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The Animal Naming Test: An Easy Tool for the Assessment of Hepatic Encephalopathy

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Screening for hepatic encephalopathy (HE) that does not cause obvious disorientation or asterixis (minimal HE [MHE]/grade 1 HE) is important. We examined if the animal naming test (ANT_1) (maximum number of animals listed in 1 minute) is useful in this context. In total, 208 healthy controls, 40 controls with inflammatory bowel disease, and 327 consecutive patients with cirrhosis underwent the ANT_1. Patients were tested for MHE by the psychometric HE score, and 146 were assessed by electroencephalography; 202 patients were followed up regarding the occurrence of overt HE and death. In the healthy controls, ANT_1 was influenced by limited education (<8 years) and advanced age (>80 years, $P < 0.001$). Using an age and education adjusting procedure, the simplified ANT_1 (S-ANT_1) was obtained. An S-ANT_1 of <10 animals was abnormal. Of the patients, 169 were considered unimpaired, 32 as having HE ≥grade 2, and 126 as having MHE/grade 1 HE. This group had lower S-ANT_1 than unimpaired patients (12 ± 0.4 versus 16 ± 0.7, $P < 0.001$) and higher S-ANT_1 than those with HE ≥grade 2 (4 ± 0.9). In grade 1 HE the S-ANT_1 was lower than in MHE. Following receiver operating characteristic analysis (Youden's index), 15 animals produced the best discrimination between unimpaired and MHE/grade 1 HE patients. Thus, a three-level score (0 for S-ANT_1 ≥15, 1 for 10 ≤ S-ANT_1 < 15, 2 for S-ANT_1 <10) was obtained. This score was correlated both to the psychometric HE score ($P < 0.0001$) and to electroencephalography ($P = 0.007$). By sample random split validation, both S-ANT_1 and its three-level score showed prognostic value regarding the 1-year risk of overt HE and death. No inflammatory bowel disease control had S-ANT <15. *Conclusion:* The S-ANT_1 is an easily obtainable measure useful for the assessment of HE. (HEPATOLOGY 2017;66:198-208).

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Hepatic encephalopathy (HE) produces a spectrum of neurological/psychiatric abnormalities ranging from subclinical alterations to coma.[1] HE that produces clear disorientation in time/space/identity or asterixis is easily detected and quantified and is labeled as HE ≥grade 2, according to the West Haven classification.[1,2] In contrast, it is difficult to detect and quantify conditions in which (1) HE is latent and asymptomatic so that only psychometric tests or neurophysiological tools can detect its occurrence, thus corresponding to what Schomerus and Hamster[3] termed *minimal* HE (MHE), or (2) HE is barely symptomatic or produces poorly defined trivial clinical signs,[1,4] which corresponds to what was called *stage 1 HE* by Conn et al.[2] The recently published American Association for the Study of Liver Diseases/European Association for the Study of the Liver practice guidelines suggest that, ideally, every patient at risk should be tested for HE that is not overtly symptomatic, i.e., for MHE/grade 1

Abbreviations: ANT, animal naming test; ANT_1, ANT at 1 minute; EEG, electroencephalogram; HE, hepatic encephalopathy; IBD, inflammatory bowel disease; MDF, mean dominant frequency; MELD, Model for End-Stage Liver Disease; MHE, minimal HE; PHES, psychometric HE score; S-ANT_1, simplified ANT.

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HE—also called by some authors *covert* HE as it sounds complementary/opposite to *overt* HE.[1] This condition requires testing to be identified and quantified because it is a relevant health problem and because even in its minimal expression (MHE) it is associated with increased caregiver burden,[5,6] poor prognosis,[7-9] increased risk of bouts of severe HE,[10] and driving inability.[11] At any rate, the recognition of HE lower than grade 2 reasonably requires quantification because it covers a rather wide spectrum of mental impairment.

Tools useful for the detection of MHE are the psychometric hepatic encephalopathy score (PHES) in countries where it is standardized,[12] quantified electroencephalography (EEG),[8,13] the critical flicker frequency,[14] and computerized tests.[7,15-17] All of these well-recognized techniques, although more or less sensitive and objective, require some kind of equipment, even if only a simple pencil-and-paper form to be completed. For grade 1 HE, no clear detection consensus exists, so Conn's criteria with the operative criteria suggested by the American Association for the Study of Liver Diseases/European Association for the Study of the Liver practice guidelines represent a reasonable approach,[1] despite the fact that they have not been validated. Ideally, a simple procedure providing an estimate of all of the mental conditions concerning HE <grade 2 would help hepatologic clinical practice.

