High efficacy of combined therapy with pegylated interferon plus ribavirin in patients with hemophilia and chronic hepatitis C

Maria Elisa Mancuso
Maria Grazia Rumi
Elena Santagostino
Silvia Linari
Antonio Coppola
Pier Mannuccio Mannucci
Massimo Colombo
for the Hepatitis Study Group of the Association of Italian Hemophilia Centers

Background and Objectives. Eradication of hepatitis C virus (HCV) is particularly difficult in patients with hemophilia. In this open, prospective, multicenter trial, the efficacy and tolerability of the combination therapy with pegylated interferon α2b (Peg-IFNα2b) plus ribavirin was evaluated in 64 human immunodeficiency virus (HIV) seronegative adult hemophiliacs with chronic hepatitis C naive to previous antiviral therapy.

Design and Methods. Peg-IFNα2b was administered at a dose of 1.5 μg/kg subcutaneously once weekly plus oral ribavirin 800-1200 mg/day, for 24 weeks to patients with HCV genotypes 2 and 3 (n=22, 34%) or for 48 weeks to those with genotypes 1 and 4 (n=42, 66%).

Results. Nine patients (14%) did not complete the study because of non-compliance (n=6) or side-effects such as decompensated diabetes, alanine aminotransferase flares and severe vomiting (n=3). Twenty-eight patients (44%) required dose reduction of either drug. Six months after stopping treatment a sustained virological response was achieved in 40 patients (63%), 19 with genotype 2 or 3 (86%) and 21 with genotype 1 or 4 (50%). A sustained virological response was significantly associated with an early virological response ($p<0.0001$), HCV genotypes 2 or 3 ($p=0.008$), no clinical evidence of cirrhosis ($p=0.02$) and higher pre-treatment serum alanine aminotransferase ($p=0.016$).

Interpretation and Conclusions. These results show that combination therapy with Peg-IFNα2b plus ribavirin is highly efficacious in hemophiliacs with chronic hepatitis C.

Key words: clinical observations, interventions, therapeutic Trials.

Haematologica 2006; 91:1367-1371

From the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Department of Medicine and Medical Specialities (MEM, ES, PMM); Division of Gastroenterology (MGR, MC), University of Milan and IRCCS Maggiore Hospital, Mangiagalli; Regina Elena Foundation, Milan, Italy; Hemophilia Center, Careggi Hospital, Florence, Italy (SL); Hemophilia Center, Federico II Hospital, Naples, Italy (AC).

Correspondence:
Pier Mannuccio Mannucci,
Via Pace 9, 20122 Milano, Italy.
E-mail: pmmannucci@libero.it

Eradication of the hepatitis C virus (HCV) with interferon-based therapy is the only approach that can halt progression of the infection to cirrhosis, clinical decompensation and perhaps hepatocellular carcinoma.[1] Eradication of HCV is particularly difficult in patients with hemophilia,[2-5] because they often present with hyporesponsiveness to interferon due to long-lasting infection with HCV, male gender, greater prevalence of HCV genotype 1 and high levels of viremia.[6] The combination of standard or pegylated interferon (Peg-IFN) and ribavirin is the mainstay of care for non-hemophilic patients with chronic hepatitis C, all the available studies showing the superiority of the combination including Peg-IFN over standard interferon.[7-9] The dose and duration of treatment depend on the HCV genotype and viral load, but with 48 weeks of treatment with Peg-IFN and ribavirin, genotypes 1 and 4 are harder to eradicate than genotypes 2 and 3, which respond to shorter treatment duration and lower ribavirin doses. Indeed, a sustained virological response was achieved in approximately 50% of the former *difficult-to-treat* patients, compared to 80% of the latter *easy-to-treat* patients.[9] So far, treatment of HCV in hemophilia has only been attempted with standard IFN at doses of 3 to 5 MU thrice weekly alone or in combination with ribavirin. This combination resulted in an approximately 30% rate of sustained virological responses in difficult-to-treat patients and 60% in easy-to-treat patients.[6,10-12] Pegylated interferons are expected to further improve the efficacy of anti-HCV therapy in hemophiliacs, because they have better pharmacokinetic and pharmacodynamic properties than standard interferons, thereby causing more efficient suppression of HCV.[13,14] This study reports the results on the effectiveness and safety of treatment with Peg-IFNα2b plus ribavirin in a cohort of 64 human immunodeficiency virus (HIV) seronegative hemophilic patients with chronic hepatitis C naïve to previous antiviral therapies.

