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HEPATITIS C VIRAL LOAD DOES NOT PREDICT DISEASE OUTCOME: GOING BEYOND NUMBERS

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SUMMARY

The analysis of 58 patients with chronic hepatitis C without cirrhosis and treated with interferon-alpha demonstrated that hepatitis C viral (HCV) load does not correlate with the histological evolution of the disease ($p = 0.6559$ for architectural alterations and $p = 0.6271$ for the histological activity index). Therefore, the use of viral RNA quantification as an evolutive predictor or determinant of the severity of hepatitis C is incorrect and of relative value. A review of the literature provided fundamental and interdependent HCV (genotype, heterogeneity and mutants, specific proteins), host (sex, age, weight, etc) and treatment variables (dosage, time of treatment, type of interferon) within the broader context of viral kinetics, interferon-mediated immunological response (in addition to natural immunity against HCV) and the role of interferon as a modulator of fibrogenesis. Therefore, viral load implies much more than numbers and the correct interpretation of these data should consider a broader context depending on multiple factors that are more complex than the simple value obtained upon quantification.

KEY WORDS: Hepatitis C; Viral Load; Interferon

INTRODUCTION

Hepatitis C is the major cause of liver transplantation and cirrhosis worldwide, affecting approximately 170 million people[14], or two percent of the global population, with regional variations being observed. Due to its asymptomatic pathology even during advanced phases of the disease, such as compensated cirrhosis, the diagnosis of hepatitis C is completely based on laboratory exams. Therefore, most patients discover their condition by chance during blood donations or routine examinations. Antibodies are routinely detected by ELISA and hepatitis C virus (HCV) infection is confirmed by the determination of viral RNA in blood using the polymerase chain reaction (PCR) and staging by anatomopathological examination of the liver.

In addition to the diagnosis, the HCV subtype and the amount of virus in plasma need to be established since the present consensus therapy varies according to these parameters[7]. After the diagnosis of chronic hepatitis C and staging, patients presenting inflammatory activity and structural alterations exceeding the portal area are indicated for the use of interferon-alpha combined with ribavirin for a period of six to 12 months, a treatment scheme that leads to a sustained virological response in 30 to 40% of cases[30]. Based on these data a series of questions should be raised: what is the benefit of treatment for most non-responders? What are the factors determining the presence or absence of a virological response? Does HCV play a direct role in disease pathogenesis (with the assumption that the higher its plasma concentration the poorer the evolutive prognosis of the patient)?

Based on these still not completely understood aspects and the massive use of costly HCV RNA quantification methods of limited diagnostic value, such as the method used to confirm the diagnosis of HCV infection, the aims of the present study were to establish the role of the amount of HCV with respect to histological evolution and to determine the factors correlated with a good course and a good therapeutic response.

MATERIAL AND METHODS

Fifty-eight patients with chronic hepatitis C confirmed by liver biopsy and detection of viral RNA in serum by nested PCR were followed up at the Hepatitis Outpatient Clinic, Department of Infectious and Parasitic Diseases, Faculty of Medicine, University of São Paulo (DMIP/FMUSP), from 1997 to 1998. The patients did not present decompensated co-morbidities, other causes of chronic liver disease, cirrhosis, or HIV infection and had not used alcohol or illegal drugs for six months before inclusion in the study. Prior to inclusion written informed consent was obtained from the patients or responsible persons and the study was approved by the Ethics Committee of DMIP/FMUSP. Demographic and epidemiological information was obtained and clinical follow-up consisted of monthly visits and examinations until the end of treatment, followed by quarterly visits, according to the care routine of the unit. The patients received 3×10^6 IU interferon-alpha subcutaneously three times per week for 12 months, according to the current consensus established by the National Institutes of Health (NIH)[22], irrespective of the therapeutic response, except in the case of adverse effects or intolerance.

