TRENDS IN INVASIVE FUNGAL INFECTIONS IN LIVER TRANSPLANT RECIPIENTS: CORRELATION WITH EVOLUTION IN TRANSPLANTATION PRACTICES

Singh, Nina1; Wagener, Marilyn M.; Marino, Ignazio R.; Gayowski, Timothy

Author Information
Author Information
1 Address correspondence to: Nina Singh, MD, VA Medical Center, Infectious Disease Section, University Drive C, Pittsburgh, PA 15240.

Accepted 5 June 2001.

Abstract
Background. The incidence of invasive fungal infections, particularly invasive candidiasis, after liver transplantation is strongly influenced by surgical factors and technical complexity of the surgery. We assessed the temporal trends in invasive fungal infections in the context of evolution in liver transplantation practices, technical developments, and other risk factors.

Methods. Demographic and clinical characteristics of the patients, transplantation-related variables, and rates of infection were longitudinally analyzed over the last 10 years in 190 consecutive liver transplant recipients at our institution. Trends for categorical data were evaluated using the Cochran-Armitage trend test and for continuous variables using analysis of variance with linear contrast.

Results. A decrease in the length of operation (p = 0.03), intraoperative transfusion requirements (p = 0.0001), cold ischemic time (p < 0.0001), use of roux-en-Y biliary anastomosis (p = 0.0015), rate of biopsy proven rejection (p < 0.0001), and retransplantation (p = 0.056) was documented over the successive years. A significant decline in Child-Pugh score (p = 0.02) and in the proportion of patients transplanted as UNOS 2a occurred (p = 0.0001). Although the incidence of cytomegalovirus infection remained unchanged, a significant increase in the frequency of primary cytomegalovirus infection (p = 0.045), and a decrease in cytomegalovirus disease (p = 0.0006) was documented. Over the same time period, a significant decrease in the incidence of invasive candidiasis (p = 0.015), and an insignificant increase in the rate of invasive aspergillosis (p = 0.29) occurred.

Conclusion. Notable technical developments in liver transplantation practices and risk profiles of patients have occurred over the decade. These variables may have a role in influencing the evolving trends in invasive fungal infections in liver transplant recipients.

INTRODUCTION
Invasive fungal infections have been reported in 5 to 42% of the liver transplant recipients with an associated mortality rate of 25 to 67% (1–7). The unique susceptibility of liver transplant recipients to invasive candidiasis is well recognized (2,4,5). Indeed, Candida species account for 62 to 91% of the fungal infections in these patients (5,8,9). The frequency of invasive fungal infections, particularly invasive candidiasis after liver transplantation is influenced strongly by surgical factors, including the technical complexity of the surgery (2,4,10). Prolonged operation time, greater transfusion requirements, type of biliary anastomosis, retransplantation, bleeding complications requiring reoperation, and posttransplant length of intensive care unit stay have been shown to portend a significantly higher risk of invasive fungal infections in liver transplant recipients (4,6,10).

The frequency and spectrum of opportunistic mycoses is continuing to evolve. Emerging data suggest that these trends are unique for different opportunistic fungi and for various subgroups of immunocompromised hosts (11,13). Although the incidence of nosocomial candidiasis has increased in the intensive care units, a decrease in candidemia, but an increase in mycotic fungal infections, has been documented in bone marrow transplant recipients (11,14,15). Evolution in risk factors, e.g., the hematopoietic transplantation practices and antimicrobial prophylactic approaches, specifically the utilization of azoles, is largely proposed to account for the changing pattern of fungal infections in bone marrow transplant recipients. There is striking paucity of data on trends in invasive fungal infections in organ transplant recipients, including those undergoing liver transplantation.

In the recent years, notable technical developments and evolution to more conservative, yet effective immunosuppressive regimens, have occurred (16). The impact of these factors on infectious complications after liver transplantation, however, has not been defined. We assessed the temporal trends in invasive fungal infections over a 10-year period in the context of evolution in liver transplantation practices, technical developments, changes in patient demographic characteristics, and other risk factors.

METHODS
A prospectively maintained database has been in place since the establishment of a liver transplant program at the Pittsburgh VA Medical Center since 1990. The study population comprised 190 consecutive liver transplant recipients transplanted between January 1990 and January 2000. All patients received tacrolimus and low-dose prednisone as immunosuppressive therapy, as previously reported (17). Rejection episodes were treated with 1 g of methylprednisolone bolus with or without steroid cycles (methylprednisolone given intravenously in four divided doses daily, tapering the dose from 200 to 20 mg/day over 6 days). OKT3 was used for steroid-resistant rejection. Since October 1995, mycophenolate mofetil (1000 mg twice daily orally) was added to the above immunosuppressive regimen of all patients undergoing liver transplantation, if neurotoxicity or nephrotoxicity suspected to be due to tacrolimus developed (18). Perioperative prophylaxis consisted of ampicillin and cefotaxime for 24 hr. Trimethoprim-sulfamethoxazole was used indefinitely as Pneumocystis carinii prophylaxis. No antifungal prophylaxis was employed in the patients. Universal prophylaxis for CMV infection was not employed. Upon detection of CMV infection, preemptive ganciclovir was administered as prophylaxis for CMV disease, as previously described (19).

