TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

HHV-6B is frequently found in the gastrointestinal tract in kidney transplantation patients

Marko Lempinen,[1] Leena Halme,[2] Johanna Arola,[3] Eero Honkanen,[4] Kaija Salmela[1] and Irmeli Lautenschlager[1,5]

1 Department of Transplantation and Liver Surgery, and Transplant Unit Research Laboratory, Helsinki University Hospital, Helsinki, Finland
2 Department of Gastrointestinal Surgery and Transplant Unit Research Laboratory, Helsinki University Hospital, Helsinki, Finland
3 Department of Pathology, Helsinki University Hospital (HUSLAB) and University of Helsinki, Helsinki, Finland
4 Department of Medicine, University Hospital, Helsinki, Finland
5 Department of Virology, Helsinki University Hospital (HUSLAB) and University of Helsinki, Helsinki, Finland

Keywords
gastrointestinal tract, HHV-6B, kidney transplantation.

Correspondence
Marko Lempinen MD, Clinic of Surgery, Department of Transplantation and Liver Surgery, Helsinki University Hospital, PO Box 340, FIN 00029, Helsinki, Finland.
Tel.: +358 50 4270437;
fax: +358 9 174975;
e-mail: marko.lempinen@hus.fi

Conflicts of Interest
The authors have declared no conflicts of interest.

Received: 15 November 2011
Revision requested: 15 January 2012
Accepted: 21 April 2012
Published online: 22 May 2012

doi:10.1111/j.1432-2277.2012.01502.x

Summary

In immunosuppressed patients human herpesvirus 6 (HHV-6) reactivations are common. The aim of the study was to determine to which extent HHV-6 can be found in the gastrointestinal tract in kidney transplant recipients and in patients on chronic dialysis. The HHV-6 and cytomegalovirus (CMV) examinations were performed on gastro duodenal and colon biopsy specimens obtained from 81 kidney transplant recipients and on 46 chronic dialysis patients. The HHV-6 and CMV were demonstrated by immunohistochemistry detecting both HHV-6A and HHV-6B, and CMV-specific antigens. The HHV-6B-positive cells, were found in gastroduodenal biopsy specimens from 34% of the transplant recipients and 28% of the patients on chronic dialysis, CMV-positive cells were found in specimens from 53% of the transplant recipients and 28% of the patients on chronic dialysis. The HHV-6B positive cells were found in the colonic mucosa specimens from 36% of the transplant recipients and 22% of the patients on chronic dialysis, CMV-positive cells were found in specimens from 36% of the transplant recipients and 17% of the patients on chronic dialysis. The HHV-6B positive cells were found equally often in the gastroduodenal as in the colorectal mucosa. The HHV-6B positive cells as well as CMV positive cells were simultaneously found in every fifth of transplant recipients.

Introduction

Human herpesvirus 6 (HHV-6) is a lymphotropic virus that belongs to the β-herpesviruses, together with its close relative cytomegalovirus (CMV). Although HHV-6 is lymphothropic, it can infect other cells such as monocytes, macrophages, endothelial cells and epithelial cells [1]. The HHV-6 is a highly prevalent virus in the Human population. Seroconversion mostly occurs before the age of 2 years, and by adulthood, more than 95% of people are seropositive [1]. The primary infection in childhood may present either as exanthemasubitum or as nonspecific infection, gastroenteritis or as diarrhoea and nausea [2]. As with the other herpesviruses, HHV-6 establishes latency following primary infection and reactivates in immunocompromised states. Two distinct types of HHV-6 have been identified, A and B, with B being the cause of exanthema subitum and the most common in transplant recipients [3]. Immunosuppressive therapy after transplantation makes the transplant recipient prone to a broad array of viral pathogens. Cytomegalovirus remains the single most important and most common viral gastrointestinal pathogen affecting organ transplant recipients. However, HHV-6 infection in immunosuppressed patients may as well be life threatening as a result of a severe end-organ disease such as pneumonitis, hepatitis, encephalitis and severe aplasia [4–7]. In stem cell transplant patients with gastrointestinal symptoms, HHV-6

DNA has been detected by PCR in gastroduodenal and colorectal mucosa [8] and by *in situ* hybridization in large bowel mucosa [9]. Occasional case reports have described HHV-6 associated colitis in lung and renal transplant recipients [10–12].