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Blood samples were collected before and 6 and 12 months after treatment, processed within 2 h and aliquots were stored at –20 ºC without unnecessary thawing, as described by DAVIS *et al.*[6], for qualitative PCR (nested PCR performed in-house) and, if positive, for quantification (AMPLICOR HCV MONITOR, ROCHE). In addition, the virus was serotyped using commercially available techniques (ABBOTT - MUREX HCV 1-6 Assay) according to manufacturer instructions. Liver biopsies were obtained with a needle by the percutaneous route and analyzed by a single, experienced pathologist who was unaware of the clinical data and of the time of puncture (before or after treatment). The samples were analyzed using the classification of ISHAK *et al.*[15]. A second biopsy was obtained 180 days after the end of treatment.

The qualitative variables were submitted to the chi-square test or to the Fisher exact test when the basic assumptions of the chi-square test were not satisfying. The response probability was analyzed by stepwise logistic regression. The following variables were compared with the histological, virological and biochemical results: age (less or more than 40 years), sex (female as the favorable variable), means of diagnosis (analysis versus donation), basal viral load and load after six months of treatment (less versus more than 500,000 copies/ml), serotype (non-1 and 1), pretreatment histological activity index - HAI (lower or higher than 3), pretreatment fibrosis (0 -1 versus higher than 2), and a history of transfusion of blood or blood derivatives. The first two options were considered the favorable ones.

Table 1
Basic characteristics of the series

	Results	n (%)
Gender	Men	39 (67.2%)
	Women	19 (32.8%)
Mean age	41 ± 9 years	-
Epidemiology	Transfusion	19 (32.8%)
	Unknown	19 (32.8%)
	Drugs	10 (17.2%)
	Tattoos	2 (3.4%)
	Infected relative	1 (1.7%)
	Professional	2 (3.4%)
	Multiple	5 (8.5%)
Diagnosis	Donation	43 (74.1%)
	Investigation	15 (25.9%)
Serotype	1	30 (one case types 1 and 4) 51.7% (1.7%)
	Non-1	20 (4 type 2; 16 type 3) 34.5%
	Not obtained	8 (13.8%)
Viral load	Median	43085.5 copies/ml
	<500,000 copies/ml	51 (87.9%)
	>500,000 copies/ml	7 (12.1%)
Liver Architecture	0-1	43 (74.1%)
	2-3	15 (25.8%)
Liver HAI	≤3	16 (27.6%)
	>3	42 (72.3%)

RESULTS

Table 1 shows the basic characteristics of the present series. A predominance of male patients with a mean age of 40 years was observed. Most of the patients acquired the infection through the transfusion of blood and blood derivatives or through drug use. However, one third of the cases did not report any suspected epidemiology. The diagnosis was mainly made upon blood donations. Serotype 1 was the most common HCV subtype (30 cases, one being co-infected with serotype 4), although 34% of the patients were non-1 (4 were type 2 and 16 type 3). In most cases, the viral load was below 500,000 copies/ml. There was a predominance of patients with elevated HAI and slight structural alterations. Table 2 summarizes the results obtained after the beginning of therapy. Median viral load decreased during the first six months of treatment, but this reduction did not persist to the end of treatment. A virological response was obtained in 21 cases and was sustained in 10 (17.2%). A biochemical response was obtained in 19 cases and was sustained in 9 (15.5%). Histological analysis revealed a reduction in inflammatory activity and maintenance of the favorable architectural profile. Despite the slow regression of the histological damage, a decrease in liver fibrosis of up to two points was noted in three non-responders, i.e., histological improvement dissociated from the virological response. In six cases, all of them non-responders, a second biopsy could not be obtained.

