatoid arthritis, 2 autoimmune hepatitis, 1 SLE and 1 autoimmune thrombocytopenic purpura. Interferon alpha has been reported to enhance *in vivo* and *in vitro* autoantibody production and may upregulate transcription of genes associated with class I major histocompatibility complex antigens[16]. It is likely that the levels of proinflammatory cytokines may trigger autoimmune phenomena in immunologically predisposed individuals when interferon is administered. Therefore, the immune system mistakenly attacks the host's tissue after recognizing a molecular epitope similar to a foreign antigen and may result in acute inflammation.

Peripheral neuropathy is a rare and uncommon side effect in patients treated with interferon alpha. A variety of peripheral neuropathies have been reported in patients treated with interferon including sensory neuropathy, autonomic neuropathy, Bell's palsy and chronic inflammatory demyelinating neuropathy (CIDP)[17-22]. PEG-IFN-2a, including interferon alpha, has been implicated in causing immune mediated CIDP during chronic hepatitis C treatment[6,23] due to cytokine-induced apoptosis in the myelin-producing oligodendrocyte, resulting in inhibition of central nervous system remyelination thus causing demyelinating neuropathy[23-25]. Interferon may evoke potential immune disease.

To the contrary, interferon alpha has also been shown to be a successful treatment in patients with CIDP[6]. However, if a patient develops demyelinating neuropathy secondary to IFN use, it should be discontinued immediately since it may cause irreversible nerve damage due to inhibition of remyelination process. It has been shown that patients with CIDP respond to prednisone, plasma exchange and IVIG[23,26,27]. The criteria for CIDP usually include the clinical deterioration of neurological symptoms for a period of greater than 8 wk as opposed to AIDP and/or Guillain-Barre syndrome (GBS) which usually has deterioration over a period of approximately 4 wk or less[27]. The pathogenesis of CIDP related to PEG-IFN-2a use is thought to be an immune-mediated progress similar to GBS[23-25].

Gressens *et al*[3] also reported a case of pericarditis during the treatment of chronic infection with HCV complicated polyneuropathy. The case of pericarditis without tamponade outside the context of a lupus-like syndrome emerged in the course of the fourth month of remission during treatment with interferon alpha in classical dosage levels. Acetylsalicylic acid had been initiated for acute pericarditis and neurological improvement was achieved through the administration of vitamin B in that case. Boonen *et al* reported a case of pericarditis during the treatment of chronic infection with HCV. This pericarditis emerged as part of a lupus-like syndrome with clinical and biological manifestations of autoimmune pathologies[1]. The patient was treated with chloroquine 250 mg per day and prednisone 40 mg per day in that case.

This case of pericarditis which we are reporting on emerged with resultant lupus-like syndrome caused by PEG-IFN-2a. The biological markers of autoimmune pathologies (anti-DNA antibody and anti-ds DNA IgM) were positive. Any effect from the HCV itself is quite unlikely; virus activity must have been low because the transaminases had normalized. We excluded other viral causes by the fact that pair serological tests were negative. We may reasonably attribute the diastolic heart failure to PEG-IFN-2a and the pericarditis to lupus-like syndrome. This case was simultaneously complicated by CIDP of the lower limbs.

In conclusion, pericarditis and CIDP developed during the interferon therapy with an increased protein concentration in the CSF and steroid and IVIG seemed to result in improvement. We suggest that interferon may induce an immune mediated pericarditis and peripheral neuropathy. Cardiac monitoring may be well advised including demyelinating neuropathy when using treatments based on interferon for chronic HCV infections.

