

## Validation of the number connection test for identifying patients with minimal hepatic encephalopathy

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### Summary

**Objectives.** Hepatic encephalopathy is a serious neuropsychiatric complication in advanced liver disease. The affected patients exhibit alterations in psychomotor and intellectual functions. The aims of this study were to identify the set of normal values for the Number Connection Tests (NCT-A and NCT-B) in a population of volunteers without liver disease, to compare the values from this reference population with those from patients with cirrhosis without hepatic encephalopathy. **Materials and methods.** This study was performed in two referral hospitals in an urban setting from the city of La Plata. We evaluated the Number Connection Tests in 112 healthy subjects and 30 patients with cirrhosis without manifestations of hepatic encephalopathy. Time for performing the tests was measured in seconds. Results were compared according to age, gender, level of education and fine motor skills in both groups. **Results.** Mean age in the control group was 45.3 years; 56 (50%) were women. Mean age in the cirrhotic group was 54.5 years; 8 (27%) were women. In the control group, the mean time for completing NCT-A and NCT-B was 60 s ± 36 s and 140 s ± 60 s, respectively. In the cirrhotic group, the mean time for completing NCT-A and NCT-B was 114 s ± 64 s y 232 s ± 87 s, respectively ( $P = 0.00001$  for both tests). 56.6% of

cirrhotic patients took more than 2 SD to perform the NCT-A and 53.3%, more than 2 SD to perform the NCT-B. **Conclusion.** We have obtained reference values for NCT-A and NCT-B completing times in our healthy population. Cirrhotic patients without overt hepatic encephalopathy took double time than controls to complete NCT-A and NCT-B and over half of our patients would have minimal hepatic encephalopathy.

**Key words.** Cirrhosis, hepatic encephalopathy, liver disease, minimal hepatic encephalopathy, number connection tests.

### Validación de los tests de conexión numérica para identificar pacientes con encefalopatía hepática mínima

#### Resumen

**Objetivos.** La encefalopatía hepática es una complicación neuropsiquiátrica grave en la enfermedad hepática avanzada. Los pacientes afectados presentan alteraciones en las funciones psicomotoras e intelectuales. Los objetivos del estudio fueron: a) determinar los valores de referencia del tiempo medio empleado en la ejecución de las pruebas de conexión numérica en nuestra población; y b) comparar los resultados de la población de referencia con los obtenidos en cirróticos sin encefalopatía hepática clínica. **Materiales y métodos.** El estudio se llevó a cabo en dos hospitales de referencia de la Ciudad de La Plata. Se evaluaron las pruebas de conexión numérica A y B (TCN-A y TCN-B) en 112 sujetos sanos

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y 30 pacientes con cirrosis sin manifestaciones clínicas de encefalopatía hepática. El tiempo para la realización de las pruebas se midió en segundos. Los resultados fueron comparados por edad, sexo, nivel de educación y las habilidades de motricidad fina en los dos grupos.

**Resultados.** La edad media en el grupo control fue de 45,3 años y 56 (50%) eran mujeres. La edad media de los pacientes con cirrosis fue de 54,5 años; 8 (27%) eran mujeres. En el grupo control, el tiempo medio para completar el TCN-A y el TCN-B fue de 60 s  $\pm$  36 s y 140 s  $\pm$  60 s, respectivamente. En el grupo con cirrosis, el tiempo medio para completar el TCN-A y el TCN-B fue de 114 s  $\pm$  64 s y 232 s  $\pm$  87 s, respectivamente ( $P = 0,00001$ ). El 56,6% de los pacientes cirróticos tomó más de 2 DS para llevar a cabo el TCN-A y el 53,3% más de 2 DS para realizar el TCN-B. **Conclusión.** Se han obtenido valores de referencia para completar el TCN-A y el TCN-B en la población sana. Los pacientes cirróticos sin encefalopatía hepática tardaron el doble de tiempo que los controles para completar el TCN-A y el TCN-B. Más de la mitad de nuestros pacientes tendrían encefalopatía hepática mínima.

