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# Nitazoxanide plus pegylated interferon and ribavirin in the treatment of genotype 4 chronic hepatitis C, a randomized controlled trial

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## Abstract

*Background and Aims:* Nitazoxanide has been proposed as a novel therapeutic agent for chronic hepatitis C virus (HCV) potentiating the effect of interferon and improving sustained virological response rates to up to 80% in genotype 4. This is an independent randomized trial to confirm the efficacy of nitazoxanide in the treatment of chronic hepatitis C genotype 4. *Methods:* This was an open-label trial. Treatment-naive genotype 4 HCV patients were recruited: Group 1 received weekly subcutaneous pegylated interferon 160 μg in addition to weight-based ribavirin (1200 mg if ≥75 kg and 1000 mg if <75 kg) for 48 weeks, Group 2 received 4 weeks lead-in therapy by nitazoxanide alone (500 mg bid) followed by triple therapy including nitazoxanide, pegylated interferon and ribavirin for a further 48 weeks. *Results:* Fifty patients were recruited in each group. Baseline characteristics were similar except for a higher BMI in group 1 (28.5 vs. 26.5, $P = 0.01$). SVR rates were similar (24/50 (48%) vs. 25/50 (50%) in groups 1 and 2 respectively, *P*: 0.84). RVR, cEVR and ETR rates were also similar (61% vs. 53% – *P*:0.4, 70% vs. 72% – *P*:0.8 and 62% vs. 58% – *P*:0.6 in groups 1 and 2 respectively). Biochemical response at week 12 was also similar (57% vs. 46% in groups 1 and 2 respectively, *P*:0.26). Complications were similar except for a higher rate of dyspepsia in the group receiving nitazoxanide (32% vs. 14%, *P*:0.03). *Conclusion:* The addition of nitazoxanide to pegylated interferon and ribavirin does not improve the virological or biochemical response rates in chronic HCV genotype 4. (clinicaltrials.gov identifier: NCT01276756).

**Abbreviations**
ALT, Alanine aminotransferase; BMI, body mass index; CEVR, complete early virological response; DMID, division of microbiology and infectious diseases; ETR, end-of-treatment response; NTZ, nitazoxanide; RVR, rapid virological response; SD, standard deviation; SVR, sustained virological response; ULN, upper limit of normal.

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Chronic hepatitis C has a significant prevalence worldwide with a remarkably high prevalence in Egypt reaching 15% (1). In Egypt, genotype 4 is the overtly prevalent genotype affecting more than 95% of patients (1). Genotype 4 has a poor response to current standard treatment by pegylated interferon and ribavirin with sustained virological response rates (SVR) ranging between 40–60% (2). There has been a relentless quest to develop new drugs to enhance such response and over the last 2 decades none has proved effective. In 2009, nitazoxanide emerged as a potential saviour as a published randomized trial showed that it improved SVR rates from 50 to 80% (3). This has triggered great enthusiasm especially in Egypt where pharmaceutical companies have rushed to the production and marketing of nitazoxanide. Although still not approved in any guidelines, nitazoxanide has been incorporated by many physicians as part of standard-of-care treatment.

In this study, we aimed at assessing the efficacy of nitazoxanide as an add-on therapy together with the standard-of-care treatment in the treatment of chronic hepatitis C virus (HCV) genotype 4. To our knowledge, this is the first investigator-driven study with no support from the industry to test the efficacy of nitazoxanide in this setting.

## Patients and methods

### Patients

Only treatment-naive chronic HCV patients with genotype 4 were included. Inclusion criteria were detectable HCV-RNA by PCR, ALT elevation above upper limit of normal and/or liver biopsy showing Fibrosis score >F1 on the Metavir grading system and age between 18 and 65 years. Exclusion criteria were inadequate haematologic indices (haemoglobin <12.5 g/dL in men or <12 g/dL in women, neutrophils <1500/mm$^3$, platelets <75 000/mm3), decompensated liver disease (bilirubin>2.5 g/dL, albumin <3.5 g/dL, INR >1.3 or clinical evidence of decompensation), BMI >35 kg/m, HBV or HIV co-infection, significant medical comorbi-

dities, drug abuse, alcohol consumption >20 g/day, uncontrolled autoimmune or psychiatric illness, pregnancy and lactation.