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CASE REPORT

Tertiary syphilis mimicking hepatic metastases of underlying primary peritoneal serous carcinoma

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Abstract

Tertiary syphilis, especially in cases involving visceral gummatous disease, can be confused with cancer of the solid organs. We report a case of tertiary hepatic syphilis that manifested with intrahepatic masses in a patient who had an underlying primary peritoneal serous carcinoma (PPSC). The patient was diagnosed with PPSC and achieved a complete remission of PPSC following six cycles of platinum-based chemotherapy. Two hepatic nodules developed during the follow-up period and were initially labeled as hepatic metastases from the underlying PPSC, based on radiological findings. A resection of hepatic nodules was performed for therapeutic and diagnostic purposes, because there were no other metastatic foci except in the liver. Unexpectedly, serology and histology confirmed tertiary syphilis. This rare case emphasizes the importance of including tertiary syphilis in the differential diagnosis of a space-occupying lesion, even with an existing diagnosis of underlying cancer.

Key words: Syphilis; Neoplasm; Peritoneal carcinoma; Liver

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INTRODUCTION

Syphilis is a unique infectious disease that presents with variable clinical manifestations[1]. Although the incidence of the disease has decreased, syphilis often presents a diagnostic dilemma, because of its variable clinical features[2]. It progresses, if untreated, through primary, secondary, and tertiary stages. In particular, tertiary syphilis often mimics cancer, because it frequently presents as a space-occupying lesion in visceral organs[3-7].

I report a rare case of hepatic nodules in a patient who had a clinical history of primary peritoneal serous carcinoma (PPSC), which was initially diagnosed as hepatic metastases of PPSC by radiological findings, but was confirmed to be tertiary hepatic syphilis by serological tests and histological findings.

CASE REPORT

A 65-year-old woman presented at our hospital with a 2-mo history of intermittent abdominal pain and distension. Past medical and familial histories were unremarkable. Physical examination showed abdominal distension and diffuse abdominal tenderness. Her Eastern Cooperative Oncology Group (ECOG) performance status score was 1. Abdominopelvic computed tomography (CT) showed a small volume of ascites and several peritoneal nodules without mass-like lesions in the visceral organs (Figure 1). Cytological examination of the ascites

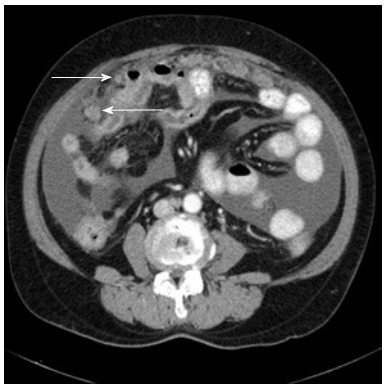

Figure 1 Abdominopelvic computed tomography scan showing ascites and contrast-enhanced peritoneal nodules.

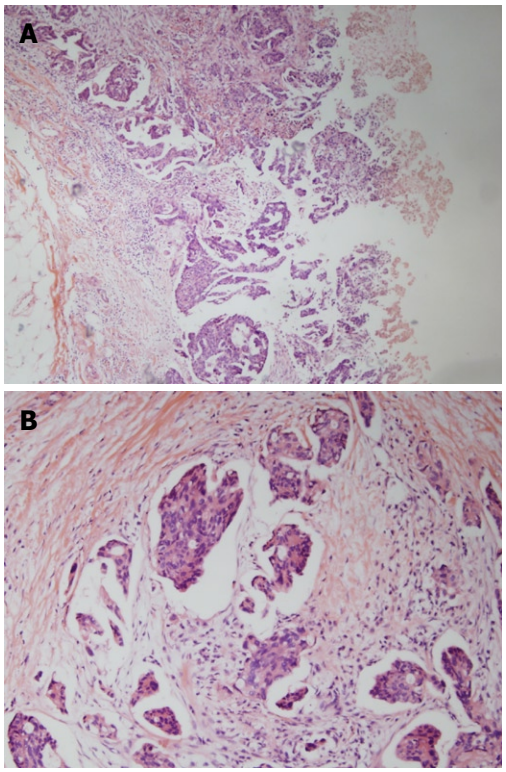

Figure 2 Microscopic findings of the peritoneal nodules revealed papillary serous adenocarcinoma with infiltrative papillary clusters (A, H&E stain × 40) and tumor nests with characteristic retraction artifacts in the stroma (B, H&E stain × 100).

