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Research Article

ACTIVE CYTOMEGALOVIRUS (CMV) INFECTION IN LIVER RECIPIENTS IN A HIGH CMV SEROPREVALENCE REGION – OUTCOMES AND THE USE OF ANTIGENEMIA

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ABSTRACT

Cytomegalovirus (CMV) is the most frequent viral infection in liver recipients, acting as immunomodulatory factor for other opportunistic infections and rejection. We assessed the outcomes of CMV infection in liver recipients in a high CMV seroprevalence region and the use of antigenemia for the diagnosis of CMV syndrome. Between March 2007 and April 2009, 44 liver recipients collected 344 samples for CMV antigenemia. Definition of active CMV infections used literature criteria. Recipients' outcomes [CMV syndrome, Hepatitis C Virus (HCV) recurrence, rejection and mortality] were analyzed. Performance of antigenemia for the diagnosis of CMV syndrome was assessed by the area under the Receiver Operating Curve (AUROC) of 52 positive samples, representing 24 recipients. CMV serology was positive (R+) in 90.9% of liver recipients. CMV syndrome occurred in 18 (40.9%) recipients. CMV negative serology (R-) recipients had lower disease-free time, as well as lower one-year and four-year survival rates ($p = 0.022$ and $p = 0.004$, respectively). HCV+ recipients presented CMV-associated indirect effects and had a tendency to lower four-year survival rate ($p=0.089$). The AUROC for CMV syndrome was 0.745 (95% CI 0.606 to 0.856, $p = 0.006$), with a cut-off of more than 8 positive cells/200,000 leukocytes, (sensitivity of 88.9% and specificity of 74.4%). CMV infection is associated to morbidity and lower survival rates in liver recipients in a high CMV seroprevalence region. Using antigenemia, the cut-off for diagnosing CMV syndrome was higher than 8 positive cells/200,000 leukocytes, with an appropriated performance through its accuracy.

Keywords: Antiviral, Cytomegalovirus, CMV, liver transplant, antigenemia
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INTRODUCTION

Cytomegalovirus (CMV) is the leading viral infection among liver transplant recipients, contributing to morbidity and mortality (Fishman, 2007; Lautenschlager, 2009; Razonable, 2008). This virus exists worldwide, and its prevalence is inversely proportional to the socioeconomic status of the studied population (Cannon; Schmid; Hyde, 2010). CMV promotes asymptomatic infections in immunocompetent hosts and becomes clinically important when there is a change in cellular immunity (Mocarski; Shenk; Pass, 2007), such as immunosupression therapy.

There are three clinical manifestations presented by liver recipients infected with CMV (LJungman; Griffiths; Paya, 2002; Razonable, 2008): CMV syndrome, end-organ disease, and indirect effects. The incidence of these manifestations varies according to the serology of both the donor and the recipient (Razonable, 2008), and is greater when the donor is seropositive for CMV and the recipient is not (D+/R-) (Bosch et al., 2011; Lautenschlager, 2009; Tryphonopoulos et al., 2011).

Two strategies for preventing CMV infections have been used in post-transplant care (Fishman, 2007; Levitsky et al., 2008): universal prophylaxis and preemptive therapy. Without prevention, CMV infection occurs in the first three months post-transplant (Lautenschlager, 2009), most patients present asymptomatic viremia, with a subgroup developing clinical manifestations (Fishman, 2007).

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Empirical treatment of the overt disease incurs risk of complications, while preventive strategies increase extra costs, in addition to exposing the recipients to the adverse effects of antiviral therapy (Fishman, 2007). Elevated costs limit the use of preventive strategies in developing countries, which have high seroprevalence of CMV (Cannon; Schmid; Hyde, 2010). Information on CMV infection and its behavior in liver transplant recipients in these regions are important in helping to streamline preventive strategies, reduce costs and provide coverage to higher risk patients.

We present a study to estimate the incidence of CMV infection, its clinical manifestations and outcomes in liver recipients in a high CMV seroprevalence population, and to evaluate the performance of CMV antigenemia in the diagnosis of CMV syndrome in liver recipients.

