The impact of clinicopathological parameters in predicting response to pegylated interferon and ribavirin in chronic hepatitis C patients

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Objectives
This study aimed to investigate the impact of clinical and histopathological changes in liver tissue of responders and nonresponders to standard pegylated interferon (Peg-IFN) and ribavirin (RBV) therapy and to determine whether they could predict treatment outcome or not.

Background
Hepatitis C virus (HCV) infection is a major health problem worldwide. Combination therapy of Peg-IFN and RBV has been recognized as a standard treatment for HCV infection. Unfortunately, this standard therapy produces a sustained virological response in only 50% of HCV-infected patients. Clinical and histological findings may play a role in predicting response to standard Peg-IFN/RBV therapy.

Patients and methods
This retrospective study included 64 patients with chronic HCV who were treated with Peg-IFNa/RBV. According to their response to treatment, they were classified into responders ($n = 34$) and nonresponders ($n = 30$). Pretreatment liver biopsies were evaluated histopathologically for necroinflammatory grade and fibrosis stage according to the modified Ishak and Metavir scoring systems for chronic hepatitis. Other pathological findings were also reported. Demographic, laboratory, and histopathological results were subjected to a statistical analysis.

Results
The current study showed that the age of the patients ($P = 0.003$), sex ($P = 0.027$), and serum α-fetoprotein level ($P = 0.046$) were the parameters that showed a statistically significant difference between responders and nonresponders to interferon therapy. However, the grade of necroinflammation, stage of fibrosis as well as other pathological changes did not show a statistically significant difference between both groups.

Conclusion
The current study showed that young age, female sex, and the baseline serum level of α-fetoprotein are the parameters that favored response to interferon and RBV therapy in chronic HCV Egyptian patients, whereas histopathological changes played no role in predicting response to treatment.

Keywords:
chronic hepatitis C, histopathological changes impact, interferon, response to therapy

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Introduction

Egypt has the highest prevalence rate of hepatitis C virus (HCV) infection in the world (15–25%) [1]. Only 20% of patients infected with HCV have an acute viral hepatitis syndrome with viral clearance. The major consequence of HCV infection is the development of chronic hepatitis C (CHC) with its potential complications such as cirrhosis or hepatocellular carcinoma (HCC) [2].

The primary aim of HCV therapy is to achieve a sustained virological response (SVR), in which HCV RNA remains undetectable at 24 weeks after the end of therapy. The current standard therapy is based on a combination of pegylated interferon α (Peg-IFNα) and ribavirin (RBV) [3]. This therapy produces a SVR in only 50% or even fewer HCV genotype 4-infected patients, the most common genotype among HCV-infected patients in Egypt. Negative HCV RNA by a sensitive test after 24 or 48 weeks of therapy refers to end-of-treatment response. Patients who fail to show clearance of HCV RNA and show a positive end-of-treatment response are termed 'nonresponders' and represent a huge challenge for clinicians worldwide. Many host and viral factors, particularly those associated with race, the genotype of HCV, and variation in specific host genes influence the treatment response to Peg-IFN and RBV combination therapy [4]. Moreover, the high costs in addition

to the side effects arising from the therapy such as influenza-like symptoms, psychiatric symptoms, and hematological abnormalities could result in a dose reduction or even the premature discontinuation of the treatment [3]. Therefore, it is necessary to predict an individual's response before or at an early stage of the treatment to increase the success rate of treatment and to avoid potential adverse events in patients who will not benefit from the treatment and also to reduce the cost of therapy [5].

Liver biopsy is considered the 'gold standard' for assessment of liver disease status and is advocated for diagnostic and prognostic purposes. Furthermore, its role is established for the decision on initiation of retreatment in nonresponders [6]. The nonresponse to therapy is an issue that should be addressed at the national level as it represents a huge medical and economic burden not only on the affected families but also the entire state at large. The histological features associated with nonresponse to therapy have been reported from western countries with a diverse ethnic and genetic profile. Therefore, we decided to report the histological features observed in responders and nonresponders of our local population as predictors of response to therapy and determine their impact on treatment-induced viral clearance in Egyptian HCV-infected patients.