suggested metastatic adenocarcinoma. We evaluated possible primary cancer origins; however, no primary site of malignancy was identified. The patient's CA-125 level was elevated, to 485 U/mL (normal range up to 35 U/mL), but all other evaluated tumor markers were negative. A laparoscopic biopsy of the peritoneum showed a papillary type of adenocarcinoma (Figure 2). Thus, the patient was finally diagnosed with PPSC and started on platinum-based combination chemotherapy. The patient achieved a complete response after six cycles of chemotherapy. Additionally, her CA-125 level returned to normal, at less than 5U/mL.

Nine mo after the last round of chemotherapy, a follow-up abdominal CT with peripheral contrast enhancement showed two low-attenuation nodules in the liver. The subsequent liver magnetic resonance imaging (MRI) findings also suggested liver metastasis (Figure 3). Laboratory tests showed elevated serum aspartate aminotransferase (AST, 260 IU/L) and alanine aminotransferase (ALT, 191 IU/L), but her CA-125 level remained within normal limits. Although PPSC recurrence was suspected, based on the clinical and radiological findings, there were no other suspicious lesions except for those found in the liver. For therapeutic and diagnostic purposes, a wedge resection was performed on the two 1-cm diameter masses in the liver.

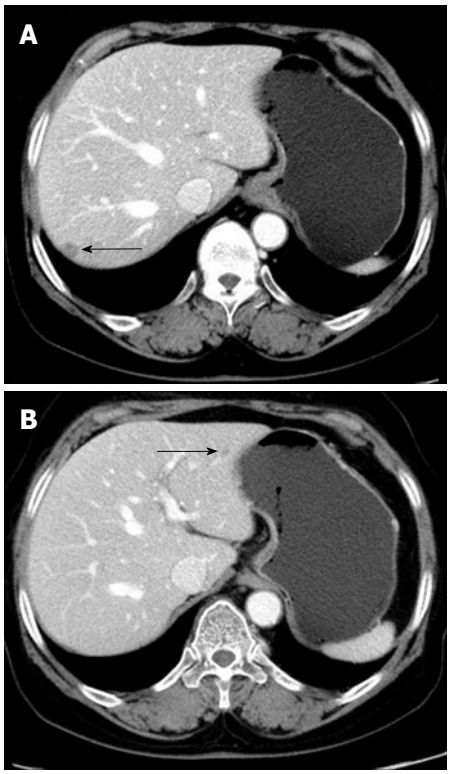

Figure 3 Computed tomography scan showing two hepatic nodules with peripheral enhancement (arrows).

Histologically, the specimens showed central necrosis, granulation tissue, and peripheral fibrosis with eosinophilic infiltration, but no evidence of malignancy (Figure 4). Periodic acid-Schiff (PAS), methenamine silver, and Warthin-Starry stains were all negative. The patient had no history of a chronic inflammatory disease, including syphilis. Evaluation for the presence of other indolent diseases which might cause granulation with necrosis in the liver, such as tuberculosis, was performed. The results of these tests, including a chest X-ray, sputum examinations for tuberculosis (staining for acid-fast bacilli and culture for *Mycobacterium tuberculosis*), and the Mantoux test were all negative. The patient was unavailable for further clinical follow-up and additional evaluations following surgery.

