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Editorial Board Member of *World Journal of Clinical Cases*, Luca Morelli, FACS, FASCRS, MD, Associate Professor, Division of General Surgery, Department of Traslational Research and of New Surgical and Medical Technologies, University of Pisa, Pisa 56124, Italy. luca.morelli@unipi.it

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WJCC mainly publishes articles reporting research results and findings obtained in the field of clinical medicine and covering a wide range of topics, including case control studies, retrospective cohort studies, retrospective studies, clinical trials studies, observational studies, prospective studies, randomized controlled trials, randomized clinical trials, systematic reviews, meta-analysis, and case reports.

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ORIGINAL ARTICLE

Case Control Study

Impact of cytomegalovirus infection on biliary disease after liver transplantation - maybe an essential factor

Jing-Yi Liu, Jian-Rui Zhang, Li-Ying Sun, Zhi-Jun Zhu, Lin Wei, Wei Qu, Zhi-Gui Zeng, Ying Liu, Xin-Yan Zhao

ORCID number: Jing-Yi Liu 0000-0002-9698-7377; Jian-Rui Zhang 0000-0002-1336-7915; Li-Ying Sun 0000-0003-1101-7994; Zhi-Jun Zhu 0000-0001-7031-2083; Lin Wei 0000-0002-0435-3829; Wei Qu 0000-0002-4484-5940; Zhi-Gui Zeng 0000-0003-1457-7495; Ying Liu 0000-0001-9087-899X; Xin-Yan Zhao 0000-0002-8016-4368.

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Jing-Yi Liu, Jian-Rui Zhang, Li-Ying Sun, Zhi-Jun Zhu, Lin Wei, Wei Qu, Zhi-Gui Zeng, Ying Liu, Xin-Yan Zhao, Liver Transplantation Center, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

Jing-Yi Liu, Jian-Rui Zhang, Li-Ying Sun, Zhi-Jun Zhu, Lin Wei, Wei Qu, Zhi-Gui Zeng, Ying Liu, Clinical Center for Pediatric Liver Transplantation, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

Jing-Yi Liu, Jian-Rui Zhang, Li-Ying Sun, Zhi-Jun Zhu, Lin Wei, Wei Qu, Zhi-Gui Zeng, Ying Liu, National Clinical Research Center for Digestive Diseases, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

Li-Ying Sun, Intensive Care Unit, Beijing Friendship Hospital, Capital Medical University, Beijing 100050, China

Corresponding author: Li-Ying Sun, MD, PhD, Chief Doctor, Professor, Liver Transplantation Center, Beijing Friendship Hospital, Capital Medical University, No. 95 Yongan Road, Beijing 100050, China. sunxlx@outlook.com

Abstract

BACKGROUND

Cytomegalovirus (CMV) infection is common in liver transplant (LT)_ recipients, and biliary complications occur in a large number of patients. It has been reported that CMV-DNA is more detectable in bile than in blood.

AIM

To investigate the effects of CMV infection on biliary complications by comparing the levels of CMV-DNA in the bile and blood of patients after LT.

METHODS

We conducted a retrospective analysis of 57 patients who underwent LT, 10 of these patients had no biliary complications and 47 patients had biliary complications. We also compared the levels of CMV-DNA in patients' bile and blood, which were sampled concurrently. We used RNAscope technology to identify CMV in paraffin-embedded liver sections.

RESULTS

CMV-DNA was not detected in bile samples and was detected in 2 blood samples

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from patients without biliary complications. In the 47 patients with biliary complications, CMV-DNA was detected in 22 bile samples and 8 blood samples, both bile and blood samples were positive for CMV-DNA in 6 patients. The identification rate of CMV-DNA in blood was 17.0%, and was 46.8% in bile. Moreover, tissue samples from 4 patients with biliary complications tested positive using RNAscope technology but were negative with hematoxylin and eosin staining. During the follow-up period, graft failure occurred in 13 patients with biliary complications, 8 of whom underwent retransplantation, and 3 died. CMV-DNA in bile was detected in 9 of 13 patients with graft failure.

CONCLUSION
In patients with biliary complications, the identification rate of CMV-DNA in bile was higher than that in blood. Blood CMV-DNA negative patients with biliary complications should still be monitored for CMV-related biliary tract diseases. Potential occult CMV infection may also be a contributing etiological factor in the development of graft failure.

Key Words: Liver transplantation; Cytomegalovirus infection; Graft failure; Biliary complications; RNAscope in situ hybridization; Retrospective study

Core Tip: For patients with biliary complications after liver transplantation, the clinical doctors should be alert to cytomegalovirus (CMV)-related biliary tract diseases even though the test of CMV-DNA in the blood is negative. The test for CMV-DNA in bile may be a novel approach for diagnosing occult CMV-related biliary disease. There has been no study on diagnosing CMV-related biliary complications after liver transplant by detecting CMV-DNA in isolated bile. Occult CMV infection in the biliary tract may be associated with biliary stenosis and a contributing factor to graft failure, leading to high mortality after surgery.

