

## Hepatitis C and liver transplantation

Tsoulfas G<sup>1</sup>, Goulis I<sup>2</sup>, Giakoustidis D<sup>1</sup>, Akriviadis E<sup>2</sup>, Agorastou P<sup>2</sup>, Imvrios G<sup>1</sup>, Papanikolaou V<sup>1</sup>

<sup>1</sup> Surgical Department of Transplantation, Aristotle University, Hippokratio General Hospital, Thessaloniki, Greece

<sup>2</sup> Department of Gastroenterology, Aristotle University, Hippokratio General Hospital, Thessaloniki, Greece

### Abstract

Cirrhosis due to chronic hepatitis C is the leading indication for liver transplantation in Europe, United States and Japan. Reinfection after liver transplantation is universal and chronic liver disease develops in at least 70% of patients at 3 years, with an accelerated course compared to the nontransplant setting. These facts underscore the need for a better understanding of hepatitis C infection and the various treatment modalities. This paper attempts a brief review of the scope of the disease, as well as the different treatment modalities, with special emphasis given to orthotopic liver transplantation. Hippokratia 2009; 13 (4): 211-215

**Key words:** hepatitis C virus, hepatocellular carcinoma, orthotopic liver transplantation

**Corresponding author:** Tsoulfas Georgios, 14 Athinas Str, NeoPanorama, 552 36 Thessaloniki, Greece, Tel: 2310 275 073, E-mail: tsoulfasg@msn.com

### Epidemiology

Hepatitis C virus (HCV) was first characterized in late 1980s. It represents an RNA flavivirus with 6 major genotypes and more than 50 subtypes<sup>1</sup>. Genotype 1 predominates worldwide, accounting for 60-75% of cases, while it is estimated that 123-170 million people are living with HCV infection, with approximately 35,000 new infections per year in the US<sup>2,3</sup>. It shows a significant variation in nucleotide sequence, as well as a propensity to mutate. The most common mode of transmission is intravenous drug abuse (IVDA), with blood transfusions at that position prior to 1990; other ways include tattoos, hemodialysis, sexual contact, perinatal transmission and occupational exposure. In an anonymous survey conducted at the annual International Liver Transplantation Society meeting in Barcelona in 2003, in a sample of 117 liver transplant surgeons that responded there was an HCV prevalence of 0.8%, whereas the respective prevalence in their patient population was 31-40%<sup>4</sup>.

In the United States approximately 4 million people are infected, most less than 50 years old. In Greece the estimated prevalence is 1-2% of the general population, one of the higher ones in Europe<sup>5</sup>. The magnitude of the problem becomes even more evident if we consider that in the US there are nearly 10,000 deaths annually due to HCV related diseases, with HCV also being responsible for nearly half of all hepatocellular carcinoma (HCC) cases, with the high risk of developing HCC since the development of cirrhosis is 3-4% per year<sup>6</sup>. As a result HCV-cirrhosis has become the most common indication for liver transplantation in the US, accounting for 35-40% of all cases<sup>7</sup>.

### Natural history of HCV infection

It has been estimated that 75% to 85% of individuals infected with HCV progress to chronic infection, per-

sisting for at least 6 months after onset, with the rate of chronic infection varying by age, sex, race, and immune system status<sup>8</sup>. Long-term infection has been associated with serious clinical sequelae, including development of hepatic fibrosis, cirrhosis of the liver, portal hypertension and HCC<sup>9-11</sup>. Although the natural history of HCV infection is believed to be variable, it is estimated that up to 20% of chronically infected individuals will develop liver cirrhosis over a 20- to 25-year period, and that these individuals are at increased risk of developing end-stage liver disease or HCC<sup>9-10</sup>. Of note, progression to cirrhosis can occur rapidly, even in patients with early-stage disease<sup>12</sup>. The pathway of acute hepatitis leading to chronic persistent hepatitis, which in turn may lead to cirrhosis, can show variations, such as spontaneous resolution, or the more threatening fulminant hepatic failure or cholestatic hepatitis<sup>12</sup>.