Five years after surgery, the patient returned to the hospital for follow-up without symptoms. Similar to her prior presentation, liver enzymes were elevated (AST 640 IU/L, ALT 380 IU/L), but other laboratory findings, including CA-125, were within normal limits. The patient had no hepatomegaly, splenomegaly, or abdominal distension. However, the follow-up abdominal CT and liver MRI showed recurrent lesions around the previous opera-

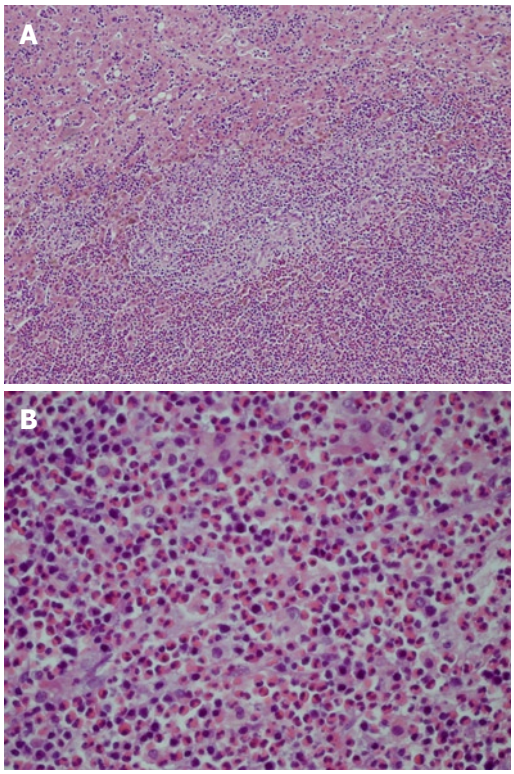

Figure 4 Liver biopsy specimens revealed an inflammatory cell infiltration among the hepatocytes and sinusoids, and partial abscess formation (A, H&E stain × 100). The inflammatory infiltrate was composed primarily of eosinophils (B, H&E stain × 400).

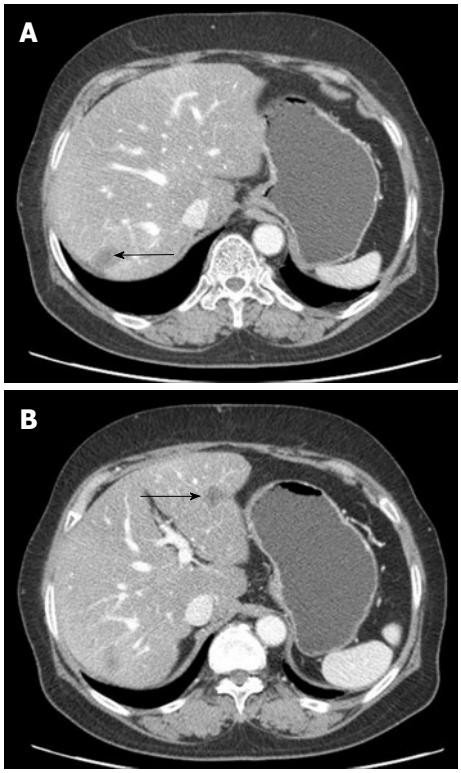

Figure 5 Computed tomography scan showing two recurrent lesions around the previous operative sites (arrows).