Our group has previously shown that HHV-6 positive cells were frequently found in the gastroduodenal mucosa of liver transplanted patients with dyspeptic symptoms [13].

The aim of the present study was to determine in which extent HHV-6 can be found in the gastrointestinal tract (gastroduodenal and colorectal mucosa) in kidney transplant recipients and patients on chronic dialysis without immunosuppressive medication who underwent endoscopy because of gastrointestinal symptoms.

Patients and methods

Study design

The HHV-6 examinations were performed on gastrointestinal biopsies taken earlier prospectively for a previous CMV study [14].

Patient population

Biopsies for HHV-6 examinations were available from a total of 127 gastrointestinal endoscopies performed between the years 1996 and 2007. Gastroduodenal biopsies during oesophagogastroduodenoscopy (OEGD, $n = 95$) were taken from 67 kidney transplant recipients and from 28 patients on chronic dialysis. Colorectal biopsies ($n = 32$) were taken from 14 kidney transplant recipients and from 18 dialysis patients. In transplant recipients, the endoscopy was performed at a median 2 years (range; 7 days to 21 years) after transplantation and in patients on chronic dialysis in median 2 years (range 1 month to 10 years) after the beginning of dialysis.

Histopathological and laboratory analysis

In all patients, biopsies for histopathological analysis were taken during OEGD and colonoscopy, as previously described [14]. Histopathological stainings included haematoxylin-eosin, Alcian blue, PAS and Giemsa (for *Helicobacter pylori*).

The presence of HHV-6 viral antigens was demonstrated by an indirect three-layer immunoperoxidase staining and monoclonal antibodies against HHV-6-specific antigens, detecting both HHV-6A and HHV-6B, as described elsewhere for tissue sections [15,16]. To demonstrate an active CMV infection in the specimens a monoclonal antibody against CMV specific antigen (pp65 matrix protein, Biotest AG, Dreieich, Germany) was used as described previously [17]. The intensity of HHV-6 and CMV infection was graded as negative, mild (1–3 positive cells/visual field), moderate (4–6 positive cells/visual field) or intense (>7 positive cells per high-power visual field).

The CMV serostatus of the kidney recipients and donors was determined prior to transplantation and in most of the patients on chronic dialysis (Table 1). In transplant recipients CMV was determined from blood by means of the CMV pp65 antigenaemia test [18] using a monoclonal antibody against the CMV pp65 antigens (BiotestPharma, Frankfurt, Germany) in response to suspected CMV infection, i.e. when patients had fever and/or their serum creatinine value was elevated without rejection.

Antiviral and immunosuppressive treatment

Transplant recipients with symptomatic CMV antigenaemia were treated with intravenous ganciclovir (5 mg/kg twice daily) for at least 2 weeks. The main immunosuppressive medication was cyclosporine for 80% of the patients and tacrolimus for 20%. These agents were used in combination with azathioprin, mycophenolatemofetil or rapamycin and methylprednisolone. Rejections were

Table 1. Characteristics of the patients.

Patient groups	Kidney transplant recipients $n = 81$	Patients on chronic dialysis $n = 46$	*P*-value
Age (median, range, years)	54 (18–73)	56 (33–81)	0.008
Gender (F/M)	29/52	15/35	0.495
Mode of dialysis			
Haemodialysis	56 (69%)*	36 (78%)	0.269
CAPD	25 (31%)*	10 (22%)	
Diagnosis of the kidney disease			
Polycystic kidney disease	13 (16%)	9 (20%)	0.615
Other kidney disease	45 (56%)	21 (45%)	0.283
Systemic disease	23 (28%)	16 (35%)	0.453
CMV serostatus		–	
R+/D+	45 (56%)	–	
R+/D–	11 (14%)	–	
R–/D+	16 (20%)	–	
R–/D–	6 (7%)		
Positive	–	30 (65%)	
Negative	–	3 (7%)	
Not done	–	13 (28%)	

CMV, cytomegalovirus; OEGD, oesophagogastroduodenoscopy; CAPD, continuous ambulatory peritoneal dialysis.

*Before transplantation. The CMV serostatus was determined before kidney transplantation in all but three patients.