Table 2
Results

Viral load at month 6	Median	6118.5 copies/ml
	Qualitative PCR	21 (36.2%)
	<500,000 copies/ml	49 (84.5%)
	>500,000 copies/ml	9 (15.5%)
Viral load at month 12	Median	54243.5 copies/ml
	Qualitative PCR	21 (36.2%)
	<500,000 copies/ml	48 (82.8%)
	>500,000 copies/ml	10 (17.2%)
Virological response	Absent	38 (65.5%)
	Not sustained	11 (18.9%)
	Sustained	10 (17.2%)
Biochemical response	Absent	39 (67.2%)
	Not sustained	10 (17.2%)
	Sustained	9 (15.5%)
Liver Architecture	0-1	40 (69%)
	2-3	12 (20.7%)
	NA	6 (10.3%)
Liver HAI	≤ 3	33 (56.9%)
	> 3	19 (32.7%)
	NA	6 (10.3%)

NA: Not available

Tables 3 and 4 show the results of the statistical analysis between the selected variables and treatment outcome. Univariate analysis revealed statistical significance only for the basal histological variables, with some demographic characteristics (female sex and age less than 40 years) being close to the alpha level of 5%. Logistic regression revealed significance only for the architectural alterations, with serotype being close to alpha in relation to the virological response. No significant difference was observed between any quantitative variable and HCV.

Although these data are not reported, separate statistical analysis grouping non-sustained and sustained responses (presence of response) versus lack of response was performed. Univariate analysis showed a good correlation between virological response and diagnosis by investigation ($p = 0.016$) and low viral load after six months of treatment ($p = 0.021$).

The fact that some variables were close to alpha indicates that the sample size was insufficient for confirmation of these data. However, the trend observed here was comparable to data reported in the literature. Although the general serotyping sensitivity was good, 13.8% of cases were lost upon serotype definition and these eight lost cases may have compromised the analysis.

DISCUSSION

This study was designed in 1997 in view of the recently published Hepatitis C Consensus of the NIH[22] and carried out during the period from 1997 to the beginning of 2000. The initial objective was to determine the role of HCV RNA quantification as a clinical-evolutive and histological predictor in chronically infected patients treated with interferon-alpha. During the development of the study and based on the

Table 3
Univariate analysis

Variable	Architectural alteration (after treatment)	HAI (after treatment)	Virological response	Biochemical response
Sex	p = 1	p = 0.076**	p = 0.472	p = 0.703
Age	p = 0.473	p = 0.077**	p = 0.726	p = 1
Serotype	p = 0.489	p = 0.300	p = 0.130	p = 1
Pre-treatment HAI	p = 0.023*	p = 0.008*	p = 0.711	p = 0.243
Pre-treatment architectural alteration	p < 0.001*	p = 0.025*	p = 0.427	p = 0.422
Basal viremia	p = 0.181	p = 0.242	p = 0.592	p = 0.296
Viremia at 6 months	p = 1	p = 1	p = 0.335	p = 0.329
Transfusion	p = 0.731	p = 0.798	p = 0.733	p = 1
Diagnosis	p = 1	p = 1	p = 0.265	p = 0.218

Table 4
Multivariate analysis/logistic regression

Variable	Architectural alteration (after treatment)	HAI (after treatment)	Virological response	Biochemical response
Sex	p = 0.6337	p = 0.0987	p = 0.1094	p = 0.4103
Age	p = 0.5848	p = 0.1967	p = 0.5601	p = 0.8542
Serotype	p = 0.1048	p = 0.2214	p =0.0714**	p = 0.6523
Pre-treatment HAI	p = 0.5165	p = 0.1597	p = 0.3838	p = 0.3648
Pre-treatment architectural alteration	p = 0.001* OR99.99 (CI 9.31; 1073.51)	p = 0.0494* OR3.91 (CI 1.0037; 15.24)	p = 0.2609	p = 0.2609
Basal viremia	p = 0.6559	p = 0.6271	p = 0.9018	p = 0.9018
Viremia at 6 months	p = 0.7880	p = 0.5600	p = 0.2214	p = 0.2609
Transfusion	p = 0.3618	p = 0.8550	p = 0.2815	p = 7503

*Significant; **Close to significance (p = 0.05)

results obtained we could define a clear and relevant role of this method as an evolutive predictor and as a predictor of the therapeutic response in the population analyzed.