There are also extrahepatic manifestations of HCV infection, which can be just as harmful. These include depression, diabetes and autoimmune-related ones, such as cryoglobulinemia, renal failure and porphyria cutanea tarda. Specifically, the cryoglobulinemia syndrome, which includes proteinuria, neuropathy and arthritis, can increase the risk of cirrhosis by a factor of 4.9 and is also associated with early recurrence after transplantation and high severity<sup>13,14</sup>.

### Treatment of chronic HCV infection

The main reason to treat patients with chronic HCV infection, no matter how imperfect the treatment may be, is the fact that 20% of chronic hepatitis patients, will develop cirrhosis over 20-30 years and have to endure a 30% risk of decompensation and a 3-4% annual risk of HCC. Regarding treatment, there is an accepted terminology, which includes:

- a) EVR (Early virologic response): > 2-log decrease in HCV-RNA within 12 weeks of treatment,
- b) ETVR (End of treatment response): absence of HCV-RNA at completion of treatment, and
- c) SVR (sustained virologic response): persistent absence of HCV-RNA 6 months after the completion of treatment.

The guidelines for treatment of HCV infection in the non-transplant population are mainly aimed at patients with high risk of cirrhosis (Genotype 1, high HCV-RNA titer, liver biopsy showing early fibrosis with inflammation, all patients with chronic hepatitis)<sup>15</sup>. The current guidelines include treatment with pegylated interferon (PEG-IFN  $\alpha$ -2a) at 180 $\mu$ g/week and Ribavirin at 1000 mg/day (<75kg) or 1200 mg/day (>75kg). Complications of therapy include those attributed to a) IFN: depression, exacerbation of autoimmune diseases/transplant rejection, bone marrow suppression and flu-like syndrome, and b) Ribavirin: hemolytic anemia (increased incidence with renal dysfunction) and teratogenicity (even in males) making contraception mandatory during and 6 months after treatment.

The problem with the medical treatment of HCV in patients with cirrhosis is that it is not highly successful and, more importantly, very hard to tolerate for the patients, who in addition to HCV have to deal with the hardships of cirrhosis (encephalopathy, fatigue, ascites, muscle wasting), all of which take a toll on the human body. The only treatment that can address both HCV and cirrhosis is liver transplantation.

#### **Considerations in liver transplant recipients with HCV infection: pre-transplantation HCV infection treatment**

The rationale for attempting treatment in the pre-transplant setting with IFN and Ribavirin, despite their significant side effects, is that there is a 30% higher risk of graft loss if the HCV-RNA titer is high at the time of transplantation<sup>16</sup>. This was shown in a prospective analysis of 166 HCV positive patients from 3 centers where 5-year survival was 57% when the HCV-RNA titer was >1x10<sup>6</sup> versus 84% when it was less than 1x10<sup>6</sup>. Another reason to attempt pre-transplant treatment is that SVR may be achieved in 30% of these patients, two-thirds of which will remain virus-free post-transplant<sup>17</sup>.

To temper the severity of the side effects some centers have proposed the use of a low accelerating dosage regimen. In a study of 124 patients with a mean Model for End-stage Liver Disease (MELD) score of 11, SVR was 13% for genotype 1 and 50% in patients infected with non-1 genotypes, whereas about 80% of patients who became HCV-RNA negative prior to the transplant remained negative following liver transplantation<sup>18</sup>.

Overall, although the benefit of pre-transplantation HCV treatment may not be universal and the side effects are significant, it certainly has application; especially in patients with lower (less than 18) MELD scores.

#### **Transplantation results**

In the United States, end-stage liver disease caused by HCV has become the most common indication for orthotopic liver transplantation (OLT)<sup>19,20</sup>. Unfortunately, it has become increasingly evident that HCV recurrence after OLT, as measured by PCR detection of HCV RNA, is nearly universal and may lead to progressive allograft injury and failure<sup>21,22</sup>. Moreover, histological evidence of HCV recurrence is apparent in approximately 50% of transplant recipients, with ensuing graft failure in 10% of patients by the fifth postoperative year<sup>23</sup>.

