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Infections in human liver recipients: different patterns early and late after transplantation

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Abstract. The first 49 consecutive patients who underwent orthotopic liver transplantation between 1984 and 1989 in our department were studied with regard to symptomatic and asymptomatic post-transplantation infections. The major infections carrying a risk of fatal outcome are presented. During the first 4 weeks, fungal and bacterial infections predominated, the percentages of patients affected being 27% and 35%, respectively. Eight patients (17%) suffered from bacterial septicemia, which in six cases was due to gram-negative micro-organisms. The bacterial septicemia was often associated with severe ischemic damage to the graft, rejection, or cholangitis. In addition, a concomitant invasive fungal infection supervened in seven out of eight septic patients, further aggravating the patients' condition. Seventeen of the 49 patients (35%) died after transplantation within 3.3 years. Infection was the cause of death in nine patients (18%), with bacterial septicemia and/or fungemia in eight of these. Cytomegalovirus (CMV) disease was the dominant cause of illness after the 1st month. While only 5 of the 49 patients developed CMV disease during the 1st month (10%), as many as 16 of the 40 recipients who survived beyond that time suffered from symptomatic CMV viremia (40%). CMV mismatching, i.e., the donation of a CMV-positive organ to a CMV-seronegative recipient, entailed the highest risk for CMV disease. *Pneumocystis carinii* pneumonia occurred within 4 months in 10% of the patients. The four liver recipients affected were among the 20 patients not receiving trimethoprim-sulfamethoxazole prophylaxis. None of the 28 patients who received this prophylaxis over a 12-month period developed this complication ($P < 0.05$). The time-related panorama of infectious complications observed in this study has immediate clinical implications for the screening, prophylaxis, and therapy of infections following liver transplantation.

Key words: Liver transplantation, infections – Infections, liver transplantation

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Introduction

The 5-year survival rate following clinical liver transplantation is now as high as 80% [21]. The improvement in results during the last decade is due to more careful patient selection, better timing of the transplantation, better organ harvesting techniques, and more effective peri- and postoperative patient management, including the use of cyclosporin. Notwithstanding the improvement in results, there remains considerable post-transplantation morbidity and mortality due to infectious complications. In this study the infectious complications occurring in the first consecutive 49 orthotopic liver transplant recipients have been analyzed in detail. The evolution of the monitoring, prophylaxis, and treatment of infections in liver transplant patients, as it has evolved during the 1980s, is also discussed. Special attention is paid to the novel diagnostic and therapeutic tools that have emerged with regard to viral and fungal infections.

Methods

Patients and donors

Orthotopic liver transplantation (OLT) was performed in 49 patients between 1984 and 1989. Ten patients were retransplanted, one of them twice, making a total case material of 60 consecutive liver transplantations. There were 43 adults (aged 18–55 years) and 6 children (aged 10 months to 14 years). The characteristics of the patient population are outlined in Table 1. In 30 of the 60 transplantations, the livers were obtained from cadaveric donors using a special, rapid in situ cooling technique [9]. This technique was used before 1988 since Swedish law at that time did not allow abdominal exploration for organ removal prior to cardiac arrest. Cooling was performed using Euro-Collins (EC) solution (Fresenius, Bad Homberg, FRG) for the first 40 livers; thereafter, University of Wisconsin (UW) solution (Du Pont Pharmaceuticals, Stevenage, UK) was used.

Operation

The OLT was performed as described by Starzl et al. [29]. Thirty-four patients had an end-to-end choledochocholedochostomy (CCS) for the biliary tract reconstruction and 15 patients received a

Table 1. Characteristics of the 49 liver recipients. CCS, Choledochocholedochostomy; CJS, choledochojejunostomy

Underlying disease	Patients (*n*)	Age range (years)	Sex M/F (*n*)	Bile duct reconstruction		Graft function > 12 months (*n*)
				CCS (*n*)	CJS (*n*)	
Chronic liver disease	35	0.8–55	14/21	22	13	29
Primary biliary cirrhosis	10	37–55	0/10	10	–	7
Sclerosing cholangitis	9	24–54	6/3	–	9	7
Chronic active hepatitis	4	25–51	4/0	4	–	3
Autoimmune chronic active hepatitis	4	14–36	1/3	4	–	4
Cryptogenic cirrhosis	3	38–44	2/1	2	1	3
Metabolic liver disease	2	18–25	0/2	2	–	2
Primary biliary atresia	3	0.8–8	1/2	–	3	3
Acute liver failure	5	19–54	2/3	4	1	3
Non-A, non-B hepatitis	4	21–54	1/3	4	–	2
Budd-Chiari syndrome	1	19	1/0	–	1	1
Primary liver malignancy	9	2.8–53	6/3	5	4	6
Total	49	0.8–55	22/27	31	18	38

choledochojejunostomy (CJS) to a Roux-en-Y loop. Three patients had CCS with their first transplant and CJS with their second graft (Table 1). With both techniques the bile was initially drained outside the abdomen by means of a standard T-tube (with CCS) or a catheter (with CJS).

