Serum levels of astroglial S100-beta and neuron-specific enolase in hepatic encephalopathy patients

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المــستويات المــصلية لأستــروغليال ــ س ــ 100 ــ بيتـا والإينـولاز النوعيـة للعـصبونات في مرضـى الاعتلال الدماغي الكبدي

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الخلاصــة: أجرى الباحثون قياسات للمستويات المصلية من الأستـروغليال ــ س ــ 100 ــ بيتا، والإينولاز النوعية للعصبونات لدى مرضى تشمُّع (تليُّف) الكبد، مع أو بدون اعتلال دماغي كبدي مصاحب، بهدف التعرف على طريقــة موثوقــة وغـير باضـعة لتـشخيص الخلـل المعرفي لـدى مرضـى تـشمع الكبـد. وقـد أظهـرت مـستويات الأستروغليال ــ س ــ 100 ــ بيتا ارتفاعاً يُعْتَدُّ به إحصائياً لدى المجموعـة المـصابة بـاعتلال دمـاغي كبـدي مـن المرحلتيْن الأولى والثانية، وذلك بالمقارنة مع مجموعة الشواهد ومجموعة التشمع الكبدي؛ في حين لم تكن مستويات الإينولاز النوعية للعصبونات في المصل مختلفة لدرجة يُعْتَدُّ بها إحصائياً بـين المجموعـات المدروسـة. وقـد كـان للأستروغليال ــ س ــ 100 ــ بيتا نوعية مقدارها 91.3٪ وحساسية مقدارها 51.7٪ لكشف الاعتلال الـدماغي الكبدي لدى مرضى تشمع الكبد. وعلى هذا فإن مـستوى الأستـروغليال ــ س ــ 100 ــ بيتـا قـد يكـون هامـاً باعتباره واسماً غير مباشر لتشخيص الخلل المعرفي لدى مرضى تشمع الكبد، قبل أن يتفاقم وضعهم إلى مراحل أكثر تدهوراً من الاعتلال الدماغي الكبدي.

ABSTRACT To find a reliable, noninvasive method for the diagnosis of cognitive impairment in patients with hepatic cirrhosis we measured serum levels of astroglial S100β and neuron-specific enolase in cirrhotic patients with and without hepatic encephalopathy (HE). S100β levels showed a significant increase in groups with HE stage 1 and 2 compared to both control and cirrhosis patients. However serum neuron-specific enolase levels were not significantly different between the studied groups. S100β levels had a specificity of 91.3% and sensitivity of 51.7% for detection of HE from cirrhosis. Serum S100β may be a useful surrogate marker for the diagnosis of mild cognitive impairment in cirrhotic patients before they progress to more advanced stages of HE.

Concentrations sériques de la protéine astrogliale S100-bêta et de l'énolase neurospécifique dans l'encéphalopathie hépatique

RÉSUMÉ À la recherche d'une méthode diagnostique fiable et non invasive du déficit cognitif associé à la cirrhose hépatique, nous avons mesuré les concentrations sériques de la protéine astrogliale S100-β et de l'énolase neurospécifique (NSE) chez des patients cirrhotiques avec et sans encéphalopathie hépatique (EH). Il a été observé une augmentation significative des taux de S100-β dans les groupes atteints d'EH de stade 1 et 2 par rapport tant aux témoins qu'aux patients cirrhotiques. Toutefois, les concentrations sériques de NSE n'ont laissé apparaître aucune différence significative entre les groupes étudiés. Les taux de S100-β font preuve d'une spécificité de 91,3 % et d'une sensibilité de 51,7 % pour la détection de l'EH à partir du sérum de patients cirrhotiques. La protéine S100-β sérique peut se révéler un marqueur de remplacement utile dans le diagnostic du déficit cognitif léger chez le cirrhotique avant une évolution vers un stade d'EH plus avancé.

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Received: 06/07/05; accepted: 09/11/05

Introduction

Cirrhosis of the liver is associated with a decreased health-related quality of life, whether physically (especially at advanced stages), mentally (subclinical encephalopathy) or both (overt hepatic encephalopathy), independent of the severity of the disease [1]. Hepatic encephalopathy (HE) is a neuropsychiatric syndrome observed in patients with liver failure and/or portal–systemic bypass. It is frequently considered to be a complex syndrome involving several behavioural manifestations, such as personality changes, memory disorders, disorientation, flapping tremor, shortened attention span, lack of muscle coordination, bradykinesia, somnolence and changes in sleep patterns [2]. However, inconsistent results regarding the behavioural, metabolic and neurochemical characteristics indicate that the pathogenesis and pathophysiology of the syndrome are still obscure [3].