Despite the risk of HCV recurrence, patients undergoing OLT for HCV have been reported to exhibit comparable overall patient and graft survival rates when compared with other indications for liver transplantation<sup>20,22,24,25</sup>. In one of the largest series from the University of California Los Angeles evaluating their 10-year experience with over 500 patients, they reported patient and graft survival rates at 1, 5, and 10 years of 84%, 68%, and 60%, and 73%, 56%, and 49% respectively, with an overall median time to HCV recurrence of 34 months<sup>26</sup>. This same group found that neither HCV recurrence, nor HCV-positive donor status significantly decreased patient and graft survival rates by Kaplan-Meier analysis. However, the use of HCV-positive donors reduced the median time to recurrence to 22.9 months compared with 35.7 months after transplantation of HCV-negative livers. The finding that the earlier the recurrence of HCV, the greater the negative impact on patient and graft survival underscores the importance of post-transplantation follow-up of these patients and treatment for the HCV recurrence.

#### **Patterns of post-transplantation recurrence**

Overall, recurrence of HCV infection after liver transplantation is almost universal. Viral titers are low immediately post-transplantation but then significantly increase, reach pre-transplant levels at 48 hours post-transplantation, and peak at 4 months. These same titers can rise up to 10- to 100-fold higher than the pre-transplantation ones, with the 4-month titer believed to be an indication of future histological activity<sup>27</sup>.

There are three patterns of recurrence. The first one is acute hepatitis with elevated transaminases mainly. The second one is the development of chronic hepatitis, which shows a more accelerated progression when compared to chronic hepatitis in the non-transplant population. It leads to cirrhosis in 25% of patients within five years<sup>28-30</sup>. The progression to fibrosis can be linear, or rapid following an initial stabilization period, or a logarithmic increase initially followed by a slower, almost linear development<sup>31,32</sup>. The rapid progression of fibrosis serves as an explanation for the more ominous natural history of the HCV recurrence in the hepatic implant, compared to the non-immunosuppressed population<sup>33,34</sup>. The faster progression of HCV disease in the transplanted patients can also be seen by the significantly greater probability of decompensation in patients that have already reached

the cirrhotic stage. The risk of decompensation during the first year after cirrhosis has developed in a transplant patient is between 17-42%, significantly higher than the equivalent in the immunocompetent population (28% in 10 years)<sup>35,36</sup>. The overall prognosis for these decompensated patients is especially bad, as their 1- and 3-year survival is only 22% and 10% respectively. Prognostic factors that have been implicated in this decompensation include advanced MELD and Child-Pugh score, low albumin levels, a short period between the time of transplantation and the development of cirrhosis and a hepatic vein pressure gradient  $>10$ <sup>35,36</sup>. The third type of recurrence, which is the most aggressive one, is fibrosing cholestatic hepatitis, which can lead to graft failure within 6 months. The latter is associated with very high immunosuppression (steroid pulse or induction treatment), very high HCV-RNA titers and ALT $>500$  IU,  $\gamma$ GT  $> 1,000$  IU and bilirubin  $> 6$  mg. Histopathologically, there is scant inflammation but central hepatocyte ballooning<sup>37</sup>. Factors that lead to an increased risk of progression to fibrosis in recurrent HCV include older donor age, high HCV-RNA titer pre-transplant, therapy with OKT3, steroid boluses for the treatment of acute cellular rejection and genotype 1b. Regarding the effects of immunosuppression it is widely believed that keeping the net immunosuppression high or making abrupt changes to the treatment regimen may be especially detrimental, as far as HCV progression is concerned.

There are several factors that contribute to the faster progression of fibrosis in patients who have undergone liver transplantation secondary to HCV. These include use of liver grafts from older donors ( $>60$ ), the use of intense immunosuppression or antithymocyte globulin for the treatment of acute rejection episodes, CMV co-infection, a high viral load pre-transplant, genotype 1, HIV co-infection, and retransplantation<sup>38-39</sup>. The role of living donor liver transplantation has not been completely elucidated, although there does not appear to be a significant difference in the results<sup>40</sup>. Moreover, although not implicated in a causal manner, the following factors are important for the prognosis of decompensation: a) early recurrence within 6 months following transplantation, b) significant steatosis and cholestasis in the initial biopsies, c) significant increase in the transaminases level in the early post-operative period, and d) mild to severe inflammatory activity or advanced fibrosis in the liver biopsy 1 year after transplantation.

Knowledge of all the above factors and the fact that they may affect the progression of HCV disease after transplantation has important implications regarding certain therapeutic maneuvers. Specifically, using organs from younger donors for HCV recipients, decreasing cold ischemia time, careful prophylaxis against CMV infection may improve results.