METHODS

Study design and population

We performed a prospective cohort study with retrospective data from liver recipients who underwent antigenemia assay for the diagnosis and monitoring of CMV infection. All transplants and follow-up were performed in a philanthropic tertiary care hospital (Hospital Português in Salvador, Brazil) which has been performing liver transplants since 2001, with a mean of 25 liver transplants per year during the period of the study (March 2007 to April 2009).

Inclusion and exclusion criteria

Liver transplant recipients with a signed informed consent form were included. Patients were excluded if they had no antigenemia assay for the diagnosis and monitoring of CMV infection.

Data collection and variables of interest

Data collection was retrospective through a review of medical records. Clinical and laboratory data of patients were evaluated at the time that samples were collected for antigenemia, which occurred at the discretion of the attending physician with the objective of monitoring and diagnosing CMV infection. Outcomes analyzed were disease-free time of CMV syndrome, transplant complications, such as Hepatitis C Virus (HCV) recurrence, infections and rejection, and survival.

We used antigenemia assay for the detection of CMV pp65 antigen by primary monoclonal antibodies (CMV Brite Turbo® kit - DPM Diagnostics). Published instructions for its use are available (Percivalle et al., 2008). Peripheral blood was collected in an EDTA-tube and was processed in a maximum of six hours after sampling.

Diagnosis of CMV syndrome

Clinical definition of CMV syndrome was the presentation of a viral profile (fever and/or asthenia), associated with leukopenia (leukocytes < 4,000/ml) and/or thrombocytopenia (platelets < 100.000/ml), and/or gastrointestinal symptoms, enteritis, hepatitis, arthralgia, retinitis, pneumatosis, colitis, esophagitis, and encephalitis (LJungman; Griffiths; Paya, 2002).

Statistical analysis

Survival functions were calculated using the Kaplan-Meier method, in which curves were estimated grouping the patients according to variables of interest, and the Log-rank (Mantel-Cox) or Breslow (Generalized Wilcoxon) tests were used for the comparison. Disease-free time of CMV syndrome was analyzed in a similar fashion.

CMV antigenemia performance for the diagnosis of CMV syndrome at the time of blood sampling was assessed by calculating the area under the Reciever Operating Curve (AUROC). Absence of positive cells in quantitative antigenemia is compatible with absence of disease. Thus, for this specific analysis, only cases with positive antigenemia were considered.

All tests were two-tailed and p values ≤ 0.05 were considered statistically significant. Data were analyzed using the Statistical Package for Social Sciences (version 20.0, USA) and MedCalc (version 12.1.4.0, Belgium) softwares.

Ethical considerations

This study conformed to ethical research principles set in Resolution 196/96 of the National Health Council and was approved by the Research Ethics Committee of Fiocruz (CEP/CPqGM/Fiocruz), and all patients included signed an informed consent form.

RESULTS

Sixty liver transplants were performed during the study period, and 44 recipients underwent at least one antigenemia assay in the post-transplant follow-up period (with a minimum of 1 and a maximum of 17 assays performed). Most recipients were male (79.5%), with a mean age of 60.8 years at the time of transplant. Forty recipients were seropositive for CMV (R+) in the pre-transplant evaluation, representing 90.9% of the total group. Data from these patients are summarized in Table 1

Implantation of CMV antigenemia occurred during this period. Recipients did not undergo preventive strategies for active CMV infection, and specific treatment for CMV was based on clinical manifestations. Antigenemia was a laboratory confirmation and monitoring of CMV infection. Treatment modalities ranged from simple reduction of immunosuppression to hospitalization for the use of intravenous ganciclovir.

Table 1: Sample characteristics and presentation of cytomegalovirus syndrome (n=44)