Five patients each in the kidney transplant group and chronic dialysis patient group had both OEGD and colonoscopy performed.

treated with high-dose methylprednisolone for 5 days and, if this treatment failed, with plasmapheresis or the monoclonal antibody OKT 3.

Ethics

The Ethics Committee of Helsinki University Hospital approved the study and all patients gave their informed consent.

Analysis of data

The variables were compared using the Mann–Whitney *U*-test, the chi-squared test and Fisher's exact test. A *P*-value of <0.05 was considered significant.

Results

Characteristics of the patients are presented in Table 1. Indications for the OEGD and colonoscopy examination in kidney transplant recipients and in patients on chronic dialysis are presented in Table 2.

Gastroduodenal mucosa

Human herpesvirus-6 positive cells were found in gastroduodenal mucosa biopsy specimens from 23 (34%) of the transplant recipients and in eight (28%) of the patients on chronic dialysis (Table 3; P = NS). All HHV-6 findings were of HHV-6B. The HHV-6B antigens were located in the mononuclear cells infiltrating the mucosa. The intensity of HHV-6B infection in the gastroduodenal mucosa was graded, based on the number of cells positive for viral antigens. In transplant recipients the intensity of HHV-6B infection in the gastroduodenal mucosa was mild in 21 patients (91%), and moderate in two patients (9%). In patients on chronic dialysis the intensity was mild in six (75%) patients and moderate in two (25%) patients. There was no statistically significant difference in the intensity between transplant recipients and dialysis patients. In neither kidney transplanted patients nor patients on chronic dialysis intense infection was observed. Histopathological findings in the HHV-6B positive mucosa were nonspecific with mild inflammation (Fig. 1).

The CMV-positive cells were found in specimens from 36 (53%) of the transplant recipients and eight (28%) of the patients on chronic dialysis (Table 3; $P < 0.05$). In transplant recipients the intensity was mild in 69% of the patients, moderate in 22% and intense in 9% respectively. All CMV infections were mild in patients on chronic dialysis. Histopathological findings in the CMV positive mucosa also were nonspecific and mild.

Of the 67 transplant recipients 14 (21%) were found to have simultaneous HHV-6B and CMV findings in the gastroduodenal mucosa (Table 3). In the gastroduodenal tract HHV-6B was found equally in the gastric mucosa as well as in the duodenal mucosa, whereas CMV positive cells were more often found in the duodenal mucosa (Table 3).

Nine (13%) of 67 transplant patients and two (6%) of 28 patients on chronic dialysis had *H. pylori* positive find-

Table 2. Indications for OEGD and colonoscopy.

Patient groups	Kidney transplant recipients	Patients on chronic dialysis	*P*-value
OEGD	*n* = 67	*n* = 28	
Pain or dyspeptic symptoms	35 (52)	7 (25)	0.015
Nausea or vomiting	4 (6)	4 (14)	0.183
Suspicion of bleeding	16 (25)	4 (14)	0.296
Gastro-oesophageal reflux	9 (13)	8 (29)	0.079
Other indications	3 (4)	5 (18)	0.032
Colonoscopy	*n* = 14	*n* = 18	
Post excision surveillance of polyps or CRC	1 (7)	3 (17)	0.402
Haematochezia	1 (7)	5 (28)	0.352
Abdominal pain	2 (14)	2 (10)	0.789
Diarrhoea	4 (29)	3 (17)	0.350
Iron-deficiency anaemia	3 (21)	1 (5)	0.210
Other indications*	3 (21)	4 (23)	0.649

CRC, colorectal cancer; OEGD, oesophagogastroduodenoscopy.
Values within the parenthesis are expressed in percentage.
*Other indications include, control endoscopy, unexplained weight loss, change in bowel habits, suspicion of diverticulosis and miscellaneous indications.

Table 3. Positive HHV-6 and CMVpp65 findings in the gastroduodenal in kidney transplant recipients and patients on chronic dialysis.

	HHV-6	CMV	HHV-6 and CMV
Transplant patients (*n* = 67)			
Gastric mucosa	14 (21)	11 (16)	1 (1)
Duodenal mucosa	17 (25)	36 (53)*	9 (13)
Gastric or duodenal mucosa	23 (34)	36 (53)*	14 (21)
Patients with on chronic dialysis (*n* = 28)			
Gastric mucosa	6 (21)	2 (7)	0
Duodenal mucosa	5 (18)	8 (28)*	4 (14)
Gastric or duodenal mucosa	8 (28)	8 (28)*	4 (14)

CMV, cytomegalovirus; HHV-6, human herpesvirus 6.
Values within the parenthesis are expressed in percentage.
*Kidney transplant recipients versus patients on chronic dialysis ($P < 0.05$).

