Prediction of a sustained viral response in chronic hepatitis C patients who undergo induction therapy with double filtration plasmapheresis plus interferon- β /ribavirin

TORU ISHIKAWA¹, SATOSHI ABE¹, YUICHI KOJIMA¹, TOMOE SANO¹,
AKITO IWANAGA¹, KEI-ICHI SEKI¹, TERASU HONMA¹, TOSHIKI YOSHIDA¹,
MIHOKO YAMAZAKI², TAKEHITO SAKAI², KAZUYUKI TASAKI² and YASUSHI SUZUKI²

Departments of ¹Gastroenterology and Hepatology, and ²Nephrology,
Saiseikai Niigata Daini Hospital, Niigata, Niigata 950-1104, Japan

Received May 1, 2014; Accepted December 8, 2014

DOI: 10.3892/etm.2015.2340

Abstract. The aim of the present study was to determine predictors of a sustained virological response (SVR) with a regimen of double filtration plasmapheresis (DFPP) combined with interferon- β plus ribavirin (IFN- β /RBV) induction therapy prior to pegylated (PEG-IFN/RBV) standard of care (SOC) therapy for patients with chronic hepatitis C who had experienced SOC treatment failure. Predictors of a SVR were analyzed in chronic hepatitis C patients with genotype 1b hepatitis C virus (HCV), who had a high viral load. The patients had been unresponsive to previous IFN therapy and underwent induction therapy with IFN- β /RBV plus DFPP, which was performed five times during the same period, followed by PEG-IFN/RBV. In total, 10 patients received the combination DFPP plus IFN- β /RBV induction therapy prior to PEG-IFN/RBV therapy for the treatment of chronic hepatitis C. Two weeks after treatment initiation, a decrease in the HCV RNA levels of ≥ 2 log IU/ml occurred in 9/10 patients (90%), while a decrease of ≥ 4 log IU/ml was observed in 4/10 patients (40%). The HCV RNA levels at week 2 after treatment initiation in the SVR and non-SVR patients decreased by 5.0 ± 0.8 and 2.9 ± 1.1 log IU/ml, respectively. Despite no response to previous IFN therapy, three of the 10 patients (30%) experienced a SVR. The results indicated that a rapid virological response ensued following IFN- β /RBV induction and DFPP supplementary therapy. Although the level of interleukin-28B is an important predictor of a SVR, a decrease in the HCV RNA volume of ≥ 4 log IU/ml at week 2 after the

initial treatment is also an important predictor. Therefore, rapid virological reduction using DFPP, in addition to IFN- β /RBV induction therapy, is an important predictor of a SVR.

Introduction

Currently, the standard antiviral therapy for patients with chronic hepatitis C is combination therapy with pegylated interferon (PEG-IFN) and ribavirin (RBV). However, only ~50% of patients infected with the genotype 1 subtype of hepatitis C virus (HCV) achieve a sustained virological response (SVR) with this regimen (1). With the use of a triple therapy combining PEG-IFN, RBV and protease inhibitors, including telaprevir, a SVR can be obtained at a high rate, even for patients with HCV genotype 1b who have a high viral load (2,3).

However, a number of adverse effects have resulted from the use of triple drug therapy, including a rash, digestive tract symptoms and psychiatric symptoms. Previous studies have reported the discontinuation of treatment in a number of patients due to these adverse effects (4,5). These patients require treatment with standard of care (SOC) centered on PEG-IFN with RBV for refractory chronic hepatitis C.

It was previously reported that twice daily administration of IFN- β in the induction phase (the start of chronic hepatitis C treatment) to patients with refractory genotype 1b chronic hepatitis C with a high viral load may improve the early virological response of HCV and increase the SVR rate with a transition to SOC (6). In addition, combination therapy with IFN- β and RBV has been covered by the National Health Insurance Program in Japan since October 2009, and an earlier virological response is expected with the introduction of this treatment.