S100β is a member of the S100 family of proteins that was termed "S100" because it was soluble in 100% saturated ammonium sulfate solution [4]. S100β is an acidic protein with a molecular weight of 21 kDA existing as a homodimer consisting of 2 beta subunits, and a biologic half-life of 0.5 hour [5,6]. S100β is produced primarily by astrocytes and exerts autocrine and paracrine effects on glia, neurons, and microglia [7]. S100β normally is low or undetectable in serum; however, elevated serum levels have been detected in a number of neuropathological conditions [8]. It is thought to be released from glial cells via a mechanism similar to that governing the secretion or release of other factors such as ciliary neurotrophic factor, interleukin-1α and 1β, or human endothelial growth factor [9].

The neuronal form of intra-cytoplasmic glycolytic enzyme enolase is called neuron-specific enolase (NSE) and it has been shown to be located in neurons and neuroectodermal cells [10,11]. After traumatic brain injury in adults, NSE has been found in the cerebrospinal fluid (CSF) [12] and serum [13], an indicator of impairment of the integrity of the blood–brain barrier (BBB). Astrocytic functions modulate neuronal ammonia toxicity because ammonia is detoxified mainly via astrocytic glutamine synthesis [14]. Abnormal BBB function frequently occurs with brain damage. S100β levels sometimes rise in the absence of neuronal damage, suggesting that S100β is a marker of BBB rather than neuronal damage, although in a variety of neurological diseases, the 2 brain-specific proteins, S100β and NSE, are released systemically [15]. In addition, S100β in serum is an early marker of BBB openings that may precede neuronal damage and may influence therapeutic strategies; this is explained by the fact that astrocytes form part of the BBB and the increase in its permeability in HE due to acute liver failure may in part be correlated to impaired astrocytic functions [16].

In clinical conditions in which an impairment of the BBB and/or astrocytic activation are implicated in the pathophysiology, elevated levels of S100β and NSE in serum and cerebrospinal fluid (CSF) have been reported. NSE and S100β are brain-specific, and their presence in the serum is a specific indicator for neuronal and astroglial cell death, respectively [17]. The aim of the present work in Cairo, Egypt, was to investigate the diagnostic efficiency of serum S100β and NSE as biomarkers of early cognitive impairment in patients with cirrhosis.

Methods

Patients
There were 52 participants in the study, divided into 3 groups:

- 14 patients with cirrhosis but without encephalopathy, with a mean age of 51.0 [standard deviation (SD) 3.5] years and male/female ratio 7/7.
- 29 patients with HE with underlying cirrhosis. These were further subdivided into 18 patients with HE stage 1, mean age 50.3 (SD 8.9) years, male/female ratio 10/8, and 11 with HE stage 2, mean age 54.2 (SD 9.0) years, male/female ratio 6/ 5. The prevalence of HE stage 1 and 2 in our HE group were 62.1% and 37.9%, respectively.
- 9 healthy age- and sex-matched controls, with a mean age of 52.2 (SD 8.9) years and male/female ratio 5/4.

The diagnosis of cirrhosis was confirmed by clinical criteria and sonography; while the diagnosis of hepatic encephalopathy was based on clinical criteria, and the severity of hepatic encephalopathy was based on the West Haven criteria for grading of mental status. This is based on changes of consciousness, intellectual function and behaviour [18]. Patients with preexisting neurological or psychiatric diseases other than hepatic encephalopathy, or sleep disorders were excluded.

The etiology of disease in the cirrhosis patients without HE was hepatitis C virus (HCV) infection in 10 patients and hepatitis B virus (HBV) infection in 4 patients. The Child–Pugh score was applied for grading of liver dysfunction in all patients. In this group, 7 cirrhotic patients were scored grade B and 7 were grade C.