Immunosuppression

Initial immunosuppression consisted of cyclosporin A (CyA; Sandoz, Basel, Switzerland), 10 mg/kg per day orally or 3 mg/kg per day i.v.. The CyA doses were adjusted during the 1st month to reach a whole blood 12-h trough level of 800–1000 ng/l or 300–350 ng/ml using the original polyclonal RIA method or RIA-specific monoclonal method, respectively. Methylprednisolone, 1 g i.v., was administered during the transplantation. All patients were then given prednisolone, 200 mg/day. The dose was tapered to 20 mg/day by day 6. All but 15 patients also received azathioprine in an initial dose of 1.5 mg/kg per day i.v., followed by 1–2 mg/kg per day orally, unless leukopenia supervened.

Acute rejection episodes were diagnosed by liver core biopsies in addition to fine needle aspiration biopsies and bile cytology [16, 20] and were usually treated with a bolus dose of 1 g methylprednisolone i.v. and steroid recycling, as described previously [10]. Steroid-resistant rejection was treated either with rabbit antithymocyte globulin (ATG; Stanford University, Calif., USA), 5 mg/kg per day i.v., usually for 7 days, or with OKT3 (Ortho Pharmaceuticals, N.J., USA), 5 mg/day i.v., usually for 10 days.

Infection prophylaxis

Beginning on the day of transplantation, cefotaxime and ampicillin, each given in a dose of 4 g/day i.v., were administered for 5 days. Nystatin, 800,000 units daily, was administered orally to all but the first 12 adults as prophylaxis against fungal colonization starting from the moment they were placed on the waiting list for OLT, and this was continued in all patients for 3–6 months after transplantation. In the last 28 patients, trimethoprim-sulfamethoxazole (T-S, Bactrim), 80 mg/400 mg daily, was administered orally during the 1st post-transplant year as prophylaxis against *Pneumocystis carinii* infection.

Patients undergoing transplantation because of chronic active hepatitis B received antihepatitis B virus hyperimmunoglobulin (HBIg; Aunativ, Kabi Vitrum, Stockholm, Sweden), 5000 IU i.v., in the operating room immediately after the anhepatic phase, followed by 2500 IU i.m. given daily for 5 days and 1250 IU i.m. given once a week thereafter for 3 months. Additional doses were given if the serum anti-HBsAg ELISA titer dropped below 100 IU/ml during the 1st year. In addition, one of these patients received prophylactic phosphonoformic acid (Foscarnet, Astra, Södertälje, Sweden) for 8 days during the 2nd week. Active immunization with H-B-Vax (Merck Sharp & Dohme, West Point, Penn., USA) was started in two patients after 3 months, but not in the first liver recipient with hepatitis B and liver malignancy. No other prophylactic antiviral therapy was administered.

Methods used for microbiological surveillance and diagnosis

Surveillance before transplantation. All but the first five donors were tested for antibodies to human immunodeficiency virus (HIV), cytomegalovirus (CMV), and hepatitis B virus (HBV) prior to donation [National Bacteriological Laboratory (NBL) or Stockholm Central Microbiological Laboratory (SCML), Stockholm, Sweden]. All recipients, excluding the first five for HIV, were serologically examined with regard to HIV, HBV, CMV, herpes simplex virus (HSV; SCML), *Legionella* species and *Pneumocystis carinii* (NBL) using the ELISA technique [30]. Virus isolation as well as antigen detection techniques were used to study urine and blood for CMV [3]. In addition, serum was analyzed for evidence of fungal infection by means of serology and the detection of free-circulating *Candida* glycoprotein antigen (C.ag.), tested by Cand-Tec (Ramco Laboratories, Tex., USA; NBL), as previously described [32]. Direct microscopy and cultures for fungi and bacteria from the throat, sputum, urine, and feces were also performed.