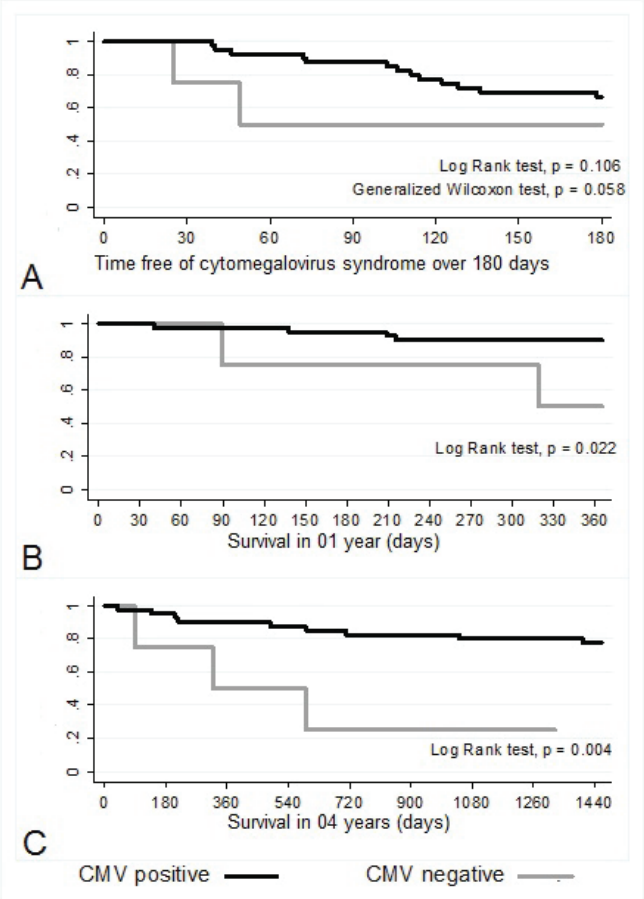
Variable	n (%)
Gender of recipient – male	35 (79.5)
Age at time of transplant (mean ± SD)	60.8 ± 7.2
Etiology of liver disease (n=64) [a]	
Hepatitis C	20 (45.5)
Alcoholic liver disease	15 (34.1)
Hepatocarcinoma	7 (15.9)
Cryptogenic	5 (11.4)
Autoimmune hepatitis	3 (6.8)
Fulminant hepatitis	1 (2.3)
Primary biliary cirrhosis	1 (2.3)
Budd-Chiari	1 (2.3)
Hepatitis B	1 (2.3)
Seropositive for HCV	20 (45.5)
HCV recurrence	5 (25.0)
Seropositive for CMV (R+)	40 (90.9)
Signs / symptoms associated with CMV syndrome	
Leukocytes (mean ± SD)	2.677.2 ± 921.4
Thrombocytopenia (< 1 x 10^5)	36 (70.6)
Weakness	22 (45.8)
Headaches	20 (43.5)
Diarrhea	18 (36.7)
Myalgia	10 (21.3)
Arthralgia	10 (21.3)
Heartburn	9 (19.1)
Abdominal pain	9 (18.8)
Colic	8 (17.4)
Vomiting	8 (17.0)
Exanthema	5 (10.6)
Fever	2 (4.1)
Development of active CMV infection up to 180 days	18 (40.9)
Disease-free time free of CMV syndrome up to 180 days (mean ± SE)	148.4 ± 7.6
Rejection	1 (2.3)
Retransplantation	1 (2.3)
Death in 01 year	6 (13.6)
Survival up to 01 year, in days (mean ± SE)	338.2 ± 11.4
Death in 04 years	12 (27.3)
Survival up to 04 years, in days (mean ± SE)	1195.2 ± 72.5
Cause of death	
Sepsis	3 (25.0)
Graft dysfunction	1 (8.3)
Metastasis of Hepatocellular carcinoma (HCC)	1 (8.3)
HCV recurrence	1 (8.3)
Cytomegalovirus disease	1 (8.3)
Acute myocardial infarction (AMI)	1 (8.3)
Stroke	1 (8.3)
Disseminated tuberculosis	1 (8.3)
Colon cancer	1 (8.3)
Leptospirosis	1 (8.3)

* SD: standard deviation; SE: standard error; [a] Some patients had more than one etiology. Hepatitis C associated with Hepatocellular carcinoma was present in 05 patients and Hepatitis C associated to Alcoholic liver disease was present in another 05 patients.

Clinical CMV syndrome occurred in 18 (40.9%) patients in a 180-day follow-up period, with a mean disease-free time of 148.4 ± 7.6 days. Using a Kaplan-Meier curves (Figure 1, Panel A) we stratified liver recipients according pre-transplant CMV serology (R+/R-) to analyze disease-free time. In the first two months, the curves tended to distance (Generalized Wilcoxon test, p=0.058), with a shorter disease-free time for R- group. However, the curves became closer to each other until the end of the period (log-rank test, p = 0.106), suggesting a greater relevance of CMV serologic status in the first two months post-transplant. Two R- recipients had clinical presentation of CMV syndrome (50% of this subgroup), and the other 16 cases were from R+ recipients (40% of this subgroup).