In the HE patients the etiology of liver cirrhosis was HCV in 20 patients, HBV in 5 patients, both HCV and HBV in 3 patients and autoimmune hepatitis in 1 patient. All 29 HE patients were scored Child–Pugh grade C. Precipitating factors for hepatic encephalopathy included haematemesis in 10 patients, spontaneous bacterial peritonitis in 8 patients, electrolyte disturbance and diuretics in 7 patients and paracentesis in 4 patients.

Data collection
Patients were subjected to the following: full history taking, general and abdominal examinations, abdominal ultrasonography, upper endoscopy and laboratory investigations.

Liver function tests, including serum aminotransferases, alkaline phosphatase, albumin, bilirubin, prothrombin time and prothrombin concentration, were measured by conventional methods. Seromarkers for HBV (hepatitis B surface antigen and hepatitis B core antibody) were assayed by enzyme-linked immunosorbent assay (ELISA) (Boehringer Mannheim) and for HCV by Murex version III ELISA (Murex Biotech, UK).

For determination of plasma ammonia levels we used an enzymatic ultraviolet-assay (Randox, UK).

Serum S100β levels were determined by a commercially available ELISA kit (CanAg Diagnostics, Gothenburg, Sweden) which is a solid-phase non-competitive assay based on the sandwich technique for optimal clinical sensitivity, specificity and non-specific interference for determination of S100ββ isoform. The assay is based on an antibody specific for the S100ββ dimer as catcher and HRP labeled monoclonal antibodies specific for S100β detection. The detection limit of this assay is 0.02 μg/L.

The levels of serum NSE were determined by a commercially available ELISA kit (CanAg Diagnostics, Gothenburg, Sweden) based on 2 monoclonal antibodies directed against 2 separate antigenic

determinants of the NSE molecule. The monoclonal antibodies bind to the γ-subunit of the enzyme and thereby detect both the γγ and the αγ form. The minimum detection limit is 1μg/L.

Statistical analysis

Numerical data were expressed as mean (SD). Multiple intergroup comparisons were made by using one-way ANOVA, post-hoc with Tukey–Kramer multiple comparisons test. Correlations were computed using Spearman's rank correlation coefficient. *SPSS*, version 10, was used for data analysis. Receiver operating characteristics (ROC) analysis was done using *Analyse-it* software. $P < 0.05$ was considered significant.

Results

The results are tabulated in Tables 1 and 2 and graphically presented in Figures 1–3.

Table 1 shows the clinical characteristics of the 3 different groups of patients (cirrhosis, HE stage 1 and HE stage 2). Fetor hepaticus was present only in HE patients (15 patients stage 1 and 9 patients stage 2). Spider and palmar erythaema were present in 13 cirrhosis patients (18 patients in stage 1 HE and in only 10 patients in stage 2 HE).

Patients with cognitive deficits showed significantly elevated serum S100β levels at $P < 0.01$ in both groups, HE stage 1 [mean 0.248 (SD 0.12)] μg/L and HE stage 2 [mean 0.311 (SD 0.12) μg/L], as compared

Table 1 **Clinical characteristics of the different groups of patients**

Characteristic	Cirrhosis (n = 14)		Hepatic encephalopathy stage 1 (n = 18)		Hepatic encephalopathy stage 2 (n = 11)	
	No.	%	No.	%	No.	%
Fetor hepaticus	0	0	15	83.3	9	81.8
Spider and palmar erythaema	13	92.9	18	100.0	10	90.9
Loss of muscle mass	9	64.3	12	66.7	8	72.7
Jaundice	4	28.6	6	33.3	4	36.4
Pallor	4	28.6	5	27.8	5	45.5
Lower limb oedema	8	57.1	15	83.3	7	63.6
Spontaneous bacterial peritonitis	4	28.6	5	27.8	3	27.3
Ascites	8	57.1	16	88.9	9	81.8
Splenomegaly	10	71.4	11	61.1	8	72.7
Liver size:						
Normal	3	21.4	2	11.1	4	36.4
Enlarged	2	14.3	5	27.8	1	9.1
Shrunken	9	64.3	11	61.1	6	54.6

n = *total number of patients.*

Table 2 **Plasma levels of ammonia, serum S100β and serum neuron-specific enolase (NSE) in the different groups of patients**