Surveillance after transplantation. During the 1st month all patients were serologically examined each week with regard to HSV, CMV, *Legionella, Pneumocystis carinii,* and various fungal species. CMV isolation from blood and urine, as well as bacterial and fungal cultures from the throat, sputum, urine, feces, bile, and drainage fluids, were also taken regularly. During the following 6 months, serology for HSV, CMV, *Legionella,* and *Pneumocystis carinii,* as well as CMV isolation in blood and urine, were repeated monthly. Patients with HBV-associated cirrhosis were monitored monthly for the 1st year regarding HBV markers (NBL) [24]. Antibodies against hepatitis C virus (HCV) [17] were retrospectively analyzed in blood obtained before transplantation and at 1 week, 3 months, 6 months, and 12 months after OLT (SCML). Thereafter, HBV, HCV, and HIV serological samples were obtained once a year. HBV, Epstein-Barr virus (EBV; NBL), CMV, and adenovirus (SCML) were analyzed by DNA hybridization and/or by using viral-specific monoclonal antibodies (mAbs) to viral antigens and the indirect immunofluorescence (IF) technique in liver biopsy material.

Table 2. Bacterial and protozoal infections during and after the first 4 weeks in 48 and 40 liver transplant patients, respectively

	Early infections				Late infections			
	Patients		Infectious episodes		Patients		Infectious episodes	
	n	(%)	*n*	(%)	*n*	(%)	*n*	(%)
Bacterial septicemia	8[a]	(16.7)	8	(25.8)	8[b]	(20.0)	12	(28.9)
Pneumonia	8	(16.7)	8	(25.8)	4	(10.0)	4	(9.5)
Cholangitis	7[c]	(14.6)	7	(22.6)	9	(22.5)	22	(52.4)
Abdominal abscess	3	(6.3)	3	(9.7)	3	(7.5)	3	(7.1)
Liver abscess	–	–	–	–	1	(2.5)	1	(2.4)
Wound infection	5	(10.4)	5	(16.1)	–	–	–	–
Total no. of infected patients	17[d]	(35.4)			14[d]	(35.0)		
Total no. of patients at risk	48				40			

[a] Gram-negative bacteria in six and gram-positive bacteria in two patients
[b] Gram-negative bacteria in four and gram-positive bacteria in four patients
[c] Four patients had a CJS performed
[d] The same patient may have had positive bacterial cultures from several sites

During febrile episodes (> 38.0 °C) three blood samples were drawn for bacterial cultures. At the same time, analyses for C. ag. in serum were also routinely performed.

All patients presenting with a dry cough, dyspnea, and fever were examined by bacteriological and fungal blood cultures, blood gases, chest films, flexible bronchoscopy with bronchoalveolar lavage (BAL), and bronchial brushing [14]. Nasopharyngeal aspirations for the detection of virus antigens for influenza A, respiratory syncytial, adenovirus, and CMV were also performed by antigen-specific mAbs and the indirect IF technique (SCML).

Criteria for diagnosis of infections. Septicemia was defined as clinical symptoms and bacterial growth in at least two blood cultures obtained during the same febrile episode. On each occasion two blood samples were obtained by venous puncture. In addition, at least one sample was obtained via a vascular access line.

The diagnosis of cholangitis required the presence of fever, right upper quadrant pain and pathological liver tests, together with histological signs of cholangitis on biopsy. Cytological evidence (granulocytes and/or intracellular bacteria) of infection in the exteriorized bile and isolation of the same micro-organism in the bile and blood were used as additional criteria.

Bacterial pneumonia was considered in the presence of the following manifestations: an acute onset (over 12–24 h) of respiratory symptoms, fever, a localized infiltrate on chest films, and positive bacterial cultures from blood or BAL fluid. Fungal pneumonia *(Candidia, Aspergillus)* was diagnosed on the basis of a positive culture of the fungus in BAL fluid or a histological demonstration of characteristic hyphae in tissue [14], associated with the detection of C. ag. (titer > 1:4) in serum. Pneumonia due to *Pneumocystis carinii* or CMV was evident in patients with a dry cough, dyspnea, interstitial and intra-alveolar infiltrates on repeated chest films, combined with the detection of *Pneumocystis carinii* or CMV antigen in BAL fluid, as previously described (NBL, SCML) [14].

Invasive fungal infection caused by *Candida albicans, crusei,* or *tropicalis* was established by positive cultures of normally sterile body fluids with or without C. ag. greater than 1:4 in serum [32]. In some cases a fungal infection was confirmed or detected by histological examination of the obtained biopsy material.