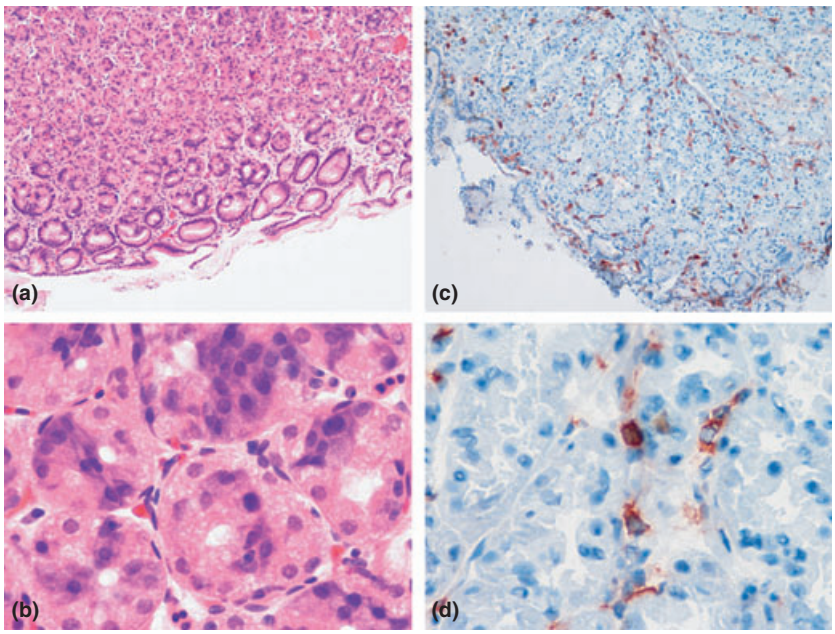

Figure 1 The gastric mucosa with intense HHV-6B infection in the gastroduodenal mucosa (a and b) Histology demonstrated in haematoxylin-eosin staining (magnification 10× and 60×). (c and d) HHV-6B positive cells in the gastric mucosa demonstrated by immunohistochemistry (magnification 20× and 100×).

ings in simultaneously obtained histopathological samples of gastric mucosa. Six (9%) of the transplant patients had simultaneous CMV and *H. pylori* infection and two (3%) patients had simultaneous HHV-6B and *H. pylori* infection. One (3%) of the patients on chronic dialysis had a simultaneous CMV and *H. pylori* infection and one patient a simultaneous HHV-6B and *H. pylori* infection.

Colorectal mucosa

All HHV-6 findings were of HHV-6B. In the colonic mucosa HHV-6B positive cells were found in five (36%) of the transplant recipients and in four (22%) of the patients on chronic dialysis (Table 4; NS). The intensity of the HHV-6B infection in the colonic mucosa was mild in all transplant recipients. Also in the patients on dialysis the intensity of the HHV-6B infection in the colonic mucosa was mild except from one patient with intense staining.

The CMV-positive cells were seen in specimens from five (36%) of the transplant recipients and in three (17%) of patients on chronic dialysis (Table 4; NS). The intensity of the CMV infection was mild in the colonic mucosa in all transplant recipients as well as in all patients on chronic dialysis. None of the kidney transplant patients was diagnosed to suffer from CMV-induced colitis.

Two (14%) of the transplant recipients had both HHV-6B and CMV positive cells in the colonic mucosa, whereas none of the patients on chronic dialysis had both HHV-6B and CMV positive cells in the colonic mucosa (Table 4; NS).

Table 4. Positive HHV-6 and CMVpp65 findings in the colorectal mucosa in kidney transplant recipients and patients on chronic dialysis.

	HHV-6	CMV	HHV-6 and CMV
Transplant patients (n = 14)			
Rectum	3 (21)	2 (14)	0
Caecum	4 (28)	4 (29)	1 (7)
Rectum or caecum	5 (36)	5 (36)	2 (14)
Patients on chronic dialysis (n = 18)			
Rectum	3 (17)	1 (5)	0
Caecum	3 (17)	2 (11)	0
Rectum or caecum	4 (22)	3 (17)	0

CMV, cytomegalovirus; HHV-6, human herpesvirus 6.
Values within the parenthesis are expressed in percentage.