Design and Methods

Study design

This was an open, prospective, multicenter study including 64 patients. It was conducted by the Association of Italian Hemophilia Centers in compliance with the

Declaration of Helsinki and with the principles of Good Clinical Practice. All patients gave written informed consent and the study was approved by the Institutional Review Board of the coordinating Center in Milan. Peg-IFNα2b (Peg-Intron®, Schering Plough, Milan, Italy) was given at doses of 1.5 μg/Kg body weight by subcutaneous injections once weekly for 24 weeks in patients with HCV genotypes 2 or 3 and for 48 weeks in those with genotypes 1 or 4. An oral dose of 800-1200 mg ribavirin (Rebetol®, Schering Plough, Milan, Italy) was given daily according to body weight (< 65 kg: 800 mg; 65-85 kg: 1000 mg; >85 kg: 1200 mg) for 24 weeks in patients with genotypes 2 and 3 or for 48 weeks in those with genotypes 1 or 4. Therapy was interrupted if HCV-RNA was still detectable within the first 6 months of therapy. The study also included a 6-month treatment-free follow-up period.

Patients

Adult patients with inherited coagulation disorders were eligible if they had been positive for serum HCV-RNA for at least 1 year, had alanine aminotransferase (ALT) values persistently or intermittently 1.5 times higher than the upper normal limit and had not been treated before with antiviral therapy. Inclusion criteria were values of hemoglobin (Hb) of ≥12 g/dL, white blood cell (WBC) count ≥3×10⁹/L, neutrophils (PMN) count ≥1.5×10⁹/L, platelet count ≥70×10⁹/L and serum bilirubin, albumin and creatinine within the normal limits. Exclusion criteria were decompensated liver disease (i.e. jaundice, ascites, gastrointestinal hemorrhage or encephalopathy), a coexisting severe medical or psychiatric illness, autoimmune diseases (including patients with serum titers of tissue autoantibodies greater than 1:80), alcohol intake greater than 60 g/day within one year before entry and coinfection with the hepatitis B virus or HIV.

A liver biopsy was not required because histological severity of hepatic disease was not a criterion for eligibility, and because this procedure is very costly in patients with hemophilia considering the need for replacement therapy and prolonged hospitalization. There was considered to be clinical evidence of liver cirrhosis in the presence of at least one feature among laboratory signs of portal hypertension (platelets <150×10⁹/L; albumin <3.5 g/L; serum cholinesterase activity <4500 U/L), endoscopic evidence of esophageal varices, portal hypertensive gastropathy, and/or abdominal ultrasound signs of irregular margins of the liver, dilated portal vein or splenomegaly.

Measurements

Biochemical and hematological measurements were obtained using standard methods at treatment weeks 2, 4 and then every 4 weeks and in the 6-month post-treatment follow-up at weeks 4, 12 and 24. Commercially available enzyme immunoassays were used for serum HBsAg, anti-HBs, anti-HBc, anti-HIV1/2 (Abbott Laboratories, Chicago, IL, USA), and anti-HCV (EIA-2 Boehringer Mannheim, Mannheim, Germany). Antibodies to nuclear, smooth muscle and liver-kidney microsomal antigens were detected on rat liver and kidney cryostat sections by immunofluorescence. Pre-treatment serum levels of HCV-RNA were quantitatively measured by a branched-DNA signal amplification assay (Versant HCV-RNA 3.0 Assay, bDNA, Bayer Diagnostics, Emeryville, CA, USA; sensitivity threshold of 615 IU/mL). HCV was genotyped by a hybridization assay (Inno-Lipa II, Innogenetics, Zwijndrecht, Belgium). Serum HCV-RNA was tested by nested reverse transcriptase polymerase chain reaction, using specific primers from the 5' non-coding region with a sensitivity limit of 25 IU/mL,[15] before treatment, at treatment week 24, 36 and 48 and during the post-treatment follow-up at weeks 4, 12 and 24.