Cytomegalovirus infection was defined as seroconversion of CMV-specific IgG and IgM in a patient previously seronegative (primary infection) or detection of a significant rise in CMV IgG antibodies with or without detectable CMV IgM antibodies (secondary infection) and/or detection of CMV viremia. CMV viremia was defined on the basis of virus isolated from peripheral blood leukocytes cocultured with human embryonic lung fibroblasts for 6 weeks and showing cytopathogenic effect.

CMV disease was evidenced by CMV syndrome, pneumonia, hepatitis, or gastroenteritis. CMV syndrome was defined as an unexplained fever (> 38.0 °C) for at least 1 week with CMV viremia or CMV antigen detection in white blood cells during that week, combined with a white blood cell count lower than 4.0×10^9/l and/or platelets lower than 100×10^9/l. CMV pneumonia was defined as radiological changes and/or hypoxia combined with CMV detection in alveolar macrophages obtained from BAL. CMV hepatitis was evidenced by pathological liver function tests and the presence of CMV in liver biopsy material. CMV gastroenteritis was defined as upper or lower gastrointestinal (GI) symptoms with CMV detected in biopsy material from the GI tract. Organ involvement required the detection of CMV antigen from the affected tissue by the indirect IF technique, positive CMV isolation from tissue, and/or histological evidence of CMV infection [3].

The diagnosis of herpes simplex virus infection was based on the presence of oral or genital mucositis and a positive viral culture or antigen detection by the indirect IF technique for HSV.

Epstein-Barr virus infection was diagnosed by seroconversion or by a significant rise in specific antibodies against EBV [1]. If there was a clinical suspicion of EBV-associated lymphoma or hepatitis, the tissue material was examined for the presence of EBV nuclear antigen by indirect IF and DNA by hybridization techniques [23].

Hepatitis B virus and hepatitis C virus infections were diagnosed by pathological liver tests, histological findings in liver biopsies combined with the occurrence of HBe-antigen and HBV-DNA or anti-HCV antibodies in blood [17, 24].

Statistical methods

Statistical analyses were performed using the chi-square or Fischer's exact test for comparison of two proportions, when applicable. For differences between mean values, Student's *t*-test was used. A *P*-value lower than 0.05 was considered to indicate a significant difference.

Results

One patient died during the liver transplant procedure. Thus, the following results concern the remaining 48 patients.

Early bacterial infections

Thirty-one bacterial infectious episodes occurred in 17 patients (35 %; Table 2). Multiple infectious foci were found in 14 of the 31 episodes, i. e., the same micro-organisms were cultured concomitantly in blood and in the liver, bile, or peritoneal fluid.

Table 3. Early and late viral infections in 48 and 40 liver transplant patients, respectively. NA, Not analyzed

Virus	Events	Early infections				Late infections			
		Patients		Infectious episodes		Patients		Infectious episodes	
		n	(%)	*n*	(%)	*n*	(%)	*n*	(%)
HSV 1	Stomatitis	9	(18.8)	11	(22.9)	5	(12.5)	6	(15.0)
2	Genital infection	4	(8.3)	5	(10.4)	6	(15.0)	15	(37.5)
CMV	Asymptomatic viremia	8	(16.7)	8	(16.7)	8	(20.0)	8	(20.0)
	Symptomatic viremia	5	(10.4)	5	(10.4)	16	(40.0)	21	(52.5)
	Pneumonia	2	(4.2)	2	(4.2)	2	(5.0)	2	(5.0)
	Hepatitis	–		–		9	(23.0)	9	(23.0)
	Gastroenteritis	–		–		1	(2.5)	1	(2.5)
HBV	Asymptomatic	–		–		1	(2.5)	1	(2.5)
	Hepatitis	–		–		2	(5.0)	2	(5.0)
HCV	Asymptomatic	–		–		1	(2.5)	1	(2.5)
	Hepatitis	–		–		2	(5.0)	2	(5.0)
EBV	Asymptomatic	NA		NA		NA		NA	
	Lymphoma	–		–		1	(2.5)	1	(2.5)
Total no. of patients at risk		48				40			

Septicemia was found in eight patients (17%); in six cases it was caused by gram-negative bacteria, such as *Enterobacter* species ($n = 2$), *Pseudomonas aeruginosa* ($n = 2$), or *Citrobacter freundi* ($n = 2$; Table 2). The septicemia emanated from cholangitis in four patients and from an abdominal abscess in two patients. In the remaining two patients, the septicemia was caused by gram-positive bacteria, *Staphylococcus epidermidis* and *Staphylococcus aureus*, respectively. These bacteria may have emanated from the vascular access lines employed since the same bacteria were isolated from blood obtained for cultures through these lines.