Patients and methods

This retrospective study included 64 consecutive CHC adult patients (41 men and 23 women) who were recruited from the Interferon Clinical National Liver Institute (NLI), Menoufia University, in the period from January 2010 to December 2010. All patients underwent antiviral treatment and follow-up protocol of 18 months according to the EASL guidelines.

Inclusion criteria

(1) Treatment-naive patients.
(2) Positive for anti-HCV antibody (ELISA) and repeatedly positive HCV RNA (by PCR).
(3) Liver biopsy performed within 3 months before the initiation of therapy.
(4) Age not younger than 18 years.
(5) Clinical criteria of patients advised by clinicians to receive treatment such as antinuclear antibody titer less than 1/60, normal thyroid-stimulating hormone and kidney functions, no evidence of decompensated liver disease or thrombocytopenia (platelet<75 000/mm^3) or moderate to severe anemia (hemoglobin<10 g/dl), and neutropenia (neutrophil count<2000/mm^3).

All patients received combined Peg-INFα and RBV therapy: Peg-IFNα at a dose of 180 mcg once weekly plus RBV. The dose of RBV was adjusted according to body weight (<75 kg, 1000 mg/day; ≥75 kg, 1200 mg/day). Patients received treatment for 48 weeks and were followed up for 6 months after the cessation of therapy.

Patients were classified into two groups according to their treatment response:

Group A: responders to interferon (INF) therapy, who had negative HCV RNA by PCR 6 months following the completion of a 48-week course.

Group B: nonresponders to IFN therapy who showed one of the following:

(1) No clearance of HCV RNA at the end of the 12th week.
(2) Partial response at the end of the 12th week, but null response at the 24th week.
(3) Complete response at the 12th week, but detectable HCV RNA at any time during the course of treatment (48 weeks).

The following clinical data were collected from patients' medical records and included age, sex, history of bilharziasis (antibodies in blood detected by ELISA), liver function tests (alanine aminotransferase, aspartate aminotransferase, total bilirubin, serum albumin), α-fetoprotein (AFP) level, and HCV RNA by a quantitative PCR at the start of therapy. Serum albumin level was within the normal range; thus, it was excluded from statistical analysis. History of bilharziasis was excluded from statistical correlation because of the limited number of confirmed cases affecting the liver (two patients only).

Histopathological evaluation

The histopathological assessment of necroinflammatory grade and fibrosis stage was scored using the modified Ishak [7] scoring system and the METAVAIR system [8]. Liver biopsies obtained at the initial visit to HCV clinic were retrieved from Pathology Archives for evaluation of histopathological changes. Serial liver sections were stained with hematoxylin and eosin and Masson trichrome.

For statistical convenience, the grades of activity were categorized as minimal (grade 0–3), mild (grade 4–8) (Fig. 1), and moderate (grade 9–12). Moreover, the stages of fibrosis were grouped into two major categories as mild (group 1) and moderate to severe (group 2) (Figs 2 and 3). Steatosis was assessed on a scale of 0–3 depending on the percentage of

hepatocytes involved [9] (Fig. 4). The presence or absence of other pathological changes such as portal lymphocyte aggregates, bile duct injury, or vascular changes was recorded if present.

The data were subjected to statistical software SPSS (version 16; SPSS Inc., Chicago, Illinois, USA) and described in terms of mean ± SD and percentages. The association of demographic, and routine laboratory and histological parameters was evaluated using the χ^2 test. *P* value of less than 0.05 was considered significant.

Results

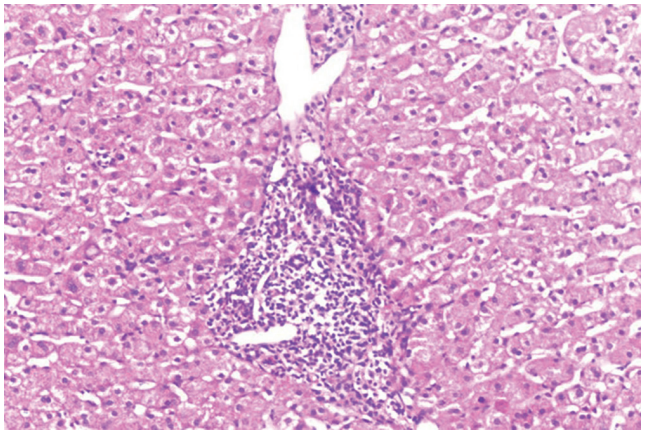
Relationship between the clinopathological parameters studied and response to therapy

The current study showed that age of the patient ($P = 0.003$), sex ($P = 0.027$), and AFP level ($P = 0.046$) were the factors that affected response to IFN, where young patients, women, and those with low AFP levels showed response to therapy in comparison with older patients, men, and those with high AFP levels (Tables 1–3).