tive sites (Figure 5). When another hepatic resection was

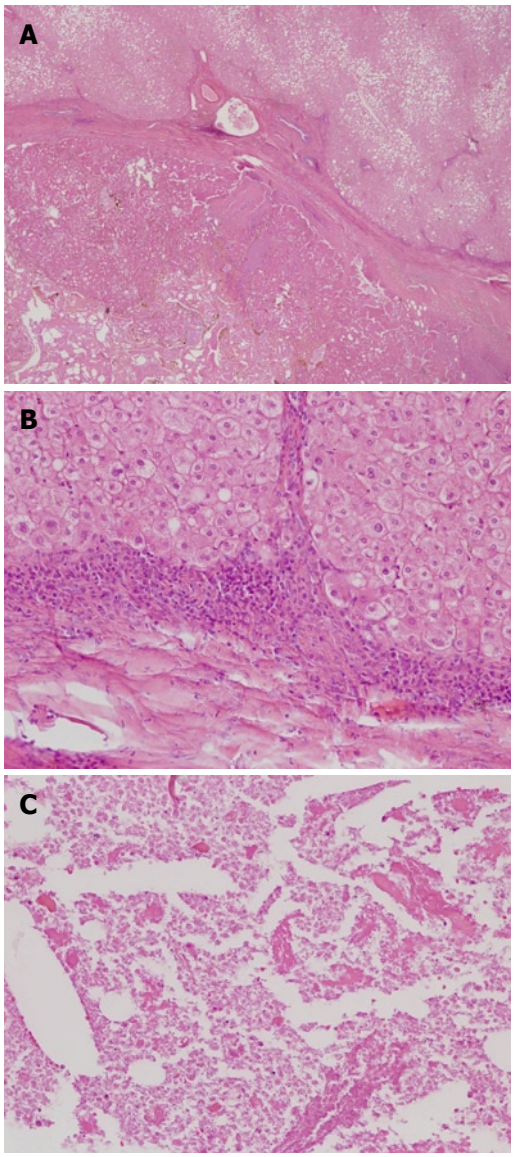

Figure 6 Pathological findings of the specimens from the secondary operation showed chronic inflammation with necrosis. A: A well-demarcated inflammatory nodule was identified within the liver parenchyma (H&E stain × 40); B: Lymphoplasma cell infiltration was also noted in the peripheral portion of the nodule (H&E stain × 100); C: The nodule was filled with inflammatory necrotic debris (H&E stain × 200).

performed to confirm the diagnosis, the histological findings were similar to the previous hepatic biopsy findings: the presence of a necrotic inflammatory mass with granulation tissue components, but without malignant cells (Figure 6). Microbiological assessments, including Gram stain, tissue culture, and fungal and mycobacterial studies, were all negative. PAS, methenamine silver, and Warthin-Starry stains were also negative. Serological testing for syphilis was also performed, which yielded the following positive results: VDRL, 1:16; *Treponema pallidum* hemagglutination assay (TPHA), 1:80; and positive fluorescent treponemal antibody absorption (FTA-ABS). Assessment of human immunodeficiency virus (HIV) serology yielded negative results. Based on these serologic and histological findings, the patient was diagnosed as having tertiary syphilis with hepatic gumma. Liver enzyme test results returned to nor-

mal following treatment with penicillin. The patient currently has no evidence of tumor or syphilis recurrence.

DISCUSSION

Syphilis, a chronic infectious disease caused by the bacterium *Treponema pallidum*, is usually acquired by sexual contact with another infected individual. The tertiary disease stage appears in approximately one-third of untreated cases. Any organ of the body may be involved, but the most common types of tertiary disease are gumma, cardiovascular syphilis, and neurosyphilis[1].

Liver involvement caused by syphilis may occur in the secondary and tertiary disease stages. Secondary hepatic syphilis usually begins within a few weeks to a few months after the initial chancre in about 0.2% of syphilis cases, typically manifesting as mild clinical hepatitis[8]. During the tertiary period, localized gumma may develop in the liver and usually develop 1-10 years after the initial infection[1].

Based on autopsy reports, the incidence of tertiary hepatic syphilis (hepatic gummas) ranges from 0.3% to 16%[3,9]. Hepatic gummas were once the most common form of visceral syphilis and often manifested as hepatosplenomegaly, anemia, and occasionally as fever and jaundice. There are usually multiple gumma lesions of variable size distributed throughout both lobes of the liver[3].

Histologically, the gumma is a granuloma[1]. Syphilitic gummas have central necrosis, similar to caseous necrosis. Other histological features of these gummas include a dense fibrous wall with adjacent chronic lymphocytic, plasmacytic and histiocytic inflammation or some epithelioid granulomas with giant cells. It is difficult to identify a causative organism in the tissue of these gummatous lesions. Spirochete identification has been reported in only 0%-5% of cases with tertiary hepatic syphilis[3].

Radiologically, a CT scan typically shows low-attenuated lesions with slight peripheral enhancement and rare calcifications[10-11]. With this radiological finding, the differential diagnosis of tertiary hepatic syphilis should also include abscess, as well as primary and metastatic tumors.

