Inosine triphosphate pyrophosphohydrolase activity: more accurate predictor for ribavirin-induced anemia in hepatitis C infected patients than *ITPA* genotype.

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ABSTRACT

Background

ITPA polymorphisms have been associated with protection against ribavirin-induced anemia in chronic hepatitis C (HCV) patients. Here we determined the association of inosine 5'-triphosphate pyrophosphohydrolase (inosine triphosphatase or ITPase) enzyme activity with *ITPA* genotype in predicting ribavirin-induced anemia.

Methods

In a cohort of 106 HCV patients, hemoglobin (Hb) values were evaluated after 4 weeks (T_4) and at the time of lowest Hb value (T_{nadir}). ITPase activity was measured and *ITPA* genotype determined. Single-nucleotide polymorphisms (SNPs) tested were c.124+21A>C and c.94C>A. ITPase activity ≥1.11 mU/mol Hb was considered as normal.

Results

After 4 weeks of treatment, 78% of the patients with normal ITPase activity were anemic and 21% of the patients with low ITPase activity ($p<0.001$). Stratified by genotype, the percentages of anemic patients were: wt/wt 76%, wt/c.124+21A>C 46% ($p=0.068$), and wt/c.94C>A 29% ($p=0.021$). At T_{nadir} virtually all patients with normal ITPase activity were anemic, compared to only 64% of the patients with low activity ($p=0.02$). Thirteen patients had wt/c.124+21A>C genotype. Within this group all five patients with normal ITPase activity and only four of eight with decreased activity developed anemia. Presence of HCV RNA did not influence ITPase activity.

Conclusions

This study is the first to report that ITPase activity predicts the development of anemia during ribavirin treatment. ITPase activity and *ITPA* genotype have high positive predictive values for development of ribavirin-induced anemia at any time during treatment, but ITPase activity predicts ribavirin-induced anemia more accurately.

INTRODUCTION

The prevalence of hepatitis C (HCV) infection is estimated at approximately 2.2%–3% of the world population (130– 170 million people).[1] The life expectancy of infected patients is reduced significantly because of high risks for liver cirrhosis and hepatocellular carcinoma.[2] HCV therefore is one of the main reasons for liver transplantation in Europe and the US.[3,4] In order to prevent these complications, patients with HCV infection have been treated with the combination of pegylated-interferon-α plus ribavirin,[5-7] in later years combined with protease inhibitors, such as telaprevir[8] or boceprevir[9] and since recently simeprevir[10] or the nucleoside polymerase inhibitor sofosbuvir.[11] Response to anti-HCV therapy is influenced by both viral and host factors as well as drug toxicity.[12] Viral genotype has been a strong predictor for treatment response. While viral remission is around 70%–80% in patients infected with HCV genotypes 2 and 3, only 50%–60% of the patients with HCV genotypes 1 and 4 acquire a sustained virological response (SVR).[13] Host factors contributing to therapeutic outcome have been identified as single-nucleotide polymorphisms (SNPs) in the *IL28B* and *LDLR* genes.[14-18] An important and common adverse drug reaction limiting optimal HCV therapy is ribavirin-induced anemia. It has been demonstrated that two functional *ITPA* SNPs, rs1127354 (c.94C>A) and rs7270101 (c.124+21A>C), are associated with protection from ribavirin-induced anemia.[19-25]

In recent years, the biological and pharmacogenetic significance of *ITPA* and its corresponding enzyme inosine 5'-triphosphate pyrophosphohydrolase (inosine triphosphatase or ITPase) have become a focal point of research, bringing many interesting and surprising data. Complete ITPase deficiency is strictly confined to erythrocytes and is considered to be a benign condition. No primary, causal, clinical symptoms are known under normal circumstances. However, ITPase activity lowering SNPs in *ITPA* are associated with adverse drug reactions to the thiopurines azathioprine and 6-mercaptopurine. This association is still subject of a lively discussion.[26-30] The pharmacogenetic significance of *ITPA* appeared not to be limited to the thiopurines, but may also be of significance for the purine analog ribavirin. In our present study we demonstrate that ITPase activity seems a more accurate predictor for ribavirin-induced anemia than *ITPA* genotype.