**Palabras claves.** Cirrosis, encefalopatía hepática, encefalopatía hepática mínima, test de conexión numérica.

Hepatic encephalopathy (HE) is a serious neuropsychiatric complication in advanced liver disease, both in the acute and the chronic presentation.<sup>1</sup> The affected patients exhibit alterations in psychomotor, intellectual, cognitive, emotional, and behavioral functioning as well as in fine motor functions. HE has been classified as either "overt" or "covert".<sup>2</sup> Overt HE (OHE) is a syndrome in which the neurologic and neuropsychiatric abnormalities can be detected by clinical tests. In patients with covert HE (CHE), although a normal mental state is indicated by clinical testing, abnormalities can only be detected by specific psychometric tests.

HE has a substantial negative effect on the quality of life, even in patients with CHE.<sup>3</sup> In fact, such a minimal form of HE still interferes significantly with daily life experiences such as working manually with machines, personal hygiene, recreational activities, and driving an automobile, with an increased risk of committing moving-vehicle infractions and even causing accidents.<sup>4-16</sup> Furthermore, in addition to these negative effects on day-to-day living in affected patients, CHE has been shown to be asso-

ciated with an elevated mortality and an enhanced risk of progression to OHE.<sup>3,17</sup> Interestingly, Prasad et al have demonstrated that the treatment of patients with CHE could improve their quality of life.<sup>4</sup>

The Number Connection Tests A and B (also known as Trail Making Tests) are included in the Psychometric Hepatic Encephalopathy Score (PHES) for Identifying Patients with Minimal Hepatic Encephalopathy (MHE) whose prevalence in cirrhotic patients is 30-84%.<sup>9</sup> As these tests only require a pencil and a sheet of paper to fill in, the PHES battery could be easily conducted by clinicians and could be translated and applied cross-culturally. A PHES validation study in Germany showed that the age of the patient affects PHES, and subsequent validation studies in Spain and Italy revealed that the level of education also influences PHES.<sup>18-20</sup>

The aim of this study was to identify the set of normal values for the Number Connection Tests A and B (NCT-A and NCT-B) in a population of volunteers without liver disease. As a second aim, the values from this reference population were compared to those from patients with cirrhosis without manifestations of low-grade OHE. This allowed the construction and validation of a reference dataset that can be used for the diagnosis of CHE in Argentina.

## Materials and methods

This study was performed in two referral hospitals in an urban setting. Adult volunteers without liver disease and patients with cirrhosis older than 18 years were eligible. The majority of chronic patients attending these hospitals come from the city of La Plata.

We compared Number Connection Tests A and B between healthy subjects (Controls) and patients with cirrhosis without manifestations of low-grade OHE (Cases). The time for performing the tests was measured in seconds. Results were compared according to age, gender, level of education and fine motor skills (FMS) in both groups. Fine motor skills were defined according to the capability of working with hands in works requiring precision like craftworkers. Education was defined as the number of complete years spent attending school: completed secondary school (CSS, 12 years of education) vs. uncompleted secondary school (USS, less than 12 years of education).

- Controls. We evaluated 112 healthy subjects. Exclusion criteria included: alcohol intake higher than 20 g/day 6 months before testing; illiterate; use of drugs with effects on the central nervous or on the peripheral neuromuscular systems 2 weeks before testing; and visual, cognitive or neuromuscular disorder.
- Cases. We evaluated 30 cirrhotic patients: 15 were alcoholics, 10 had chronic HCV infection, 2 had PBC, 2 had cryptogenic cirrhosis and 1 had AIH. Exclusion criteria included: alcohol intake higher than 20 g/day 6 months before testing; illiterate; use of drugs with effects on the central nervous or on the peripheral neuromuscular systems 2 weeks before testing; overt hepatic encephalopathy; and visual, cognitive or neuromuscular disorder.

EPI INFO 6 software was used for statistical analysis. We considered normal values those between  $\pm 2$  SD of the mean value. Bivariate analyses were performed using the Pearson correlation, Student's t-test and the chi-square test, as appropriate. A two-tailed  $P$  value of  $< 0.05$  was considered statistically significant.