### Methods

This was an open-label single-centre randomized trial that took place at the National Railway Hospital Center in Cairo, Egypt between November 2010 and November 2012. The trial was fully investigator-driven with no funding or support from the industry, all medications were supplied by the national railway hospital. After a written informed consent, eligible patients were sequentially randomized in a 1:1 ratio into two groups: Group 1 received standard-of-care treatment in the form of subcutaneous pegylated interferon alfa-2a 160 μg once weekly (Reiferon retard, Minapharm, Cairo, Egypt) plus ribavirin (Ribavirin, Minapharm, Cairo, Egypt) 1000 mg or 1200 mg daily (based on body weight <75 kg or ≥75 kg respectively) for 48 weeks, group 2 received oral nitazoxanide 500 mg twice daily (Xerovirin-C, Minapharm, Cairo, Egypt) for 4 weeks as a lead-in therapy and continued for 48 weeks further together with pegylated interferon alfa-2a and ribavirin in the same doses as described in group 1. Patients were evaluated clinically together with laboratory testing every 2 weeks during the first 4 weeks and every 4 weeks thereafter and every 12 weeks after the end of the treatment. HCV-RNA was recorded at baseline and at week 4, 12, 24, 48 and 6 months after the end of the treatment (Cobas Amplipre/Cobas Taqman, lower limit of detection 15 IU/mL, Roche Molecular Systems, Pleasanton, CA, USA). This study was approved by the local ethics committee and the trial was registered at www.clinicaltrials.gov (registration number: NCT01276756).

### Efficacy assessment

Our primary outcome of efficacy was the rate of sustained virological response as defined by an undetectable serum HCV-RNA 24 weeks after the end of the treatment. Secondary outcome parameters included rates of rapid virological response (RVR), complete early virological response (CEVR) and end-of-treatment response (ETR) defined as serum HCV-RNA below the limits of detection at 4 weeks and 12 weeks of combination treatment and at the end of the treatment respectively. Biochemical response (as defined by an ALT< 30U/L) was also evaluated at 12 weeks and compared with baseline levels. Patients who had less than 2 log drop in serum HCV-RNA at 12 weeks of combination therapy or detectable serum HCV-RNA at 24 weeks of combination therapy were considered as treatment failures and treatment was discontinued.

### Safety assessment

Safety assessment was done throughout the treatment and follow-up including clinical examination and laboratory assessment. Adverse events, dose reductions and withdrawals because of intolerance were recorded and documented. Adverse events were graded as mild, moderate, severe or life-threatening according to the US division of microbiology and infectious diseases (DMID) adult toxicity tables (4). Significant neutropenia was considered when the absolute neutrophil count was below 1000/mm, significant anaemia when haemoglobin level was below 10 g/dL and significant thrombocytopenia when platelets were below 100 000/mm. Epoetin beta (Recormon, Roche, Basel, Switzerland) was the initial management of choice for haemogloblin levels between 8.5 and 10 g/dL, failure of epoetin was then managed by stepwise reduction in ribavirin dose by 200 mg decrements down to 600 mg/day and discontinued if haemoglobin fell below 8.5 g/dL. Filgrastim (Neupogen, Roche, Basel, Switzerland) was the initial management of choice for neutrophil counts between 750 and 1000/mm. The dose of pegylated interferon was reduced to 80 μg weekly for neutrophil counts between 500 and 750/mm or platelet counts below 50 000/mm and discontinued for neutrophil counts below 500/mm and platelet counts below 30 000/mm.

### Statistical analysis and sample size

A sample size of 45 patients in each group was calculated with assumptions including 95% confidence level (1-alpha) with α-error of 5 and 80% power of the study to detect an expected response of 50% in the control group and 80% in the group receiving nitazoxanide. An additional 5 patients were recruited in each group to account for possible dropouts. Quantitative variables were expressed by mean and standard deviation (SD) and compared using the Mann–Whitney test. Qualitative variables were expressed by numbers (frequency) and per cent and compared between groups using the chi-square test and the Fisher's exact test when appropriate. Univariate and multivariate regression analysis models were applied, all significant variables at the univariate level were entered into the stepwise logistic regression model. *P*-value was considered to be significant if less than 0.05. All patients' data were analysed using SPSS 17.0 for windows 7 (SPSS Inc., Chicago IL, USA).