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INTRODUCTION

Biliary complications occur in up to 40% of patients after liver transplantation (LT) and cause significant mortality[1-3]. Post-LT biliary complications include strictures (anastomotic and non-anastomotic), leaks, stones, sphincter of Oddi dysfunction, and recurrence of primary biliary diseases. Cytomegalovirus (CMV) infection is a common opportunistic infection in LT recipients[4-5] and can occur in the liver, lungs, and gut and as a systemic infection[6-7]. CMV infection in LT recipients has been associated with many complications in addition to an increased risk of graft loss and death. CMV disease in LT patients most frequently presents as CMV syndrome, with the constellation of fever, neutropenia, and/or thrombocytopenia or as CMV hepatitis, as evidenced by elevated levels in liver function tests.

In our clinical work, we have found that in some patients with biliary complications after LT, CMV-DNA in the bile was positive but was negative in blood[8]. Rauber *et al* [9] reported that CMV was more frequent in bile than in liver biopsy or serum. Bile is not routinely obtained in patients after LT if there are no biliary complications. We obtained the bile from the patients without biliary complications using a T-tube during surgery following the surgeon's judgement of the biliary system. Although it is difficult to obtain bile, this phenomenon is not observed in patients without biliary complications.

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This finding suggests that serum CMV-DNA may be negative in patients with CMV biliary tract disease after LT. Testing for CMV in the biliary tract may be a novel approach for diagnosing occult CMV biliary disease. The present study aimed to determine the role of CMV infection in biliary complications after LT.

MATERIALS AND METHODS

Patients

From December 2012 to January 2020, 57 patients who underwent LT in Beijing Friendship Hospital were retrospectively analyzed; 10 patients did not have biliary complications and 47 patients did have biliary complications.

Patients had routine blood CMV-DNA tested every 1-2 mo according to different risk levels during the first year and had blood CMV-DNA tested every 3-6 mo according to previous CMV infection in the late postoperative period. Patients had not routinely tested CMV-DNA in body fluid such as urine and bile.

All patients received universal prophylaxis or pre-emptive therapy for CMV infection. For high-risk patients (CMV IgG D+/R-), universal prophylaxis with intravenous ganciclovir or oral valganciclovir therapy was applied immediately after surgery. Pre-emptive treatment was be used to prevent CMV infection from progressing to CMV disease when patients were positive for CMV-DNA in blood or body fluid.

Methods

When patients presented to the hospital with nonspecific symptoms, such as jaundice, abdominal pain, and fever, the diagnosis of biliary complications was confirmed by laboratory tests, like abnormal liver function and increased bilirubin, and imaging examination. Some of the biliary complications were proven by pathologic biopsy.

We routinely collected the bile samples weekly for patients with biliary complications by endoscopic nasobiliary drainage, percutaneous transhepatic cholangial drainage, or indwelling T-tube. For patients without complications, we obtained the bile samples by indwelling T-tube when it was removed. We analyzed the bile and blood samples for the presence of CMV-DNA.

We conducted a retrospective analysis of all patients and compared the levels of CMV-DNA in bile and blood, which were sampled concurrently. We also used RNAscope technology to identify CMV in paraffin-embedded liver sections from patients with biliary complications.

Ethics

The study was conducted following the Declaration of Helsinki and was approved by local ethics committees. All patients gave informed consent.

Bile CMV-DNA analysis

Bile samples were analyzed for the presence of CMV by polymerase chain reaction (PCR) after DNA extraction. Nucleic acid was isolated from bile samples with a Diagnostic Kit for the Quantification of Human Cytomegalovirus DNA (PCR-fluorescence method) (Zhongshan University, Daan Gene, China). At the beginning of the experiment, 1 mL of each bile sample was transferred to a 1.5 mL centrifuge tube. The tubes were centrifuged at 12000 rpm for 5 min, and the supernatants were removed. Fifty microliters of DNA extraction buffer were added, and the samples were incubated at 100 ℃ for 10 min and centrifuged at 12000 rpm for 5 min. Then, two of each resulting filtrate was used for PCR amplification. Amplification and detection were performed on a LightCycler instrument (Roche, Shanghai, China) with a thermocycling profile of 93 ℃ for 2 min followed by 40 cycles at 93 ℃ for 5 s and 57 ℃ for 45 s.

Blood CMV-DNA analysis

Blood samples were analyzed for the presence of CMV by PCR after DNA extraction. Nucleic acid was isolated from blood samples with a Diagnostic Kit for the Quantification of Cytomegalovirus DNA (PCR-fluorescence method). At the beginning of the experiment, 1 mL of each blood sample was transferred to a 1.5 mL Eppendorf tube. The samples were incubated at 4 ℃ overnight, and the filtrate (serum) was transferred into a fresh 1.5 mL Eppendorf tube. Fifty microliters of serum were transferred to a 1.5 mL Eppendorf tube, and 50 mL of DNA extraction buffer was added. The samples

were incubated at 100 ℃ for 10 min and centrifuged at 12000 rpm for 5 min. Then, 2 mL of each resulting filtrate was used for PCR amplification. The amplification and detection procedures were the same as those for the bile samples.

RNAscope in situ hybridization

The RNAscope assay uses a novel and proprietary method of *in situ* hybridization to detect single RNA molecules from virtually any gene in various tissue samples, including formalin-fixed paraffin-embedded (FFPE) tissues. The sensitivity and specificity of RNAscope *in situ* hybridization (RISH) have been determined with a variety of viral entities, including high-risk human papillomaviruses, hepatitis E virus, and hepatitis C virus.