#### **Treatment of HCV recurrence**

After transplantation factors other than HCV infection may lead to fibrosis, such as cytokine interactions

secondary to rejection, the effect of certain viruses, such as cytomegalovirus (CMV), and modulation of fibrogenesis/fibrosis by immunosuppressive agents. Therefore changes in histology as well as the viral status need to be documented in response to antiviral therapy. Moreover, as in nontransplant setting, clinical benefit may occur even when viral clearance is not achieved, leading to histological and clinical improvement.

The first option is that of pre-emptive therapy, early post-transplant, with the rationale that low HCV RNA titers are likely to be more susceptible. The difficulty with this strategy is that it is poorly tolerated, mainly due to associated problems such as leucopenia and renal failure, which in turn lead to a low SVR, because of frequently required dose reductions. Furthermore, there is no difference in the histological outcome when compared with treatment of established, recurrent HCV.

Antiviral therapy for recurrent HCV infection and disease after liver transplantation has only been evaluated in 16 randomized studies (534 patients) and thus robust data to evaluate efficacy is scanty. However, it is clear from both these randomized and the 74 nonrandomized studies (2061 patients), that treatment is far less effective and has more side effects than chronic HCV hepatitis pre-transplant therapy<sup>41</sup>. Moreover the data concerning combinations of either interferon or pegylated interferon with ribavirin mainly reflect EVR (maximum 36%) or ETVR (maximum 32%) with very little data on SVR. Thus, currently there is neither easily applicable, nor reasonably effective antiviral therapy for HCV recurrence after liver transplantation, considering the frequency of side effects and the need to reduce doses or to discontinue therapy. This has led to the use of protocols which combine PEG-IFN and Ribavirin, which however are not tolerated by around 40% of patients and show a 26-45% SVR, including a histological response in some<sup>42,43</sup>. Finally, genotype 1 is less likely to be susceptible to treatment.

Finding the right treatment for established, recurrent HCV infection after liver transplantation is intimately associated with two other issues: the effect of immunosuppression and how to confidently identify the severity of the recurrence. Specifically, although there are conflicting findings regarding immunosuppression strategies between different studies, most agree that the recent era of liver transplantation (since year 2000) presents the worst recurrence and survival rate for HCV infected patients. The main culprit appears to be changes in immunosuppression strategies, and especially the increased use of induction medications (OKT3, Thymoglobulin) and high pulse steroids for episodes of rejection<sup>44</sup>. The question of what type of an effect low-dose maintenance steroids have on HCV recurrence has not been fully answered.

The issue of correctly identifying the severity of HCV recurrence is both a clinical and histopathological one. Specifically, clinically the elevation of the liver transaminases is the first sign that there is a change. It does not, however, answer the question of whether this is because of HCV infection recurrence or because of acute cellular

rejection (or even worse, both). An elevation of the HCV RNA titer would certainly point to one direction, but the ultimate diagnosis may have to rest on a liver biopsy. That presents its own problems, as there is no infallible histopathological marker. Acute cellular rejection is suspected if there is significant ductitis, portal endothelitis and the presence of eosinophils, whereas the diagnosis of recurrent HCV infection is more likely if there is sinusoidal dilatation and lymphoid aggregates. HCV-RNA in the liver biopsy specimen more than 10,000 copies/mg of tissue may also point towards HCV infection. Part of the problem is that the two may coexist to a certain extent and at that point any adjustments in immunosuppression are more of an art than a science. Specifically, treating acute cellular rejection in the background of HCV may require the more graceful application of increased doses of existing medications (tacrolimus or cyclosporine), rather than the heavy-handed approach of multiple steroid pulses, or even stronger medications, such as thymoglobulin.

#### Future directions and needs

Despite the difficulties that the physician faces in the treatment of HCV infection, it cannot be ignored because of the vast number of patients it affects worldwide. Indeed, all efforts need to focus on better designed multicenter trials, instead of small, sporadic studies that leave conflicting trails and better diagnostic accuracy in distinguishing between recurrent HCV infection and acute cellular rejection. Some encouraging news may arise from the direction of new medications, such as new HCV protease inhibitors or long acting types of interferon, which may be combined in order to improve the results and, possibly, decrease the side effects<sup>45</sup>. These are being evaluated in ongoing human trials, initially in the pre-transplant chronic HCV population, with the transplant data yet to come.