Double filtration plasmapheresis (DFPP) has been used for a number of years as a therapeutic method for autoimmune diseases and hyperlipidemia. The therapy functions by eliminating high molecular weight substances, mainly immunoglobulins and lipids, from plasma based on a molecular sieving effect using hollow fiber membranes with two different pore sizes. Since April 2008, DFPP has also been covered by

Correspondence to: Dr Toru Ishikawa, Department of Gastroenterology and Hepatology, Saiseikai Niigata Daini Hospital, 280-7 Teraji, Niigata, Niigata 950-1104, Japan
E-mail: toruishi@ngt.saiseikai.or.jp

Key words: double filtration plasmapheresis, hepatitis C virus, interferon- β

the National Health Insurance Program for the treatment of chronic hepatitis C due to its ability to directly eliminate HCV from the plasma. Among attempts to improve the efficacy of IFN therapy for chronic hepatitis C, an approach that differs from the idea of combined drug use to achieve a rapid virological response is promising (7).

Therapeutic outcomes for chronic hepatitis C patients with genotype 1b, who have a high viral load and are considered to have the most difficult subtype to treat, have remained unsatisfactory. Virus eradication in the early stages of the disease is important, which can be achieved through the use of IFN. Specifically, a SVR is known to be high when the virus is eliminated from the blood within two weeks from the start of administration (8). Therefore, whether virological reduction is associated with treatment efficacy when these combination therapies are used, and whether a virological response occurs at an early stage are important issues that require further study.

Through using DFPP in addition to IFN- β /RBV induction therapy, an early virological response may be ascertained. In addition, predictions can be formed with regard to which patients may achieve a SVR. The present study retrospectively analyzed treatment outcomes following IFN- β /RBV induction and DFPP therapy prior to SOC for chronic hepatitis C patients with the genotype 1b subtype. These patients had a high viral load and were cases of relapse or non-response to SOC. Furthermore, whether the extent of virus reduction at week 2 after treatment initiation was able to predict the development of SVR was analyzed.

Materials and methods

Patients and treatment protocol. In total, 10 patients with chronic hepatitis C of genotype 1b were included in the study. The patients had a high viral load and had not responded to previous IFN therapy. IFN- β (Feron; Toray Industries, Inc., Tokyo, Japan; Daiichi Sankyo Co., Ltd., Tokyo, Japan) and RBV (Rebetol; MSD K.K., Tokyo, Japan) induction therapy with DFPP was performed five times during two weeks. During DFPP induction therapy, the patients were treated with IFN- β at 3 million units (MU) twice daily for two weeks. The 3 MU of IFN- β (Feron; Toray Industries, Tokyo, Japan) was dissolved in 100 ml physiological saline or 100 ml of 5% glucose for injection, and the solution was administered twice daily by intravenous drip infusion over 30 min in the morning and evening. Subsequent to IFN- β treatment, 1.5 μ g/kg PEG-IFN- α 2b (Pegintron; Schering-Plough, Tokyo, Japan) was administered once a week. RBV (Rebetol; Schering-Plough) was administered at a dose of 600 mg/day (200 mg after breakfast, 400 mg after dinner) to patients weighing <60 kg, 800 mg/day (400 mg after breakfast, 400 mg after dinner) to those weighing 60–8 kg, and 1,000 mg/day (400 mg after breakfast, 600 mg after dinner) to those weighing >80 kg. The daily dose of RBV was reduced by 200 mg once the hemoglobin levels had decreased to <10 g/dL, and RBV was discontinued once the hemoglobin levels had decreased to <8.5 g/dL. When side effects made continued administration difficult, or when HCV RNA levels did not decrease, IFN was discontinued at the discretion of the physician.

DFPP. For DFPP, a double lumen catheter (Unitika Ltd., Osaka, Japan) was placed in the femoral vein from the right inguinal region as vascular access. A Plasmauto iQ21 (Asahi Kasei Medical Co., Ltd., Tokyo, Japan) apheresis system was used for DFPP, with a Plasmaflo OP (Asahi Kasei Medical Co., Ltd.) plasma separator (primary membrane) and a Cascadeflo EC-50 W (Asahi Kasei Medical Co., Ltd.) plasma component separator (secondary membrane). The plasma processing volume used for DFPP was 50 ml/kg and the blood flow rate was 120–130 ml/min, with one session lasting ~2 h. Nafamostat mesilate was used as an anticoagulant for the first DFPP session only, after which heparin sodium was used for the second and subsequent sessions. DFPP replacement fluid was not required. DFPP was performed a total of five times over a period of two weeks, but never on more than two consecutive days in order to prevent the blood fibrinogen levels decreasing to <100 mg/dl (9).