Average one-year survival rate was 338.2 ±11.4 days, with 6 deaths in this period, and average four-years survival rate was 1195.2 ± 72.5 days, with a total of 12 deaths (Figure 1, Panels B and C). Death causes are listed in Table 1. R- group had lower one-year and four-year survival rates (p = 0.022 and p = 0.004, respectively).

Figure 1. Curves showing time free of cytomegalovirus syndrome over 180 days (A) and survivals in 01 (B) and 04 (C) years, according to the CMV serology of the recipient.

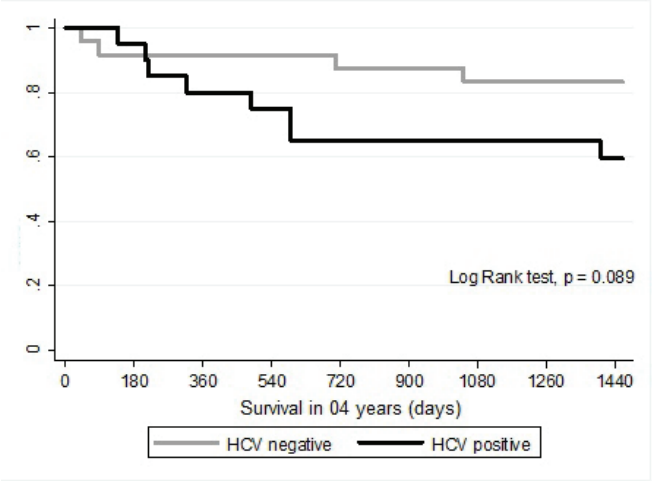


HCV infection was the main cause of liver

disease, occurring in 20 (45.5%) recipients (Table 1). These recipients presented complications that are possible CMV-associated indirect effects (LJungman; Griffiths; Paya, 2002). Five (25%) of HCV+ recipients were R+ and had HCV recurrence, but none had manifestations of CMV syndrome. CMV antigenemia was positive in four of these recipients. Three of these patients died (one died because of graft cirrhosis, another by a stroke, and a third by disseminated tuberculosis).

Besides recurrence, HCV+ recipients presented other CMV-associated indirect effects with positive CMV antigenemia. One R-/HCV+ recipient presented rejection and had presented clinical manifestations of CMV syndrome. Another R+/HCV+ recipient required retransplantation due to biliary stenosis. This patient presented clinical manifestations of CMV syndrome and developed non-anastomotic biliary stricture. She died of primary dysfunction after re-transplant. Overall, HCV+ recipients had a tendency toward lower four-year survival rate (p = 0.089), with 40% mortality at the end of the period (Figure 2).

Figure 2. Survival curve over 04 years according to recipient serology for HCV.



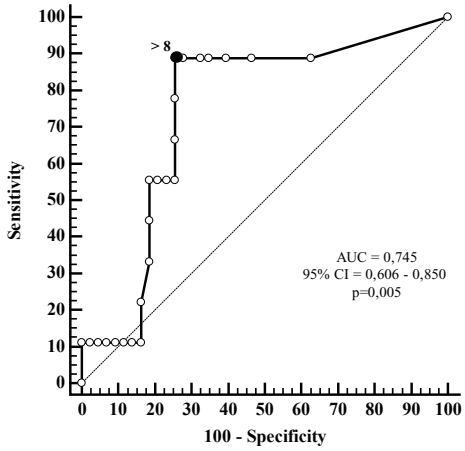
Half of all deaths (6/12) was caused by infections. Three recipients died from bacterial sepsis, one by disseminated tuberculosis, one by leptospirosis, and one by severe cytomegalovirus syndrome. Four of these patients (66.7%) had clinical manifestations of CMV syndrome and were treated with ganciclovir. Five patients had a positive CMV antigenemia (83.3%), and two were R- recipients.

The other six deaths occurred from non-infectious causes. Two recipients had complications associated with CMV (graft cirrhosis caused by HCV recurrence and delayed anastomotic biliary stricture) with positive antigenemia during follow-up. Two more recipients suffered from cardiovascular diseases (AMI and stroke), and another two died from neoplastic disease (metastasis of HCC and advanced colon cancer). None of these four patients had clinical manifestations of CMV syndrome or positive antigenemia.