There was no statistically significant difference among responders and nonresponders to IFN therapy in the score of portal inflammation interface hepatitis, spotty necrosis, confluent necrosis, and the stage of fibrosis.

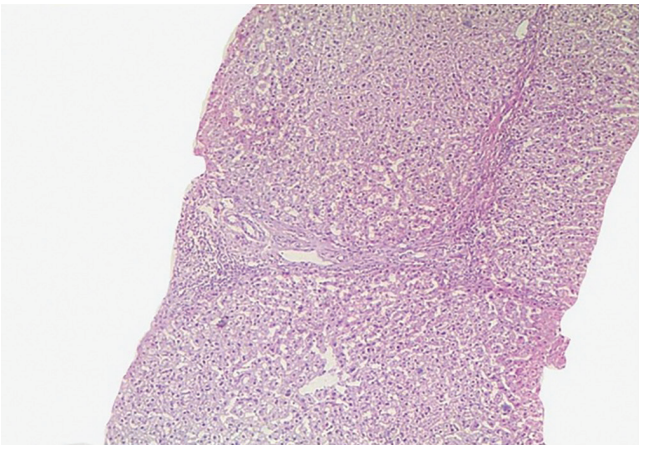
Similarly, steatosis was present in less than 50% of the biopsies studied, and less than 10% showed higher steatosis grades 2 and 3 that led to no significant difference between either group. There was no statistically significant difference in other nonspecific pathological changes such as vascular changes or bile duct injury between either group.

Figure 1



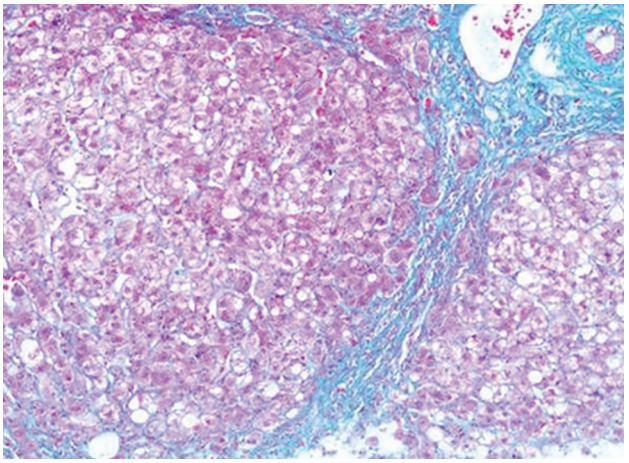
A case of chronic hepatitis C virus showing a mild degree of fibrosis (2/6) and portal tract inflammation according to the Ishak score (H&E, ×200).

Figure 2



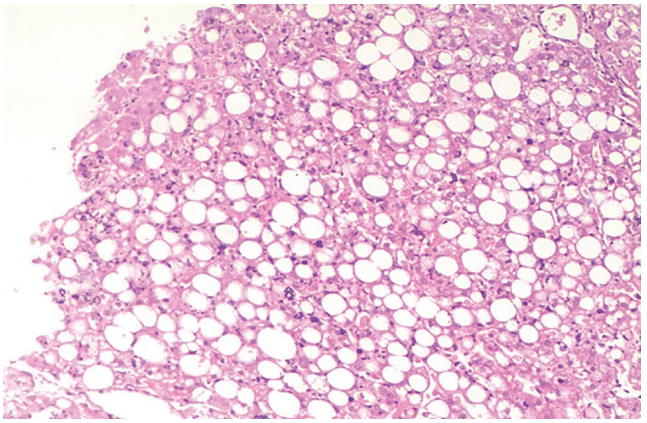
A case of chronic hepatitis C virus showing a moderate degree of fibrosis (3/6) according to the Ishak score (H&E, ×100).