Cholangitis was caused by enterococci in combination with gram-negative or gram-positive enteric bacteria (*Escherichia coli, Citrobacter freundi, Klebsiella pneumoniae, Clostridium perfringens,* streptococci) in seven patients (14.6%; Table 2). In four out of seven patients *Candida albicans* was concomitantly cultured in the bile. Four of the seven patients with cholangitis had a CJS performed. In six out of seven patients, cholangitis developed at the same time as a rejection episode. Positive bacterial bile cultures without cytological or histological evidence of cholangitis were found in an additional ten patients; in nine cases the causative agent was *Staphylococcus epidermidis* or *Staphylococcus aureus* and in one case enterococci. However, in no case did these bacteria cause clinical evidence of cholangitis. Moreover, these patients did not have an increased number of leukocytes or phagocytized bacteria in the bile.

Pulmonary infection was diagnosed in eight patients (17%; Table 2). A bacterial etiology was found in four of them. *Klebsiella pneumoniae* ($n = 1$), alpha streptococci ($n = 1$), and *Legionella* ($n = 2$) species were verified by examination of BAL fluid in each case, respectively. In the remaining four cases the pneumonia was discovered at autopsy and the causative micro-organism was not established. One of the eight patients with pneumonia suffered from liver cirrhosis associated with intrapulmonary arteriovenous shunts and reduced oxygen saturation (hepatopulmonary syndrome) before OLT. The other patients with pneumonia had had a primary nonfunctioning graft ($n = 2$) and/or severe rejection ($n = 5$), or they had been subjected to reoperation because of post-transplant bleeding complications ($n = 2$).

Deep bacterial wound infections and abdominal abscesses developed in five and three recipients, respectively (Table 2). Two wound infections were associated with abdominal abscesses, while the other three infections were decubital ulcers infected by staphylococci, enterococci, *Enterobacter* species, and *Candida albicans*. All three patients with abdominal abscesses were found to have a septicemia due to the same bacteria that were later cultured from the abscesses (*Pseudomonas* species, *Enterobacter* species and *Citrobacter* species or *Staphylococcus aureus*).

Early fungal infections

In seven of the eight patients, the bacterial septicemia was associated with fungemia. Invasive fungal infections were diagnosed by means of cultures obtained from blood, heart, lung, liver, peritoneal fluid, kidneys, or brain, and were found to be *Candida albicans* in 13 (27%) of the recipients. In patients with invasive fungal infections, the main transplantation operative time was 15 h, while in patients without such infections, a shorter transplantation time of 11 h was found ($P < 0.02$). Moreover, patients with invasive fungal infections needed more reoperations after transplantation than did those without fungemia [32]. A significantly larger number of the former patients died – 8 out of 13 (62%) versus 4 out of 35 (11%) with a reduced operation time ($P < 0.001$). In addition, an early postoperative liver graft malfunction, as manifested by a high peak alanine-aminotransferase level (> 40 µkat/l; normal < 0.7 µkat/l) and a low initial bile production (< 30 ml/24 h), was found in all patients with an invasive fungal infection.

Early viral infections

Recurrent stomatitis caused by HSV type 1 was diagnosed in nine patients (19%) and genital ulcers due to HSV type 2 in four patients (8%), but the infection did not

become disseminated in any of them. CMV infection, verified by viremia, was found in 13 (27%) of the recipients, but only five (10%) had clinical signs of CMV disease (Table 3).

Late infections

Forty patients were alive 4 weeks post-transplantation. A change in the panorama of infectious complications, with an increasing incidence of viral infections, was observed after the first 4 weeks.

Late bacterial and protozoal infections

Septicemia was found in 8 of the 40 patients (20%; Table 2). In four of them the biliary tract was the primary focus of a gram-negative septicemia. In one patient simultaneous cultures from blood and a subphrenic abscess showed *Pseudomonas aeruginosa* and *Bacteroides fragilis.* In the remaining three patients a central venous line gave rise to the septicemia with gram-positive bacteria *(Staphylococcus epidermidis).*

Nine patients (22.5%) suffered from recurrent episodes of cholangitis, due to multiple micro-organisms mainly emanating from the intestinal bacterial flora (Table 2). In four patients the dominant bacterial findings were enterococci and gram-negative organisms, such as *Escherichia coli* or *Klebsiella pneumoniae,* which were diagnosed in cultures taken simultaneously from the bile and blood. Occasionally, anaerobic bacteria, such as *Clostridium perfringens* and *Bacteroides fragilis,* were isolated, in addition to streptococci. Eight of the nine patients (89%) had bile duct abnormalities secondary to a hepatic artery thrombosis ($n = 4$), severe rejections ($n = 2$), liver abscess ($n = 1$), or stenosis in the choledochocholedochostomy ($n = 1$). Among the 31 patients without cholangitis, 9 had bile duct abnormalities (29%; $P < 0.01$).