Analysis of FFPE tissues was conducted following the RNAscope® 2.5 HD Detection Kit (BROWN) Quick Guide for FFPE Tissues (Advanced Cell Diagnostics, Newark, CA, United States). These samples underwent deparaffinization, proteolytic digestion with enzyme denaturation, and hybridization with probes. RNAscope target RNA was retrieved by initial incubation at 98 ℃ for 15 min followed by incubation with the RNAscope enzyme at 40 ℃ for 30 min. V-CMV, peptidylpropyl isomerase B (positive control), and DapB (negative control) probes were added and allowed to hybridize for 2 h at 40 ℃. Then, AMP1 3,3′-diaminobenzidine (DAB) (40 ℃ for 30 min), AMP2 DAB (40 ℃ for 15 min), AMP3 DAB (40 ℃ for 30 min), AMP4 DAB (40 ℃ for 15 min), AMP5 DAB (room temperature for 30 min), and AMP6 DAB (room temperature for 15 min) were incubated for the noted amount of time, and the slides were washed with the BOND reagent. The slides were incubated with DAB for 15 min and then counter-stained with hematoxylin for 2 min. Finally, the samples were rinsed with water, and coverslips were affixed.

Statistical analysis

All analyses were performed with SPSS 23.0 (Armonk, NY, United States). Parametric variables are expressed as the mean ± SD, whereas nonparametric variables are given as the median (interquartile range). Continuous data were compared with the nonparametric Mann-Whitney U test. Differences between actuarial estimates were analyzed with the log-rank test. Frequency differences were compared with the chi-square test. For expected frequencies less than 5, Fisher's exact test was used. $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

The demographics and clinical characteristics of the 57 patients are described in Table 1, and Table 2 describes the clinical data of adult patients. The median interval between LT and the procedure for obtaining bile was 14.35 mo (range = 1.0-134.3 mo) and 0.5 mo (range = 0.2-27.5 mo) in patients with and without biliary complications, respectively. The Pediatric End-Stage Liver Disease score of children was 16.7 ± 13.5, and the Model for End-Stage Liver Disease score of adults was 14.6 ± 6.8, which had no statistically significant difference with patients without complications. The biliary reconstructions and drainage technique were not statistically different, neither was the incidence of graft rejection and hepatic artery thrombosis. The *P* value of biliary CMV status and cold/warm ischemia time was less than 0.05 in Table 1, which included the children and adults. However, the *P* value of cold/warm ischemia time was more than 0.05 in Table 2 with adults only.

All the patients without biliary complications had negative CMV-DNA in bile, and only 2 patients had positive blood CMV-DNA.

Of the 47 patients with biliary complications, the median age was 33.0 years (3.3-51.8 years), including 30 male patients and 17 female patients. Ten patients received living donor liver transplantation (LDLT), 34 patients received deceased donor liver transplantation (DDLT), and 3 patients received cross-assisted liver transplantation.The mean follow-up period was 57.2 mo (range = 1.3-159.7 mo). During the follow-up period, graft failure occurred in 13 patients with biliary complications, 5 patients died due to graft failure, 8 patients underwent retransplantation, and 3 patients died. Ten patients without biliary complications and 39 patients with biliary complications were alive at the end of the follow-up period.

Table 1 Demographics and clinical characteristics comparisons by biliary complications

	Patients with biliary complications (*n* = 47)	Patients without biliary complications (*n* = 10)	*P* value
Age (yr)	33.0 (3.3-51.8)	51.6 (40.6-54.5)	0.021
Sex (M/F)	30/17	10/0	0.059
Primary disease (child)			/
Biliary atresia	11	0	
Metabolic disease	6	0	
Other	2	0	
PELD	16.7 ± 13.5	/	/
Primary disease (adult)			
Liver failure	3	0	
Decompensated liver cirrhosis	14	10	
HCC	9	0	
Other	2	0	
MELD	14.4 ± 6.3	15 ± 5.5	0.533
Liver transplantation			0.056
LDLT	10	0	
DDLT	34	10	
Cross-assisted liver transplantation	3	0	0.016
Cold ischemia time (min)	361.9 ± 244.4	582.1 ± 150.9	
Warm ischemia time (min)	5 (3-5)	5 (5-7)	0.025
Biliary reconstruction (Duct-to-duct / Roux-en-Y)	23/9	1/9	0.404
Blood CMV status (P/N)	8/39	8/2	0.822
Biliary CMV status (P/N)	22/25	0/10	0.016
Rejection (Yes/No)	29/4	9/1	0.855
HAT (Yes/No)	1/32	0/10	0.578
Biliary drainage			1
PTCD	30	0	
ENBD	12	0	
T-tube	5	10	
Outcome (alive/died)	39/8	10/0	0.365

HCC: Hepatocellular carcinoma; LDLT: Living donor liver transplantation; DDLT: Deceased donor liver transplantation; CMV: Cytomegalovirus; HAT: Hepatic artery thrombosis; PTCD: Percutaneous transhepatic cholangial drainage; ENBD: endoscopic nasobiliary drainage; PELD: Pediatric end-stage liver disease; MELD: Model for end-stage liver disease; M: Male; F: Female; P: Positive; N: Negative.