Figure 3



A case of chronic hepatitis C virus showing a moderate degree of fibrosis (4/6) according to the Ishak score (Masson trichrome, ×200).

Figure 4



A case of chronic hepatitis C virus with a severe degree of steatosis grade 3 (H&E, ×200).

Table 1 Baseline characteristics of patients

	n (%) (*n* = 64)
Age (years)	
Mean ± SD	35.8 ± 10.0
Median	34
Range	19–57
Sex	
Male	41 (64.1)
Female	23 (35.9)
Bilharziasis	
Present	17 (26.6)
Absent	47 (73.4)
Viremia	
Mild	35 (54.7)
Moderate	26 (40.6)
High	3 (4.7)
AST	
Mean ± SD	49.8 ± 39.1
Median	38
Range	20–256
ALT	
Mean ± SD	56.5 ± 39.5
Median	42
Range	10.3–231
Bilirubin	
Mean ± SD	0.7 ± 0.3
Median	0.6
Range	0.3–1.4
Albumin	
Mean ± SD	4.4 ± 0.3
Median	4.35
Range	3.5–5.3
AFP	
Mean ± SD	3.6 ± 5.2
Median	2
Range	0.4–37
Ishak score	
Mean ± SD	5.4 ± 1.8
Median	5
Range	2–11
Ishak grade	
I	10 (15.6)
II	44 (68.8)
III	10 (15.6)
Nature of infiltrate (type of inflammatory cells)	
I (lymphocytes only	28 (43.8)
II (lymphocytes+plasma cells)	17 (26.6)
III (lymphocytes+plasma cells+eosinophils)	19 (29.7)
Interface hepatitis	
0	1 (1.6)
I	39 (60.9)
II	24 (37.5)
Spotty necrosis	
0	1 (1.6)
I	33 (51.6)
II	30 (46.9)
Portal tract inflammation	
I	53 (82.8)
II	11 (17.2)
Fibrosis	
I	49 (76.6)
II	15 (23.4)
Fibrosis Metavir	
I	50 (78.1)
II	10 (15.6)
III	4 (6.2)
Steatosis grade	
0	36 (56.2)
I	22 (34.4)
II	4 (6.2)
III	2 (3.1)
Steatosis	
Present	28 (43.8)
Absent	36 (56.2)
Lymphoid aggregates	
Present	35 (54.7)
Absent	29 (35.4)
Confluent necrosis	
Present	27 (42.2)
Absent	37 (57.8)
Bile duct injury	
Present	19 (29.7)
Absent	45 (70.3)
Vascular changes	
Present	11 (17.2)
Absent	53 (82.8)

AFP, α-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Discussion

HCV genotypes are of considerable clinical significance because they affect responses to antiviral therapy [10]. Genotype 4 predominates throughout the Middle East and parts of Africa, often in association with a high population prevalence as in Egypt [11,12]. More than 90% of Egyptian HCV isolates belong to genotype 4 [12,13]. Subtype 4a is the dominant Egyptian HCV strain, but other subtypes (provisionally named 4α, 4β, and 1g) are also present at a lower prevalence rate [14]. Peg-IFNα plus oral RBV has become the current standard of care for CHC because of the higher efficacy of Peg-IFN-based regimens in inducing yielding SVRs [15,16].

The reason for the differences in the response of various genotypes to antiviral therapy remains unclear as it is not known why certain genotypes respond more favorably to antiviral therapy than other genotypes [17]. The outcome of therapy may be influenced by a dynamic complex relationship that exists between the pharmacological characteristics of the therapeutic regimen, viral kinetics, and host immune responses [18].

Table 2 Demographic and routine laboratory data for responders and nonresponders to standard combination therapy in chronic hepatitis C Egyptian patients