Evaluation of IFN therapy. The quantity of HCV RNA was measured using the COBAS® TaqMan® HCV test (detection limit, 1.2 log IU/ml; Roche Diagnostics, Tokyo, Japan) and the high-range AMPLICOR™ HCV monitor method (detection limit, 5 kIU/ml; Roche Diagnosis).

A rapid virological response (RVR) was defined as undetectable serum levels of HCV RNA at week 4, while an early virological response was defined as undetectable serum levels of HCV RNA at week 12. A late virological response was defined as detectable serum levels of HCV RNA at week 12 and a SVR was defined as an undetectable serum level of HCV RNA at week 24 after the initiation of treatment. No virological response was defined as being HCV RNA-positive throughout the treatment period.

Ethical considerations. Prior to the initiation of the study, written informed consent was obtained from each patient. The present study was approved by the Ethical Committee of Saiseikai Niigata Daini Hospital (Niigata, Japan), and was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis. Statistical analyses were performed using χ^2 or Fisher's exact tests for categorical data, and Student's t-test for continuous data. Subgroup analyses were performed to determine the contributing factors to EVR and SVR, and these factors were evaluated by logistic regression analysis. Multivariate analysis was conducted to identify independent prognostic factors using the Cox proportional hazards model to calculate the adjusted hazard ratio and 95% confidence interval. Statistical analyses were performed using StatView version 5.0 software (SAS Institute, Inc., Cary, NC, USA). All reported P values are 2-sided, and P<0.05 was considered to indicate a statistically significant difference.

Results

Clinical characteristics. In total, 10 subjects were included, of which six were male and four were female. The mean age of the patients was 63.0 $\pm$ 6.6 years (range, 54–73 years), and the average HCV RNA level was 7.0 $\pm$ 0.5 log IU/ml (range, 5.5–7.1), as shown in Table I.

Table I. Patient characteristics.

Characteristics	Patients
Gender, male/female (n)	6/4
Age (years)	63.0±6.6 (54-73) ^a
BMI (kg/m ²)	22.0±2.4 (20-27) ^a
Liver biopsy (n)	
Stage (F0/F1/F2/F3/F4)	0/4/4/0/1
Grade (A0/A1/A2/A3)	0/4/4/1
ALT (IU/l)	80.5±76.9 (23-282) ^a
AST (IU/l)	63.5±46.4 (30-173) ^a
PLT (x10 ⁴ /μl)	13.6±4.8 (8.7-23.0) ^a
Hb (g/dl)	13.0±1.4 (11.0-16.0) ^a
γGTP (IU/l)	41.0±24.8 (14.0-92.0) ^a
HbA1c (%)	5.1±1.1 (4.9-8.4) ^a
Fibrinogen (mg/dl)	209.3±31.2 (176-257) ^a
TC (mg/dl)	145.5±25.6 (125-207) ^a
LDL-C (mg/dl)	80.8±14.7 (54-100) ^a
HDL-C (mg/dl)	43.0±12.7 (28-63) ^a
TG (mg/dl)	116.5±67.7 (57-265) ^a
HCV RNA (log IU/ml)	7.0±0.5 (5.5-7.1) ^a

^aData are presented as the median (minimum-maximum). BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PLT, platelet count; Hb, hemoglobin; γGTP, γ-glutamyl transpeptidase; HbA1c, glycated hemoglobin; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; HCV, hepatitis C virus.

Figure 1. Decrease in the levels of HCV RNA at week 2 after the start of treatment. HCV, hepatitis C virus; SVR, sustained virological response.

No proteinuria or depressive symptoms were observed in any of the patients during IFN-β/RBV administration; thus, the treatment was continued for two weeks.