Biliary CMV-DNA detection

Biliary CMV-DNA was detected in 22 of 47 patients. Table 3 shows the patients' baseline demographics with biliary complications and compares demographic and clinical parameters based on the biliary CMV-DNA status of the patients. The detected biliary CMV-DNA levels were between 100 copies/mL and 1.5 × 106 copies/mL. The median interval between surgery and biliary CMV-DNA detection was 9.7 mo (range = 1.6-91.7 mo).

Among the 47 patients with biliary complications after LT, 21 had biliary anastomotic strictures (Figure 1), of whom 14 patients were positive for CMV-DNA. Only 8 patients with other biliary complications had positive bile CMV-DNA (Table 4). This difference was statistically significant (P = 0.020).

Table 2 Demographics and clinical characteristics comparisons by biliary complications in adults

	Patients with biliary complications (*n* = 28)	Patients without biliary complications (*n* = 10)	*P* value
Age (yr)	49.3 (37.8-57.5)	51.6 (40.6-54.5)	0.619
Sex (M/F)	19/9	10/0	0.079
Primary disease (adult)			0.047
Liver failure	3	0	
Decompensated liver cirrhosis	14	10	
HCC	9	0	
Other	2	0	
MELD	14.6 ± 6.8	15 ± 5.5	0.63
Liver transplantation			1
LDLT	1	0	
DDLT	25	10	
Cross-assisted liver transplantation	2	0	
Cold ischemia time (min)	477.9 ± 193.7	582.1 ± 150.9	0.208
Warm ischemia time (min)	5 (3.25-5)	5 (5-7)	0.069
Biliary reconstruction (Duct-to-duct/Roux-en-Y)	16/2	9/1	0.927
Blood CMV status (P/N)	6/22	2/8	1
Biliary CMV status (P/N)	10/18	0/10	0.038
Rejection (Yes/No)	3/16	1/9	0.667
HAT (Yes/No)	1/18	0/10	0.46
Outcome (alive/died)	25/3	10/0	0.552

HCC: Hepatocellular carcinoma; LDLT: Living donor liver transplantation; DDLT: Deceased donor liver transplantation; CMV: Cytomegalovirus; HAT: Hepatic artery thrombosis; MELD: Model for end-stage liver disease; M: Male; F: Female; P: Positive; N: Negative.

Among the 13 patients with graft failure, 9 patients had positive bile CMV-DNA. Of the 8 patients who died, 5 patients had positive bile CMV-DNA.

Blood CMV-DNA detection

Of the 47 patients with biliary complications, CMV-DNA in blood was detected in 8 patients. Six patients tested positive for CMV-DNA in both blood and bile simultaneously. CMV-DNA was positive only in blood in 2 patients. Furthermore, both bile and blood were negative for CMV-DNA in 23 of 47 patients (Table 5). The positive identification rate of CMV-DNA in blood was 17.0% (8/47), and the positive identification rate in bile was 46.8% (22/47); the *P* value was 0.123. The difference was not statistically significant.

RNAscope

We performed hematoxylin eosin staining of liver tissue in patients after LT. Although the results were negative, pathological manifestations were identified in patients who tested positive for bile CMV-DNA. These pathological manifestations included infiltration of inflammatory cells in the portal tract area, vacuolar degeneration in the portal tract area, deletion in the portal tract area, dilation of the lumen, and epithelial disorder or degeneration in the small bile duct. However, the patients who tested negative for CMV-DNA did not exhibit these manifestations; thus, we believe these manifestations were related to CMV infection. In recent years, some studies have reported that RISH is more sensitive than immunohistochemistry (IHC) in detecting CMV. Therefore, we used RISH to test for the presence of CMV in paraffin-embedded liver sections from 8 patients who tested positive for biliary CMV-DNA. Four patients tested positive using RNAscope technology (Figure 2).

Table 3 Demographics and clinical parameter comparisons by biliary cytomegalovirus status in patients with biliary complications