We believe that HCV viral load does not play a predictive role in the histological evolution of the disease or, at least as observed in the present study, in the therapeutic response. The initial objective was therefore reached since we defined interpretative quantification limits and demonstrated solid and feasible alternatives that would provide information about the possible course of the disease to both doctors and patients, i.e., demographic and histological factors.

In Brazil, difficulties exist in performing quantitative or non-quantitative exams involving molecular biology and the results are frequently interpreted erroneously based on the assumption that the higher the HCV viremia the poorer the disease progression. This is based on a parallelism drawn between HIV and HCV viral load. In fact, HIV viral load is a reliable predictor of disease progression, while plasma HCV viremia does not reflect the amount of virus in the liver and the plasma-liver correlation is not well established[29]. In addition, technical limitations still exist for HCV quantification, such as the standardization of the results between different methodologies, as extensively discussed by PAWLOTSKY[26], and still not completely understood aspects of virus-host interaction, which make the interpretation of "a high viral load as an index of severe disease" a naive and incorrect simplification.

So, which role does HCV RNA quantification play and what is its use? Recent studies on viral dynamics answer this question, complemented by the understanding of the role of interferon as a therapeutic agent and its impact on different viral subpopulations within the context of the host response to HCV. We discuss below different aspects concerning HCV dynamics and the role of interferon for a better understanding of our results.

Viral dynamics. The understanding of HCV kinetics is fundamental. It is known that trillions of virions are produced and eliminated each day[27], remaining in a steady state, an aspect that can be studied using interferon therapy or plasmapheresis[19], procedures that lead to alterations in the established steady state. Studies on viral dynamics, encouraged by similar investigations carried out on HIV, have regularly been conducted since 1995[39], and the data have been solidified and successively reproduced in order to better understand HCV pathogenesis and its response to antiviral therapy. The initial aim was to obtain the optimal time point to predict a sustained virological response, and 12 weeks after interferon treatment was established as the best time point to carry out HCV RNA quantification[20]. However, quantification during the fourth week was found to be reliable and able to predict a sustained virological response[2, 4,10]. ZEUZEM *et al.*[37] considered undetectability of the virus during week 4 to be a more important predictor than basal viremia or genotype. According to LAM *et al.*[17], the early clearance of HCV is due to the dose-dependent action of interferon. This result was later confirmed and great emphasis has been placed on viral dynamics since the study by NEUMANN *et al.*[23] at the end of 1998. These authors showed a biphasic viral response after the beginning of interferon therapy and clearly demonstrated a rapid response during the first 24 to 48 h of therapy accompanied by a marked reduction in viral load. This reduction was dependent on interferon and related to the inhibition of virion production and release, being influenced by the efficacy of the drug and directly dose related. Subsequently, the HCV genotype was also found to be a determining factor of a better response during this early period[18]. During a second slower phase with a variable decrease in viremia, infected cells, which represent reservoirs for de novo infection, are eliminated. This second phase is known to depend on the dose and effectiveness of interferon, on the rate of elimination by infected hepatocytes (ranging from 1.7 to more than 70 days), on T cell cytokines that inhibit HCV, and on viral genotype[18]. A decline in viral load higher than 0.3 log/week during the first four weeks[18] or > 3 log[37] is correlated with a sustained virological response. HCV RNA quantification after 4 weeks of treatment can therefore be used to predict a response. A sustained virological response thus results from the efficient action of interferon within a favorable immunologic scenario that leads to the destruction of infected hepatocytes[18]. According to NEUMANN *et al.*[23], interferon exerts multiple effects, but one vital effect is the blockade of the production or release of virions during the initial phase of therapy, while other mechanisms of action may become relevant at other time points during treatment. However, further studies using combination therapy with ribavirin and the new peg interferons have become necessary. According to LAYDEN & LAYDEN[18], the effect of ribavirin on viral kinetics is considerable and the drug does not alter the response curve to interferon, although further studies employing this combination should be carried out. Analyzing viral dynamics in patients treated with standard versus peg interferon, this author emphasized the absence of any modification in the response curve or between the different drug curves, although ZEUZEM *et al.*[36] observed superiority of peg interferon for the elimination rate of genotype 1. Based on the identical results regarding HCV dynamics obtained by ZEUZEM *et al.*[36], LAYDEN & LAYDEN[18] established a paradox: how to explain the superiority of peg interferon over standard interferon? The author raised the hypothesis that a constant concentration of interferon stabilizes the inhibition of virus production and/or increases the degradation of infected cells. In addition, non-1 (2 or 3) genotypes show rapid clearance during phase 2, thus supporting, from the point of view of viral kinetics, shorter treatment periods for patients infected with these virus subtypes[18].