CMV antigenemia was performed in 344 samples (minimum of 1 sample and maximum of 17 samples/patient) from 44 liver recipients. Positive antigenemia occurred in 52 samples (15.1%), representing 24 recipients (54.5%). Median positive antigenemia had 3 positive cells / 200,000 leukocytes, with an interquartile range of 1.0 – 26.75.

The AUROC was 0.745 (CI 95% 0.606 – 0.856, p = 0.006), which represents a good discriminatory capacity. The threshold for positivity in the diagnosis of CMV syndrome was established at an antigenemia value greater than 8 positive cells / 200,000 leukocytes, with a sensitivity of 88.9 (CI 95% 51.8 – 99.7) and specificity of 74.4 (CI 95% 58.8 – 86.5) (Figure 3). This threshold resulted in a positive probability ratio (PR) of 3.47 (95% CI 2.6 to 4.6) and negative PR of 0.15 (95% CI 0.02 to 1.0).

Figure 3. ROC (Receiver Operating Curve) for quantitative antigenemia and the diagnosis of cytomegalovirus syndrome. AUC: Area under the curve.



DISCUSSION

Our study assessed CMV infection in liver transplant recipients, their outcomes and the use of CMV antigenemia for the diagnosis of CMV syndrome in a liver transplant center in the city of Salvador, in northeastern Brazil. Our center is located at a high CMV prevalence region (Cannon; Schmid; Hyde, 2010), which was confirmed by a 90.9% CMV positive serology (R+) in pre-transplant evaluation of recipients.

At the beginning of the study, this transplant center had already performed liver transplantations in 40 recipients, and completed 100 recipients by the end of the study period. From the 60 liver recipients of this period, 44 underwent at least one quantitative CMV antigenemia. During the implantation of CMV antigenemia, recipients did not undergo preventive strategies for active CMV infection, as specific treatment for CMV was based on clinical manifestations of CMV syndrome (LJungman; Griffiths; Paya, 2002). Antigenemia was used as laboratory confirmation or

monitoring of CMV infection. Treatment modalities ranged from simple reduction of immunosuppression to hospitalization for the use of intravenous ganciclovir (Fishman, 2007; Razonable, 2008), according to clinical settings.

During the study period, 18 recipients developed clinical manifestations of CMV syndrome, although 24 patients had positive antigenemia at some point during follow-up. All 18 patients underwent treatment for CMV with reduced immunosuppression and the use of ganciclovir. These manifestations occurred in the first months of transplant, at an average time of 148.4 ± 7.6 days (Table 1). These findings are consistent with the descriptions of manifestations of CMV infection in liver transplant patients (Lautenschlager, 2009; LJungman; Griffiths; Paya, 2002).

R- recipients were at a higher risk of developing clinical manifestations of CMV infection (Bosch et al., 2011; Lautenschlager, 2009; Tryphonopoulos, 2011), especially in a high CMV prevalence region (75% positivity in liver donors – data not shown). These patients do not develop immunity to CMV and, in addition to the risk for CMV infection, often have more severe manifestations. Only 4 recipients (9,1%) were R-, as expected (CANNON, 2010). Two of these recipients (50%) had clinical manifestations of CMV syndrome, and tended to have a shorter disease-free time in the first 2 months post-transplant. One and four-year survival rate of R- recipients was lower (50% and 25%, respectively), with a clear worse overall outcome for these patients. Only one patient of this group remained alive.

R+ recipients are less likely to develop manifestations of CMV infection (Bosch, 2011; Lautenschlager, 2009; Tryphonopoulos, 2011). Even so, this immunity is not completely effective (Mocarski, 2007). In this study, 40% of R+ recipients developed manifestations of CMV syndrome. Consequences, however, are less significant due to some protection from acquired immunity (Mocarski 2007; Razonable, 2008).
Indirect effects of CMV infection in liver transplant recipients arise from immunomodulation promoted by the virus (Kumar et al., 2009; Razonable, 2008). Thus, opportunistic infections, rejection, biliary stenosis and HCV recurrence may be consequences of CMV infection (Fishman, 2007; Lautenschlager, 2009; LJungman; Griffiths; Paya, 2002; Razonable, 2008). We observed these manifestations in HCV+ recipients, which presented a 25% of HCV recurrence, mostly associated to positive CMV antigenemia without CMV syndrome. Three of these patients died. Besides that, other two HCV+ recipients had possible indirect effects of CMV infection: one rejection and one non-anastomotic biliary stricture, who died at re-transplantation.