Assessment of efficacy

The primary measure of efficacy was a sustained virological response, defined as undetectable HCV-RNA in serum at the end of the 6-month treatment-free follow-up. Analyses were done on the whole treated population, i.e. all patients who received at least one dose of the study medication. Non-response was defined as the persistence of HCV-RNA in serum at treatment week 24. End-of-treatment response was defined as undetectable serum HCV-RNA at the end of treatment. A virological breakthrough was defined as reversion to positive HCV-RNA any time during treatment. A relapse was defined as positive HCV-RNA occurring at any time after the end of treatment. The early virological response, defined as undetectable HCV-RNA at treatment week 12, was retrospectively evaluated in the available frozen serum samples.

Assessment of safety

Adverse events were graded as mild, moderate, severe and potentially life-threatening according to the WHO grading system. For severe adverse events other than anemia, the dose of Peg-IFNα2b was decreased by 25-50% and the dose of ribavirin was lowered to 600 mg/day. Full doses were restarted when the event was no longer present. If the event persisted, both drugs were discontinued. In patients with anemia, the dose of ribavirin was lowered to 600 mg/day for Hb drops below 10 g/dL, and discontinued if Hb dropped below 8.5 g/dL. The Peg-IFNα2b dose was reduced by 25-50% in patients with moderate neutropenia (PMN count 0.99-0.5×10⁹/L) and discontinued in those with severe neutropenia (< 0.5×10⁹/L). The Peg-IFNα2b dose was decreased by 50% if platelet counts dropped below 70×10⁹/L and discontinued for counts lower than 50×10⁹/L.

Statistical analysis

Continuous variables were expressed as median values and ranges, and were compared by the Mann-Whitney U test. Categorical variables were expressed as frequency and percent values, and compared by using Fisher's exact test. Response to treatment was evaluated by intention-to-treat analysis. All *p* values reported are two-sided.

Results

Between October 2001 and February 2004, 64 patients naïve to interferon therapy were enrolled at the Hemophilia Centers of Milan, Florence, Naples, Perugia, Pescara, Castelfranco Veneto, Udine and Vicenza. Their main characteristics are shown in Table 1.

Efficacy

Nine patients (14%) did not complete the study, six (9%) withdrew because of non-compliance to treatment and three (5%) because of side effects (Table 2). An end-of-treatment response was achieved in 21 patients with genotype 2 or 3 and in 22 with genotype 1 or 4 (95% *vs* 52%, $p<0.001$), and none of the responders had a virological breakthrough during the treatment period. During the post-treatment follow-up period, three patients (5%) relapsed, two with genotype 3 and one with genotype 1b. Therefore, a sustained virological response was ultimately achieved in 40 patients (63%), including 19 with genotype 2 or 3 (86%) and 21 with genotype 1 or 4 (50%) (Table 3). A sustained virological response was achieved in 39 of 57 patients without clinical evidence of cirrhosis and in one of seven patients with clinical evidence of cirrhosis (68% *vs* 14%, $p=0.009$), and was associated with higher pre-treatment serum ALT (111 *vs* 75 IU/L in non-responders, $p=0.016$). Rates of sustained virological response did not differ in relation to the patient's age, pre-treatment viremia, median disease duration and compliance with full-dose treatment (Table 3).

The early virological response was retrospectively evaluated in serum samples obtained at week 12 of treatment in a subgroup of 45 patients (27 with HCV genotype 1 or 4, and 18 with HCV genotype 2 or 3); HCV-RNA was negative in 33 of them (73%), 15 with HCV 1 or 4 (56%) and 18 with HCV 2 or 3 (100%). A sustained virological response was achieved in 28 of 33 early virological responders (75%), 12 (80%) with HCV genotype 1 or 4 and 16 (89%) with HCV genotype 2 or 3. Hence, an early virological response was a significant predictor of a sustained virological response ($p<0.0001$), regardless of the HCV genotype (Table 3). By multivariate analysis, the early virological response remained the only significant predictor of sustained virological response after adjusting for all the other variables ($p=0.01$). No significant differences were found between the doses of Peg-IFN and/or ribavirin given to patients who responded at week 24 compared to those given to patients who did not respond at week 24 (median cumulative Peg-IFNα2b dose: 2205 μg, range 1200-3019 *vs* 2156 μg, range 1181-3600, respectively; median ribavirin dose: 13.5 mg/kg/day, range 9.1-17.8 *vs* 13.5 mg/kg/day, range 9.3-16, respectively). In the 36

Table 1. Characteristics of the 64 patients included in the study.