Pneumocystis carinii pneumonia was diagnosed within 4 months after OLT in four patients (10%; Table 2). All of them were among the 20 patients who did not receive T-S prophylaxis. CMV was also isolated from BAL fluid and/or the buffy coat obtained from these four patients. None of the 28 liver recipients who were given T-S prophylaxis developed a *Pneumocystis carinii* pneumonia ($P < 0.05$).

Late fungal infections

Invasive fungal infection was caused by *Candida albicans.* Fungal infection occurred in seven patients (18%) after the 1st month, as compared to an incidence of 27% during the 1st month. In addition to an increase in the C. ag. titer ($> 1{:}4$), a disseminated *Candida* infection was demonstrated by cultures from the peritoneal fluid ($n = 3$), bile duct ($n = 5$), and lung ($n = 1$).

Late viral infections

Cytomegalovirus was the predominant viral agent after the 1st post-transplantation month. CMV infection, verified by viremia, was diagnosed in 24 of the 40 patients (60%). In 8 patients (20%) without clinical symptoms, CMV was isolated from the blood on routine controls. In contrast, symptomatic CMV disease occurred in 16 patients (40%); CMV hepatitis was found in 9, pneumonia in 2, and ulcerative gastroenteritis in 1 patient. The remaining patients suffered from only minor manifestations of the CMV disease (Table 3). Two of 40 patients (5%) died with CMV pneumonia and hepatitis, respectively.

CMV was detected in blood significantly earlier after OLT in patients who developed CMV disease than in those without clinical illness (28 ± 7 days vs 42 ± 18 days, $P < 0.05$). The serological CMV status was known for 35 donor-recipient pairs prior to OLT. The frequencies of CMV disease were similar in both CMV-seropositive and CMV-seronegative recipients (30% and 33%, respectively) when the donor was seronegative. On the other hand, CMV disease was found in 41% of CMV-seropositive patients and in 80% of CMV-seronegative patients receiving a CMV-positive liver graft.

Four patients were transplanted because of chronic hepatitis B virus infection; three patients had developed cirrhosis and one had developed a hepatoma. All four patients were HBsAg-positive and HBeAg-negative before OLT. One of them died early after transplantation for reasons unrelated to HVB. In the three remaining patients, HBV became reactivated within 5 months after OLT; two had clinical symptoms and the third developed an asymptomatic, persistent HBV infection in the liver graft (Table 3). One of the patients died of rapidly progressing hepatitis in combination with the recurrence of her hepatoma. The remaining two patients received active and passive immunoprophylaxis for 1 year and are doing well 3.3 and 3.5 years, respectively, after transplantation.

Hepatitis C virus infection was diagnosed in three patients after OLT (Table 3). One of them was anti-HCV-positive prior to transplantation but remained asymptomatic. The other two patients seroconverted to anti-HCV-positivity 9 and 16 months, respectively, after OLT. Both had slightly elevated, fluctuating transaminases 5.5 and 2.5 years post-transplantation, without histological signs of rejection.

A localized monoclonal Epstein-Barr virus lymphoma of recipient origin was found in the liver graft of one patient within 4 months after transplantation (Table 3). Following treatment with acyclovir and cytostatic drugs, a successful hemihepatectomy was performed [4]. The patient is well 4 years later.

None of the 49 liver recipients developed markers for HIV-1 infection after transplantation. All were HIV-1-seronegative before OLT.

Mortality associated with infections

Seventeen liver recipients (35%) died during the follow-up study. A tumor recurrence was the cause of death in four patients (8%). In 9 of the 17 patients (53%), i.e., 18% of the study population, an infection was the cause of death, often with several micro-organisms being found in the same patient. Fungemia occurred in 8 patients, combined with bacteremia or CMV in 5 and 2 patients, respectively. In the 1st month, four patients died of bacterial sep-

ticemia and abscesses in the abdomen or liver. Two patients had a fatal outcome because of CMV hepatitis or pneumonia. All of these patients also had an invasive fungal infection documented at autopsy. Several reasons could be identified for the fatal outcome from infections. Four of the nine recipients were retransplanted within the 1st month because of a vascular thrombosis or a primary nonfunctioning graft. The remaining five patients were reoperated for bleeding or bile leakage or because their graft had been damaged by ischemia.