HCV+ recipients form a special group in liver transplant. Despite not reaching statistical significance, the absolute difference of four-year survival rates is relevant (Figure 2). They are patients with a high risk of complications, such as HCV recurrence, graft impairment, and risk of retransplantation. Even without presenting clinical manifestations of CMV syndrome, its presence in antigenemia was associated with complications that arose in HCV+ recipients.

Mortality is also associated to CMV infection in liver recipients (Fishman, 2007; Lautenschlager, 2009; Razonable, 2008). In a four-year follow-up, twelve recipients have died, six (50%) from infectious diseases (two R- recipients). Incidence of positive CMV antigenemia was high (83.3%), as well as CMV syndrome (66.7%), which is expected in a setting with no preventive treatment (Fishman, 2007). Even non-infectious deaths have CMV infection association (Fishman, 2007; LJungman; Griffiths; Paya, 2002; Razonable, 2008). Two recipients with positive CMV antigenemia presented indirect effects of CMV infection and died as consequence.

The use of antigenemia helps to identify cellular inclusions in peripheral blood leukocytes related to CMV replication, quantifying the number of positive cells in a standard base number of leukocytes. Its limitations include the need to be performed with whole blood, short time for preparation and limitations in leukopenic patients (Marchetti et al., 2011). Comparatively, the use of the polymerase chain reaction (PCR) is more sensitive and specific in liver transplant recipients with clinical manifestations of CMV (Cortez et al., 2003; Marchetti et al., 2011), allows the use of serum samples, has a longer preparation time, does not depend on the number of leukocytes and is quantitative for the number of viral copies. However, PCR is more costly than antigenemia and many services in developing countries do not have access to it.

In this study, antigenemia was positive in 52 of the 344 samples obtained (15.1%), and in 24 of the 44 recipients (54.5%). Clinical manifestations of CMV syndrome were present in 18 of the 44 recipients (40.9%). Using only positive antigenemia assays, since the negative assays are a strong indicator of the absence of the disease, we found a cut-off of 8 positive cells per 200,000 leukocytes to determine the presence of clinical manifestations of CMV syndrome.

Marchetti compared rt-PCR (real-time PCR) and antigenemia in the diagnosis of CMV infection and found a significantly higher AUROC curve for the rt-PCR ($p = 0.0001$) (Marchetti et al., 2011). However, the authors used 793 samples of 230 recipients from various transplants (126 recipients of bone marrow, 92 kidney recipients, 11 liver recipients, and 1 heart recipient). This mixes patients with different immunosuppressive regimens and different levels of risk for CMV infection based on the transplanted organ or tissue, and did not establish a cut-off for the diagnosis of CMV syndrome.

The use of analysis of probability ratios (PR)

has greater relevance to the clinical applicability of a diagnostic test. In this study, we found a positive PR 3.5, with a relatively narrow confidence interval, reflecting a moderate increase on the pretest probability of disease. As for negative PR (0.15), this also had a moderate effect on the pre-test probability, but with a wide confidence interval. Thus, the use of this cut-off of > 8 positive cells / 200,000 leukocytes in quantitative antigenemia would have a greater applicability for the clinical diagnosis than for the exclusion of CMV syndrome in liver transplant recipients.

CONCLUSIONS

There was a high incidence of CMV infection in liver transplant recipients in a high CMV seroprevalence region, diagnosed by both clinical manifestations and antigenemia. This infection had a strong association with poorer outcome in R- recipients and HCV+ recipients. The use of antigenemia is diagnostic for CMV syndrome, with a cut-off of > 8 cells/200,000 leukocytes, and its performance was deemed adequate based on its accuracy.

Despite their costs, preventive strategies need to be developed for liver transplant recipients in high CMV prevalence regions, mostly for R- recipients and HCV+ recipients.

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