Variable	Control (n = 9)		Cirrhosis (n = 14)		Hepatic encephalopathy stage 1 (n = 18)		Hepatic encephalopathy stage 2 (n = 11)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ammonia (μmol/L)	28.9	9.7	105.6	10.7[a]	111.6	11.5[a]	117.8	11.0[a]
S100β (μg/L)	0.099	0.040	0.086	0.060	0.248	0.120[a, b]	0.311	0.120[a,b]
NSE (μg/L)	12.6	1.1	13.3	2.1	13.9	1.9	14.5	2.3

[a]*P < 0.01 versus control group; *[b]*P < 0.01 versus cirrhosis group.*
n = total number of patients; SD = standard deviation.

to controls [mean 0.099 (SD 0.04) μg/L] and cirrhosis patients [mean 0.086 (SD 0.06) μg/L] (Table 2).

Because all the HE patients have underlying cirrhosis, it seemed to be more important and practical to distinguish HE from cirrhosis rather than from healthy controls. In our study, the sensitivity and the specificity for each value of S100β were calculated and then the ROC curve was constructed by plotting the sensitivity against [1–specificity] at each value (Figure 1). At the optimum cut-off point of 0.198 μg/L the specificity of serum S100β for the
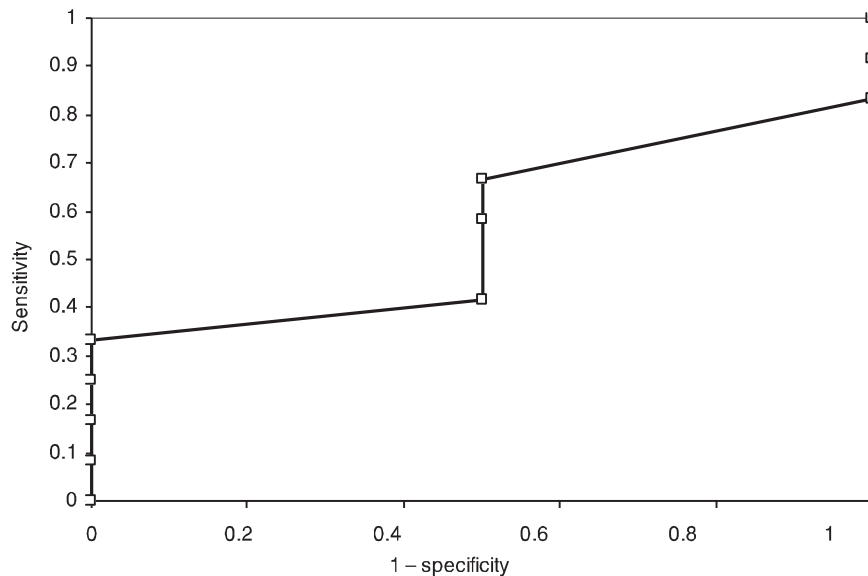
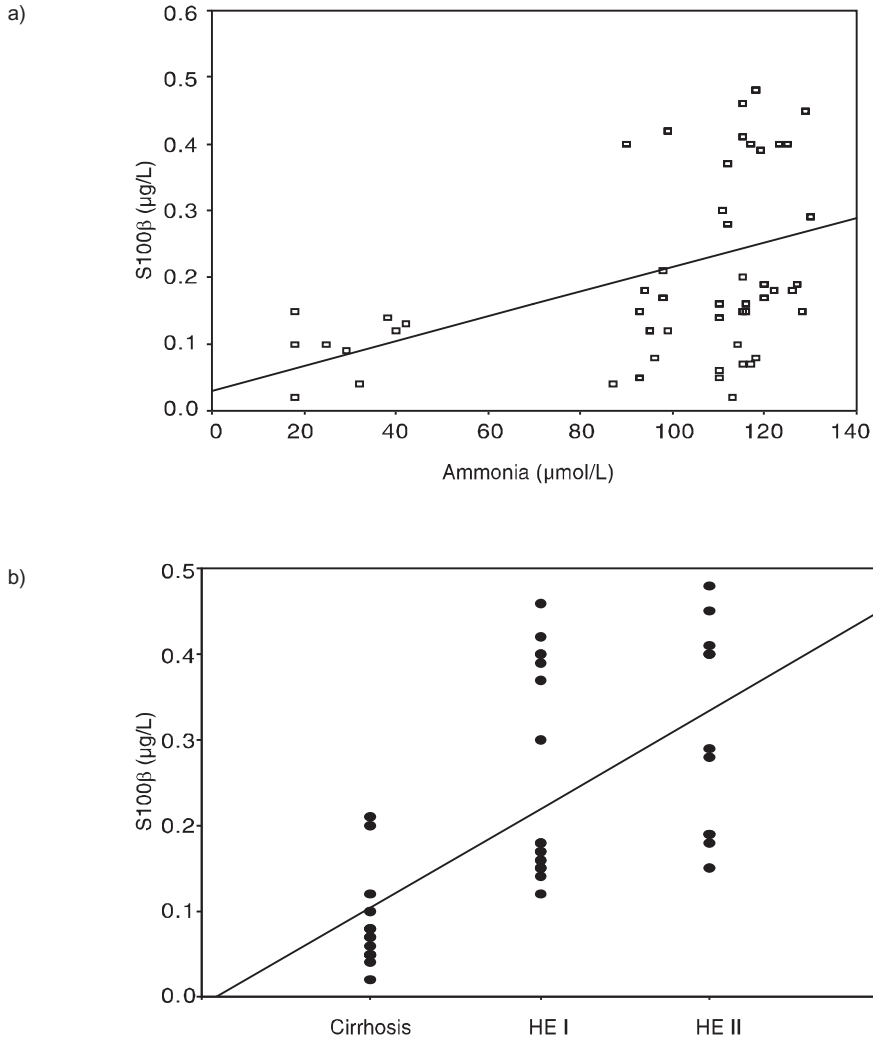Figure 1 **Receiver operating characteristic (ROC) curve for S100β**

