DURABILITY OF THE RESPONSE TO PEGINTERFERON ALFA2b AND RIBAVIRIN IN CHRONIC HEPATITIS C PATIENTS: A COHORT STUDY IN THE ROUTINE CLINICAL SETTING.

DURABILITY OF THE RESPONSE TO PEGINTERFERON ALFA2b AND RIBAVIRIN IN CHRONIC HEPATITIS C PATIENTS: A COHORT STUDY IN THE ROUTINE CLINICAL SETTING.

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Running title: Long-term response to PEG IFN and RBV in HCV + pts

ABSTRACT

Objectives: to evaluate whether in chronic hepatitis C-positive naïve patients recruited in the **routine** clinical setting and treated with pegylated-interferon-alfa2b (Peg-IFN) and ribavirin (RBV) the sustained virologic response (SVR) is durable over the long-term and if it is associated to a decrease of liver complications and incidence of glucose abnormalities.

Methods: prospective long-term follow-up of 182 naïve patients **enrolled in 2001-2002 and** treated with Peg-IFN and RBV and followed **up to December 2010** with clinical, biochemical and virological evaluations every 6-12 months.

Results: none of the 115 (63.2%) sustained responders showed late viremic relapse during the follow-up. SVR was better defined at 24 weeks (**16/16 relapsers**, 100%) than at 12 weeks after the end of therapy (**14/16 relapsers**, 87.5%). At multivariable analysis, viral genotype (OR 0.16, 95% CI 0.07-0.36, p= 0.0001) and a >20% RBV reduction (OR 5.21, 95% CI 1.54-17.67, p= 0.008) independently predicted long-term response (LTR). Incidence of cirrhosis was significantly higher among non responders (21.3%) compared with long-term responders (0.9%, p≤0.0001) but risk of **developing glucose abnormalities was not significantly reduced in long-term responders (HR=1.36, p= 0.363). Hepatocellular carcinoma occurred in three cases only.**

Conclusions: SVR obtained in patients treated in the **routine** clinical setting with Peg-IFN and RBV is durable over the long-term and LTR significantly reduces the risk of progression to cirrhosis; however, in a

population with mild liver fibrosis the clinical impact of LTR on the risk of glucose abnormalities seems negligible.

INTRODUCTION

Despite well established response rates in patients with chronic hepatitis C treated with standard Interferon (IFN) with or without ribavirin (RBV) in international controlled trials (1-5), knowledge is still incomplete regarding the long term clinical, virologic, histologic outcomes in the clinical setting. Even more limited data exist regarding the long term durability of a sustained virologic response (SVR) following Pegylated-IFN (Peg-IFN) treatment (6-9). In particular, the risk of late virologic relapse and late complications of HCV infection (decompensated liver disease, hepatocellular carcinoma) after treatment courses with Peg-IFN and RBV are scarcely known and the few studies which addressed these issues either come from randomized controlled trials (6,9) or have a retrospective design (8) without fully reporting the type of patients recruited. Moreover, these studies used HCV-PCR assays with different degrees of sensitivity and heterogeneous patients populations, resulting in variable late relapse rates, ranging from 0% to 8.7%. The wide range of sensitivity regarding Hepatitis C Virus -RiboNucleic Acid (HCV-RNA) testing may also account for the controversy concerning the optimal timing for the definition of SVR: according to a recent study (10) assessment of serum HCV-RNA 12 weeks after the end of treatment is as relevant as after 24 weeks to predict SVR but this conclusion was not confirmed by a Japanese study (11).

Finally, the clinical impact of HCV eradication on the incidence of glucose metabolism derangements is still unclear. Curing HCV seems to have beneficial effects on the level of insulin sensitivity (12,13) and to significantly reduce the incidence of diabetes mellitus (DM) (14,15), although this may not be the rule (16).

Primary aims of this study were 1) to determine the durability of SVR in patients recruited in the clinical setting and treated with Peg-IFN alfa2b and ribavirin, comparing the clinical outcome of patients with a long-term response (LTR) with that observed in non responders (NR)/relapsers (RR) 2) to estimate the cumulative incidence rate of glucose abnormalities in patients with and without LTR.