## Results

### Patients characteristics

Between November 2010 and January 2011, 100 patients were enrolled (50 in each group, Fig. 1). Baseline characteristics were similar in both groups except for a significantly lower BMI in group 2 (Table 1). Two patients from group 1 dropped out after finishing the 48 weeks of treatment. All patients who received at least one dose of interferon were included in the intention-to-treat safety and efficacy analysis.

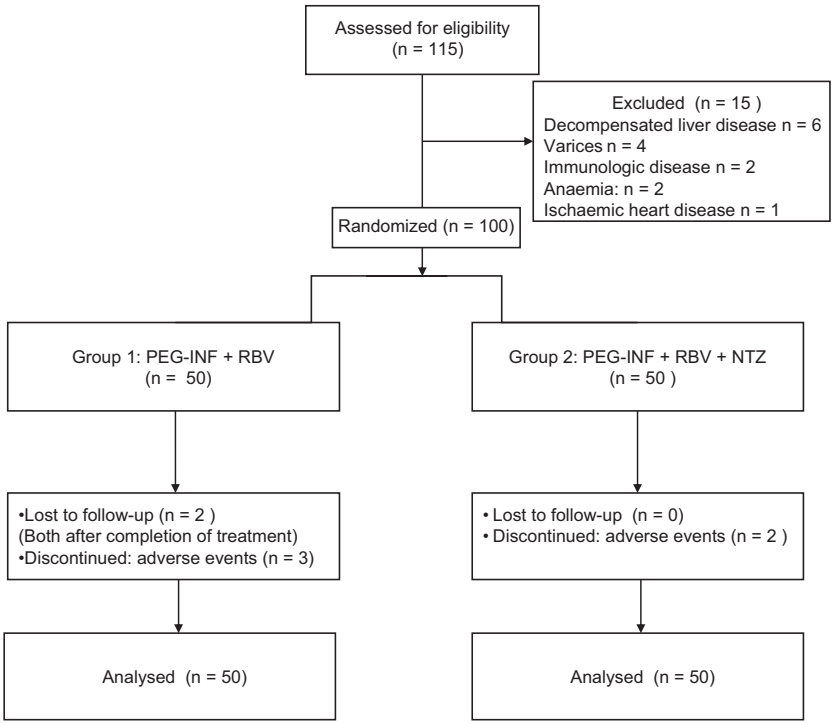


**Fig. 1.** Flow diagram of the study: study enrollment and disposition of patients. PEG-INF, Pegylated interferon; RBV, ribavirin; NTZ, nitazoxanide.

**Table 1.** Baseline characteristics of patients

| | Group 1 (PEG-INF + RBV) | Group 2 (PEG-INF + RBV + NTZ) | *P*–value |
|---|---|---|---|
| Sex (F/M) | 2/48 | 1/49 | 0.5 |
| Age | 45.46 ± 6.88 | 45.42 ± 8.36 | 0.97 |
| BMI | 28.50 ± 3.15 | 26.7 ± 3.26 | 0.01 |
| Significant NAFLD* | 15 (30) | 9 (18) | 0.08 |
| DM | 2 (4) | 7 (14) | 0.08 |
| ALT U/L | 89.1 ± 54.18 | 83.62 ± 62.32 | 0.4 |
| HCV-RNA IU/L x$10^5$ | 7.6 ± 11.19 | 7.4 ± 8.7 | 0.66 |
| High viral load (>400 000 IU/L) | 22 (44) | 28 (56) | 0.23 |
| Advanced Fibrosis (F3, F4) | 12 (24) | 10 (20) | 0.6 |

Categorical parameters are numbers (percentages); continuous parameters are means (±SD). PEG-INF: Pegylated interferon, RBV, ribavirin; NTZ, nitazoxanide; DM, Diabetes mellitus; NAFLD, Non-alcoholic fatty liver disease.
*Defined as moderate or severe steatosis on liver biopsy.

### Efficacy of treatment

On an intention-to-treat analysis, the rates of RVR, CEVR, ETR and SVR were similar in both the groups (Table 2). SVR rate was 48% in group 1 and 50% in group 2 ($P = 0.84$). Biochemical response as defined by a normalized ALT at 12 weeks (<30 U/L) was also similar between both the groups (57.1% vs. 45.8%, $P = 0.26$ for groups 1 and 2 respectively).