Patients' characteristics	
Median age, years (range)	36 (20-64)
Male sex	58 (91%)
Hemophilia A	53 (82%)
Hemophilia B	1 (2%)
Other inherited bleeding disorders*	10 (16%)
Median disease duration, years (range)°	27 (15-53)
Median body mass index, kg/m2 (range)	24.5 (18.9-29.7)
Median ALT, IU/L (range)	99 (41-405)
Cirrhosis	7 (11%)
Median HCV-RNA, IU/mL (range)	6.0×10^5 (1.7×10^4-2.8×10^6)
HCV genotype	
1a	15 (23%)
1b	25 (39%)
1a/1b	1 (2%)
2	11 (17%)
3	11 (17%)
4	1 (2%)

**von Willebrand disease (n=8) , prothrombin deficiency (n=1), afibrinogenemia (n=1). °The onset of HCV infection was assumed to coincide with the first infusion of a non-virus inactivated coagulation factor concentrate manufactured from large pools of plasma.*

Table 2. Rates of discontinuation, dose reduction and side effects during treatment.

	Patients (%)
Withdrawal from treatment	9 (14%)
non-compliance	6 (9%)
side effects	3 (5%)
Dose reduction of both drugs	9 (14%)
Dose reduction of ribavirin for	
Anemia	11 (17%)
Dose reduction of Peg-IFN for	
Leukopenia/neutropenia	12 (19%)
Severe flu-like syndrome	5 (8%)
Severe fatigue	1 (2%)
AST/ALT increase	1 (2%)
Irritability	1 (2%)
Side effects	
Weight loss	26 (41%)
Fatigue	21 (33%)
Headache	8 (12%)
Irritability	6 (9%)
Depression	5 (8%)
Nausea	5 (8%)
Peg-IFN injection-site reactions	4 (6%)
Thyroid disorders	3 (5%)

Table 3. Epidemiological, clinical and virological characteristics of the patients according to virological response to combination therapy.

Patients' characteristics	*Sustained virological responders (n=40)*	*Non responders (n=24)*	*p value*
Median age, years (range)	36 (20-59)	31 (20-64)	ns
Median disease duration, years (range)	28 (18-53)	26 (15-43)	ns
Median ALT, IU/L (range)	111 (41-405)	75 (48-316)	0.016
Median HCV-RNA, IU/mL (range)	5.5×10^5 (1.7×10^4-2.8×10^6)	6.6×10^5 (1.6×10^5-1.2×10^6)	ns
HCV type 1 or 4	21/42 (50%)	21/42 (50%)	0.008
HCV type 2 or 3	19/22 (86%)	3/22 (14%)	
Cirrhosis	1 (2.5%)	6 (25%)	0.02
Compliant to full dose	20/28 (75%)	8/28 (29%)	ns
Early virological response	28/33 (75%)	5/33 (15%)	< 0.0001

patients non compliant to the full-dose therapy regimen, the median duration of full-dose therapy was 1 month (range: 3 days-7 months).

Safety

Twenty-eight (44%) patients required a dose reduction of either drug. The causes for Peg-IFN and/or ribavirin dose reduction and the most frequently observed side effects are shown in Table 2. Hb levels transiently decreased by a median value of 3.0 g/dL (range 0.4-6.0 g/dL) in 62 patients (97%). A decrease in Hb to less than 10 g/dL occurred in three patients (5%). No treatment discontinuation for anemia was needed. Decreases in platelet counts from baseline values were observed in 21 patients (33%), however none required treatment discontinuation. Mild and moderate neutropenia occurred in 23 (36%) and 26 (41%) patients, respectively. Side-effects requiring treatment discontinuation were decompensated diabetes, ALT flares and vomiting not responding to anti-emetic drugs.