MATERIALS AND METHODS

Patients

Consecutive HCV infected patients attending the outpatient clinic of the Erasmus Medical Center in Rotterdam, The Netherlands were included during 6 months with the aim of inclusion of 100 patients. The following data were collected: gender, age, hemoglobin (Hb),

white blood cell count (WBC), HCV genotype and HCV RNA in serum, type of medication, start and end of treatment, and treatment outcome. Treatment was given according to the Dutch national guidelines at that time[31] with peginterferon-α-2a or peginterferon-α-2b (in a dosage of 180 μg or 1.5 μg/kg once a week), in combination with ribavirin 800–1200 mg a day depending on patient weight. One patient also received boceprevir and one patient also received miravirsen in addition to the ribavirin and peginterferon-α.

Plasma HCV-RNA levels were measured with the use of the COBAS AmpliPrep/COBAS TaqMan HCV assay, version 1.0 (Roche molecular systems) from October 2008 until March 2012, version 2.0 (Roche molecular systems) was used from March 2012 until June 2012. Anemia was defined as Hb reduction of ≥1.9 mmol/L compared to pre-treatment values and/or Hb concentrations <7.5 mmol/L for females and <8.0 mmol/L for males.[22,32]

The study was performed according to the Helsinki Declaration and approved by the Medical Ethical Committees of the Erasmus Medical Center, Rotterdam, The Netherlands. The participants provided their written informed consent to participate in this study.

ITPase activity

ITPase activity was determined as described previously with some minor modifications[33] and measured by formation of inosine 5'-monophosphate (IMP) from ITP. Briefly, 3 μL whole blood was incubated with 2.00 mM ITP, 50 mM $MgCl_2$, 0.5 mM Dithothreitol (DTT) and 0.2 mM α-,β-methyleneadenosine 5'-diphosphate (AMP- CP) in 75 mM Tris in a final volume of 200 μL. All chemicals were of the highest grade and purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands).

Samples were prepared and analyzed in duplicate. In addition, blanks (negative controls) and pool samples (positive controls) were also analyzed for ITPase activity to confirm correct sample preparation, analysis and quality control. High performance liquid chromatography (HPLC) separations were performed on a Supelcosil LC-18 S column (Sigma-Aldrich, Zwijndrecht), using an Alliance separation system (Waters, Etten-Leur, The Netherlands) coupled to a Jasco multi-wavelength detector (Jasco Benelux, IJsselstein, The Netherlands). Data were analyzed with TotalChrom data acquisition and handling software (Perkin-Elmer, Groningen, The Netherlands). ITPase activity was expressed as milliUnits of IMP formed from ITP per mol hemoglobin (mU/mol Hb). The intra-assay variation coefficient was <5%, and the inter-assay variation coefficient was <10%. The cut-off value discriminating between normal or decreased ITPase activity was set at 1.11 mU/mol Hb (=4 mmol IMP/mmol Hb/h), which is the lowest value within the 95% CI for *ITPA* wild type (wt/wt) carriers.[34,35]

ITPA genotype analysis

Genomic DNA was isolated from whole blood using the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA) and genotyped for two *ITPA* polymorphisms; wt/c.94C>A (p.Pro32Thr, rs1127354) and wt/c.124+21A>C (rs7270101). When no polymorphisms were detected at both positions and the ITPase activity was within the wt/wt reference intervals, the genotype was considered to be wt/wt. M13-tagged primers forward 5'-TGTAAAACGACGGCCAGTCTTAGGAGATGGGCAGCAG and 5'-CAGGAAACAGCTATGACCCACAGAAAGTCAGGTCACAGG reverse were used in a PCR reaction, which consisted of 1xAmplitaq Gold Mastermix (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands), 8% glycerol, and 200 nM of each primer. PCR conditions were 40 cycles with an annealing temperature of 60°C. The resulting 241 bp PCR product was purified and directly sequenced in both directions using the Big Dye Terminator kit and was subsequently analyzed on an ABI 3720 Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). The resulting sequence was aligned with *ITPA* reference sequence NM_033453.2. All sequences were evaluated by two independent laboratory experts.