Biliary cytomegalovirus status	Children with biliary complications		*P* value	Adults with biliary complications		*P* value
	Positive (*n* = 12)	Negative (*n* = 7)		Positive (*n* = 10)	Negative (*n* = 18)	
Age	25.8 (7.9-126.3) mo	9.0 (6.0-94.1) mo	0.612	51.6 ± 8.3 yr	44.7 ± 14.5 yr	0.250
Sex (M/F)	6/6	5/2	0.667	7/3	12/6	0.856
Primary disease (child)			0.414			/
Biliary atresia	7	4		/	/	
Metabolic disease	4	2		/	/	
Other	1	1		/	/	
Primary disease (adult)			/			0.487
Liver failure	/	/		1	2	
Decompensated liver cirrhosis	/	/		4	10	
HCC	/	/		5	4	
Other	/	/		0	2	
Liver transplantation			0.351			0.241
LDLT	6	3		0	1	
DDLT	6	3		10	15	
Cross-assisted liver transplantation	0	1		0	2	
Cold ischemia time (min)	169 (57.5-547.5)	94 (55.5-296)	0.429	445.5 (195.5-583.8)	490.0 (402.5-672.5)	0.604
Warm ischemia time (min)	3 (3-6)	3.5 (1.5-5.5)	0.788	5	5 (2-5)	0.145
Biliary reconstruction (Duct-to-duct/Roux-en-Y)	4/3	3/4	0.710	6/0	10/2	0.529
Laboratory parameters before bile drainage						
Bilirubin (mol/L)	100.4 (35.2-390.8)	14.9 (9.5-36.9)	0.009	91.0 (35.9-203.0)	37.0 (24.1-97.9)	0.150
AST (U/L)	287.1 (58.2-635.0)	66.1 (54.3-123.0)	0.118	115.2 (69.2-189.8)	66.1 (36.5-146.4)	0.292
ALT (U/L)	106.0 (71.8-445.8)	79.0 (44.0-98.0)	0.205	117.0 (71.5-151.5)	60.5 (39.0-183.0)	0.502
ALP (U/L)	702.0 (177.0-1096.8)	256.0 (183.0-968.0)	0.735	368.0 (168.8-908.0)	388.5 (199.5-628.25)	0.943
GGT (U/L)	469.5 (388.25-847.25)	478.0 (206.0-1206.0)	0.866	334.5 (160.5-731.5)	289.5 (229.5-492.5)	0.811
Bac Inf. (biliary tract) (P/N)	9/3	5/2	0.865	4/4	9/3	0.503
Biliary stricture (P/N)	12/0	5/2	0.237	9/1	18/0	0.761
Rejection (Yes/No)	1/7	0/6	0.755	2/5	1/11	0.523
HAT (Yes/No)	0/8	0/6	1.000	0/7	1/11	1.000
Outcome (Alive/Died)	9/3	5/2	0.865	8/2	17/1	0.585

ERCP: Endoscopic retrograde cholangiopancreatography; PTCD: Percutaneous transhepatic cholangial drainage; AST: Aspartate aminotransferase; ALT: Alanine transaminase; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transferase; CRP: C-reactive protein; HCC: Hepatocellular carcinoma; LDLT: Living donor liver transplantation; DDLT: Deceased donor liver transplantation; Bac Inf.: Bacterial infection; HAT: Hepatic artery thrombosis; M: Male; F: Female; P: Positive; N: Negative.

DISCUSSION

CMV infections are the primary cause of illness and death in immunocompromised patients, and CMV infection usually has no clinical symptoms[10-11]. Although pp65 antigen and CMV-DNA in serum are useful and early markers of CMV infection, it was reported that CMV detection in body fluids and tissues was more sensitive than in blood[12-14].

Table 4 Comparison of biliary complications and bile cytomegalovirus-DNA

		Biliary complications		Total
		Anastomotic stricture	Non-anastomotic stricture	
Bile cytomegalovirus-DNA	Positive	14	8	22
	Negative	7	18	25
Total		21	26	47

Table 5 Comparison of biliary and blood cytomegalovirus-DNA in patients with biliary complications

		Biliary CMV-DNA		Total
		Positive	Negative	
Blood cytomegalovirus-DNA	Positive	6	2	8
	Negative	16	23	39
Total		22	25	47

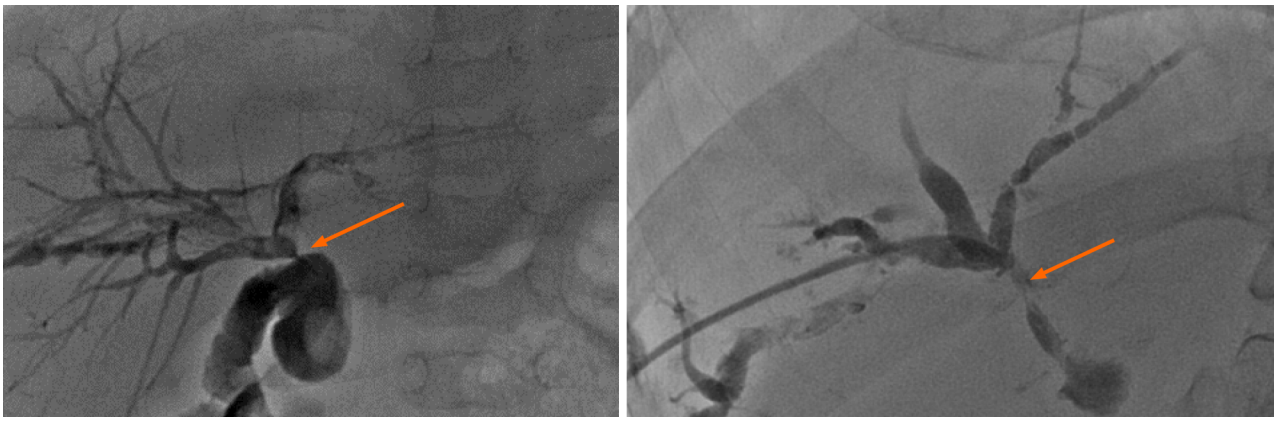

Figure 1 Endoscopic cholangiogram showing anastomotic strictures after liver transplantation.

It is worth noting that only 10 adult patients without biliary complications were included in this study. Only the patients who presented to the hospital with biliary complications would receive endoscopic nasobiliary drainage, percutaneous transhepatic cholangial drainage, or biliary surgery to resolve their problem. In 10n patients without biliary complications, T-tube was used to get the bile. The T-tube was placed during LT following the surgeon's judgment of the biliary system. We obtained the bile specimens at the time when the T-tubes were removed. A T-tube is not routinely inserted in children. This is the reason why the group of patients without biliary complications did not include children.