	Groups		Test of significance	*P* value
	Responders (*n* = 34)	Nonresponders (*n* = 30)		
Age(mean ± SD) (years)	33.3 ± 9.2	38.8 ± 10.2	*t* = 2.26	0.027 (S)
Sex [*n* (%)]				
Male	16 (47.1)	25 (83.3)	χ^2 = 9.11	0.003 (S)
Female	18 (52.9)	5 (16.7)		
Viremia [*n* (%)]				
Mild	21 (61.8)	14 (46.7)	χ^2 = 2.11	0.349
Moderate	11 (32.4)	15 (50.0)		
High	2 (5.9)	1 (3.3)		
AST (mean ± SD)	57.6 ± 49.6	40.9 ± 19.4	Mann–Whitney = 1.49	0.135
ALT (mean ± SD)	67.3 ± 48.6	44.3 ± 20.3	Mann–Whitney = 1.82	0.068
Bilirubin (mean ± SD)	0.7 ± 0.2	0.7 ± 0.3	Mann–Whitney = 0.51	0.909
α-Fetoprotein	2.5 ± 2.3	4.9 ± 7.1	Mann–Whitney = 1.99	0.046 (S)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; S, significant; *Significant.

In the present study, all patients were selected to be eligible to receive IFN therapy; thus, all patients were known to have compensated liver, which was confirmed clinically and biochemically. Also, all our patients showed noncirrhotic liver or severe inflammation according to the histopathological findings of liver biopsy.

Our study showed that younger age of patients (P = 0.0027) and female sex (P = 0.0003) were significant predictors of treatment response. This was in agreement with previous reports [19] that found that older age (≥ 40 years) was one of the unfavorable markers of the elimination of HCV RNA from the serum of patients with CHC infection and also in agreement with the study of Bresci *et al.* [20] and Horiike *et al.* [21], who reported that older age was not an unfavorable marker for IFNa treatment, and Garson *et al.* [22], who reported that patients who showed a complete response were significantly younger than those who showed no response.

However, Adnan *et al.* [23] reported a nonsignificant relationship between age and response to IFN therapy. Moreover, Horiike *et al.* [21] reported that elderly patients with a low level of HCV RNA respond well to IFNa treatment. These conflicting results on the predictive role of age could be attributed to differences in population, racial differences, and HLA genes [24]. The latter study found that the response to IFNα treatment was better in women than in men among patients younger than 40 years and that the response decreased markedly in women aged 40 years and older. Why HCV appears to act differently in younger and older women, however, is unclear. During perimenopause (from age 40 years), ovulation can be erratic and plasma gonadotropin levels frequently reach menopausal levels, even when plasma estrogen levels are within the menstrual range [25,26]. These data suggest that the decreased rate of a complete response to IFNα treatment may correspond with decreases in estrogen levels. Interleukin 1, associated with an inflammatory response, is stimulated by low concentrations of estrogen and progesterone. A low concentration of estrogen allows peripheral blood monocytes to secrete more interleukin 1 [27]. The spontaneous production of interleukin 1b by peripheral mononuclear cells has been shown to be significantly higher in patients with CHC than in healthy control individuals, and then decreased in those with a complete response after the administration of IFNα [28]. This cytokine production may alter the effectiveness of IFNα treatment in perimenopausal and menopausal women with CHC infection.

Other studies showed that hormonal activity, in particular, the level of estrogen, may be associated with the sustained elimination of HCV in patients undergoing IFNα treatment for HCV infection. Hormone levels should be measured before treatment [29].

Our study observed a predictive role of AFP with respect to treatment response in CHC and showed that a low baseline AFP level could be a potential predictor for response to treatment, which was reported by Mabrouk *et al.* [30]. Serum AFP is a well-known clinical serum marker for diagnosing HCC, which was found to be elevated in patients with chronic liver disease in the absence of HCC [31]. The rate of HCV patients with elevated AFP ranged between 10 and 43% [32,33]. Previous studies have shown that AFP can be considered one of the serological predictors of response to antiviral therapy [34,35]. Peg-INFα/RBV was associated with a decrease in serum AFP with respect to virological response to treatment [36,37].

Table 3 Histopathological findings in liver tissue of responders and nonresponders to standard combination therapy in chronic hepatitis C Egyptian patients