Statistical analysis

Results were analyzed using GraphPad Prism 5.01 (Graphpad Software for Windows, San Diego, CA, USA), Microsoft Excel (Microsoft, Redmond, WA, USA) software and IBM SPSS Statistics 20 (IBM Corporation, New York, NY, USA) software. Pearson's χ^2-tests, Fisher's exact tests (in the case of small sample sizes) or T-tests for independent samples were used to determine significant differences. p-Values <0.05 were considered to be statistically significant.

RESULTS

Patient characteristics

A total of 106 HCV infected patients in various stages of infection were included (Table 1). In our cohort there was a male predominance and HCV genotype 1 was most prominent. In total 69 patients were treated for chronic HCV infection and SVR was reached in 30 (43.5%) of the patients. The most prominent *ITPA* genotype was wt/wt (68.9%). The occurrence of wt/c.124+21A>C and wt/c.94C>A *ITPA* genotype variants were 19.8% and 9.4%, respectively. One patient was homozygous for c.124+21A>C and one was compound heterozygous, i.e. c.124+21A>C/c.94C>A. Our cohort showed expected allele frequencies for both loci and did not differ from the reference population.[35,36] The population was in Hardy-Weinberg equilibrium. Age, pre-treatment Hb levels and white blood cell counts were not significantly different between patients with different *ITPA* genotypes. As expected, mean ITPase activity correlated with *ITPA* genotype (Table 1).

Table 1. Characteristics of the study population

Characteristic	Total Population (n=106)	Treated Population (n=69)
Age, median years (min-max)		
Total	51 (20-88)	52 (20-79)
Wt/wt	51 (20-88)	52 (20-79)
Wt/c.124+21A>C	51 (33-76)	52 (39-76)
Wt/c.94C>A	55 (41-65)	54 (41-59)
Gender, n (%)		
Male	68 (64.2)	51 (73.9)
Female	38 (35.8)	18 (26.1)
HCV genotype, n (%)		
Genotype 1	63 (59.4)	44 (63.8)
Genotype 2/3	28 (26.4)	20 (28.9)
Genotype 4	8 (7.5)	5 (7.2)
Unknown	7 (6.6)	-
***ITPA* genotype,** n (%)		
Wt/wt	73 (68.9)	49 (71.0)
Wt/c.124+21A>C	21 (19.8)	13 (18.8)
Wt/c.94C>A	10 (9.4)	7 (10.1)
Other	2 (1.8)	-
HCV genotype 1, n (%)		
Total	63 (59.4)	44 (63.8)
Wt/wt	45 (71.4)	33 (75.0)
Wt/c.124+21A>C	11 (17.5)	7 (15.9)
Wt/c.94C>A	7 (11.1)	4 (9.1)
ITPase mean activity[a] ± SD		
Wt/wt	1.64 ± 0.47	1.67 ± 0.50
Wt/c.124+21A>C	1.01 ± 0.29	1.03 ± 0.37
Wt/c.94C>A	0.46 ± 0.16	0.50 ± 0.17
Other	0.33 ± 0.19	-
Absolute ITPase activity (%)		
Wt/wt	100%	
Wt/c.124+21A>C	62%	
Wt/c.94C>A	28%	
Other	20%	
ITPase mean activity[a] ± SD		
HCV RNA <5 copies/ml	1.44 ± 0.69	
HCV RNA ≥ 5 copies/ml	1.34 ± 0.53	
ITPase activity[a]**,** n (%)		
< 1.11	29 (27.4)	16 (23.2)
≥ 1.11	77 (72.6)	53 (76.8)