## Results

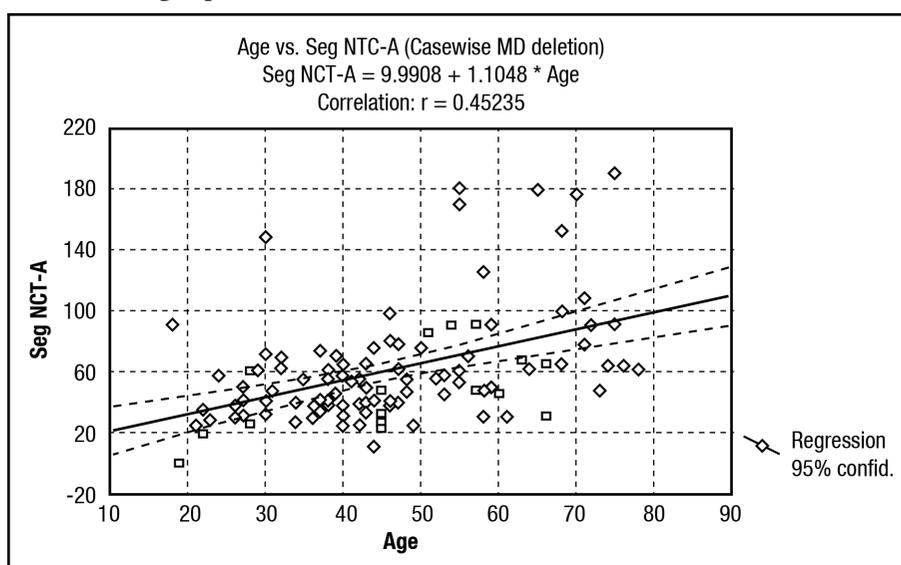
Mean age in the control group ( $n=112$ ) was  $45.3 \pm 14.7$  years; 56 (50%) were women. Se-

venty four subjects (66%) completed secondary school (CSS), and 85 (76%) with fine motor skills (FMS). Mean age in the cases group ( $n=30$ ) was  $54.5 \pm 11.5$  years; 8 (27%) were women. Five subjects (16%) completed secondary school and 13 (43%) with FMS.

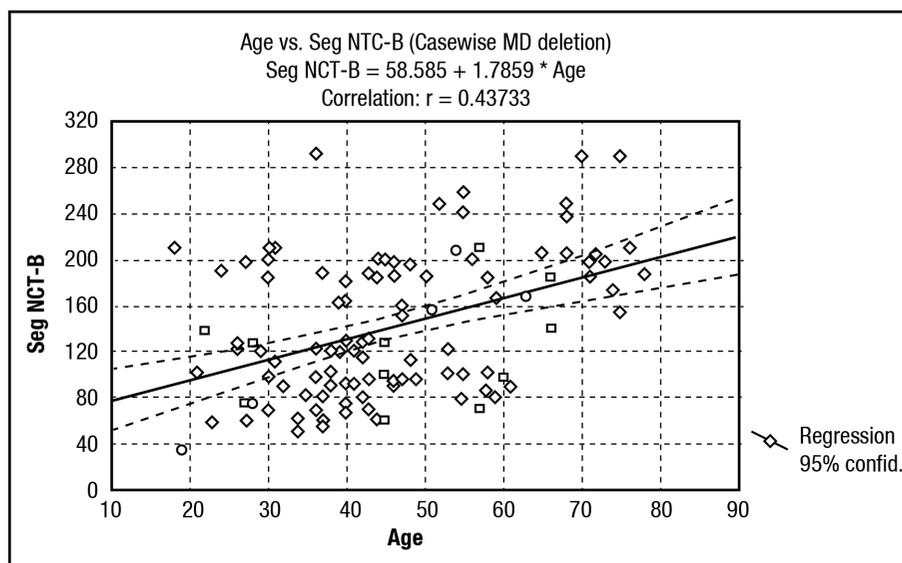
In the control group, the mean time for completing NCT-A and NCT-B was  $60 \text{ s} \pm 36 \text{ s}$  and  $140 \text{ s} \pm 60 \text{ s}$ , respectively. It was  $50 \text{ s} \pm 21 \text{ s}$  for the NCT-A, and  $120 \text{ s} \pm 49 \text{ s}$  for the NCT-B in women; it was  $70 \text{ s} \pm 44 \text{ s}$  for the NCT-A, and  $159 \text{ s} \pm 64 \text{ s}$  for the NCT-B in men (women vs. men for NCT-A  $P = 0.003$ , and for NCT-B  $P = 0.005$ ). According to educational level, it was  $49 \text{ s} \pm 22 \text{ s}$  for NCT-A, and  $116 \text{ s} \pm 51 \text{ s}$  for NCT-B in CSS subjects; and it was  $82 \text{ s} \pm 46 \text{ s}$  for NCT-A and  $185 \text{ s} \pm 50 \text{ s}$  in USS ( $P = 0.00001$  for NCT-A, and  $P = 0.00001$  for NCT-B). The correlation between age and time to complete tests was significantly different (NCT-A:  $r = 0.452$ ;  $P = 0.0001$ , and NCT-B:  $r=0.437$ ;  $P = 0.0001$ ) (Figures 1 and 2). Also, correlation between educational level and time to complete tests was significantly different (NCT-A:  $r = 0.438$ ,  $P = 0.001$ , and NCT-B:  $r = 0.421$ ,  $P = 0.002$ ). There was no correlation between gender (NCT-A:  $r = 0.06$ , NS; NCT-B:  $r = 0.25$ , NS), and fine motor skills (NCT-A:  $r = 0.03$ , NS; NCT-B:  $r = 0.15$ , NS) with the time to complete tests.