Irrespective of the type of interferon, the dose or scheme employed, and viral factors, 30 to 40% of patients treated with interferon show a triphasic decrease in viral load after the beginning of therapy, with the curve reaching a plateau at the beginning of the second phase. This fact can be explained by the initial absence of a significant reduction in viremia, which, if remaining within the "limit of inhibition" (i.e., 100,000 ± 60,000 IU/ml), is followed by a discontinuation in the decline of viral load (plateau) which may last 3 to 21 days due to the lack of an efficient immune response. Once immunity has been restored, viremia starts to fall again[3]. Therefore, a triphasic response should be considered before predicting a sustained virological response based on phase 2 of the response curve. In addition, phase 1 of the curve determines the presence or absence of this plateau. Thus, the presence or absence of a sustained virological response can be predicted as early as within a few hours of therapy. However, further clinical studies are necessary to thoroughly validate these data, although the initial argumentation is highly convincing.

Based on the above considerations, we conclude that HCV RNA quantification plays a role in viral dynamics and can provide an early prediction (24 to 48 h) of a sustained virological response. On the other hand, disease progression is more complex and does not simply depend on viremia.

The role of interferon. Interferon-alpha acts in two distinct and complementary manners. On the one hand, it induces a nonspecific antiviral state in the infected cell, thus impairing HCV replication, and on the other it exerts an immunomodulatory action that potentiates the host immune response[25]. These actions are induced by binding to target cell surface receptors, triggering a cascade of intracellular reactions that lead to the activation of numerous genes whose products mediate the action of interferon. Although several interferon-induced proteins are involved, only three have been extensively studied: 2'-5' oligoadenylate synthethase (2'5'OAS), Mx proteins (MxA and MxB), and double-strand RNA-dependent protein kinase (PKR). The Mx proteins seem to be less important for HCV (25), while 2'5'OAS and PKR, nonspecifically activated by virus replication products (RNA), are fundamental. Both proteins interfere with the synthesis of new virus particles - 2'5'OAS through endoribonucleases (Rnase L) that destroy virus precursors and PKR by inactivating the eukaryotic initiation translation factor eIF2, thus blocking virus replication.

The immunomodulatory action of interferon-alpha occurs after binding to receptors on the surface of immune cells, triggering four main actions: induction of antigen expression through MHC I, activation of effector cells (natural killer cells, macrophages, cytotoxic T lymphocytes), interaction with the cytokine cascade stimulating the production of T helper cells (Th1) (producing INF-γ and IL-2) and reducing Th2 cells (main producers of IL-4 and IL-5), and anti-inflammatory actions inhibiting the production of IL-1, IL-8 and TNF-α and stimulating the production of IL-10 at the periphery.

Based on the lack of a completely efficient therapy and the fact that the results in terms of virus eradication are in fact quite unsatisfactory, it is important to evaluate and understand which mechanisms of interferon resistance are used by HCV. An outstanding line of research during recent years was initiated by ENOMOTO *et al.*[8], who described a region associated with the response to interferon - called interferon sensitivity determining region (ISDR) - located between amino acids 2209 and 2248 of the NS5A protein in an HCV 1b strain, called HCV-J. Strain HCV-J was considered to be the wild type, with mutations (4 to 11 amino acid substitutions) being related to a higher probability of therapeutic response, a fact that defines the presence of an ISDR mutant as a predictive factor for response.