Table 1. Characteristics of the study population (continued)

Characteristic	Total Population (n=106)	Treated Population (n=69)
Mean white blood cell count[b] ± SD	5.9 ± 2.4	5.5 ± 2.4
Mean Hb pre-treatment[c] ± SD		9.0 ± 1.0
Treatment outcome, n (%)		
SVR[d]		30 (43.5)
Relapse		22 (31.9)
Non-response / termination		17 (24.6)
Spontaneous clearance	7 (6.6)	

[a] mU/mol Hb; [b] $x10^9$/L; [c] mmol/L; [d] SVR, Sustained virological response

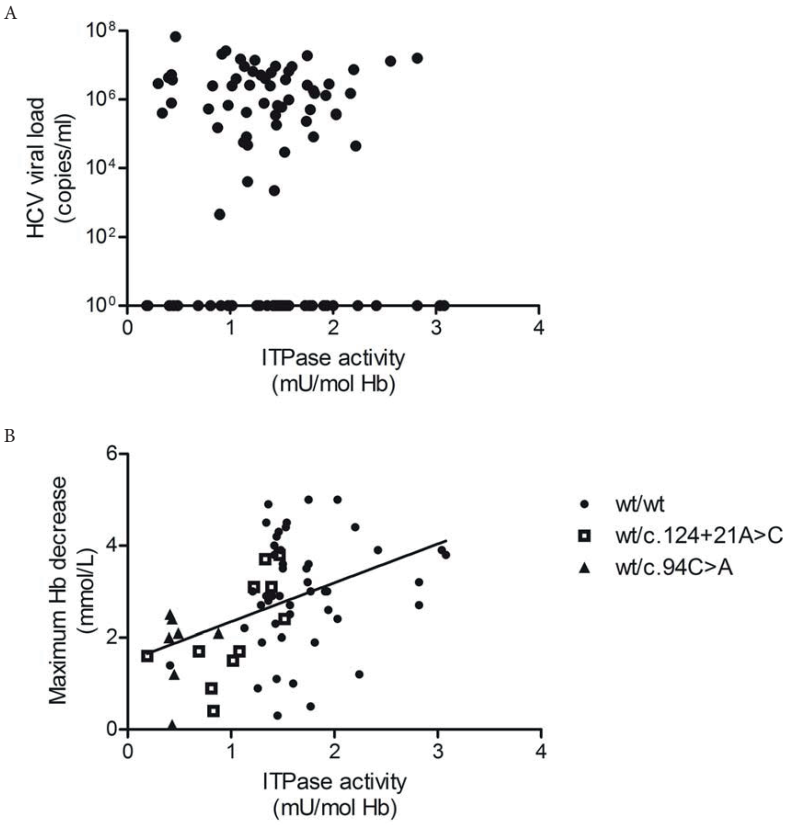

Figure 1: (A) ITPase activity and HCV viral load. Plasma HCV viral load (copies/ml) is plotted against ITPase activity (milliUnits IMP/mol Hb). Neither the presence nor the level of plasma HCV viral load is correlated to ITPase activity. HCV, hepatitis C virus; ITPase, inosine triphosphate pyrophosphohydrolase. (B) Association of ITPase activity and Hb decrease. ITPase activity (milliUnits IMP/mol Hb) is plotted against maximum Hb decrease during therapy. *ITPA* genotypes are displayed in different shapes (circles: wt/wt, squares: wt/c.124+21A>C, triangles: wt/c.94C>A). Higher ITPase activity is associated with increased Hb decline. ITPase, inosine triphosphate pyrophosphohydrolase; Hb, Hemoglobin.

Hepatitis C and ITPase activity

ITPase activity was not statistically significantly different in HCV-infected patients and non-HCV controls (data not shown).[35] Presence or absence of HCV-RNA was not associated with ITPase activity (Figure 1A), regardless of genotype. In patients with a detectable HCV-RNA, the viral load was not associated with ITPase activity (Figure 1A).