	Groups [*n* (%)]		Test of significance	*P* value
	Responders (*n* = 34)	Nonresponders (*n* = 30)		
Ishak score (mean ± SD)	5.5 ± 1.9	5.3 ± 1.8	Mann–Whitney = 0.01	0.989
Ishak grade				
I	4 (11.8)	6 (20.0)	χ^2 = 0.92	0.632
II	24 (70.6)	20 (66.7)		
III	6 (17.6)	4 (13.3)		
Nature of infiltrate				
I	14 (41.2)	14 (46.7)	χ^2 = 0.33	0.846
II	10 (29.4)	7 (23.3)		
III	10 (29.4)	9 (30.0)		
Interface hepatitis				
I	0 (0.0)	1 (3.3)	χ^2 = 1.39	0.497
II	22 (64.7)	17 (56.7)		
III	12 (35.3)	12 (40.0)		
Spotty necrosis				
0	0 (0.0)	1 (3.3)	χ^2 = 2.38	0.305
I	20 (58.8)	13 (43.3)		
II	14 (41.2)	16 (53.3)		
Portal tract inflammation				
I	29 (85.3)	24 (80.0)	χ^2 = 0.31	0.575
II	5 (14.7)	6 (20.0)		
Fibrosis				
I	28 (82.4)	21 (70.0)	χ^2 = 1.36	0.244
II	6 (17.6)	9 (30.0)		
Fibrosis Metavir				
I	28 (82.4)	22 (73.3)	χ^2 = 1.48	0.878
II	5 (14.7)	4 (16.7)		
III	1 (2.9)	3 (10.0)		
Steatosis				
0	18 (52.9)	18 (60.0)	χ^2 = 3.49	0.322
I	13 (38.2)	9 (30.0)		
II	3 (8.8)	1 (3.3)		
III	0 (0.0)	2 (6.7)		
Lymphoid aggregates				
Present	21 (61.8)	14 (46.7)	χ^2 = 1.47	0.226
Absent	13 (38.2)	16 (53.3)		
Confluent necrosis				
Present	16 (47.1)	11 (36.7)	χ^2 = 0.71	0.401
Absent	18 (52.9)	19 (63.3)		
Bile duct injury				
Present	10 (29.4)	9 (30.0)	χ^2 = 0.003	0.959
Absent	24 (70.6)	21 (70.0)		
Vascular changes				
Present	8 (23.5)	3 (10.0)	χ^2 = 2.05	0.152
Absent	26 (76.5)	27 (90.0)		

The pretreatment viral load is another factor that is considered a predictor of response to therapy. A viral load of more than 600 000 IU/l is associated with an unfavorable response [9]. The viral load of the majority of our patients was less than 600 000 IU/l; therefore, the degree of viremia did not show statistically significant differences between responders and nonresponders.

Results from the current study showed that the response to therapy was not associated with the severity of inflammation according to the Ishak score statistically (P = 0.989) or the degree of fibrosis (P = 0.878); these findings were also reported in other studies [38]. In contrast to our results, Dienstag *et al.* [39] reported that inflammatory scores greater than 4 represented severe disease and piecemeal necrosis on any scoring system predicting response to antiviral therapy. In the study by Batool *et al.* [40], 9.55% of patients had severe disease and those who responded had mild to moderate disease on liver biopsy. Most of the nonresponders in

this study had moderate to severe degree of disease, but the difference was not statistically significant. Hence, the possibility still exists that lower scores of inflammation grade and fibrosis stage in our series could be attributed to the results obtained on no significant difference between responders and nonresponders to IFN therapy.

The impact of steatosis on treatment response in CHC infection is controversial. In this study, steatosis was not associated with response to therapy, which is not in agreement with the result of Yaginuma *et al.* [41], who reported that hepatic steatosis is an important predictor of poor response to therapy of (IFNα-2b and RBV) in CHC patients. However, our results were in agreement with those of Cross *et al.* [42], who reported that only steatosis (without steatohepatitis) was not significantly important as a predictor for treatment response. There was no statistically significant difference in other nonspecific pathological changes such as vascular changes or bile duct injury between either group.

Taylor *et al.* [43] reported that patients who showed a vigorous early virological response to treatment with Peg-INFα and RBV showed concurrent vigorous alterations in peripheral blood mononuclear cells gene mRNA levels, including genes whose levels were induced and repressed. However, gene expression responses were relatively blunted in patients with a poor viral response. Taylor *et al.* [43] concluded that the relative lack of viral response to IFN therapy of HCV infection is associated with blunted IFN cell signaling. In addition, no specific regulatory gene could be identified as responsible for this global blunting or racial differences.

Finally, the current study showed the importance of age, sex of the patients, and the serum level of AFP in determining response to IFN and RBV in CHC patients.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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