Of the 81 transplant recipients 34 had received antiviral treatment with ganciclovir and 25 (31%) had received rejection treatment during the 3 months before the endoscopy. No correlation was observed between the time of the appearance of HHV-6B infection or the intensity of the HHV-6B infection and the use of antiviral medication or rejection treatment.

Discussion

For the first time the occurrence of HHV-6B is studied in the upper as well as in the lower gastrointestinal tract in kidney transplant recipients. Immunosuppressive therapy predisposes kidney transplant recipients to various gastrointestinal problems [19–21]. In contrast to the described effects of CMV infection on the gastrointestinal tract [22], the role and impact of HHV-6 infection is, however, much less clear. In the present series, HHV-6B positive cells were equally often found in the gastroduodenal mucosa as in the colorectal mucosa of the kidney transplant recipients. On the other hand, CMV was more often found in the upper than in the lower gastrointestinal tract. In patients on chronic dialysis without immunosuppressive medication, HHV-6B and CMV were, however, observed almost as often in the gastroduodenal mucosa as in the colorectal mucosa. In respect of the intensity of the HHV-6B findings in transplant recipients all intense findings were found in the upper gastrointestinal tract.

In the present and in our previous studies, CMV was found more commonly in the upper than in the lower GI tract and more often in the duodenum than in the stomach [14,23]. These findings are in accordance with other series in which CMV has been examined from gastroduodenal biopsies from bone marrow or solid organ transplanted patients [24–26]. On the other hand, epidemiological studies on the occurrence of HHV-6 in the gastrointestinal tract are scarce. In liver transplant recipients HHV-6B positive cells were found in the gastroduodenal mucosa in 23% of the patients [13]. In patients with inflammatory bowel disease (IBD) up to 76% of colonic mucosa specimens have shown HHV-6 positivity, and also in non-IBD patients 40–86% of mucosa specimens have shown HHV-6 positivity [27,28].

The HHV-6 is considered to be a relatively harmless β-herpes virus that may, however, cause life threatening severe end-organ diseases in immunosuppressed patients [4–6]. Diarrhoea and gastroenteritis-like symptoms are frequently observed in association with primary HHV-6 infection in infants [2,29]. In stem cell transplant recipients HHV-6 has been detected in the gastroduodenal as well as in the colorectal mucosa in patients with diarrhoea and gastrointestinal symptoms [8,9]. These patients were reported, however, to have other simultaneous viruses such as EBV, CMV, adenovirus, HSV and rotavirus respectively. The HHV-6 reactivations are common in transplant recipients, but clinical significance is thought to be minor as clinical disease is estimated to occur only in 1% of patients [30]. In the previous study on liver transplant patients, the symptoms of HHV-6 GI-infection were mild and often associated with concurrent CMV-findings of GI-tract [13]. Likely, the main causes for upper gastrointestinal symptoms were *H. pylori* induced gastritis, gastro-oesophageal reflux etc. Although there have been a few case reports describing HHV-6 colitis [10–12], a recent study on HHV-6 in over a thousand samples has demonstrated several cases with gastrointestinal biopsies infected with HHV-6, mostly HHV-6B and having significant clinical symptoms such diarrhoea, fever and colitis [31]. The experience of antiviral treatment of HHV-6 infections is very limited and mostly based on the treatment of encephalitis [7]. Antivirals could also be indicated in other severe diseases, such as colitis, especially in the case of co infection with CMV. In our material, however, the symptoms were mild, if any, and no antiviral treatment was given.

The HHV-6 is often associated with co-infection with other viruses, especially CMV [32,33]. In most studies HHV-6 precedes CMV infection, but the viruses have been found also concomitantly. In a recent study on lung transplant recipients, the association between CMV and HHV-6 was found in the minority of the patients [34]. In the study by Halme *et al.* 13% of liver transplant recipients and none of the immunocompetent patients had simultaneously HHV-6 and CMV positive cells in the gastroduodenal mucosa [13]. In the present study, CMV positive cells, in the gastroduodenal mucosa, were found simultaneously with HHV-6B in 21% of the transplant recipients and in 14% of patients on chronic dialysis. In 14% of the transplant recipients but in none of the patients on chronic dialysis HHV-6B and CMV positive cells were found simultaneously in the colorectal mucosa.