Definitions of infections.

Rigorous and previously established criteria were used for defining invasive fungal infections (3-5). Invasive candidiasis required histopathological evidence of tissue invasion by biopsy, or on autopsy, or isolation of Candida species in one or more blood cultures, or isolation of Candida in normally sterile body fluid or sites, with samples collected intraoperatively or by percutaneous needle aspirate (3-5). Yeast colonization at isolated sites, e.g., oral cavity, sputum, upper respiratory tract, stool or urine, in the absence of tissue invasion, was considered insufficient evidence of infection. Diagnosis of Cryptococcus neoformans was established by isolation in culture. Invasive mycelial infections, including aspergillosis required evidence of tissue invasion on biopsy or autopsy plus isolation of the mold in culture (3-5). CMV infection and disease were defined as previously reported in liver transplant recipients (19).

Statistical analysis.

Statistical analysis was performed using Prophet Statistics (version 6.0, ABTech Corporation, Charlottesville, VA) and SAS (version 8.01, SAS Institute, Cary, NC). Temporal trends for categorical data were evaluated using the Cochran-Armitage trend test. Odds ratios were calculated compared to the reference years of 1990-1992. The analysis of variance (ANOVA) with linear contrast was used to assess trends in continuous variables.

RESULTS

A total of 190 consecutive patients underwent liver transplantation during the study period. The mean follow-up was 48 months and ranged up to 9.8 years. The demographic characteristics of the patients are outlined in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Demographic characteristics of the study patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range)</td>
</tr>
<tr>
<td>Gender, % male</td>
</tr>
<tr>
<td>UNOS status</td>
</tr>
<tr>
<td>2a</td>
</tr>
<tr>
<td>2b</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>Underlying liver diseasea</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>Alcoholic</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
</tr>
<tr>
<td>Primary sclerosing cholangitis</td>
</tr>
<tr>
<td>Cryptogenic</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
</tr>
<tr>
<td>Metabolic</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>CMV status</td>
</tr>
<tr>
<td>Recipient +/donor +</td>
</tr>
<tr>
<td>Recipient +/donor −</td>
</tr>
<tr>
<td>Recipient −/donor +</td>
</tr>
<tr>
<td>Recipient −/donor −</td>
</tr>
</tbody>
</table>

* Some patients had more than one liver disease.

Evolution in patient characteristics and transplantation related variables.

The age of the recipients (P < 0.0024) and the donors (P < 0.005) increased significantly over time. Whereas, the number of patients undergoing liver transplantation as UNOS 2a candidates, declined significantly (P = 0.001), the number of patients transplanted as UNOS status 2b candidates increased significantly (P < 0.001). The overall Child-Pugh score of the patients declined (P < 0.02). A trend toward an increase in hepatitis C virus as an indication for liver transplantation was documented (P = 0.099); the proportion of patients undergoing liver transplantation for alcoholic liver disease, hepatitis B virus, hepatocellular carcinoma, or other liver diseases did not change significantly over time (Table 2).
A decrease in the length of transplant operation ($P=0.03$), intraoperative transfusion requirements ($P=0.0001$), cold ischemic time ($P<0.0001$), use of roux-en-Y biliary anastomosis ($P=0.0015$), biopsy-proven rejection episodes ($P<0.0001$), and rate of retransplantation ($P=0.056$) were documented over the successive years (Table 2). No significant change in the length of posttransplant intensive care unit stay, requirement of pre- or posttransplant dialysis or mortality were noted.

### Trends in invasive fungal infections.

Fungal infections occurring in the study patients included invasive candidiasis in nine patients (Candida albicans six, Candida glabrata two, Candida tropicalis one), invasive aspergillosis in eight, Cryptococcus neoformans infection in eight, and dematiaceous fungal infection in two patients. The overall incidence of invasive fungal infections remained unchanged ($P=0.22$) (Table 3). However, there was a significant decline in the incidence of invasive candidiasis ($P=0.015$). Candida infections occurred in 9% of the patients between 1990-1992, in 1.5% between 1993-1995, and in 1.7% of the patients from 1996 onward. There was an insignificant increase in invasive aspergillosis ($P=0.20$) over time. Because the rate of invasive mycoses may be influenced by CMV infection, we also assessed the trends in the frequency of CMV infection and disease. The incidence of CMV infection remained unchanged, however, a significant increase in primary CMV infection ($P=0.045$), but a decrease in the rate of CMV disease ($P=0.0006$) was documented.