To reduce statistical error, we compared baseline data not only between patients with and without biliary complications but also between groups of adults. The demographics and clinical characteristics of the 57 patients are described in Table 1, and Table 2 describes the clinical data of adult patients. It is understandable that the *P* value related to age and cold/warm ischemia time in Table 1 was less than 0.05, but in Table 2 it was more than 0.05. LDLT is more commonly used in pediatric LT, resulting in a significant difference in cold/warm ischemia time compared with adult LT. The *P* value of biliary CMV status was less than 0.05, which also confirmed the relationship between biliary CMV status and biliary complications. There were no statistically significant differences in other baseline data in patients with or without biliary complications.

Table 3 shows the demographics and clinical parameters of patients with biliary complications and different biliary CMV status. In children with biliary complications, the *P* value of bilirubin showed that a higher bilirubin level might indicate positive biliary CMV status.

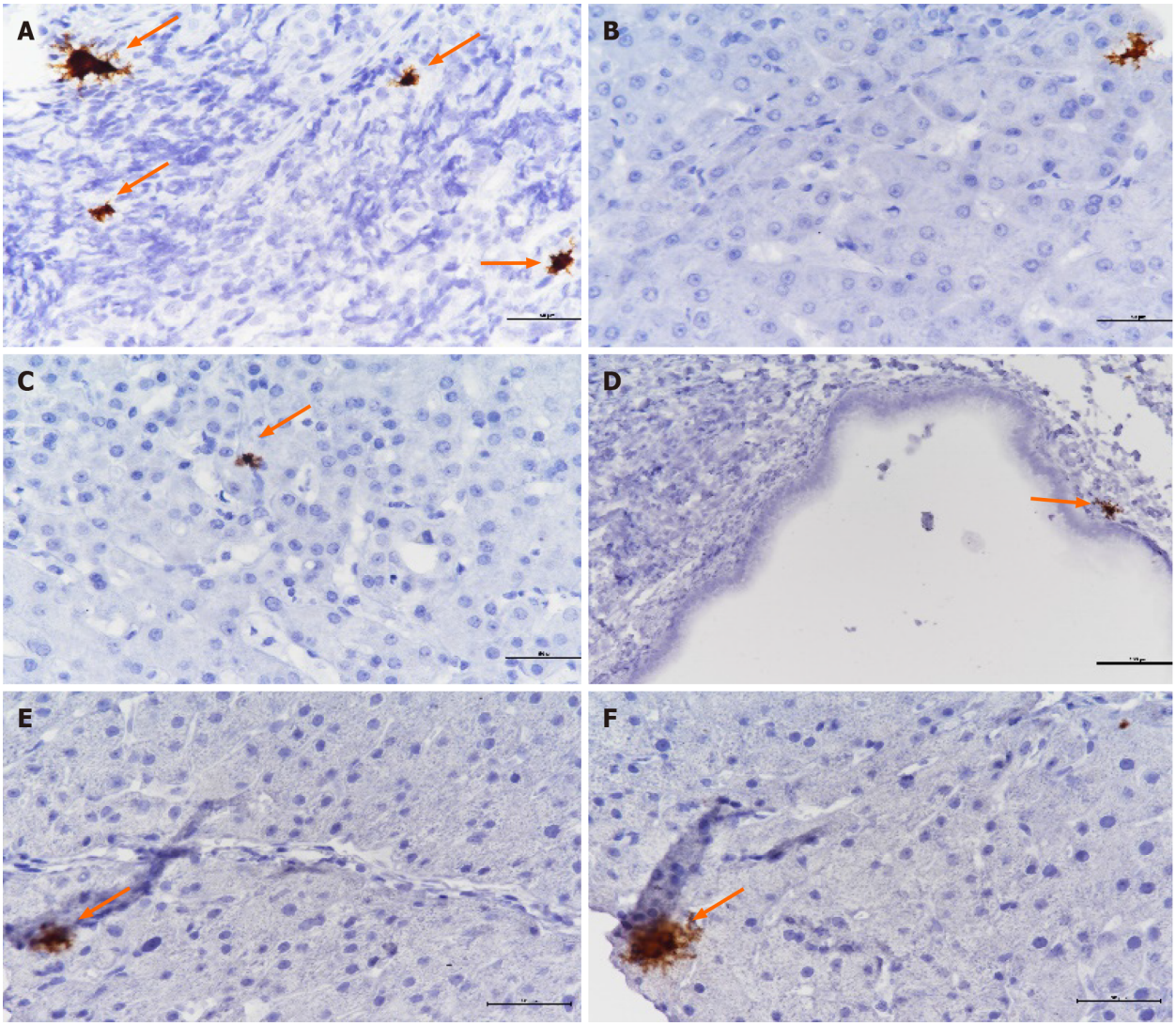

Figure 2 RNAscope *in situ* hybridization tests for the presence of cytomegalovirus in paraffin-embedded liver tissue samples. A: Positive control; B-D: Patient 3, Patient 6, Patient 10; E and F: Patient 13. We used RNAscope *in situ* hybridization to test for the presence of cytomegalovirus in paraffin-embedded liver sections from 8 patients who tested positive for biliary cytomegalovirus-DNA. Four patients tested positive using RNAscope technology.