In conclusion, HHV-6B positive cells were found equally often in the gastroduodenal as in the colorectal mucosa, whereas CMV positive cells were more often seen in the gastroduodenal than in the colorectal mucosa. The HHV-6B positive cells as well as CMV positive cells were simultaneously found in every fifth of transplant recipients. Although CMV, and even HHV-6, may cause in some transplantation patients colitis, in a series of patients with unspecific symptoms and endoscopic findings HHV-6 and CMV can be detected in the gastrointestinal tract without any clinical significance.

Authorship

ML: participated in the research design, writing of the manuscript, data collection and data analysis. LH: participated in the research design, writing of the manuscript, data collection and data analysis. JA: participated in writing of the manuscript and data analysis. EH: participated in writing of the manuscript. KS: participated in writing of the manuscript. IL: participated in the research design, writing of the manuscript and data analysis.

Funding

This study was supported by grants from Helsinki University Hospital Funds (EVO).

Acknowledgements

The authors thank Marjatta Palovaaraara for excellent technical assistance.

References

1. De Bolle L, Naesens L, De Clercq E. Update on human herpesvirus 6 biology, clinical features, and therapy. *Clin Microbiol Rev* 2005; **18**: 217.
2. Yamanishi K, Okuno T, Shiraki K, *et al.* Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988; **1**: 1065.
3. Ljungman P, Singh N. Human herpesvirus-6 infection in solid organ and stem cell transplant recipients. *J Clin Virol* 2006; **37**(Suppl. 1): S87.
4. Singh N, Carrigan DR, Gayowski T, Marino IR. Human herpesvirus-6 infection in liver transplant recipients: documentation of pathogenicity. *Transplantation* 1997; **64**: 674.
5. Rossi C, Delforge ML, Jacobs F, *et al.* Fatal primary infection due to human herpesvirus 6 variant A in a renal transplant recipient. *Transplantation* 2001; **71**: 288.
6. Benito N, Ricart MJ, Pumarola T, Marcos MA, Oppenheimer F, Camacho AM. Infection with human herpesvirus 6 after kidney-pancreas transplant. *Am J Transplant* 2004; **4**: 1197.
7. Razonable RR. Infections due to human herpesvirus 6 in solid organ transplant recipients. *Curr Opin Organ Transplant* 2010; **15**: 671.
8. Hentrich M, Oruzio D, Jager G, *et al.* Impact of human herpesvirus-6 after haematopoietic stem cell transplantation. *Br J Haematol* 2005; **128**: 66.
9. Amo K, Tanaka-Taya K, Inagi R, *et al.* Human herpesvirus 6B infection of the large intestine of patients with diarrhea. *Clin Infect Dis* 2003; **36**: 120.
10. Delbridge MS, Karim MS, Shrestha BM, McKane W. Colitis in a renal transplant patient with human herpesvirus-6 infection. *Transpl Infect Dis* 2006; **8**: 226.
11. Lamoth F, Jayet PY, Aubert JD, *et al.* Case report: human herpesvirus 6 reactivation associated with colitis in a lung transplant recipient. *J Med Virol* 2008; **80**: 1804.
12. Pilmore H, Collins J, Dittmer I, *et al.* Fatal human herpesvirus-6 infection after renal transplantation. *Transplantation* 2009; **88**: 762.
13. Halme L, Arola J, Höckerstedt K, Lautenschlager I. Human herpesvirus 6 infection of the gastroduodenal mucosa. *Clin Infect Dis* 2008; **46**: 434.
14. Lempinen M, Halme L, Sarkio S, *et al.* CMV findings in the gastrointestinal tract in kidney transplantation patients, patients with end-stage kidney disease and immunocompetent patients. *Nephrol Dial Transplant* 2009; **24**: 3533.
15. Lautenschlager I, Höckerstedt K, Linnavuori K, Taskinen E. Human herpesvirus-6 infection after liver transplantation. *Clin Infect Dis* 1998; **26**: 702.