Hemoglobin levels and ITPase activity

In total 53 of 77 patients with normal ITPase activity, and 16 of 29 patients with decreased ITPase activity were treated with pegylated-interferon-α plus ribavirin. ITPase activity was significantly associated with Hb decrease (Table 2, Figure 1B, p<0.001). Of the patients having normal ITPase activity, 78.0% (n=39) were anemic after 4 weeks of therapy (T_4), compared to 21.4% (n=3) of the patients with reduced ITPase activity, see Table 2 (p<0.001). Exactly 92.2% of patients with normal ITPase activity developed anemia at any moment during therapy (T_{nadir}), compared to 64.3% of patients with low ITPase activity (p=0.02, Table 2). Test characteristics are shown in Table 3. The positive predictive value (PPV) of normal ITPase activity was 78% for anemia at 4 weeks and 92% for the development of anemia at any time during therapy. The negative predictive value (NPV) of a decreased ITPase activity for the development of anemia was 79% and 36%, respectively, for T_4 and T_{nadir}.

Table 2. Comparison of ITPase activity and *ITPA* genotype in development of anemia, and percentage of successful treatment outcome (SVR) in the treated population (n=69).

	ITPase activity (mU/mol Hb)			***ITPA* genotype**				
	≥ 1.11 (n=53)	**< 1.11 (n=16)**	**p-value**	**Wt/wt (n=49)**	**Wt/c.124+21A>C (n=13)**	**p-value**	**Wt/c.94C>A (n=7)**	**p-value**
Pre-treatment								
Hb[a]	9.0 ± 1.0[b]	8.9 ± 1.0	0.84	9.0 ± 1.0[b]	8.9 ± 0.7	0.84	9.3 ± 1.3	0.45
T_4								
Hb[a]	7.3 ± 1.0[c]	8.7 ± 1.0[b]	<0.001	7.3 ± 1.0[c]	7.9 ± 1.2[b]	0.06	8.8 ± 1.4	0.001
Anemia; n (%)	39 (78.0)[c]	3 (21.4)[b]	<0.001	35 (76.1)[c]	5 (45.5)[b]	0.07	2 (28.6)	0.02
Reduction[a]	1.7 ± 1.1[c]	0.4 ± 0.4[b]	<0.001	1.7 ± 1.1[c]	1.1 ± 1.3[b]	0.17	0.5 ± 0.4	0.006
T_{nadir}								
Hb[a]	5.9 ±1.1[b]	7.5 ±1.2[b]	<0.001	6.0 ± 1.1[b]	6.9 ± 1.1[b]	0.02	7.5 ± 1.5	0.002
Anemia; n (%)	47 (92.2)[b]	9 (64.3)[b]	0.02	43 (91.5)[b]	9 (81.8)[b]	0.32	4 (57.1)	0.04
Reduction[a]	3.1 ± 1.1[b]	1.5 ± 0.7[b]	<0.001	3.0 ± 1.2[b]	2.2 ± 1.1[b]	0.04	1.8 ± 0.9	0.012
SVR; n (%)	23 (43.4)	7 (43.8)	0.98	22 (44.9)	5 (38.5)	0.68	3 (42.9)	0.99

[a]Mean ± SD (mmol/L); [b] Values missing from 2 patients; [c] Values missing from 3 patients

Hemoglobin levels and *ITPA* genotype

Treatment with pegylated-interferon-α plus ribavirin was started in 49 of 73 patients with the *ITPA* wt/wt genotype, in 13 of 21 patients with *ITPA* wt/c.124+21A>C genotype and

Table 3. Comparison of ITPase activity and *ITPA* genotype in occurrence of anemia after 4 weeks of therapy (T_4) and at any time during therapy (T_{nadir}) and positive (PPV) and negative (NPV) predicting test characteristics.