a)

b)

Figure 2 **Correlation between S100β level and (a) plasma ammonia level in all diseased groups and (b) stage of cognitive impairment (cirrhosis, hepatic encephalopathy (HE) stage 1 and HE stage 2)**

diagnosis of HE was 91.3% and sensitivity was 51.7%. The positive predictive value, negative predictive value and diagnostic efficiency were 87.5%, 58.3% and 67.3% respectively.

We observed a significant positive correlation ($r = 0.478$, $P < 0.001$) between plasma ammonia levels and serum S100β concentration in all patients (Figure 2a). Also, a significant positive correlation ex-

isted between S100β levels and the stage of cognitive impairment ($r = 0.70$, $P < 0.001$) (Figure 2b).

Serum NSE levels showed a non-significant increase in parallel with the degree of cognitive impairment (Table 2). Values were as follows: control group [mean 12.6 (SD 1.1) µg/L], cirrhotic patients [mean 13.3 (SD 2.1) µg/L], HE stage 1 [mean 13.9 (SD 1.9) µg/L] and HE stage 2 [mean 14.5 (SD 2.3) µg/L].

Plasma ammonia levels (µmol/L) were significantly elevated in cirrhosis patients [mean 105.6 (SD 10.7) µmol/L], HE stage 1 [mean 111.6 (SD 11.5) µmol/L] and HE stage 2 [mean 117.8 (SD 11.0) µmol/L] as compared to controls [mean 28.9 (SD 9.7) µmol/L] ($P < 0.01$). However, there was no significant increase in plasma ammonia levels in HE stage 1 and HE stage 2 groups as compared to the cirrhosis group (Table 2).

Discussion

HE is a diverse group of neuropsychiatric disorders caused by liver dysfunction, usually associated with advanced cirrhosis and portal hypertension. An increased severity of liver disease is associated with decreased physical aspects of quality of life and there is accumulating evidence about the clinical significance of patients with HE compared to cirrhosis patients who have normal psychometric test performance. At the advanced stage, HE adversely affects both the physical and mental aspects of patients, whereas at stage 1 HE affects mainly the mental aspects, independently of liver disease severity [19].

Dichotomization of the HE group according to West Haven criteria for grading of mental status demonstrated that patients with cognitive deficits showed significantly elevated serum S100β levels in both HE stage 1 and HE stage 2 groups as compared to controls and cirrhosis patients.