Secondary aim was to establish if measurement of serum HCV-RNA at 12 weeks post-treatment to assess LTR is as relevant as at 24 weeks post-treatment in patients with an end of treatment response (ETR).

To answer these questions, we prospectively followed up **from 2001 up to 2010** 182 chronic hepatitis C naive (CHC) patients recruited in the **routine** clinical setting treated with Peg-IFN alfa2b plus RBV.

PATIENTS AND METHODS

Between March 2001 and March 2002, all naive patients with histologically proven chronic hepatitis C consecutively collected in 8 Hepatologic Units of North Western of Italy who fulfilled the inclusion criteria were asked to participate into this prospective cohort study.

Inclusion criteria were: age > 18 years; positive results for HCV-RNA by polymerase chain reaction (PCR); CHC at liver biopsy; documented baseline informations regarding: gender, Body Mass Index (BMI), viral load, genotype, liver function tests, liver histologic staging, glucose. Exclusion criteria were: age > 65 years, inclusion in trials with IFN-based and ribavirin therapies, major contraindications to IFN or RBV therapy, haemoglobin level < 12 g/dL, platelet count < 100000/mm^3, granulocyte count < 1500/mm^3, decompensated liver disease, active alcohol intake, **psychoactive legal and illegal drug abuse**, presence of

other concomitant diseases or conditions representing a contraindication to therapy, unwillingness to participate or to give consent.

Since the only pegylated interferon licensed in Italy and reimbursed by the National Health System in 2001 was pegylated interferon alfa2b (Peg-Intron, Schering Plough, USA), each patient was treated with Peg-IFN (1.5 μg/kg once weekly) plus RBV (800-1200 mg/daily) for 24 or 48 weeks according to genotype **(genotype 2-3: 24 weeks, genotype 1 and 4: 48 weeks).**

When severe side effects occurred, the dose of Peg-IFN was decreased by 20%-50% and the dose of RBV was lowered to 600-800 mg daily, depending on the baseline dosage.

After week 24 post-treatment, all patients were followed up every 6 months for the first 3 years and once a year thereafter if they achieved a sustained response. NR/RR and cirrhotics were followed up every six months. Routine follow-up studies included clinical assessment, conventional biochemical tests, HCV-RNA detection and HCC screening using serum alfafetoprotein and liver ultrasonography. The starting date of follow-up for each patient began at the end of antiviral treatment. The end of follow-up was the date of death or the closing date of the study, December 31, 2010, **or the date of the last available visit for patients lost to follow-up**. At the last follow-up visit, each patient was invited to undergo transient elastography.

A sustained virologic response (SVR) was defined as the HCV-RNA clearance **24 weeks** after the end of treatment. Patients who maintained viral eradication throughout the follow-up were defined as long-term responders (LTR). Patients who did not show HCV-RNA clearance at the end of therapy were considered NR; those who showed a viral reactivation after 12 or 24 weeks after treatment were defined as RR. The median duration of follow-up was 9.2 years (**interquartile range, IQR: 9.0-9.5).** Written informed consent was obtained from all the patients prior to recruitment. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by our local Ethical Committee.

Definitions

According to the criteria recommended by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, diabetes was defined as fasting plasma glucose ≥ 126 mg/dl (7 mmol/l) in two separate measurements and impaired fasting glucose (IFG) was defined as baseline glucose levels 100-125 mg/dl (5.6-6.9 mmol/l) (17).

Virology

At baseline, genotypes were identified using a line probe hybridization assay (Line Probe assay, LIPA HCV, Innogenetics, Zwijndrecht, Belgium). Immediately before the inclusion into the study, HCV-RNA was quantified by a branched-DNA assay (Quantiplex, Bayer Diagnostics, Tarrytown, NY, USA) with a lower limit of detection of 615 IU/ml. Serum samples below this cut-off were evaluated for HCV-RNA by the COBAS-AMPLICOR assay (Roche Diagnostic Systems, Branchburg, NJ, USA) which had a sensitivity of 50 IU/ml. The COBAS AMPLICOR assay was also used to test viraemia in each patient at the end of therapy and during follow-up until the advent of a more sensitive assay using real-time PCR-based methods (Cobas Taqman/CAP-CTM, Roche Molecular Systems, Basel, Switzerland) with a limit of detection of 15 UI/ml.