In the cirrhotic group, the mean time for completing NCT-A and NCT-B was  $114 \text{ s} \pm 64 \text{ s}$  and

**Figure 1.** Correlation between age and mean NCT-A and NCT-B completion time in the control group ( $P = 0.0001$ ).



**Figure 2.** Correlation between age and mean NCT-A and NCT-B completion time in the control group ( $P = 0.0001$ ).



**Table 1.** Mean time for NCT-A and B completion in both groups according to education, motor skills and gender.

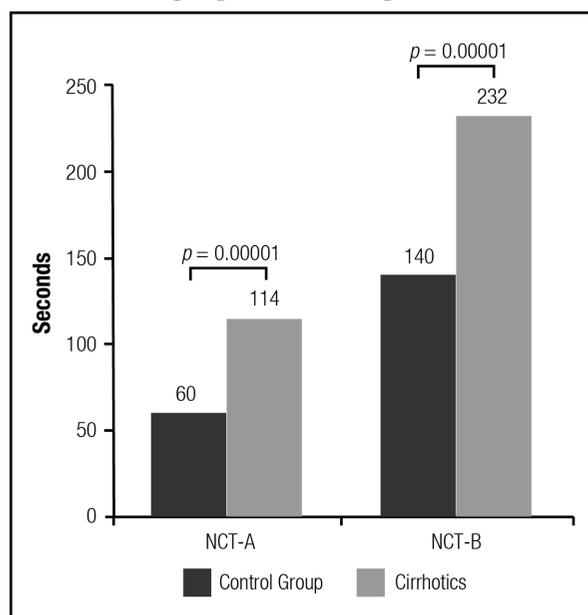
		Controls (n = 112)					
Test	Total	CSS (n 74)	USS (n 38)	WFMS (n 86)	WOFMS (n 27)	Female (n 56)	Male (n 56)
NCT-A	60±36	49±22 P = 0.00001	82±47	54±29 P = 0.0001	86±55	50±21 P = 0.001	70±44
NCT-B	140±50	116±51 P = 0.00001	189±56	132±50 P = 0.009	163±58	120±49 P = 0.0002	159±54
		Cases (n = 30)					
Test	Total	CSS (n 5)	USS (n 25)	WFMS (n 13)	WOFMS (n 17)	Female (n 8)	Male (n 22)
NCT-A	114±64	79±46 P = 0.00001	118±41	71±34 P = 0.00001	142±39	87±36 P = 0.003	120±23
NCT-B	232±87	161±31 P = 0.00001	253±35	224±23 P = 0.003	248±28	201±39 P = 0.005	251±34

CSS: completed secondary school, USS: uncompleted secondary school, WFMS: with fine motor skills, WOFMS: without fine motor skills.

232 s ± 87 s, respectively. Mean time according to gender, FMS and CSS are shown in Table 1. Mean time for completing NCT-A and NCT-B was significantly longer in cirrhotic patients than in the control group (NCT-A  $P = 0.00001$  and, NCT-B  $P = 0.00001$ ). Mean time for completing both tests is shown in Figure 3. There was no correlation

between age (NCT-A:  $r = 0.02$ , NS; NCT-B:  $r = 0.24$ , NS), gender (NCT-A  $r = 0.08$ , NS; NCT-B:  $r = 0.21$ , NS), educational level (NCT-A:  $r = 0.07$ , NS; NCT-B:  $r = 0.19$ , NS), and fine motor skills (NCT-A:  $r = 0.11$ , NS; NCT-B:  $r = 0.09$ , NS) with the time to complete tests in the cirrhotic group. 56.6% of cirrhotic patients took more than 2 SD to perform in the NCT-A, and 53.3% more than 2 SD in the NCT-B.