	T_4 (n=64)			T_{nadir} (n=65)		
	Anemia	**No anemia**	**Predictive value**	**Anemia**	**No anemia**	**Predictive value**
ITPase activity[a]						
≥1.11	39	11	PPV: 78%	47	4	PPV: 92%
<1.11	3	11	NPV: 79%	9	5	NPV: 36%
***ITPA* genotype**						
Wt/wt	35	11	PPV: 76%	43	4	PPV: 91%
Wt/c.124+21A>C+Wt/c.94C>A	7	11	NPV: 61%	13	5	NVP: 28%

[a] mU/mol Hb

in 7 of 10 patients with *ITPA* wt/c.94C>A genotype. At Week 4 of therapy, anemia was observed in 76.1% of the patients carrying *ITPA* wt/wt, 45.5% of the patients carrying *ITPA* wt/c.124+21A>C (p=0.07) and 28.6% of the patients with *ITPA* wt/c.94C>A genotype (Table 2, p=0.02).

Anemia at any time during treatment (T_{nadir}) occurred significantly less frequently in patients with the wt/c.94C>A genotype (57.1%) compared to patients with the wt/wt genotype (91.5%) (p=0.04, Table 2). Hb at T_{nadir} was significantly higher in patients with the wt/c.124+21A>C *ITPA* genotype, and Hb reduction was significantly less compared to wt/wt *ITPA* genotype, but there was no difference in frequency of anemia (p=0.32).

ITPase activity vs. *ITPA* genotype

Of the patients with the wt/c.124+21A>C genotype, 38.5% (5 of 13) had a normal ITPase activity (Table 4). Of these five patients, four developed anemia at T_4 (80%), whereas in the eight patients with the same genotype, but with decreased ITPase activity, only one patient became anemic. At T_{nadir} all wt/c.124+21A>C patients with normal ITPase activity developed anemia in contrast to only four of the eight patients with decreased ITPase activity.

In all patients carrying wt/c.94C>A *ITPA* genotype ITPase activity was decreased and anemia was present in four of seven patients. Of the wt/wt genotype carrying patients, 48 of 49 had a normal ITPase activity and the patient with low ITPase activity developed anemia.

PPV for wt/wt genotype and ITPase activity were not different for both T_4 and T_{nadir} (Table 3). NPV for the ITPase activity lowering *ITPA* genotypes (wt/c.124+21A>C and wt/c.94C>A together) was lower compared to NPV for ITPase activity <1.11 mU/mol Hb for both T_4 (61% vs. 79%) and T_{nadir} (28% vs. 36%).

Table 4. Occurrence of anemia in treated patients according to *ITPA* genotype and ITPase activity after 4 weeks of therapy (T_4) and at any time during therapy (T_{nadir}).

ITPA genotype	Total, n	T_4, n (%)	T_{nadir}, n (%)
Wt/wt	49		
Activity[a] <1.11	1		
No anemia		1 (100)	
Anemia			1 (100)
Activity[a] ≥1.11	48		
No anemia		10 (21)	4 (8)
Anemia		35 (73)	42 (88)
Unknown		3 (6)	2 (4)
Wt/c.124+21A>C	13		
Activity[a] <1.11	8		
No anemia		5 (63)	2 (25)
Anemia		1 (13)	4 (50)
Unknown		2 (25)	2 (25)
Activity[a] ≥1.11	5		
No anemia		1 (20)	
Anemia		4 (80)	5 (100)
Wt/c.94C>A	7		
Activity[a] <1.11	7		
No anemia		5 (71)	3 (43)
Anemia		2 (29)	4 (57)
Activity[a] ≥1.11	0		
No anemia			
Anemia			

[a] mU/mol Hb

Treatment outcome

SVR was achieved in 43.5% of the patients (n=30), whereas in 56.5% (n=39) treatment failed due to relapse, serological non-response, or termination of therapy because of adverse events. SVR was not associated with ITPase activity or *ITPA* genotype (Table 2), nor with anemia (data not shown). Table 5 shows the treatment outcome stratified by HCV genotype. Taking into account HCV genotype, still SVR was not associated with ITPase activity (Figure 2A) or *ITPA* genotype (Figure 2B). Also per-protocol analysis of the patients adherent to the entire treatment regimen, did not show an association between SVR and ITPase activity or *ITPA* genotype (data not shown).