Discussion

This study demonstrates that the combination of Peg-IFNα2b and ribavirin is an efficacious and safe treatment for hemophilic patients with chronic hepatitis C. The 50% rates of sustained virological response obtained in patients infected with difficult-to-treat HCV genotypes 1 or 4 mimic the results of the phase III trials in non-hemophilic patients that led to the licensing of Peg-IFN and ribavirin for the treatment of treatment-naïve patients with chronic hepatitis C.[7,9] A 24-week treatment course was sufficient to treat patients with genotypes 2 and 3, who had a sustained virological response rate as high as 86%. A sustained virological response was strongly associated with an early virological response, also in patients with unfavorable predictors as genotypes 1 or 4.[16] These good efficacy findings were somewhat unexpected, because hemophilic patients with chronic hepatitis C have long been considered difficult-to-treat patients, since they often carry more than one predictor of poor therapeutic response,[6,10] such as male gender, genotype 1 and cirrhosis.[17] Why males and carriers of genotype 1 (and 4) resist interferon-based therapies more than females and carriers of genotypes 2 and 3 is still unclear. Males are suspected of having more aggressive hepatitis C and to cluster more co-morbidities that attenuate the effectiveness of interferon.[18] Interferon hyporesponsiveness to genotype 1 may be related to virus structure[19] and/or to pre-treatment HCV-RNA load.[20,21] However, our findings of no correlation between pre-treatment load and sustained virological response in hemophilic patients with genotype 1 or 4 suggests that factors other than the degree of HCV load are involved in interferon hyporesponsiveness of these difficult-to-treat patients.

This study confirms that cirrhosis predicts interferon hyporesponsiveness in hemophilic patients,[22,23] because only 14% of those with clinical evidence of cirrhosis responded to combination therapy compared to 69% of those without. It cannot be ruled out, however, that such a large difference in response between cirrhotic and non-cirrhotic patients reflects a bias in patient selection, the small size of the study and the relatively low accuracy of a diagnosis of cirrhosis made on clinical evidence. There was a positive relationship between pre-treatment ALT levels and rates of sustained virological response, a finding that links interferon responsiveness in some way to the degree of hepatic inflammation.[24]

As expected, there was a trend for higher rates of sustained virological response among patients compliant to the scheduled doses and duration of therapy than in those who were non-compliant. In non-hemophilic patients intention-to-treat analysis of phase III trials clearly demonstrated that rates of sustained virological response to ribavirin and standard interferon were higher for patients who did receive more than 80% of scheduled drug dosages for periods longer than 80% of the expected treatment duration.[25] The low rates of treatment discontinuation (14%) in our study may be partially accounted for by the peculiar characteristics of

hemophilic patients, who are highly compliant with HCV treatment because they are accustomed to comply with the treatment for their chronic disease. An additional factor that perhaps kept the rates of treatment discontinuation low was the close co-operation between hematologists and hepatologists, resulting in early treatment of hematologic side-effects such as hemolytic anemia and neutropenia.

In conclusion, treatment with Peg-IFNα2b and ribavirin at dosages comparable to those offered to non-hemophilic patients was highly efficacious, well tolerated and safe in hemophilic patients with chronic hepatitis C.

Appendix

Hepatitis Study Group of the Association of Italian Hemophilia Centers. A. Rocino, Hemophilia Center, San Giovanni Bosco Hospital, Naples; A Iorio, Hemophilia Center, Policlinico Hospital, Perugia; A. Dragani, Hematology Department, Ospedale Civile dello Spirito Santo, Pescara; G. Barillari, Transfusional Unit, "S. Maria della Misericordia" Hospital, Udine; G. Tagariello, Hemophilia Center, Castelfranco Veneto; R. Nicolin, Division of Infectious Diseases, S. Bortolo Hospital, Vicenza.

MEM, MGR, ES, PMM and MC conceived and designed the study; MEM, SL, AC and all the members of the Hepatitis Study Group listed in Appendix contributed to the collection of clinical and laboratory data; MEM, SL and AC contributed to the interpretation and the analysis of clinical and laboratory data; MEM, MGR and ES wrote the article; PMM and MC revised it critically. All authors approved the final version of the manuscript. The authors declare that they have no potential conflicts of interest.

Manuscript received May 5, 2006. Accepted July 18, 2006.