Discussion

During the 1st month after transplantation we found six cases of gram-negative septicemia originating from cholangitis or an abdominal abscess. In all cases the graft had sustained severe ischemic damage and/or acute rejection. This finding is in accordance with previous reports showing that ischemia or rejection increases the risk of septicemia [6, 15]. Interestingly, half of the liver grafts in this study suffered some warm ischemia because they were harvested before the concept of brain death was regulated by law in Sweden. Early infection may also follow vascular complications and results in a high mortality [34]. In our study, four out of seven patients with early thrombosis of the hepatic artery or portal vein developed septicemia and died. These dramatic experiences have prompted us to repeatedly follow the patency of the vessels during the first 3 weeks by means of Doppler ultrasonography.

Fever and pain in the right upper quadrant should arouse suspicion of cholangitis, especially in patients who still have bile duct drainage catheters. These symptoms should call for a cytological examination of the bile with direct staining, as well as for cultures for fungi and gram-negative bacteria. The risk of cholangitis is presumably greater during episodes of rejection with a decreased bile production or if a CJS has been performed. Four of our seven patients with an early gram-negative cholangitis, probably originating in the small bowel, were found to have had CJS. Consequently, these patients are given prophylactic oral ciprofloxacin (250–500 mg twice daily), ampicillin (375–500 mg twice daily), or T-S (80 mg/400 mg twice daily) for 6–12 months. Contrary to these findings, an additional ten patients had positive bacterial bile cultures without an increased number of granulocytes on cytology. In the majority of these patients, various strains of staphylococci were found, but none of them developed cholangitis. Therefore, the presence of staphylococci in drained bile without cytological signs of infection seems to indicate a skin contamination of no clinical significance. The incidences of late-occurring septicemia and cholangitis (> 4 weeks after OLT) were almost equal, affecting 20% and 22% of the patients, respectively. Most of the patients with late septicemia also had radiological findings showing cholestasis, liver abscesses, or late-occurring hepatic arterial thrombosis, indicating a graft-related genesis. These patients needed, in addition to intravenous antibiotics, a percutaneous, transhepatic catheter for diversion of the bile.

Liver recipients with remittent fever and septicemia who do not respond to antibiotic treatment should be examined for abdominal infections, even when abdominal symptoms are scarce. Abdominal abscesses in our septic patients were associated with myocarditis secondary to infected subphrenic hematomas or with reoperations for bile leak and vascular thrombosis. Well-functioning abdominal drainage for a few postoperative days and an early re-exploration to empty the abdominal hematomas may prevent liver recipients from developing such abscesses.

The importance of abdominal foci, including the biliary tract, for the development of septicemia after OLT formed the basis of our prophylactic antibiotic regimen with ampicillin and cefotaxime for 5 days. Bacterial infections occurred in 35% of our patients. This may be compared with 68% of patients receiving cefoxitin for 2 days, as reported by George et al. [12]. In that study most of the *Enterobacteriaceae* organisms isolated during the 1st week after OLT were resistant to cefoxitin but susceptible to a third generation cephalosporin, such as cefotaxime.

In this study, early bacterial pneumonia occurred in 16.7% of our patients, a figure similar to that reported by Kusne et al. [18]. An increased risk of pneumonia has been reported when the patient requires a ventilator for a long time, e.g., because of a hepatopulmonary syndrome [11]. In our series, one patient who had this syndrome also developed an early post-transplant pneumonia. Other conditions that predispose one to pneumonia are graft dysfunction with ascites and the development of atelectases, as well as encephalopathy with a risk for aspiration [27]. These conditions may explain the other seven early pulmonary infections. All cases of pneumonia occurring after 1 month were due to *Pneumocystis carinii* and/or CMV, which often create life-threatening conditions. Clinically, *Pneumocystis carinii* pneumonia is often indistinguishable from the interstitial pneumonia caused by CMV [14]. It is therefore essential to perform early and repeated blood gas and bronchoscopy analyses. Since we started to give prophylactic T-S to all recipients, no patient has developed *Pneumocystis carinii* pneumonia, as compared to a 20% risk in non-T-S-treated OLT patients. This is in agreement with previous reports on bone marrow transplant recipients in Seattle [36].