Table 5. Treatment outcome per hepatitis C genotype, ITPase activity and *ITPA* genotype.

HCV genotype	Treatment outcome	ITPase activity[a]		*ITPA* genotype		
		≥ 1.11 (n=53)	< 1.11 (n=16)	Wt/wt (n=49)	Wt/c.124+21A>C (n=13)	Wt/c.94C>A (n=7)
1						
	SVR[b]	11	3	11	2	1
	Non-response	6	3	5	2	2
	Relapse	16	2	14	3	1
	Termination	3	0	3	0	0
2/3						
	SVR[b]	11	4	10	3	2
	Non-response	1	0	1	0	0
	Relapse	0	2	0	1	1
	Termination	0	2	0	2	0
4						
	SVR[b]	1	-	1	-	-
	Non-response	2	-	2	-	-
	Relapse	2	-	2	-	-
	Termination	0	-	0	-	-

[a] mU/mol Hb; [b] SVR, sustained virological response

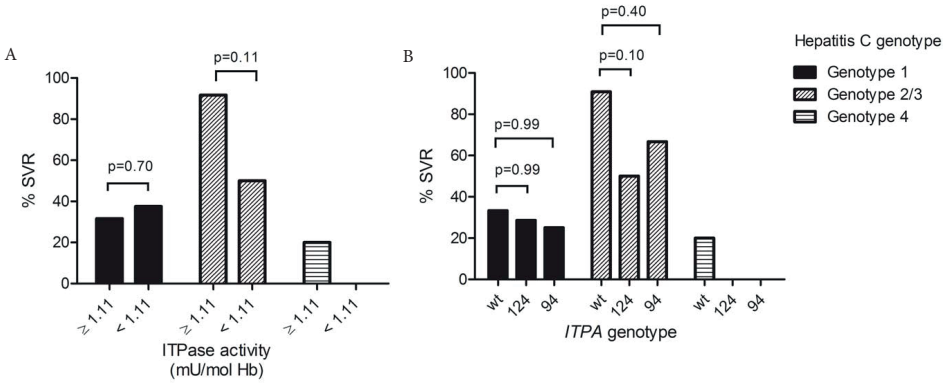

Figure 2. (A) The percentages of patients reaching SVR are shown when patients are stratified by hepatitis C genotype. No significant differences were observed between patients with ITPase activity ≥1.11 mU/mol Hb and with patients with ITPase activity <1.11 mU/mol Hb. SVR, sustained virological response; ITPase, inosine triphosphate pyrophosphohydrolase. (B) The percentages of patients reaching SVR are shown when patients are stratified by hepatitis C genotype. No significant differences were observed between patients with *ITPA* genotype wt/wt and patients with *ITPA* genotypes wt/c.124+21A>C and wt/c.94C>A. SVR, sustained virological response; wt, wt/wt; 124, wt/c.124+21A>C; 94,wt/c.94C>A.

DISCUSSION

This is, to our knowledge, the first study evaluating the association of ITPase enzyme activity and ribavirin-induced anemia and comparing it to *ITPA* genotype in patients treated for chronic HCV. All studies evaluating the association of *ITPA* polymorphisms with ribavirin-induced anemia assumed that in HCV patients, the reported ITPase activity directly corresponds to a specific *ITPA* polymorphism.[19,20,22,23,37] Here we show that an *ITPA* variant such as wt/c.124+21A>C leads to a variety of ITPase activities ranging from as low as 0.19 to as high as 1.52 mU/mol Hb and association is less direct as has previously been assumed. More in depth analysis showed that negative predicting value for ribavirin-associated anemia of the wt/c.124+21A>C genotype was only 18%. Within this group, all the patients with normal ITPase activity developed anemia throughout the treatment period, compared to 50% of the patients with decreased ITPase activity.