16. Lautenschlager I, Linnavuori K, Höckerstedt K. Human herpesvirus-6 antigenemia after liver transplantation. *Transplantation* 2000; **69**: 2561.
17. Halme L, Höckerstedt K, Lautenschlager I. Cytomegalovirus infection and development of biliary complications after liver transplantation. *Transplantation* 2003; **75**: 1853.
18. The TH, van den Berg AP, Harmsen MC, van der Bij W, van Son WJ. The cytomegalovirus antigenemia assay: a plea for standardization. *Scand J Infect Dis Suppl* 1995; **99**: 25.
19. Sarkio S, Halme L, Kyllönen L, Salmela K. Severe gastrointestinal complications after 1,515 adult kidney transplantations. *Transpl Int* 2004; **17**: 505.
20. Ponticelli C, Colombo D, Novara M, Basilisco G, Group CS. Gastrointestinal symptoms impair quality of life in Italian renal transplant recipients but are under-recognized by physicians. *Transpl Int* 2010; **23**: 1126.
21. Chan H, Cheung C, Chan Y, *et al.* Intestinal tuberculosis as a cause of gastrointestinal bleeding in a renal transplant recipient. *Transpl Int* 2010; **23**: 657.
22. Goodgame RW. Gastrointestinal cytomegalovirus disease. *Ann Intern Med* 1993; **119**: 924.
23. Halme L, Lempinen M, Arola J, Sarkio S, Höckerstedt K, Lautenschlager I. High frequency of gastroduodenal cytomegalovirus infection in liver transplant patients. *APMIS* 2008; **116**: 99.
24. Hackman RC, Wolford JL, Gleaves CA, *et al.* Recognition and rapid diagnosis of upper gastrointestinal cytomegalovirus infection in marrow transplant recipients. A comparison of seven virologic methods. *Transplantation* 1994; **57**: 231.
25. Muir SW, Murray J, Farquharson MA, Wheatley DJ, McPhaden AR. Detection of cytomegalovirus in upper gastrointestinal biopsies from heart transplant recipients: comparison of light microscopy, immunocytochemistry, *in situ* hybridisation, and nested PCR. *J Clin Pathol* 1998; **51**: 807.
26. Kaplan B, Meier-Kriesche HU, Jacobs MG, *et al.* Prevalence of cytomegalovirus in the gastrointestinal tract of renal transplant recipients with persistent abdominal pain. *Am J Kidney Dis* 1999; **34**: 65.
27. Wakefield A, Fox J, Sawyerr A, *et al.* Detection of herpesvirus DNA in the large intestine of patients with ulcerative colitis and Crohn's disease using the nested polymerase chain reaction. *J Med Virol* 1992; **38**: 183.
28. Sipponen T, Turunen U, Lautenschlager I, Nieminen U, Arola J, Halme L. Human herpesvirus 6 and cytomegalovirus in ileocolonic mucosa in inflammatory bowel disease. *Scand J Gastroenterol* 2011; **46**: 1324.
29. Asano Y, Nakashima T, Yoshikawa T, Suga S, Yazaki T. Severity of human herpesvirus-6 viremia and clinical findings in infants with exanthem subitum. *J Pediatr* 1991; **118**: 891.

30. Humar A, Kumar D, Caliendo A, *et al.* Clinical impact of human herpesvirus 6 infection after liver transplantation. *Transplantation* 2002; **73**: 599.
31. Revest M, Minjolle S, Veyer D, Lagathu G, Michelet C, Colimon R. Detection of HHV-6 in over a thousand samples: new types of infection revealed by an analysis of positive results. *J Clin Virol* 2011; **51**: 20.
32. Humar A, Malkan G, Moussa G, Greig P, Levy G, Mazzulli T. Human herpesvirus-6 is associated with cytomegalovirus reactivation in liver transplant recipients. *J Infect Dis* 2000; **181**: 1450.
33. Härma M, Höckerstedt K, Lyytikainen O, Lautenschlager I. HHV-6 and HHV-7 antigenemia related to CMV infection after liver transplantation. *J Med Virol* 2006; **78**: 800.
34. Bauer C, Jaksch P, Aberle S, *et al.* Relationship between cytomegalovirus DNA load in epithelial lining fluid and plasma of lung transplant recipients and analysis of coinfection with Epstein-Barr virus and human herpesvirus 6 in the lung compartment. *J Clin Microbiol* 2007; **45**: 324.