HCV RNA levels. The mean decrease in the level of HCV RNA at week 2 after the start of treatment was 3.6±1.4 log IU/ml. The

achievement rate of a decrease of ≥2 log IU/ml after two weeks was 90% (9/10 patients), while a decrease of ≥4 log IU/ml was achieved in 40% of the patients (4/10 patients). The virus reduction in the SVR patients was 5.0±0.8 log IU/ml, while that in the non-SVR patients was 2.9±1.1 log IU/ml; the difference of which statistically significant (Fig. 1).

None of the background factors were found to be clinically significant in contributing to SVR (Table II).

Safety and adverse effects. No serious complications, including a catheter infection, were observed during extra-corporeal circulation and DFPP. However, one adverse effect of the DFPP therapy was decreased blood pressure, which was observed in four patients and occurred a total of six times. However, this adverse effect was managed with an infusion of ~100-200 ml physiological saline. In addition, in certain patients, the fibrinogen level was found to decrease temporarily to <100 mg/dl at the end of the DFPP treatment; however, the fibrinogen level was ≥100 mg/dl in all the patients at the final termination of DFPP.

Discussion

Almost 20 years have elapsed since IFN therapy was initiated for the treatment of chronic hepatitis C (10). Advances over the past several years have been marked, such that a SVR is currently obtained in approximately half of all patients who receive PEG-IFN/RBV combination therapy, even in patients with genotype 1b HCV and a high viral load. Direct antiviral agents that target HCV, including HCV protease inhibitors and polymerase inhibitors, have also been developed. There are high expectations for these new agents in significantly improving future treatment outcomes for patients with chronic hepatitis C. However, there are a number of problems associated with adverse effects in using these new agents. Considering the current status of chronic hepatitis C in Japan, which has a particularly large elderly population, questions of whether the new agents can be used and whether treatment with these agents can be delayed until they are approved may be problematic.

In addition to these pharmaceutical advances, there are expectations for improved SVR rates with combination therapies that contribute to virus reduction. DFPP has long been used as a method for treating diseases by predominantly eliminating immunoglobulins and other disease-associated proteins. HCV particles are hypothesized to have diameters of 55-65 nm; therefore, selective elimination of substances of this size may enable the efficient elimination of HCV particles. The Cascadeflo EC-50 W, which is already used in DFPP therapy, has a maximum pore size of 30 nm. Therefore, theoretically, HCV particles are unable to pass through this membrane and can be directly eliminated from extracorporeally circulating blood. In addition, the safety of this extracorporeal circulation equipment has previously been established (10).

In the developmental stage, a RVR is expected from DFPP therapy; thus, DFPP may be performed at the start of IFN administration. Various other usage methods have also been considered, including using DFPP as a supplementary treatment in patients who do not obtain a virological response

Table II. Factors contributing to a SVR.

Characteristics	SVR (n=3)	non-SVR (n=7)	P-value (t-test)
Gender, male/female (n)	1/2	5/2	-
Age (years)	57.0±9.6 (54-72) ^a	63.0±5.1 (61-73) ^a	0.3137
BMI (kg/m ²)	24.0±2.8 (21-27) ^a	22.0±2.2 (20-26) ^a	0.3632
Liver biopsy (n)			
Stage (F0/F1/F2/F3/F4)	0/1/2/0/0	0/3/2/0/1	-
Grade (A0/A1/A2/A3)	0/1/1/1	0/3/3/0	-
ALT (IU/l)	41.0±44.6 (23-282) ^a	91.0±38.4 (30-140) ^a	0.5445
AST (IU/l)	48.0±48.2 (30-121) ^a	68.0±48.1 (31-173) ^a	0.5758
PLT (x10 ⁴ /ml)	14.8±3.4 (12.4-19.2) ^a	11.5±5.4 (8.7-23.0) ^a	0.8118
Hb (g/dl)	12.5±2.1 (11.7-15.7) ^a	13.4±1.3 (10.7-14.5) ^a	0.7221
γGTP (IU/l)	37.0±40.1 (14.0-92.0) ^a	41.0±19.4 (31.0-87.0) ^a	0.8104
HbA1c (%)	5.4±0.8 (4.9-6.5) ^a	5.1±1.3 (5.1-8.4) ^a	0.8604
Fibrinogen (mg/dl)	241.4±25.9 (201-249) ^a	201.9±33.3 (176-257) ^a	0.4007
TC (mg/dl)	169.0±15.0 (143-169) ^a	137.0±29.7 (125-207) ^a	0.6485
LDL-C (mg/dl)	81.0±7.0 (79-92) ^a	77.0±17.1 (54-100) ^a	0.5397
HDL-C (mg/dl)	54.0±12.7 (45-63) ^a	38.0±11.0 (28-54) ^a	0.2184
TG (mg/dl)	110±34 (77-145) ^a	123±79 (77-145) ^a	0.5445
HCV RNA (log IU/ml)			
Baseline	6.4±0.5 (6.2-7.1) ^a	7.0±0.6 (5.5-7.1) ^a	0.6072
Week 2	1.4±0.4 (1.2-2.0) ^a	3.9±1.5 (1.8-5.8) ^a	0.0315