Most studies[20,22,25,37,38] only investigated Hb values after 4 weeks of therapy as at this time point many patients may start with erythropoietin treatment to stimulate red blood cell production. In our cohort, mean time to nadir was 4 months with 61% of patients having anemia at T_4, and 84% having anemia at T_{nadir}. ITPase activity was statistically significantly associated with anemia at both T_4 and T_{nadir}.

In two studies *ITPA* genotype polymorphisms were found to be protective for anemia during the course of the entire treatment.[39,40] The predictive value of *ITPA* genotype was similar to that reported in the literature in our hands, despite the small sample size.[22,24,37] Differences in occurrence of anemia were only statistically significant for *ITPA* wt/wt compared to wt/c.94C>A.

No influence of HCV presence or titer could be detected, this is in contrast with our observation in human immunodeficiency virus (HIV)-infected population, in which the geno-phenotype correlation differs significantly from the reference population.[35] Similar to *ITPA* polymorphisms in other studies, ITPase activity was not predictive for SVR in our cohort.[19,21,37] Some studies report higher SVR rates for patients with ITPase activity decreasing *ITPA* genotypes,[25,41] and a recent study reported reduced relapse risk following treatment for HCV genotype 2/3 in these genotypes.[42] However, probably due to small sample size, we were not able to confirm these findings in our study. Addition of the new protease inhibitors telaprevir or boceprevir improves response rates to 70% in patients with genotype 1.[8,9] Although the influence of protease inhibitors and the nucleoside polymerase inhibitor sofosbuvir on ITPase activity needs to be established, ribavirin is still a part of these treatment regimens and it might be cost saving to prevent adverse events like severe anemia by more tailor-made treatment.

Other purines are still widely used in the treatment of other diseases (i.e., abacavir and tenofovir in HIV, azathioprine and 6-mercaptopurine in inflammatory bowel disease and acute lymphoblastic leukemia) and may be also influenced by ITPase activity. So even though ribavirin is becoming less important in the treatment of HCV infection, further research to the impact of *ITPA* genotype and ITPase activity on the degradation of purine analogs will still be important.

Despite the fact that ITPase activity was measured in whole blood, the activities measured correlated with the genotype-specific reference values established in erythrocytes in our laboratory and by others.[35,43,44] Although markedly more men were included in this study, this did not influence the assessment of anemia, as gender-specific Hb reference values were used.

It is not clear why decreased ITPase activity protects from ribavirin-induced anemia. A direct association between ribavirin levels, ITPase activity and anemia has been hypothesized but could not be proven.[37] Although it has been reported that ITPase deficiency decreased the need for ribavirin dose reduction,[40] this could not be confirmed in HCV mono-infected patients[19] nor in HIV/HCV co-infected patients.[45] Thus, direct influence of ITPase activity on ribavirin levels does not seem to be a plausible explanation.

Another possible explanation for the assumed protective effect of *ITPA* SNPs has been suggested by Hitomi et al.,[46] who stated that *ITPA* polymorphisms resulted in decreased ITPase activity causing accumulation of ITP. However, ITP was found to only accumulate in the erythrocytes of patients homozygous for c.94C>A *ITPA* genotype variant.[44,47] Furthermore, Hitomi et al.[46] stated that ITP was a substitute for GTP in the generation of AMP by adenylosuccinate synthetase (ADSS). If this were to be correct, the proposed protective effect could only be effective in erythrocytes of patients who are homozygous for c.94C>A. However, neither Hitomi and coworkers, nor any other author (including our group) has, to the best of our knowledge, demonstrated ADSS activity in erythrocytes. The exact mechanism is still not elucidated and needs a more mechanistic approach.

In conclusion, this study is the first to describe the direct correlation of ITPase activity and decrease in Hb values during treatment with ribavirin. In addition, we demonstrated that ITPase activity is a better pre-treatment parameter to predict ribavirin-induced anemia than *ITPA* genotype.

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