The lack of reproducibility of these data outside Japan and inconsistent *in vivo* results aroused some initial suspicion and even irony (could this be a "Japanese effect"?)[13]. In fact, most European researchers did not find the same relevance of ISDR mutations as predictors of response[16,32,38], except for one study[12]. In this regard, data were reproduced[21] and refined, demonstrating that the larger the number of mutations, the stronger the correlation with a sustained virological response, especially when the mutations occurred in amino acids 2209, 2216 and 2227. In addition, the relevance of ISDR found in the Japanese study was categorically reaffirmed, irrespective of the discrepancies from western studies[35].

The differences in the data between Japan and the western world were subsequently investigated more extensively and meta-analysis of the available studies revealed that the ISDR is only valid as a predictor for genotypes 1a and 1b[24], with three possibilities being proposed to explain the conflicting results: ISDR is not the only region of the NS5A gene involved in the inhibition of the antiviral effects of interferon, other factors (e.g., race) may have interfered with the European studies, and an undetectable number of ISDR resistant quasispecies are present before treatment which eventually predominate during therapy[24].

Despite the controversy, the mechanism of interference of ISDR with the response to interferon was unknown until GALE Jr. *et al.*[9] demonstrated an interaction between the carboxy terminal portion of NS5A and PKR. The interferon-induced PKR gene possesses multiple functions activated by double-strand RNA, including pro-apoptotic actions, growth control and differentiation and, obviously, the previously discussed inhibition of translation in response to viral infection[9]. Interaction between PKR and NS5A inhibits the functions of this kinase, while an ISDR mutant loses this binding capacity and therefore maintains the effects of the PKR kinase.

Based on the fact that the ISDR studies on European patients were conducted after treatment, PATERSON *et al.*[24] performed an *in vivo* analysis of the NS5A region in western patients using samples collected before and during treatment and using different genotypes. The following observations were made: genotype 1 possessed the same behavior as the HCV-J strain; no differences in the NS5A region were observed between non-1 genotypes and HCV-J, while the criteria for ISDR mutation were noted (these being therefore "sensitive" genotypes); the ISDR of genotype 3 differed from that of genotype 4, with genotype 3 being responsive and 4 being non-responsive; a higher amino acid diversity was observed for genotype 1 compared to non-1; genotype 1 showed higher ISDR diversity before treatment, with interferon exerting selective pressure and reducing diversity during treatment; alterations in more than three ISDR amino acids predicted a response for genotype 3 but not for genotype 4, and, finally, a specific model is required to understand the NS5A/PKR interaction in the case of non-1 genotypes, since the model of Enomoto was found to be inadequate. These observations led to the following two conclusions. First, no resistant quasispecies occurred during treatment, thus excluding an explanation for the differences observed between Japan and the western world, and, second, the ISDR should not be considered as the only response determining factor among European patients. In fact, mutations in a region comprising amino acids 2356 to 2379 of the NS5A gene, called V3, are correlated with an interferon response through an as yet unknown mechanism[33].

Recently, PODEVIN *et al.*[28] have extended the discussion about the role of NS5A, questioning previous models that did not analyze the role of NS5A in hepatocytes and whose results should therefore be considered with caution. In their study, the first one on hepatocytes, the authors analyzed human hepatocytic cell lines (Huh 7) expressing NS5A sequences derived from one responder and two non-responders in the presence of interferon-alpha and exposed to virus strains sensitive to the drug. When the antiviral action of interferon was measured, NS5A was found to confer resistance. The ISDR of the responder corresponded to the interferon-sensitive virus, while a non-responder represented the wild type and the other possessed 8 amino acid mutations. Exposure to interferon resulted in virus multiplication in the wild type (ISDR resistant) but, surprisingly, also in the mutants (ISDR sensitive), although to a lesser extent. Thus, NS5A plays an ISDR-independent role in the escape from interferon, since mutations in this region are not associated with treatment efficacy. The authors therefore concluded that neither the mutant nor the wild type depend on PKR and proposed NS5A-induced

IL-8 expression as a possible mechanism for the inhibition of the antiviral actions of interferon.