We noticed that the number and proportion of patients with advanced fibrosis in our study was higher than that in the similar published trial by Rossignol *et al.* (22% vs. 5%) (3), to exclude that the presence of advanced fibrosis could have altered the results we compared the response rates within the subset of patients without advanced fibrosis. SVR rates were also similar

**Table 2.** Virological response rates

| | RVR | CEVR | ETR | SVR |
|---|---|---|---|---|
| Group 1 | 30/49* (61.2%) | 35/50 (70%) | 31/50 (62%) | 24/50 (48%) |
| Group 2 | 25/47* (53.2%) | 36/50 (72%) | 29/50 (58%) | 25/50 (50%) |
| *P*-Value | 0.44 | 0.82 | 0.68 | 0.84 |

*One patient from group 1 and three patients from group 2 missed the PCR test at week 4.

in this subset of patients 21/37 (56.7%) and 23/40 (57.5%) in groups 1 and 2 respectively ($P = 0.94$).

As the baseline BMI was lower in the group receiving nitazoxanide, we performed a separate comparison of SVR rates among the different BMI categories. Within each BMI category, the SVR rates did not differ between both treatment groups (Table 3).

### Adverse events

No serious or life-threatening adverse events were observed in either group. The most common complications were fever, fatigue, headache, myalgias and arthralgias. The occurrence of complications was similar in both the groups except for a higher rate of dyspepsia in the group receiving nitazoxanide (32% vs. 14%, $P = 0.03$). Dose reductions and discontinuation of treatment because of adverse events were similar in both the groups. The dose of interferon was reduced in seven patients in group 1 and four patients in group 2 ($P = 0.2$). The dose of ribavirin was reduced in three patients in group 1 and none in group 2 ($P = 0.12$). In group 1, treatment was discontinued because of adverse events in three patients (two because of neutropenia at weeks 2 and 24 and one because of severe facial palsy at week 24), in group 2 treatment was discontinued in two patients (one because of anaemia and one because of neutropenia, both at week 2).

The use of growth factors and haematopoietic stimulants was also similar between both the groups. Filgrastim was administered in 10 (20%) and 16 (32%) of the patients in group 1 and 2 respectively ($P = 0.1$). Epoetin was administered in 11 (22%) of the patients in each group ($P = 0.99$).

### Predictors of response

The following variables were assessed as potential predictors of SVR: Age <50 years, BMI <30, nitazoxanide, baseline ALT>2 folds upper limit of normal, fibrosis score ≤2, HCV-RNA <400 000 IU/L, RVR and biochemical response at 12 weeks. On univariate analysis, significant predictors of an SVR were an RVR and fibrosis score ≤2. Stepwise logistic regression revealed the following as predictors of an SVR: RVR, fibrosis score ≤2 and ALT≥2 fold ULN (Table 4).

**Table 3.** Comparison of SVR rates in different BMI categories

| BMI category | Group 1 | Group 2 | *P*-value |
|---|---|---|---|
| <25 | 5/8 | 4/14 | 0.16 |
| 25–30 | 12/26 | 15/28 | 0.6 |
| >30 | 7/16 | 6/8 | 0.18 |

**Table 4.** Predictors of response on multivariate analysis

| | Odds ratio | CI 95% | *P*-value |
|---|---|---|---|
| RVR | 10.24 | 3.42 - 30.65 | <0.001 |
| ALT≥2xULN | 5.0 | 1.47 - 16.67 | 0.010 |
| Fibrosis score <2 | 6.05 | 1.25 - 29.64 | 0.027 |

ULN: upper limit of normal.

## Discussion

We aimed in this study to test the efficacy of adding nitazoxanide to pegylated interferon and ribavirin in the treatment of genotype 4 chronic hepatitis C. To our knowledge, this is the first investigator-driven study with no support from the industry to test nitazoxanide in this setting. The addition of nitazoxanide resulted in no difference or even a trend for a difference in RVR, EVR, ETR or SVR. Similarly, no difference was observed in biochemical response as measured by ALT levels at week 12. These results came despite a significantly lower BMI in the group of patients receiving nitazoxanide in addition to pegylated interferon and ribavirin which would normally favour a better outcome. Our results come in stark contrast to the previous reports on the *in vitro* and *in vivo* success of nitazoxanide in the treatment of HCV.