#### References

- Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science*. 1989; 244: 359-362.
- Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis*. 2005; 25: 5: 558-567
- WHO. Hepatitis C – global prevalence (update). *Wkly Epidemiol Rec*. 1999; 74: 425-427.
- Thorburn D, Roy K, Wilson K, et al. Anonymous study of hepatitis C virus (HCV) prevalence in liver transplant surgeons. *Liver Transpl*. 2006; 12: 1084-1088.
- Gogos CA, Fouka KP, Nikiforidis G, et al. Prevalence of hepatitis B and C virus infection in the general population and selected groups in South-Western Greece. *Eur J Epidemiol*. 2003; 18: 551-557.
- Davis GL, Albright JE, Cook SF, Rosenberg DM. Projecting future complications of chronic hepatitis C in the United States. *Liver Transpl*. 2003; 9: 331-338.
- Szabo E, Lotz G, Paska C, Kiss A, Schaff Z. Viral hepatitis: new data on hepatitis C infection. *Pathol Oncol Res*. 2003; 9: 215-221.
- Hoofnagle JH. Course and outcome of hepatitis C. *Hepatology*. 2002; 36: (Suppl 1): S21-S29.
- National Institutes of Health. Management of Hepatitis C: 2002. NIH Consensus Statement. 2002; 19: 1-46.
- Strader DB, Wright T, Thomas DL, Seeff LB. American Association for the Study of Liver Diseases practice guideline: diagnosis, management and treatment of hepatitis C. *Hepatology*. 2004; 39: 1147-1171.
- Hoofnagle JH. Hepatitis C: the clinical spectrum of disease. *Hepatology*. 1997; 26 (Suppl 1): 15S-20S.
- Shiffman M, Di Bisceglie AM, Lindsay KL, et al. Peginterferon alpha-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology*. 2004; 126: 1015-1023.
- Kayali Z, Buckwold V, Zimmerman B, Schmidt W. Hepatitis C, cryoglobulinemia, and cirrhosis: A meta-analysis. *Hepatology*. 2002; 36: 978-985.
- Rayhill S, Kirby P, Voigt M, et al. Positive serum Cryoglobulin is associated with worse outcome after liver transplantation for hepatitis C. *Transplantation*. 2005; 80: 448-456.
- Kim AI, Saab S. Treatment of hepatitis C. *Am J Med*. 2005; 118: 808-815.
- Charlton M, Seaberg E, Wiesner R, et al. Predictors of patient and graft survival following liver transplantation for hepatitis C. *Hepatology*. 1998; 28: 823-830.
- Forns X, Garcia-Retortillo M, Serrano T, et al. Antiviral therapy of patients with decompensated cirrhosis to prevent recurrence of hepatitis C after liver transplantation. *J Hepatol*. 2003; 39: 389-396.
- Everson GT, Trotter J, Forman L, et al. Treatment of advanced hepatitis C with a low accelerating dosage regimen of antiviral therapy. *Hepatology*. 2005; 42: 255-262.
- Wright TL. Liver transplantation in patients with chronic hepatitis B and C. In: Maddrey WC, Sorrell MF, eds. *Transplantation of the liver*, 2d ed. Norwalk, CT: Appleton & Lange; 1995: 477
- Ghobrial RM, Farmer DG, Baquerizo A, et al. Orthotopic liver transplantation for hepatitis C: outcome, effect of immunosuppression and causes of retransplantation during an eight-year single center experience. *Ann Surg*. 1999; 6: 824-833.
- Wright TL, Donegan E, Hsu HH, et al. Recurrent and acquired hepatitis C viral infection in liver transplant recipients. *Gastroenterology* 1992; 103: 317-322.
- Rosen H, Gretch DR, Oehlke M. Timing and severity of initial hepatitis C recurrence as predictors of long-term liver allograft injury. *Transplantation*. 1998; 65: 1178-1182.
- Bizollon T, Ducer C, Trepo C. Hepatitis C virus recurrence after liver transplantation. *Gut*. 1999; 44: 575-578.
- Boker KHW, Dalley G, Bahr MJ, et al. Long-term outcome of hepatitis C virus infection after liver transplantation. *Hepatology*. 1997; 25: 203-210.
- Gane EJ, Portmann BC, Naoumov NV, et al. Long-term outcome of hepatitis C infection after liver transplantation. *N Engl J Med*. 1996; 334: 815-820.
- Ghobrial RM, Steadman R, Gornbein J, et al. A 10-year experience of liver transplantation for hepatitis C: analysis of factors determining outcome in over 500 patients. *Ann Surg*. 2001; 234: 384-394.
- Sreekumar R, Gonzalez-Koch A, Maor-Kendler Y, et al. Early identification of recipients with progressive histologic recurrence of hepatitis C after liver transplantation. *Hepatology*. 2000; 32: 1125-1130.
- Wiesner RH, Sorrell M, Villamil F. Report of the First International Liver transplant Society Consensus Conference on liver transplantation and hepatitis C. *Liver Transplant*. 2003; 9(Suppl 3): S1-S9.
- Gane E. The natural history and outcome of liver transplantation in hepatitis C virus-infected recipients. *Liver Transpl*. 2003; 9(Suppl 3): S28-S34.
- Samuel D, Forns X, Berenguer M, et al. Report of the Monothematic EASL Conference on liver transplantation for viral hepatitis (Paris, France, January 12-14, 2006). *J Hepatol*. 2006; 45: 127-143.