^aData are presented as the median (minimum-maximum). BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PLT, platelet count; Hb, hemoglobin; γGTP, γ-glutamyl transpeptidase; HbA1c, glycated hemoglobin A1c; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; HCV, hepatitis C virus; SVR, sustained virological response.

during IFN administration. There have also been notable studies investigating the possibility of preventing HCV reinfection in liver transplant recipients, and marked effects have been obtained against post-transplant fibrosing cholestatic hepatitis (11). However, further studies with a greater sample size are required to investigate whether a SVR can be obtained with retreatment using a combination of DFPP in patients who did not achieve a SVR with SOC using PEG-IFN, which is currently the mainstream treatment.

To date, SVR rates have increased to 40-50% with SOC. When the timing of the virological response is considered, the treatment effect has increased to ~60% with treatment protocols that extend the treatment duration. Using any treatment method, the period up to four weeks from the start of administration has been shown to be important, and the SVR rate in patients in whom a virological response had previously been obtained is known to be much higher. Based on these observations, treatment strategies that aim to eliminate the virus from the blood at an earlier stage are being tried. Okushin *et al* reported that an HCV RNA response was obtained with a high rate early in treatment of administering IFN-β twice daily at the time of IFN therapy induction places importance on hepatic migration from venous administration and drug accumulation with consideration of pharmacokinetics (12).

Fujiwara *et al* reported a much higher viral elimination rate in the second week after the start of twice daily IFN-β therapy compared with a once daily regimen (13). Similarly, Izumi *et al* reported a more notable decrease in the level of HCV RNA in the patient group who received twice daily administration of IFN-β when compared with the patient group who received once daily administration (14). These observations demonstrate that while the half-life of venously-administered IFN-β in the blood is very short, the blood levels are maintained in the effective treatment range with twice daily administration (15). In addition, Asahina *et al* analyzed HCV dynamics in serum and peripheral blood mononuclear cells and demonstrated that twice daily administration of IFN-β exhibited a strong antiviral effect until the second phase (16).

Based on these studies, we previously investigated the efficacy of IFN-β induction therapy for patients with HCV of genotype 1b with a high viral load (6). In addition, DFPP supplementary treatment was found to contribute to early virus reduction, even in patients who relapsed following previous IFN-β induction therapy (17).

Using IFN-β in combination with RBV has become possible, and with a combination that also includes DFPP, virus reduction is hypothesized to occur in a shorter time.

Although the present study had the limitation of a small number of patients that exhibited a relapse or non-response with SOC, a SVR of 30% was obtained. There were no statistically significant differences observed in the background factors between the patients with and without SVR; however, the extent of virus reduction alone is hypothesized to be an important factor in therapeutic efficacy. Various factors are associated with treatment efficacy in chronic hepatitis C, including drug factors, virus factors and host factors. Among these, the host factor, interleukin (IL)-28B, is considered to be one of the most important. However, a decrease in the level of HCV RNA of ≥ 4 log IU/ml at week 2 after the start of induction therapy, obtained through using combination therapy with DFPP and IFN- β /RBV for patients with genotype 1b and a high viral load, is also important. If the decrease were to be < 4 log IU/ml, IFN therapy for chronic hepatitis C should aim to not only produce a SVR, but also improve hepatic function reserve.