The biliary complications observed were primarily biliary strictures and obstructions. Biliary strictures complicate approximately 2%-14% of LT cases and are classified as anastomotic and non-anastomotic[15]. Gotthardt *et al*[16] reported that biliary CMV was associated with non-anastomotic stenosis after LT. This study showed that biliary anastomotic strictures were related to biliary CMV infection (Figure 3). The positive rate of bile CMV-DNA was higher in patients with biliary anastomotic strictures than in patients with non-anastomotic strictures (See Table 4). The *P* value also showed this difference was statistically significant. Positive biliary CMV DNA led to biliary strictures that caused an elevation of bilirubin. As children have a smaller biliary tract, when they develop biliary complications they are more likely to show high hyperbilirubinemia.

Murine models suggest that CMV latency occurs in epithelial and endothelial cells. Latent CMV in hepatic sinusoidal endothelial cells leads to its reactivation in the liver [17]. CMV infection promotes fibroblast proliferation during the bile duct's healing process, resulting in anastomotic scarring, which leads to biliary anastomotic stenosis. In scar tissue, myofibroblasts are active. The relevant literature confirms that myofibroblasts play an important role in forming a benign biliary stricture, which is an important cause of anastomotic contracture and postoperative anastomotic biliary strictures[18]. In addition, CMV infection reduces the expression of monocyte chemoattractant protein-1 in fibroblasts and promotes inflammation by binding to macrophage inflammatory protein (MIP)-1 MIP-1α, and MIP-1β[19]. Given the small sample size in this study, future studies should expand the sample size.

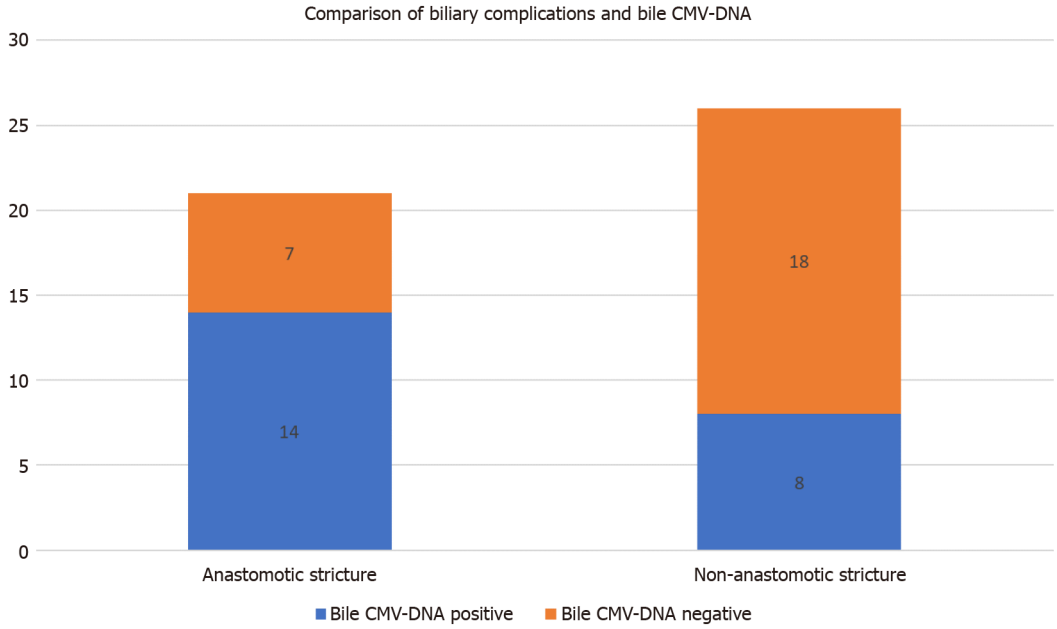

Figure 3 Comparison of biliary complications and bile cytomegalovirus-DNA. In 21 patients with anastomotic stricture, 66.7% (14/21) of patients had positive bile cytomegalovirus (CMV)-DNA. In 26 patients with other biliary complications, 30.8% (8/26) of patients had positive bile CMV-DNA. These results showed that biliary anastomotic stricture was more relevant to biliary CMV infection in this study.

Our research demonstrated that in 10 patients without biliary complications, none had positive CMV-DNA in bile, and 2 patients had positive CMV-DNA in blood. In patients with biliary complications, the positive rate of CMV-DNA in bile was much higher than that in blood. However, the difference between the positive rate in bile and blood of patients with biliary complications was not statistically significant (P = 0.123, See Table 5).

CMV infection may present only as viremia without clinical symptoms. In patients with biliary complications after LT, the phenomenon that CMV DNA in bile is positive, but CMV-DNA in blood is negative, may be highly suggestive of CMV biliary disease. According to a summary of these results, serum pp65 antigen and CMV-DNA negative patients with biliary complications should still be monitored for CMV-related biliary diseases. Testing for CMV in the biliary tract may be a novel approach for diagnosing occult CMV biliary diseases.