According to PODEVIN *et al.*[28], NS5A participates in at least four actions:

1. It interferes with the double-strand RNA-dependent interferon-induced PKR kinase.

2. NS5A-mediated PKR inhibition may abolish PKR-dependent apoptosis (a fundamental process for the destruction of infected hepatocytes which occurs during the second phase of the viremia reduction curve in response to interferon, as emphasized by WATANABE *et al.*[35] who showed that apoptosis is also induced by TNF-alpha which in turn is mediated by interferon-alpha) and induce phenotype alterations in NIH3T3 cells (carcinogenesis?).

3. Cis transactivation of various elements such as Grb2 - abolished in mutant strains.

4. NS5A may interfere with nuclear RNA and protein transport by interacting with karyophenin ß3.

Therefore, NS5A expression may play a crucial role in the control of the antiviral and proliferative state of HCV-infected hepatocytes through interaction with multiple cell signaling pathways. This protein should be thoroughly studied in other models since it contains domains (ISDR, PKR-binding domain, V3) that are fundamental for the understanding of the interaction with interferon. TAYLOR[33] observed that in all models available NS5A inhibited the action of interferon regardless of sequence or genotype. The author thus proposed that a basal level of NS5A expression confers some protection on the virus, opening the possibility that other viral genes or host factors determine resistance to interferon, for example, the highly conserved sequences of the E2 region of interferon-resistant genotypes which act by inhibiting PKR. Therefore, E2 may confer a basal level of resistance on resistant genotypes and new therapies should consider the neutralization of this basal inhibition of endogenous or exogenous interferon which is certainly mediated by genes other than NS5A and E2.

PAWLOTSKY[25] divided resistance-related factors into factors associated with treatment, patient, disease and viral factors, as follows:

Treatment-related factors:

1. Treatment scheme during induction: three times a week is inadequate in terms of viral kinetics since viremia increases before the second dose (this effect is reduced by ribavirin and daily interferon doses or peg interferon).

2. Treatment duration: a longer duration is associated with fewer relapses.

Patient-related factors:

1. Age (the older the patient the poorer the response)

2. Sex (females show a better response)

3. Race (Hispanics and Africans show a poorer response than Caucasians and Asians) - the first three races show genetic, hormonal and/or immunological determinants.

4. Weight (alters drug distribution, reducing its concentration in the receptors).

5. Anti-interferon antibodies.

6. Habits: alcoholism, drug use.

7. Treatment compliance.

Factors related to disease and associated with a poor response:

1. Advanced fibrosis and compensated cirrhosis.

2. HIV-HCV co-infection

3. Extra-hepatic manifestations.

4. Non-response to previous treatments.

5. Normal ALT and poorly active chronic hepatitis (which are in fact "patients for whom it is difficult to decide about a therapy" rather than patients "difficult to treat").

HCV-associated factors:

1. Diverse and numerous populations of quasispecies: absence of intrinsic resistance since at a certain time viremia is always reduced in response to interferon. The modifications result from the adaptation of these populations to the environment created in the presence of interferon, bearing in mind that interferon does not act directly on HCV but stimulates an unfavorable environment for the virus. Therefore, no resistance to the drug but rather adaptation to the hostile environment exists, in contrast to the previously discussed proposal of TAYLOR[33] with respect to the basal protection level conferred by NS5A and E2, among other probable factors.

2. Genotypes. Probably related to differences in nucleotides which result in different proteins among isolates, with a greater or lower capacity to escape the actions of interferon. Again, a role for NS5A, E2 and even viral polyprotein is proposed, which may inhibit the Jak-Stat pathway, the main interferon signal transduction pathway.