**Figure 3.** Mean time for NCT-A and NCT-B completion in control group and cirrhotic patients..



## Discussion

Patophysiologic mechanisms involved in covert hepatic encephalopathy (previously known as minimal hepatic encephalopathy, MHE) are not well understood. Most patients develop attention deficit and impairment in fine motor skills. Psychometric tests, easily performed with a pencil and a sheet of paper, are very sensitive to detect extrapyramidal signs. A recent study showed that CHE presented extrapyramidal signs, independently of the test used for its diagnosis.<sup>21</sup>

Diagnosis of CHE has a major impact on cirrhotic patients since it is related with diminished quality of life, impairment in the capacity to drive a car, higher risk of falls and higher risk to progress to OHE.<sup>15-16,22-23</sup> Psychometric and neurophysiologic tests are required for CHE diagnosis. Multiple combination of psychometric tests had been used, from NCT-A to the Wechsler Adult Intelligence Scale. Psychometric tests can be easily made and they do not require sophisticated equipment. Although there is no agreement on how many tests are required, it is widely accepted that a combination of 3 tests might be enough to detect neuropsychological manifestations in cirrhotic patients.<sup>24</sup>

Age, gender and educational level might influence the results of psychometric tests. It is controversial how to stratify educational level in a patient. It may be a continuous variable (years of education) or a discrete variable, rating the different levels of education accomplished by the patient (primary, secondary or tertiary education). Even though there is no agreement on the classification, level of education must be taken into account when making normality tables in a given population.

Weissenborn et al reported the usefulness of 5 psychometric tests for the diagnosis of MHE using a sample of 249 healthy subjects to define normal values.<sup>18</sup> After defining normal values, MHE was evaluated in a study involving 120 healthy subjects, 63 patients with cirrhosis without HE and 25 cirrhotic patients with grade I-II HE. MHE was diagnosed whenever a deviation of  $<-4$  SDs occurred across the five tests comparing with an age matched control population. Romero Gómez et al evaluated 884 Spanish healthy volunteers with the same 5 tests, and defined the normality table for Spain. Multivariate analysis showed that age and years of education independently influence on the tests results.<sup>19</sup> These normal values had recently been validated for the Mexican population with similar results.<sup>25</sup> However, these normality tables cannot be extrapolated to other populations since there might be geographical and educational variations in different countries.

We have designed this study to validate NCT-A and NCT-B in a cohort of 112 healthy subjects from the city of La Plata and its surroundings, to use these values as a reference for comparison with cirrhotic patients in our population. In the bivariate analysis in the control group, we found significant differences in the time to complete tests according to gender and level of education. In the correlation test, we found that age and educational level might interfere with tests results. We also found, as had been reported, statistically significant differences between cirrhotics and the control group in the time to complete tests. These findings let us note that over half of our patients would have MHE, and can help us to validate normal values in the healthy population, and can guide us in obtaining reference values to evaluate cirrhotics with suspected MHE.

One drawback of psychometric tests is time: it requires at least 15 minutes of more to complete the 5 tests. This makes it difficult to implement them in everyday consultation. It had been debated if all

cirrhotic patients must be evaluated, or if it requires some kind of patient selection. This selection can be done according to signs of neurologic deterioration, in candidates to liver transplantation, or according to the patient risk at work.<sup>24</sup> Future studies and consensus documents will define the most suitable diagnostic method for CHE/MHE and its applicability in daily practice.

We have evaluated a small sample of healthy subjects and we have an ongoing multicenter study to evaluate the 5 test of PHES.<sup>20,26</sup> Meanwhile, NCT-A and NCT-B might be a useful tool for the evaluation of our cirrhotic patient population with suspected CHE.

In conclusion, we have obtained reference values for NCT-A and NCT-B completing times in our healthy population. Healthy young women, subjects with completed secondary school used a shorter time to complete both formularies. Cirrhotic patients without overt hepatic encephalopathy took double time than controls to complete NCT-A and NCT-B and over half of our patients would have MHE. The Number Connection Tests A and B could be used in our population to diagnose covert hepatic encephalopathy.

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