Tertiary syphilis is treated with benzathine-penicillin G. Symptoms disappear and serologic tests normalize within a few weeks or months after treatment. In addition to histological findings and positive blood syphilis reactions (VDRL and FTA), therapeutic response has been used as confirmation of tertiary syphilis[3].

PPSC has been used to describe serous carcinoma of the peritoneum. PPSC is characterized by peritoneal carcinomatosis with pathological and clinical features that are similar to ovarian cancer, although there is no ovarian primary tumor[12-13]. The optimal treatment for PPSC is initial cytoreductive surgery, if indicated, followed by platinum/paclitaxel combination chemotherapy, similar to patients with ovarian cancer[13].

CA-125 is elevated in 80%-85% of women with advanced epithelial ovarian cancer[14]. Follow-up measurement of the patient's CA-125 level is recommended if the level was initially elevated. Hepatic metastasis of primary ovarian cancer eventually occurs in nearly one-half of patients[15]. With the current patient, the results of imaging studies were highly suggestive of metastatic hepatic carcinoma. However, her CA-125 levels were within normal limits. This clinical discordance supported the need to resect the hepatic nodules for both diagnostic purposes and treatment. The pathological findings were not compatible with a hepatic metastasis from PPSC. Instead, tertiary hepatic syphilis was diagnosed, which is a readily treatable disease.

Cases involving gummas are increasingly infrequent, due to the availability of penicillin treatment. However, gummas most often elicit medical attention as space-occupying lesions in the liver, stomach, brain and oropharynx, mimicking neoplastic conditions[3-7]. Differentiation of a cancer from a gumma is essential to facilitate appropriate patient management.

In conclusion, this case illustrates several important points. Firstly, this is the first report on tertiary hepatic syphilis mimicking hepatic metastases in a patient who showed a complete response after treatment for an underlying PPSC. Although syphilis has decreased in incidence, tertiary hepatic syphilis should still be considered in the differential diagnosis of space-occupying lesions of the liver. Additionally, histological diagnosis is critical, especially in patients treated with a curative aim and with any discordance between the clinical and imaging diagnoses.

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International Conference on Medical Negligence and Litigation in Medical Practice

January 25-29
Waikoloa, HI, United States
Selected Topics in Internal Medicine

January 26-27
Dubai, United Arab Emirates
2nd Middle East Gastroenterology Conference

March 04-06
Bethesda, MD, United States
8th International Symposium on Targeted Anticancer Therapies

March 05-07
Peshawar, Pakistan
26th Pakistan Society of Gastroenterology & Endoscopy Meeting

March 12-14
Bhubaneswar, India
18th Annual Meeting of Indian National Association for Study of the Liver

March 25-28
Beijing, China
The 20th Conference of the Asian Pacific Association for the Study of the Liver

March 27-28
San Diego, California, United States
25th Annual New Treatments in Chronic Liver Disease

April 07-09
Dubai, United Arab Emirates
The 6th Emirates Gastroenterology and Hepatology Conference, EGHC 2010

April 14-18
Vienna, Austria
The International Liver Congress™ 2010

May 01-05
New Orleans, LA, United States
Digestive Disease Week Annual Meeting

May 06-08
Munich, Germany
The Power of Programming: International Conference on Developmental Origins of Health and Disease

June 04-06
Chicago, IL, United States
American Society of Clinical Oncologists Annual Meeting

June 16-19
Hong Kong, China
ILTS: International Liver Transplantation Society ILTS Annual International Congress

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Montreal, Canada
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ICAAC: Interscience Conference on Antimicrobial Agents and Chemotherapy Annual Meeting

September 16-18
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Prague Hepatology Meeting 2010

September 23-26
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October 15-20
San Antonio, TX, United States
ACG 2010: American College of Gastroenterology Annual Scienitfic Meeting

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Organization as author

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Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1948-5182/g_info_20100107115140.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and

on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

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