Routine detection of CMV in liver tissue is not sensitive. We used RISH to test paraffin-embedded liver sections from 8 patients with positive biliary CMV-DNA. All the tissues tested negative with CMV IHC, but the tissues of 4 patients tested positive using RISH. The results of our study suggest that RISH is more sensitive than IHC, which is consistent with previous studies[20-21]. Moreover, it also demonstrated that the identification rate of CMV-DNA in bile was higher in patients with CMV diseases.In this study, graft dysfunction occurred in 13 patients with biliary complications, 5 patients died, 8 patients underwent secondary transplantation, and 3 patients died after retransplantation. CMV-DNA in the biliary tract was detected in 9 of 13 patients with graft failure. The 1-year cumulative survival rate was 96.0%, the 3-year cumulative survival rate was 91.6%, and the 5-year cumulative survival rate was 86.2% in patients with CMV-DNA negative bile. The 1-year cumulative survival rate was 90.9% in patients with CMV-DNA positive bile, and the cumulative 3- and 5-year survival rates were 75.7% (Figure 4). Occult CMV infection is a risk factor for chronic graft failure and mortality after kidney transplantation[22-25]. Our study suggested that occult CMV infection might be related to chronic graft dysfunction and death in patients after LT. CMV infection in the biliary tract leads to increased biliary obstruction, which in turn increased bilirubin level, and the likelihood of graft dysfunction increased. Verdonk *et al*[26] showed that CMV-DNA positive recipients were more likely to be CMV-DNA positive in bile after surgery, which may be related to CMV reactivation *in vivo* after LT following treatment with immunosuppressive agents. Therefore, it is important to detect CMV-DNA regularly after transplantation [12]. For patients with these risk factors, close monitoring, adjustment of the immunosuppressive regimen, and targeted prevention may reduce the risk of chronic graft failure[24]. Graft dysfunction after LT has been reported to occur in 50% of

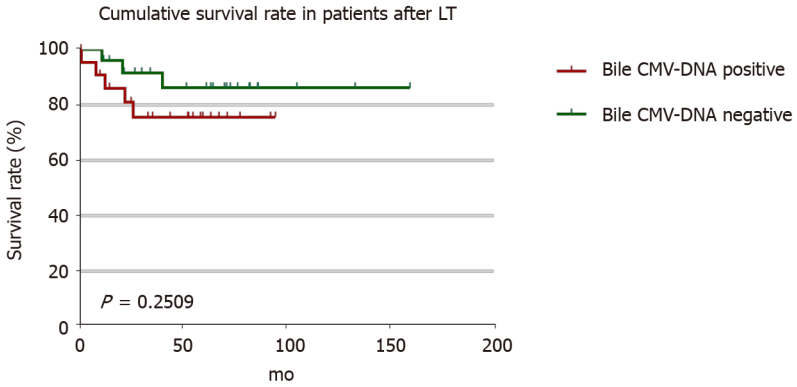

Figure 4 Cumulative survival rate in patients after liver transplantation. In patients with negative cytomegalovirus (CMV)-DNA bile, 1-year cumulative survival rate was 96.0%, 3-year cumulative survival rate was 91.6%, and 5-year cumulative survival rate was 86.2%. In patients with CMV-DNA positive bile, 1-year cumulative survival rate was 90.9%, and the cumulative 3- and 5-year survival rates were 75.7%. Occult CMV infection is a risk factor for chronic graft failure and mortality after liver transplant.

patients with biliary tract non-anastomotic strictures[26]. Among the 13 patients with graft failure, 6 had an anastomotic stricture and 7 had no anastomotic stricture. These results are consistent with previous studies.

Currently, prophylactic or preemptive treatment with ganciclovir or valganciclovir has partly reduced the incidence of CMV disease[27-28]. Patients in this study were given antiviral drug therapy, such as ganciclovir or valganciclovir, after CMV infection, and CMV-DNA gradually turned negative in bile and blood.

CONCLUSION

In this study, the positive rate of CMV-DNA in bile was higher than that in the blood of patients with biliary complications after LT. Therefore, CMV-DNA negative patients with biliary tract complications should be monitored for CMV-related biliary tract diseases and tested for CMV-DNA in bile. RISH is more sensitive than traditional immunohistochemical methods to detect CMV infection in liver tissue. Furthermore, occult CMV infection may be associated with biliary anastomotic stenosis and a contributing factor in graft failure, leading to high mortality after surgery. Improving CMV prevention strategies and treatment options is a priority.

ARTICLE HIGHLIGHTS

Research background

The association of cytomegalovirus (CMV) with biliary complications after liver transplant (LT) is an essential topic in clinical practice.

Research motivation

In clinical work, we have found that CMV-DNA in the bile and blood was inconsistent in patients with biliary complications after LT, and the positive rate of CMV-DNA in bile was higher than that in the blood.

Research objectives

To investigate the impact of CMV infection on biliary disease.

Research methods

We conducted a retrospective analysis of the clinical data from 57 patients with or without biliary complications.

Research results

CMV detection in bile is more sensitive than in blood. RNAscope *in situ* hybridization is more sensitive than traditional methods to detect CMV infection in liver tissue. Biliary CMV infection is definitively associated with biliary complications and poor

prognosis after LT, especially anastomotic stenosis.

Research conclusions

Patients with negative CMV-DNA in blood should still be monitored for bile CMV-DNA. Bile CMV infection maybe a contributing etiological factor in the development of graft failure.

Research perspectives

Current prevention strategies for CMV infection are inadequate and clinical doctors should be more vigilant of biliary CMV infection.

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