Nitazoxanide came first into the spotlight by Korba *et al.* who first reported the *in vitro* success of nitazoxanide in inhibiting genotype 1 HCV replication (5). *In vivo* studies are still scarce with most being presented only in the abstract form and to date there are only three fully published studies including one brief communication (3, 6, 7). When considering genotype 4, three studies reported evidence of the efficacy of nitazoxanide (3, 6, 8). Rossignol *et al.* tested nitazoxanide monotherapy in 23 patients with chronic HCV genotype 4 and compared them with 24 patients receiving placebo (6). They reported an undetectable HCV-RNA during treatment in seven patients receiving nitazoxanide and eventually an SVR in four patients (17%) compared to nil in the placebo group. In a later open-label randomized trial, the same group of investigators compared 28 patients who received triple therapy by nitazoxanide, pegylated interferon and ribavirin (following a 12-week lead-in with nitazoxanide) with 40 patients receiving standard of care pegylated interferon and ribavirin. They reported an SVR of 79% in comparison with 50% in controls ($p = 0.023$) (3). The investigators then tested the efficacy of a 4-week lead-in instead of 12 weeks. In an uncontrolled trial with a 4-week lead-in by nitazoxanide followed by triple therapy for 48 weeks, 80% SVR was achieved in the tested 44 patients and this led the authors to conclude that a 4-week lead-in phase is sufficient (8).

For genotype 4 which is known to have a poor response to therapy, the previous reports were considered a breakthrough. We hoped our results could solidify this evidence but this was not the case. Other than the drug being not effective, we tried to search for possible explanations for the discrepancy in results. One of the first elements to consider was the possibility of the presence or emergence of resistance. Korba *et al.*

reported the creation of nitazoxanide-resistant HCV-infected replicon cell lines by repeated passage of nitazoxanide and its metabolite tizoxanide, the same study and further studies revealed that HCV resistance to nitazoxanide is not because of viral mutations, is not transferable and is rather related to host factors (9, 10). If it could be argued that some of the previous studies were performed on different populations (North American) and therefore had different host immune responses with less development of resistance (11), then this will not apply to the three trials that were performed on typically the same population as ours (Egyptian, genotype 4 patients) (3, 6, 8). As regards patient selection, our study included more patients with significant fibrosis than the previously published trial by Rossignol *et al.* (22% vs. 5%) (3), whether the presence of fibrosis specifically hampers the antiviral effect of nitazoxanide or favours the development of resistance is not known, but even when considering only the subset of patients without advanced fibrosis in our study nitazoxanide still had no effect on SVR rates (56.7% vs. 57.5% in groups 1 and 2 respectively). Apart from the higher fibrosis levels in our study, both studies included a significant proportion of relatively difficult-to-treat patients with high viral loads and older ages, reproducibility of the results on patients with more favourable profiles should be confirmed with further studies. Another possibly noteworthy difference between our study and the one by Rossignol *et al.* is that we used 20 kDa Hansenula-derived interferon alpha-2a (Reiferon retard, Minapharm, Cairo, Egypt) as opposed to the 40 kDa E.coli-derived interferon alpha-2a (Pegasys, Roche, Basel, Switzerland). The two interferons differ in the method of preparation with the Hansenula-derived interferon being significantly cheaper (12). The mechanism of action of nitazoxanide is the induction of phosphorylation of eukaryotic initiation factor 2$\alpha$, a mechanism which is independent of the action of interferon and therefore should not differ between different types of interferon (13). Hansenula-derived interferon has also shown typically similar clinical outcomes to those produced by other interferons (14). These factors, together with the fact that our control group produced an SVR similar to that of the control group in the study by Rossignol *et al.*(50%), greatly eliminate the argument that the use of a different interferon could have influenced the results.

Despite the presence of several studies supporting the efficacy of nitazoxanide, our study is not the only one negating such evidence. Recently Laufer *et al.* reported no reduction in HCV-RNA levels after 4 weeks of nitazoxanide in HCV/HIV coinfected patients (7). They also noted no improvement in ALT or AST levels. Similarly in an ongoing multicentre trial in HCV/HIV coinfected patients with published online interim results, after 4 weeks of nitazoxanide monotherapy in 64 patients they reported only 0.1 log drop in HCV-RNA, although no statistical analysis is provided but such mediocre drop is probably of no statistical or clinical significance (15). Interestingly, this study also measures the interferon-responsive proteins present in stored peripheral blood mononuclear cells throughout the treatment, therefore testing the hypothesized mechanism of action of nitazoxanide, the full results of this trial are thus eagerly awaited.