References

1. Dienstag JL, McHutchison JG. American Gastroenterological Association technical review on the management of hepatitis C. Gastroenterology 2006;130:231-64.
2. Rumi MG, Colombo M, Gringeri A, Mannucci PM. High prevalence of antibody to hepatitis C virus in multitransfused hemophiliacs with normal transaminase levels. Ann Intern Med 1990;112:379-80.
3. Darby SC, Ewart DW, Giangrande PLF, Spooner RJ, Rizza CR, Dusheiko GM, et al. Mortality form liver cancer and liver disease in haemophilic men and boys in UK given blood products contaminated with hepatitis C. Lancet 1997;350:1425-31.
4. Goedert JJ, Eyster ME, Lederman MM, Mandalaki T, De Moerloose P, White GC 2nd, et al. End-stage liver disease in persons with hemophilia and trasfusion-associated infections. Blood 2002; 100:1584-9.
5. Lindsay KL. Introduction to therapy of hepatitis C. Hepatology 2002;36:S114-S20.
6. Meijer K, Smid WM, van der Meer J. Treatment of chronic hepatitis C in haemophilia patients. Haemophilia 2000;6:605-13.
7. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon α-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958-65.
8. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon α-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975-82.
9. Hadziyannis SJ, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-α2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. Ann Intern Med 2004;140:346-55.
10. Rumi MG, Santagostino E, Morfini M, Gringeri A, Tagariello G, Chistolini A, et al. A multicenter controlled, randomized, open trial of Interferon α2b treatment of anti-human immunodeficiency virus-negative hemophilic patients with chronic hepatitis C. Blood 1997; 89: 3529-33.
11. Fried MW, Peter J, Hoots K, Gaglio PJ, Talbut D, Davis PC, et al. Hepatitis C in adults and adolescents with hemophilia: a randomized, controlled trial of interferon α-2b and ribavirin. Hepatology 2002;36:967-72.
12. Santagostino E, De Filippi F, Rumi MG, Colombo M, Mannucci PM. Sustained suppression of hepatitis C virus by high doses of interferon and ribavirin in adult hemophilic patients. Transfusion 2004; 44:790-4.
13. Glue P, Fang JW, Rouzier-Panis R, Raffanel C, Sabo R, Gupta SK, et al. Pegylated interferon-α2b: pharmacokinetics, pharmacodynamics, safety, and preliminary efficacy data. Hepatitis C Intervention Therapy Group. Clin Pharmacol Ther 2000;68:556-67.
14. Kozlowski A, Charles SA, Harris JM. Development of pegylated interferons for the treatment of chronic hepatitis C. BioDrugs 2001;15:419-29.
15. Soffredini R, Rumi MG, Parravicini ML, Ronchi G, Del Ninno E, Russo A, et al. Serum levels of hepatitis C virus core antigen as a marker of infection and response to therapy. Am J Gastroenterol 2004;99:1738-43.
16. Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with Peginterferon α-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38:645-52.
17. Di Bisceglie AM, Hoofnagle JH. Optimal therapy of hepatitis C. Hepatology 2002;36 Suppl 1:S121-S7.
18. Davis GL, Lau JYN. Factors predictive of a beneficial response to therapy of hepatitis C. Hepatology 1997; 26 Suppl 1: S122-S7.
19. Neumann AU, Lam NP, Dahari H, Gretch DR, Wiley TE, Layden TJ, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of IFN-α therapy. Science 1998;282:103-7.
20. Zeuzem S, Buti M, Ferenci P, Sperl J, Horsmans Y, Cianciara J, et al. Efficacy of 24 weeks treatment with peginterferon α-2b plus ribavirin in patients with chronic hepatitis C infected with genotype 1 and low pretreatment viremia. J Hepatol 2006;44:97-103.
21. Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon α2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet 1998; 352:1426-32.
22. Dalgard O, Bjoro K, Hellum KB, Myrvang B, Ritland S, Skaug K, et al. Treatment with pegylated interferon and ribavirin in HCV infection with genotype 2 or 3 for 14 weeks: a pilot study. Hepatology 2004;40:1260-5.
23. Sherman IA, Pappas SC, Fisher MM. Hepatic microvascular changes associated with development of liver fibrosis and cirrhosis. Am J Physiol 1990;258: H460-H5.
24. Zeuzem S, Feinman SV, Rasenack J. Peginterferon α-2a in patients with chronic hepatitis C. N Engl J Med 2000; 343:1666-72.
25. McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, et al. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 2002; 123:1061-9.