Liver-related events

Patients were considered to have liver failure if they met any of the following criteria: ascites confirmed by ultrasound or computed tomography (CT), bleeding oesophageal varices, jaundice with bilirubin > 35 µmol/L or hepatic encephalopathy. Patients were considered to have HCC if the diagnosis was cyto-histologically confirmed, or if two coincident imaging techniques (ultrasound, CT or magnetic resonance imaging) showed a focal lesion > 2 cm with arterial hypervascularization or if one imaging technique showed a focal lesion > 2 cm with arterial hypervascularization in the presence of alpha-fetoprotein > 400 ng/ml.

Histology

The degree of liver fibrosis was assessed blindly by one pathologist according to the Ishak scoring system (18). During the follow-up, subjects were classified as cirrhotic by a second liver biopsy or, in the absence of bioptic examination, if they showed unequivocal evidence of portal hypertension (presence of esophageal varices not associated with portal vein thrombosis), or a particular ultrasound pattern (nodular contour, diminished hepatopetal flow, collaterals) or a typical serology (platelets < 80000/mm^3, albumin < 3.5 g/l, clotting factors < 50%) or by liver elastography adopting the stringent recommendations suggested by Kettaneh et al. and showing a liver stiffness > 14.5 KPascal (19).

Statistical analysis

Patients' characteristics at baseline were described with means and standard deviations (or with medians and inter quartile ranges if not normally distributed) or with frequencies (and percentages). **To analyze the prognostic role of clinical and virological features on LTR we used logistic regression models (since after the first 24 weeks after the end of therapy no relapses have occurred during the rest of follow up), including all relevant baseline characteristics chosen "a priori", without any further selection.** The association between the long-term virological response and the development of clinical outcomes at the end of follow-up (cirrhosis, HCC) was assessed with the Fisher exact test. The risk of developing IFG or DM during the follow-up was estimated in 151 patients with normal glycemic values at baseline and with a complete follow-up. We estimated the cumulative incidence of IFG or DM with the Kaplan Meier method, since no competing events occurred during the follow-up. To explore the role of the baseline patients' characteristics on the risk of occurrence of IFG or DM we compared the survival curves with the log-rank test. **Separate Cox proportional hazard models were then fitted for each explanatory variable, including as potential confounders age (in years) and baseline viral load (in log IU/ml) in all models, since these**

two variables were the strongest predictors of the outcome and the limited number of failures analysed (n= 19) prevented a more careful adjustment.

RESULTS

Of 224 consecutively collected naïve patients, 182 (81.2%) accepted to participate into the study; of the remaining 42 (18.8%), 28 did not fulfil inclusion/exclusion criteria and 14 did not give their informed consent. Demographic, clinical, virological and histological features of recruited patients are reported in Table 1. Out of 182 patients, 131 (72%) were HCV-RNA-negative at the end of therapy while 51 (28%) were non responders. Viremic relapse was observed in 16 of 131 (8.8%), 14 at 12 weeks and 2 at 24 weeks after the end of therapy. Overall, 115 (63.2%) patients were sustained responders and none showed late relapse during the follow up. Of 182 enrolled patients, 11 (6%) prematurely discontinued treatment due to side effects and were lost to long term follow-up; four of them showed IGF/DM at baseline.

Thirty-nine (44.8%) of 87 genotype 1 patients, 45 (86.5%) of 52 genotype 2 patients, 26 (81.2%) of 32 genotype 3 patients and 5 (45.4%) of 11 genotype 4 patients were LTR; a significant difference in outcome between unfavourable (1-4) and favourable (2-3) genotypes was observed (44.9% vs 84.5%, OR 0.15, 95% CI 0.07-0.30, p= 0.0001). Other baseline features associated to LTR are reported in Table 2. Patients with a LTR were younger than NR/RR and showed a lower viral load and BMI at baseline; a >20% reduction of Peg-IFN dosage negatively influenced the LTR. The presence of IFG or overt DM was associated with a lower LTR rate (37.5% vs 67.1%, OR 0.29, 95% CI 0.12-0.72, p=0.007). At the multivariable logistic regression analysis (Table **2**), most of the variables showed weaker associations with LTR and only the viral genotype (OR 0.16, 95% CI 0.07-0.36, p= 0.0001) and a >20% RBV reduction (OR 5.21, 95% CI 1.54-17.67, p= 0.008) strongly predicted LTR.