Early fungal infections are common and can become life-threatening. The observed 27% frequency of invasive *Candida albicans* infections during the 1st month is comparable to previous reports from our center and from Pittsburgh [32, 35]. Eight of these 13 patients with invasive fungal infection died within 2 months after transplantation. A risk evaluation indicated that patients with invasive fungal infection spent significantly more time in the operating room than did patients without fungemia [32]. The majority of these patients had positive cultures for *Candida* in the peritoneal fluid, bile, or liver. The GI tract constitutes the source of *Candida* colonization in 30%–60% of normal subjects [7]. A CJS in an immunosuppressed liver recipient may increase the risk of an ascending fungal infection. This is evidenced by the fact all four patients with fungal cholangitis in our series had a CJS performed. In addition to regular cultures, prophylactic intravenous and oral antifungal treatment may help to minimize the risk of fungal dissemination [33].

Viral infections may develop as early as in the first weeks; in the present study, HSV predominated, followed by CMV. However, only minor, local HSV infections were observed and dissemination was prevented by early treatment with acyclovir for 5 days or longer because of simultaneous antirejection therapy [27]. After the 1st months, CMV accounted for the highest incidence (60%) and morbidity (4%) in viral infections. These figures are similar to those reported by Singh et al. [28]. During the 1st month, many of the patients were treated for one or more rejection episodes, and those entering the 2nd month were, therefore, in most cases heavily immunosuppressed. It has been shown that viral infections are related to the type and amount of immunosuppression given [27]. CMV was detected by virus isolation from the blood of patients who developed CMV disease significantly earlier (≤ 28 days) than from those who developed an asymptomatic CMV infection (≥ 42 days). However, by using the polymerase chain reaction (PCR), we detected CMV genome in mononuclear cells as early as 4 days after transplantation [26]. This indicates that viral replication may begin at the time of transplantation. In the beginning of our OLT program, foscarnet combined with hyperimmune CMV globulin was used with good results [3]. Today, the rapid diagnosis of CMV antigenemia (48–72 h) [3] or viremia by PCR [26] is used as a guideline for the early introduction of antiviral treatment with the less toxic drug, ganciclovir [8].

In the case of patients transplanted for HBV cirrhosis, the major problem has been the recurrence of the virus in the new liver. In some preliminary trials, short-term passive and active immunization against HBV reinfection has had no preventive effect [19], while others have demonstrated promising results [5, 31]. Although the numbers in this study are small, the results tend to support the view that the combined therapy may moderate the infectious course.

HCV has been reported to be less virulent than HBV in liver graft recipients [13], and our results are in accordance with this finding. None of our anti-HCV-positive patients developed symptoms of hepatitis up to 5.5 years after transplantation. However, rapidly progressive cirrhosis with fatal outcome after re-OLT has been previously reported [22]. In the future, the risk of HCV transmission may be minimized by screening blood and organ donors by more sensitive and specific virological tests.

EBV, together with CMV, has been reported to constitute up to 50% of the late-occurring viral infections [27]. EBV may be involved in potentially fatal post-transplant lymphoproliferative diseases, which are found in 2.2% of OLT patients [23]. For these patients, reduced immunosuppression and treatment with acyclovir or ganciclovir has proved to be successful [23, 25]. Chemotherapy has only been given in cases of malignancy [23]. Accordingly, in our series, one (2.5%) malignant EBV-associated lymphoma in the graft was successfully treated [4].

We conclude that there is a difference in the infectious panorama before and after the first 4 weeks following OLT. During the early period, bacteria and fungi are mainly found. Later, viral infections predominate, although in a few patients recurrent graft-related bacterial infections may be common. In patients with biliary tract abnormalities, i.e., after hepatic artery thrombosis or severe rejections, we use long-term prevention with antibiotics against recurrent, ascending cholangitis. The common occurrence of invasive fungal infections, especially in patients with graft dysfunction, argues for intensive, prophylactic, antifungal therapy [33]. Trimethoprim-sulfamethoxazole is routinely used as a prophylactic agent against *Pneumocystis carinii* pneumonia in our program. The role of prophylactic measures for HBV carriers remains controversial [31], but our policy still is to give passive and active immunization for the 1st year. In CMV-seronegative patients receiving a CMV-positive graft and/or receiving additional immunosuppression, acyclovir probably provides some prophylaxis [2]. In cases of CMV viremia, we give early treatment with ganciclovir.

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