Effect of *NOD2/CARD15* variants in T-cell depleted allogeneic stem cell transplantation

Miquel Granell
Alvaro Urbano-Ispizua
Juan Ignacio Aróstegui
Francesc Fernández-Avilés
Carmen Martínez
Montserrat Rovira
Josefa Rius
Susana Plaza
Anna Gaya
Alfons Navarro
Carme Talarn
Enric Carreras
Mariano Monzó
Emili Montserrat
Jordi Yagüe

Background and Objectives. Three single nucleotide polymorphisms (SNP) in the *NOD2/CARD15* gene have been associated with the incidence and the severity of acute graft-versus-host disease (GVHD) following allogeneic stem cell transplantation (SCT). We hypothesized that the clinical effect of SNP in *NOD2/CARD15* might be different in patients submitted to T-cell-depleted allogeneic SCT, in which donor T cells, the main effectors of GVHD, are eliminated.

Design and Methods. SNP 8, 12 and 13 in *NOD2/CARD15* were studied using a Taqman protocol in 85 patients undergoing HLA-identical, T-cell-depleted SCT and in 71 of their sibling donors.

Results. *NOD2/CARD15* variants were present in nine (11%) patients and six (8%) donors. The incidences of acute GVHD and chronic GVHD were not associated with either the donors' or recipients' *NOD2/CARD15* variants. In contrast, these genetic variants were associated with a lower disease-free survival (17% vs. 48%, p=0.03). Death due to pulmonary infection was more frequent in the group of patients with *NOD2/CARD15* variants. In the multivariate analysis, only *NOD2/CARD15* variants (RR 2.3, p=0.04) and older age (RR 2.2; p=0.04) were independent prognostic factors for disease-free survival.

Interpretation and Conclusions. *NOD2/CARD15* variants have a deleterious effect on clinical outcome in T-cell-depleted allogeneic SCT, which is independent of GVHD. These results supports the hypothesis that the detrimental effect of *NOD2/CARD15* variants in such a transplant setting might be produced by an alteration of the innate immune system more than by activation of the adaptive immune system.

Key words: *NOD2/CARD15*, polymorphisms, stem cell transplantation.

Haematologica 2006; 91:1372-1376

From the Departments of Hematology (MG, AU-I, FF-A, CM, MR, AG, CT, EC, EM) and Immunology (JIA, JR, SP, JY), Hospital Clínic of Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Faculty of Medicine, University of Barcelona, Spain (AN).

Correspondence:
Alvaro Urbano-Ispizua, M.D., Institute of Hematology and Oncology, Department of Hematology, Hospital Clínic Villarroel 170, 08036 Barcelona Spain. E-mail: aurbano@clinic.ub.es

Gastrointestinal mucosal damage and subsequent bacterial translocation seem to play a pivotal role in the initiation and maintenance of the inflammatory status that leads to graft-versus-host disease (GVHD).[1] In this sense, gut decontamination has been shown to protect against GVHD in animal models[2] and also in the clinical setting.[3] The *NOD2/CARD15* gene is involved in the innate immune response against bacterial infections in the gastrointestinal tract; it has recently been shown that variants of this gene are associated with a higher incidence of acute GVHD and transplant-related mortality after allogeneic stem cell transplantation.[4,5] Nod2 is a cytosolic protein belonging to the nucleotide-binding oligomerization domain (NOD) family. It is involved in the maintenance of commensal and gastrointestinal mucosal homeostasis;[6] it recognizes muramyl dipeptide (MDP), a component of peptidoglycan present in the cell wall of Gram-positive and Gram-negative bacteria. Upon ligand binding, Nod2 oligomerizes and activates the nuclear factor κB (NF-κB) signaling pathway inducing the transcription of several genes encoding proinflammatory cytokines.[7] Nod2 is expressed mainly in monocytes, macrophages, dendritic cells and Paneth cells.[8] Nod2 contains three distinct functional domains: multiple carboxy-terminal leucine-rich repeats, a centrally nucleotide binding domain and two amino-terminal caspase recruitment domains. Three common single nucleotide polymorphisms (SNP) in the leucine-rich repeats domain - SNP8 (R702W), SNP12 (G908R) and SNP13 (L1007finsC) - have been previously associated with an increased risk of Crohn's disease.[8] It has been suggested that these variants might be associated with an abnormal inflammatory response against normal bacterial flora.[9] These *NOD2/CARD15* variants have also been associated with the incidence of grades III-IV acute GVHD.[4] Holler *et al.* suggested that such an association was due to an increased release of cytokines for recipients with the polymorphisms, causing a stimulation of recipient dendritic cells and donor T cells.[4] We hypothesized that an abnormal inflammatory response associated