Out of 168 patients without baseline cirrhosis, 14 (8.3%, 95% CI 5.03-13.50) developed cirrhosis throughout the follow up. Among 107 LTR without cirrhosis at baseline, only one (0.9%) developed liver cirrhosis compared with 13 “de novo” cirrhosis in NR/RR (21.3%, p<0.0001). Three out of 8 (37.5%) LTR with cirrhosis at baseline showed a significant improvement of their liver stiffness; all of them were re-biopsed: two of them were classified as stage 4 according to Ishak's scoring system, the remaining patient as stage 5.

Three patients developed HCC during follow up (1.6%, 95% CI: 0.42-6.29). One out of 115 LTRs had HCC detected on ultrasound performed for his scheduled clinic visit 6 years after achieving SVR; two of the 67

NR/RR developed HCC respectively 4.5 and 7 years after the end of therapy. The risk of developing episodes of liver decompensation was very low: ascites was detected in only one NR during the follow-up.

At baseline, 24 patients (13.2%) showed IGF/DM; four of them were lost to follow-up.

After a median follow-up of 9.2 years, the cumulative risk of IFG "de novo"(N= 17) or DM (N= 4) among the 151 patients without baseline glucose abnormalities still in follow-up was 13.9% (95% CI 8.2-18.0). The cumulative incidence of IFG or DM during follow-up is shown in Figure 1.

Mean BMI of patients prior to therapy was 24±3.2; patients with a higher baseline BMI (>=24) showed a lower probability of achieving a LTR (OR= 0.47, 95% CI 0.23-0.98, p= 0.044) but this association was weaker, and no more statistically significant, when adjusted by other potential confounders (OR=0.67, p=0.375). At the end of follow-up, LTR showed a mean weight increase of 0.80 Kg (range -0.31 / +1.92) compared to a mean weight loss of 0.55 (range -1.95 / + 0.85) observed among NR/RR (p= 0.13).

In order to individuate baseline features predicting the incidence of IFG/DM during the long term follow-up we performed univariable log-rank test comparisons and a multivariable Cox proportional hazard regression analysis (Table **3**): age >50 at diagnosis emerged as a strong independent risk factor of IFG/DM (**HR 3.59, 95% CI 1.34-9.57, p= 0.011)**; high baseline viral load was significantly associated to development of glucose abnormalities, **both at univariable analysis (p= 0.015) and after adjustment for age in the Cox multivariable analysis (p= 0.0280). Out of 106 LTRs without baseline IGF/DM, 15 developed glucose abnormalities (13 IFG and 2 DM) compared to 6 out of 45 NR (4 IFG and 2 DM, logrank p-value: 0.840).**

DISCUSSION

The main finding of this long term prospective follow up study is that an SVR obtained with Peg-IFNalfa2b plus RBV in clinical practice is durable over the long term and that late viremic relapse is absent.

Our data show that SVR rates achieved in the clinical setting are similar to those observed in randomized clinical trials, addressing the question of whether data obtained in randomized, controlled trials are representative for an average patient treated outside a major expert center. A recent study (20) raised this issue showing that patients not included in therapeutic trials receiving the standard of care had more advanced liver disease, a more frequent history of psychiatric disorders and often were on drug-substitution therapy. In contrast to this finding, a prospective cohort study (21) demonstrated that treatment for HCV infection delivered in the community-based setting induced therapeutic results similar to those observed in academic medical centers.

According to our results, patients with CHC treated with Peg-IFNalfa2b plus RBV in the clinical setting during the years 2001-2002 in different Centres of North Western Italy showed a high percentage of genotype 2-3 (46.2%) and this may account for the good therapeutic results obtained.