31. Berenguer M, Aguilera V, Prieto M, et al. Delayed onset of severe hepatitis C-related liver damage following liver transplantation: a matter of concern? *Liver Transpl.* 2003; 9: 1152-1158.
32. Neumann UP, Berg T, Bahra M, et al. Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. *J Hepatol.* 2004; 41: 830-836.
33. Berenguer M, Prieto M, San Juan F, et al. Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. *Hepatology.* 2002; 36: 202-210.
34. Burak KW, Kremers WK, Batts KP, et al. Impact of cytomegalovirus infection, year of transplantation, and donor age on outcome after liver transplantation for hepatitis C. *Liver Transpl.* 2002; 8: 362-369.
35. Kalambokis G, Manousou P, Samonakis D, et al. Clinical outcome of HCV-related graft cirrhosis and prognostic value of hepatic venous pressure-gradient. *Transpl International.* 2009; 22: 172-181.
36. Berenguer M, Wigh T. Liver transplantation in the management of chronic hepatitis C. In: H Thomas, S Lemon, AJ Zuckermann: *Viral Hepatitis*, 2005; p. 345-369.
37. International Liver Transplantation Society HCV Consensus Conference on Liver Transplantation and Hepatitis C. *Liver Transpl.* 2003; 9(Suppl 3): S1-S19.
38. Berenguer M. What determines the natural history of recurrent hepatitis C after liver transplantation. *J Hepatol.* 2005; 42: 448-456.
39. Samonakis D, Triantos CK, Thalheimer U, et al. Immunosuppression and donor age with respect to severity of HCV recurrence after liver transplantation. *Liver Transpl.* 2005; 11: 384-385.
40. Berenguer M. Live donor liver transplantation for hepatitis C: new data, old story. *Liver Transpl.* 2006; 12: 516-519.
41. Triantos C, Samonakis D, Stigliano R, Thalheimer U, Patch D, Burroughs A. Liver transplantation and hepatitis C virus: systematic review of antiviral therapy. *Transplantation.* 2005; 79: 261-268.
42. Rodriguez-Luna H, Khatib A, Sharma P, et al. Treatment of recurrent hepatitis C infection after liver transplantation with combination of pegylated interferon [alpha]2b and ribavirin: an open-label series. *Transplantation.* 2004; 77: 190-194.
43. Dumortier J, Scoazec JY, Chevallier P, Boillot O. Treatment of recurrent hepatitis C after liver transplantation: a pilot study of Peginterferon alfa-2b and ribavirin combination. *J Hepatol.* 2004; 40: 669-674.
44. Brown RS. Hepatitis C and liver transplantation. *Nature.* 2005; 436: 973-978.
45. De Clerq E. Emerging antiviral drugs. *Expert Opin Emerg Drug.* 2008; 13: 393-416.