In our study, at the optimum cut-off point of 0.198 µg/L the specificity and sensitivity of serum S100β for the diagnosis of HE were 91.3% and 51.7% respectively. The positive predictive value, negative predictive value and a diagnostic efficiency were 87.5%, 58.3% 67.3% respectively. Accordingly, elevated serum S100β levels reflect only specific aspects of the pathophysiology underlying HE, because a high specificity of serum S100β in the diagnosis of HE is paralleled by a comparatively low sensitivity. Similarly, Wiltfang et al. [20] found that S100β levels had a specificity and sensitivity of 100% and 56.5% respectively for predicting subclinical portal systemic encephalopathy. They also concluded that although S100β was significantly dependent on the Child–Pugh score, it was more closely related to cognitive impairments than the score.

A significant positive correlation existed between S100β levels and the stage of cognitive impairment ($r = 0.70$). Others found that S100β correlated with the severity of brain injury and is a sensitive non-invasive marker of injury [21]. Various stimuli cause astroglial activation resulting in releases of S100β by these cells so it is a well established marker for this activation [22].

Elevated serum S100β levels in HE can be used as a noninvasive marker of disturbances in BBB function and brain lesions [15,16]. Massive elevations in S100β are indicators of prior brain damage and can be used to differentiate extensive damage from minor, transient impairment. This can in part be explained by subtle post-traumatic impairments of the BBB [23]. S100β is involved in the regulation of energy metabolism in brain cells. It modulates the proliferation and the differentiation of neu-

rons and glia. Furthermore, it interacts with many immunological functions of the brain. Quite clearly, S100β exerts a protective effect as long as it is kept within the cells at physiological levels. However, once it is secreted or released, its local concentration dictates its beneficial or detrimental effects. Nanomolar concentrations appear to exert neuroprotective effects while micromolar concentrations produce neurodegenerative or apoptosis-inducing effects [24].

A number of routes of S100β leakage into the peripheral circulation have been suggested. One possible route consists of disruption of the brain–CSF interface, leading to increased levels of S100β in CSF that are reabsorbed into the cerebral venous system. A second, more direct route is provided by disruptions on the capillary level that allow drainage of perivascular S100β directly into the circulation [15]. The second route is more likely in patients with brain tumours or other lesions [16].

Serum NSE levels showed a non-significant increase in parallel with cognitive impairment in HE. We conclude that serum NSE has no value in diagnosis of HE in cirrhotic patients as it did not show a significant difference between the diseased groups. Others found that NSE was only slightly higher in patients with mild traumatic brain injury whereas S100β levels were significantly higher [25]. NSE does not seem to act as a peripheral marker of brain damage and BBB dysfunction [15]. In Parkinson's disease, Schaf et al. concluded that S100β and NSE levels were not useful diagnostic markers, but that S100β may be a signal of disease progression [26].

Plasma ammonia levels (μmol/L) were elevated in cirrhotic patients, HE stage 1 and HE stage 2 as compared to controls. However, there was no significant increase in plasma ammonia levels in HE stage 1 and stage 2 groups as compared to the cirrhosis group. We observed a significant positive correlation ($r = 0.478$, $P < 0.001$) between plasma ammonia levels and serum S100β concentration in all patients. This contrasts with the results of Wiltfang et al. who did not observe any correlation between arterial ammonia levels and serum S100β concentration [20]. Despite the significant correlation between the partial pressure of ammonia and HE, Nicolao et al. suggested that neither was more useful clinically than venous ammonia levels and that all 3 have a limited role in the diagnosis of HE and clinical management [27].

Due to the high prevalence of liver diseases in Egypt, early diagnosis of HE in cirrhosis patients is of great importance to allow proper management of HE patients, thus preventing further deterioration of their mental status. Serum S100β increased with progression of HE, indicating that enhanced cerebral release due to HE and impaired metabolism due to liver cirrhosis may act synergistically in elevating serum S100β. Moreover, S100β is clearly superior to NSE and ammonia in terms of diagnostic value in HE. While S100β seems to be a promising biochemical surrogate marker for mild cognitive impairment due to HE, studies with repetitive measurements of serum S100β are not yet available. Future studies will be valuable to determine to what extent a systematic displacement of serum S100β is influenced by therapeutic strategies and to investigate the relation of serum S100β to the etiology of liver disease (hepatocellular versus/cholestatic and HCV versus non-HCV).

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