The relatively high number of treated patients with favourable genotypes does not represent a selection bias but reflects the epidemiological distribution of HCV genotypes in this part of Italy (22). Moreover, indications to treatment in 2001 were still influenced by the concern regarding the potential prolonged effect of Peg-IFN on neutrophils and platelets and this may account for the low number of cirrhotics included.

In our study, LTR was accompanied by a significant decrease of cirrhosis incidence compared with NR/RR (0.9% vs 21.3%, $p \leq 0.0001$); however, liver decompensation among NR/RR with cirrhosis was rarely observed. The relative good outcome found among NR/RR may appear surprising but it reflects the favourable baseline histologic features of patients included.

At variance with previous studies which reported a significant improvement of HCC incidence in cirrhotics obtaining SVR (23-29), the relatively low number of cirrhotics at baseline and the very low number of those developing HCC did not allow us to analyze with sufficient statistical power this association. However, the incidence of HCC observed among our cirrhotics not responding to therapy was similar to that reported by

a recent epidemiological review (30). The results of our study stress the importance of continuing ultrasound monitoring in cirrhotics, even though they obtain a long term viral eradication.

Interestingly, a >20% reduction of Peg-IFN dosage negatively influenced the LTR rate as reported by some re-treatment studies (31,32) while patients who received <80% of the total RBV dose showed a significantly higher rate of LTR, confirming the results of a previous study (33) where patients who required a RBV-dose reduction due to anemia had a higher rate of SVR.

Our study failed to show a statistically significant lower incidence of both IFG and DM in LTR compared to NRs, confirming our previous observations (16,34). The discrepancy with the results obtained by other Authors (14,35) showing a decrease in the incidence of DM among SVRs may be explained by the different baseline features of our patients (low number of cirrhotics, predisposed to hepatogenous DM) and by the weight increase observed in our SVR. The beneficial effect of SVR on insulin resistance (36,37) was probably counterbalanced by the weight increase as previously demonstrated (36,38).

Secondary aim of this study was to establish if measurement of serum HCV-RNA at 12 weeks post-treatment to assess LTR was as relevant as at 24 weeks post-treatment in patients with an end of treatment response. According to our data, 2 out of 16 (12.5%) relapsers were HCV-RNA-negative 12 weeks after the end of treatment; these results were obtained by assays with a low sensitivity (50 IU/ml) but are similar to those reported by Kanda and coll. (11) who used more sensitive assays, suggesting that current definition of SVR is still appropriate.

Our results may be affected by some methodological weaknesses. The main limitation was that we could not assess the clinical outcome of 11 (6%) NR/RR who were dropped out from the study. We therefore cannot exclude the possibility that these patients may have developed cirrhosis and/or its complications or IFG/DM. Second, the number of recruited patients may not be sufficient to detect as statistically significant small/medium effects; for this reason, larger prospective observational studies are needed to better establish the long-term effect of viral eradication in patients treated in the clinical practice.

In conclusion, SVR achieved with PegIFN and RBV is durable among patients treated in the clinical setting and such patients may be considered cured from a virologic standpoint with the exception of cirrhotics who should be monitored for the risk of HCC. LTR is associated with a significantly reduction of cirrhosis incidence but in patients with baseline mild forms of HCV-related liver disease there is no clear evidence that LTR will result in significantly reduced morbidity and mortality compared with NR/RR. Conversely, in a population predominantly composed by non-cirrhotic patients the incidence of glucose abnormalities is not significantly different between LTR and NR/RR over the long term.

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Legend

Figure 1. Cumulative incidence of IFG or diabetes during the follow-up in a cohort of 151 chronic hepatitis C patients

Table 1. Baseline characteristics of the cohort and length of follow-up.