RVR rates in both the groups were quite high (collectively 57%) in comparison with other studies on genotype 4 where RVR rates ranged between 22% and 45% (16, 17). We suspected this could be related to the rigorous measures taken in this study to ensure compliance especially in the early weeks of treatment, but this argument fails to explain why the high RVR did not translate to higher ETR and SVR rates. Could this be a characteristic of the 20KDa interferon? This explanation, however, is not supported by the EVR, ETR and SVR rates being typically similar to 40KDa interferon. Most importantly, the high RVR rates were similar in both tested cohorts, precluding the possibility of nitazoxanide affecting the rapid virological response.

As expected and in line with previous reports, the only significant side effect of nitazoxanide was gastrointestinal in the form of dyspepsia. There was no discontinuation of treatment because of adverse effects related to nitazoxanide. The occurrence of all other complications was similar in both the groups and generally similar to the usually reported complications with standard-of-care treatment (18). This reassuring safety profile may support the rationale for trying nitazoxanide at higher doses in future trials. A considerable proportion (about one-fourth) of our patients was administered haematopoietic growth factors. Our treatment protocol involved the liberal use of haematopoietic growth factors aiming at avoiding dose reductions/discontinuation as much as possible. This policy led to the frequent use of growth factors but also a low rate of dose reduction/discontinuation: our collective rate of treatment discontinuation was only 5% compared to 14–22% in the largest published trials (18, 19). Similarly, our rate of dose modification was 14% compared to 14–42% reported in the literature (18, 19). Therefore, the frequent use of haematopoietic growth factors was not because of the severity of haematological adverse events but rather because of the predetermined treatment protocol. The possibility of haematopoietics causing a drug interaction and altering response to treatment is partly omitted as haematopoietics were used equally used in both cohorts, whether it selectively counteracts the action of nitazoxanide is not known but our search of literature did not reveal any known interaction.

Although it was not the main aim of this study, we assessed the predictive factors of response. In line with previous reports, RVR, a lower fibrosis score and a higher baseline ALT were predictive of an SVR. RVR showed the strongest correlation with an Odds ratio of 10.2. It is important to note that nitazoxanide also had no effect on RVR rates further supporting the fact that

nitazoxanide had no effect on viral response. Unexpectedly age, baseline viral load and BMI were not predictive of response, however, our study was not designed originally to detect these values and sample size might not have been appropriate in this setting.

Our study has some limitations. The sample size could have been larger, nevertheless, our results definitely point that if there exists any difference in response because of nitazoxanide then it is definitely much lower than the previously reported 30%, at least in our studied population. Baseline patient characteristics were similar in both the groups except for a lower BMI in the nitazoxanide group which could have favoured a better response in the nitazoxanide group. However, even with a lower BMI patients who received nitazoxanide did not have better viral responses confirming the lack of efficacy of nitazoxanide in this study. The very low number of females in both cohorts is also a worthy observation (3%), this finding is very similar to the study by Rossignol *et al.* (also conducted in Egypt) in which only 8% of their patients were females (3). This could be explained by the low educational/economic level of the studied population where cultural barriers still hamper women from seeking medical care, travelling for long distances to reach our hospital is still difficult for many women and unfortunately many have unnecessary fears of lifelong sterility from ribavirin. Strictly speaking, this means that our results apply mainly to men and interpretation for female patients should be done with caution. We assessed the virological response in a categorical manner comparing RVR, EVR, ETR and SVR rates in the two groups, unlike other studies we did not measure quantitatively viral loads before and after the lead-in phase with nitazoxanide or viral loads at week 4 as it would have posed an additional cost and we believed it was a less clinically significant criterion for the assessment of response. Nevertheless, viral loads could have provided supportive evidence on the efficacy of nitazoxanide. We now also know that IL28 genotypes have a significant effect on outcome and therefore should have been assessed in both groups to ensure this wasn't a source of bias. Other limitations of our study include the absence of blinding and lack of a placebo control which should be avoided in a further larger confirmatory trial.

In conclusion, this study has demonstrated lack of efficacy of nitazoxanide when added to pegylated interferon and ribavirin in the treatment of chronic HCV genotype 4. The enthusiasm shown towards the use of nitazoxanide (especially in Egypt) has to be curbed until the emergence of more concrete evidence. We suggest that a larger randomized placebo-controlled trial perhaps with higher doses of nitazoxanide may put an end to the conflicting results.

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*Conflicts of interest*: None of the authors has any conflict of interest to disclose.

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