### DISCUSSION

Surgical technique, patient characteristics, and immunosuppressive drug regimens in transplant recipients have continued to evolve (16, 20, 21). To our knowledge, our study is the first to document these temporal trends after liver transplantation longitudinally and to analyze their impact on infectious morbidity, particularly invasive fungal infections. Our data show that significant surgical technological advances, e.g., decline in operation time, blood loss, cold ischemic time, use of roux-en-Y biliary anastomosis, and the rate of retransplantation have occurred in the last decade. Transplant centers elsewhere have documented similar trends in advances in surgical practices (16, 20, 21).

Division of study duration into time periods allowed not only the trends, but also the likely risk (odds ratio) to be assessed (Table 2). However, identical results were obtained when examining the correlation between the year of transplant and the data for all patients, i.e., the slope of the best fit line was significantly different from zero.

Over the same time period, a significant decline in the incidence of invasive candidiasis was documented. In addition to surgical factors, CMV infection, particularly primary CMV infection, has previously been shown to be a significant risk factor for invasive fungal infections in liver transplant recipients (22). Fungal infections decreased despite an unchanged rate of CMV infection and a significant increase in the rate of primary CMV infection in our patients. Although the number of CMV seropositive donors remained constant ($P=0.89$), the number of CMV seronegative recipients increased significantly ($P=0.017$), which likely accounted for the increase in the rate of primary CMV infection. Child-Pugh scores of the patients decreased over time; a lower transfusion requirement during candidacy may have led to a higher proportion of liver transplant recipients being CMV seronegative.

Although our approach to prophylaxis for CMV disease (preemptive therapy with ganciclovir initiated upon detection of CMV infection on surveillance cultures) remained the same, a precipitous decline in the incidence of CMV disease since 1996 was likely due to the availability of newer diagnostic tests, e.g., CMV antigenemia, that can detect
Candida infection earlier and more reliably, as compared to the older cytopathology-based diagnostic methods, e.g., shell vial assay.

The incidence of invasive aspergillosis remained unchanged and, in fact, showed an insignificant increase. Similar trends have been documented from tertiary care centers in the nontransplant setting and in bone marrow transplant recipients (11–13, 23). An overall increase in invasive aspergillosis, non-\textit{fumigatus} Aspergillus species, and mycelial fungal infections has been documented in hematopoietic stem cell transplant recipients in the later part of the 1990s (11). An autopsy study at a university hospital in Europe between 1978 and 1992 revealed an increase in invasive fungal infections, due largely to a rise in \textit{Aspergillus} infections (P <0.001) (13). The frequency of invasive aspergillosis (cases/autopsies performed) in transplant recipients in this study, increased from 6% between 1983–1987, to 11% between 1988–1992, although the prevalence of invasive candidiasis declined from 12 to 3% during the same time period (13).

Traditionally, invasive mold infections in liver transplant recipients have almost exclusively been due to \textit{Aspergillus} (6, 8, 9). However, in a prospective, multicenter, ongoing study initiated in 1998, we have documented that fungi other than \textit{Aspergillus} now account for 37% of all mold infections in liver transplant recipients and for 43% of all deaths in patients with mold infections (24). Mortality in patients with mold infections other than \textit{Aspergillus} was noted to exceed that due to invasive aspergillosis (24).

We point out that during the time period for which the study was conducted, the surgical, anesthesia, and infectious disease team, and antimicrobial prophylactic practices remained unchanged. Furthermore, no antifungal prophylaxis was used; nine patients who participated in an antifungal prophylaxis trial were excluded from the analyses for this study (25). An obvious limitation of our study, however, is that the observed trends represent data from a single institution and are based on a relatively small sample size. Nevertheless, the evolving trends in invasive mycoses in liver transplant recipients have a number of relevant implications; our findings therefore warrant replication in future studies at other centers.

Universal prophylaxis for Candida infections with fluconazole is being widely used at many liver transplant programs. A declining incidence of invasive candidiasis suggests that wide utilization of prophylaxis may not be necessary, and that the need for fluconazole prophylaxis should be assessed based on the institutional trends in the incidence of invasive candidiasis. With the advent of newer antifungal agents, it has become apparent that rapid and accurate identification of the mycelial fungi can be pivotal in selecting the appropriate therapy. An increase in the incidence of mold infections in the transplant setting would suggest that mycological and laboratory expertise may need to be readily and more widely available. Finally, amidst a rising incidence of invasive mycelial infection, particularly aspergillosis, a critical need for more effective antifungal drugs for the optimal management of these patients is evident.

REFERENCES