			Normal values
Age (years): mean (SD)	45.9	(12.4)	
Gender (males) : n (%)	109	(59.9)	
BMI: mean (SD):	24.0	(3.2)	
Fasting glucose (mg/dl): mean (SD)	90.9	(19.1)	70-100
IFG: n (%)	16	(8.8)	
Diabetes mellitus: n (%)	8	(4.4)	
IFG or diabetes mellitus: n (%)	24	(13.2)	
Haemoglobin (g/dl): mean (SD)	14.7	(1.33)	13-17
Leucocytes (x 10^3): mean (SD)	6.2	(1.6)	4.5-11
Neutrophils (x 10^3): mean (SD)	3.3	(1.2)	1.8-9
TSH (mU/l): mean (SD)	1.85	(1.1)	0.1-3.5

ALT (IU): median (IQR)	94	(76)	10-35
GGT (IU): median (IQR)	41.5	(49)	10-50
Histology:			
Grading: mean (SD)	4.8	(2.2)	
Staging: mean (SD)	2.5	(1.2)	
Cirrhosis: n (%)	14	(7.7)	
Viral load (x 10^5 UI/ml): median (IQR)	7.8	(17.3)	
Viral genotype: n (%)			
1	87	(47.8)	
2	52	(28.6)	
3	32	(17.6)	
4	11	(6.0)	
Follow-up length (years): median (IQ range)	9.2	(9.0-9.5)	

Table 2. **Clinical and virological features associated to long term virological response: univariable and multivariable logistic regression analyses**

			Univariable analysis			
	n/tot	**(%)**	**OR**	**(95% CI)**	**p**	**OR**
Age group:						
< 50	75/111	(67.6)	1	-	-	0.99[a]
>=50	40/71	(56.3)	0.62	(0.34-1.15)	0.127	
Gender:						
Females	47/73	(64.4)	1	-	-	1
Males	68/109	(62.4)	0.92	(0.50-1.70)	0.784	1.57
BMI:						
<24	52/71	(73.2)	1	-	-	1
>=24	35/62	(56.5)	0.47	(0.23-0.98)	0.044	0.67
Missing	28/49	(57.1)	0.49	(0.22-1.06)	0.068	0.54
IFG or diabetes mellitus:						
Absent	106/158	(67.1)	1	-	-	1
Present	9/24	(37.5)	0.29	(0.12-0.72)	0.007	0.47
Cirrhosis:						
Absent	107/168	(63.7)	1	-	-	1
Present	8/14	(57.1)	0.76	(0.25-2.29)	0.626	0.94
Viral load (UI/ml):						
< 800000	64/94	(68.1)	1	-	-	0.80[b]
>=800000	51/88	(58.0)	0.65	(0.35-1.18)	0.158	
Viral genotype:						
2-3	71/84	(84.5)	1	-	-	1
1-4	44/98	(44.9)	0.15	(0.07-0.30)	<0.0001	0.16
Peg-IFN reduction (>20%):						
No	110/169	(65.1)	1	-	-	1
Yes	5/13	(38.5)	0.34	(0.11-1.07)	0.065	0.32
RBV reduction (>20%):						
No	91/150	(60.7)	1	-	-	1
Yes	24/32	(75.0)	1.95	(0.82-4.62)	0.132	5.21

a) Age in years

b) Viral Load in log (UI/ml)

Table 3. Clinical and virological features associated to incidence of IFG or diabetes during the follow-up: univariable logrank-test comparisons and multivariable Cox proportional hazard regression analysis.