In conclusion, although long-term observations are required in the future, treatment with DFPP combined with induction therapy of IFN- β /RBV for chronic hepatitis C was demonstrated to be a useful induction method. In addition, the results revealed that the SVR can be predicted from an early decrease in the viral load. Thus, in addition to IL-28B measurements, the extent of virus reduction may also be a potential therapeutic diagnostic indicator in response-guided therapy.

References

1. Manns MP, McHutchison JG, Gordon SC, *et al*: Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 358: 958-965, 2001.
2. Kumada H, Toyota J, Okanoue T, Chayama K, Tsubouchi H and Hayashi N: Telaprevir with peginterferon and ribavirin for treatment-naïve patients chronically infected with HCV of genotype 1 in Japan. *J Hepatol* 56: 78-84, 2012.
3. Chayama K, Hayes CN, Ohishi W and Kawakami Y: Treatment of chronic hepatitis C virus infection in Japan: update on therapy and guidelines. *J Gastroenterol* 48: 1-12, 2013.
4. Chopra A, Klein PL, Drinnan T and Lee SS: How to optimize HCV therapy in genotype 1 patients: management of side-effects. *Liver Int* 33 (Suppl 1): 30-34, 2013.
5. Cacoub P, Bourlière M, Lübke J, *et al*: Dermatological side effects of hepatitis C and its treatment: patient management in the era of direct-acting antivirals. *J Hepatol* 56: 455-463, 2012.
6. Ishikawa T, Kubota T, Abe H, *et al*: Efficacy of the regimen using twice-daily β -interferon followed by the standard of care for chronic hepatitis C genotype 1b with high viral load. *Hepatol Res* 42: 864-869, 2012.
7. Schettler V, Monazahian M, Wieland E, *et al*: Reduction of hepatitis C virus load by H.E.L.P.-LDL apheresis. *Eur J Clin Invest* 31: 154-155, 2001.
8. Neumann AU, Lam NP, Dahari H, *et al*: Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. *Science* 282: 103-107, 1998.
9. Yamashita T, Arai K, Sakai A, *et al*: Virological effects and safety of combined double filtration plasmapheresis (DFPP) and interferon therapy in patients with chronic hepatitis C: A preliminary study. *Hepatol Res* 36: 167-175, 2006.
10. Strader DB, Wright T, Thomas DL, Seeff LB; American Association for the Study of Liver Diseases: Diagnosis, management, and treatment of hepatitis C. *Hepatology*. 39: 1147-1171, 2004.
11. Taniguchi M, Furukawa H, Shimamura T, *et al*: Impact of double-filtration plasmapheresis in combination with interferon and ribavirin in living donor liver transplant recipients with hepatitis C. *Transplantation* 81: 1747-1749, 2006.
12. Okushin H, Morii K, Kishi F and Yuasa S: Efficacy of the combination therapy using twice-a-day IFN β followed by IFN α 2b in treatment for chronic hepatitis C. *Kanzo* 38: 11-18, 1997 (In Japanese).
13. Fujiwara K, Mochida S, Matsuo S, *et al*: Randomized control trial of interferon- β injections at 12-h intervals as a therapy for chronic hepatitis C. *Hepatology Res* 12: 240-251, 1998.
14. Izumi N, Kumada H, Hashimoto N, *et al*: Rapid decrease of plasma HCV RNA in early phase of twice daily administration of 3 MU doses interferon-beta in patients with genotype 1b hepatitis C infection: a multicenter randomized study. *Dig Dis Sci* 46: 516-523, 2001.
15. Hilfenhaus J, Damm H, *et al*: Pharmacokinetics of human interferon-beta in monkeys. *J Interferon Res* 1: 427-436, 1981.
16. Asahina Y, Izumi N, Uchiyama M, *et al*: A potent antiviral effect on hepatitis C viral dynamics in serum and peripheral blood mononuclear cells during combination therapy with high-dose daily interferon alfa plus ribavirin and intravenous twice-daily treatment with interferon beta. *Hepatology* 34: 377-384, 2001.
17. Ishikawa T, Higuchi K, Kubota T, *et al*: Complete early virological response was highly achieved by double filtration plasmapheresis plus IFN-beta induction therapy for HCV-1b patients with relapse or no response after previous IFN therapy. *Ther Apher Dial* 15: 400-405, 2011.