Based on this vast panorama and the absence of new clarifying experiments confirming *in vivo* results, PAWLOTSKY[25] reached three conclusions regarding interferon resistance: i) resistance to interferon is multifactorial, ii) is characterized by significant qualitative and quantitative population alterations, and iii) such alterations can have consequences for the prognosis of hepatic disease.

One aspect that should be considered at the end of this discussion is the fact that patients with advanced fibrosis due to hepatic cirrhosis show an unfavorable disease course. In view of the difficulties in eradicating HCV, various reports have emphasized the importance of interferon as a modulating agent of fibrogenesis in non-responders, such as the recent

study by ALRIC *et al*.[1]. Furthermore, some studies have reported complete resolution of hepatic cirrhosis[5]. WANLESS[34] partially agreed with this study by emphasizing that vascular lesions remained despite the removal of collagen. However, clinical studies have demonstrated the effective modulation of fibrogenesis. SIME & O'REILLY[31] postulated the hypothesis that fibrosis is the result of a disequilibrium in the Th1/Th2 immune response, with the type 1 response being associated with tissue architecture restoration and type 2 with exuberant fibroblast activation and proliferation, thus causing fibrogenesis. **Interferon-**γ - the prototype of the Th1 response - possesses various potential actions on fibrogenesis (i.e., inhibition of fibroblast proliferation and collagen deposition, promoting apoptosis of fibroblasts and inhibiting **TGF-**β, a fibrogenic cytokine) and its action is increased by other type 1 cytokines. Experimental and animal models have confirmed this hypothesis, thus opening a field for the study of treatments of diseases that result in tissue fibrosis[31]. As mentioned before, interferon-alpha favors a Th1 response, an effect that might modulate fibrogenesis in the models proposed by SIME & O'REILLY[31].

Based on a study in which the nowadays inadequate interferon monotherapy was used, our results and the review of the literature demonstrated that hepatitis C evolution depends on multiple factors related to histological, host and viral aspects, including not only quantitative factors but mainly the diversity and dynamics of the viral population, in addition to aspects of treatment. We were able to demonstrate that viremia is an important aspect but should not be used alone for the prediction of disease outcome since its interpretation differs from that of HIV viremia. Although our study design might be criticized and considered to be old-fashioned due to the use of monotherapy, our model was found to be strong and sufficient to suggest what subsequent investigations confirmed - such as the recent study by GERVAIS *et al.*[11] who demonstrated in a similar study that viremia is correlated with genotype and response to treatment but not with histological lesions - and to broaden the set of possibilities to study and better understand the different aspects of hepatitis C. In this respect, the professionals involved with hepatitis C should have an ample vision and should be aware of the extreme velocity at which knowledge about the pathogenesis of the disease and implications for patient follow-up and treatment accumulate.

RESUMO

A carga viral do vírus da hepatite C não prediz a evolução: indo além dos números

Através da análise de 58 pacientes tratados com Interferon Alfa em função de hepatite C crônica e sem cirrose, demonstramos que a carga viral do Vírus da Hepatite C (VHC) não se correlacionou com a evolução histológica da doença ($p = 0,6559$ para alterações arquiteturais e $p = 0,6271$ para o Índice de Atividade Histológica-IAH). Assim a utilização da quantificação do RNA viral como preditor evolutivo ou determinante da gravidade da hepatite C é incorreto e de valor relativo. Revisando o tema encontramos variáveis do VHC (genótipo, heterogeneidade e mutantes, proteínas específicas), do hospedeiro (sexo, idade, peso, etc) e dos medicamentos (posologia, tempo de tratamento, tipo de Interferon) fundamentais e interdependentes, inseridas no contexto mais amplo da cinética viral, da resposta imunológica mediada pelo Interferon (além da imunidade natural em resposta ao VHC) e do papel do Interferon como modulador da fibrogênese. Assim, há muito mais que números por trás da Carga Viral e sua correta interpretação deve ser feita considerando-se um horizonte mais amplo dependente de múltiplos fatores mais complexos que o simples valor obtido na quantificação

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