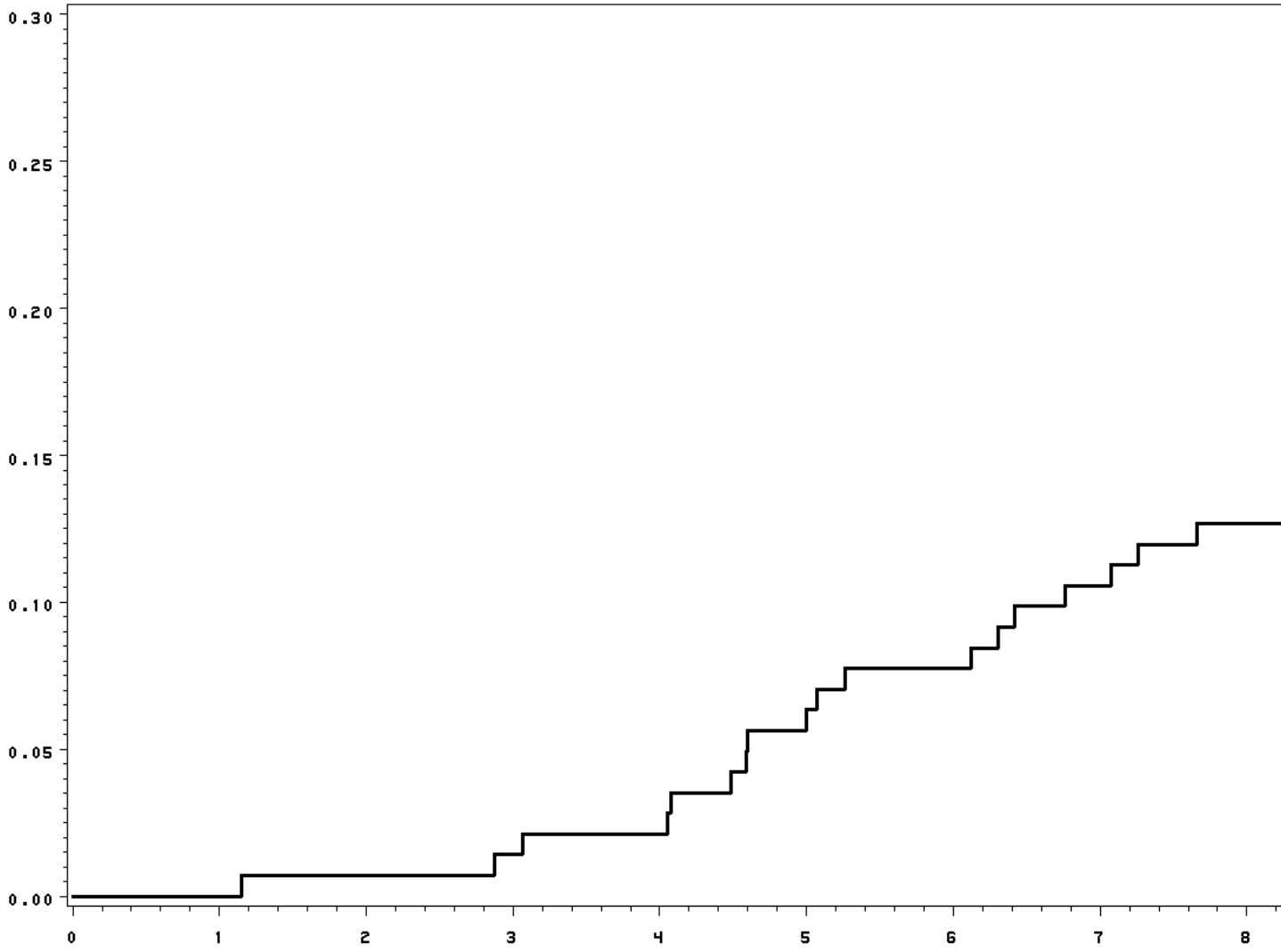
	Incident Cases/Total	(%)	Log-Rank Test (p-value)	HR[(a)]
Age group:				
< 50	6/102	5.9		1 [(b)]
>=50	13/56	23.2	0.003	3.59
Gender:				
Females	10/65	15.4		1
Males	9/93	9.7	0.327	0.87
BMI:				
<24	7/65	10.8		1
>=24	10/50	20.0	0.110	1.71
Missing	2/43	4.7		0.63
Cirrhosis:				
Absent	16/147	10.9		1
Present	3/11	27.3	0.070	1.67
Viral load (UI/ml):				
< 800000	5/86	5.8		1 [(c)]
>=800000	14/72	19.4	0.015	3.14
Viral genotype:				
2-3	12/78	15.4		1
1-4	7/80	8.8	0.177	0.46
Peg-IFN reduction (>20%):				
No	19/148	12.8		1
Yes	0/10	0.0	0.242	Not estimable
RBV reduction (>20%):				
No	14/130	10.7		1

Yes	5/28	17.9	0.312	1.54
Sustained virological response				
No	5/52	9.6		1
Yes	14/106	13.2	0.840	1.63

Adjusted for age and baseline viral load (log transformed) as continuous variables, unless otherwise specified

Adjusted for baseline viral load (log transformed) as continuous variable

Adjusted for age, as continuous variable


0.30
0.25
0.20
0.15
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2
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8