In this regard, it is well known and accepted that mental function can be evaluated by simple verbal questions (such as those concerning orientation in time/space/identity),[1] which have obvious intercultural applicability and can be easily used in clinical practice. We, therefore, focused on the applicability of a simple verbal question to obtain a routinely and rapid assessment of patients with cirrhosis who do not have HE causing frank disorientation, both in the office and at the bedside. We hypothesized that the animal naming test (ANT) would be useful for this aim. The ANT is a semantic fluency test that consists of listing as many names of animals as possible in 1 minute (ANT_1).

Adequate performance of the ANT requires efficient organization of verbal retrieval and recall, as well as self-monitoring aspects of cognition (the participant must keep track of responses already given), effortful self-initiation, and inhibition of responses, when appropriate.[18] These cognitive skills require efficient executive functions in addition to adequate memory. Therefore, the presence of the initial stage of HE, which impairs executive functions,[19,20] is expected to damage semantic fluency. The ANT is sensitive to cognitive functions related to prefrontal cortex/anterior cortical areas,[21,22] which are particularly vulnerable to HE in its initial stages.[23,24] Accordingly, use of the ANT can be reasonably hypothesized to function as an easy first-line tool to investigate HE in patients with cirrhosis. The rationale behind the choice of the ANT is that knowledge of animal names is reasonably widespread in humans of every culture, and the influence of age, education, and gender, if any, might be limited.

Toward this aim, we performed a study (1) to standardize the ANT in a sample of healthy subjects, (2) to compare the ANT to standard tools for the assessment of HE, (3) to produce a simple scoring system based on the ANT useful for the assessment and scoring of HE <grade 2, and (4) to assess the prognostic value of the ANT.

Materials and Methods

PARTICIPANTS

Three groups of subjects underwent the ANT. The first was composed of healthy individuals and served to standardize the ANT. The second was a consecutive sample of patients with cirrhosis who had been admitted to the university hospital of Padua or Rome, while the third was a consecutive sample of patients with inflammatory bowel disease (IBD) admitted to the university hospital of Padua or Rome.

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Healthy Controls

This group was a convenience sample of 208 healthy volunteers (42% males) who were enrolled by students in university courses in health education, comprising people living in the urban and rural areas around Padua. The volunteers underwent a structured interview to exclude alcohol misuse, consumption of psychotropic drugs, insulin-dependent diabetes, or diseases that can negatively affect cognitive function. The selection of the sample was stratified according to age in order to have participants in each of the following age classes: 18-40, 41-60, 61-70, 71-80, and >80 years. Four educational levels were considered: elementary school (5 years or lack of achievement of middle school qualification), middle school (8 years or lack of achievement of high school qualification), high school (13 years or lack of achievement of a university degree), university degree (≥16 years). After obtaining informed consent, each individual underwent the ANT_1, which measures the number of animals listed in 1 minute (see the instruction for the test provided in the Supporting Information). A subgroup of 41 healthy volunteers (46 ± 15 years of age, 54% males, education 14 ± 4.6 years) was retested for the ANT_1 after 1 week to assess learning, and 20 of them repeated the test daily for 1 week, to obtain the day-to-day coefficient of variation of the measure.

Control Sample of Subjects With IBD

A group of 40 patients with IBD without liver disease was enrolled as a control group of individuals with chronic inflammatory disease of the gastrointestinal tract. The same exclusion criteria as for the patients with cirrhosis (see below) were adopted.

Patients With Cirrhosis

The patient population was consecutively recruited from two university hospitals (Padua and Rome, Italy). It comprised 327 consecutive patients (69% males) with cirrhosis (172 from Padua, 155 from Rome; age 60 ± 13 years, mean ± standard deviation; Child A, 40%; B, 37%; C, 23%). Their clinical and biochemical findings are shown in Table 1. Subjects were considered eligible if they had cirrhosis with or without clinical signs of HE. Exclusion criteria were age <18 years; neurological comorbidities (e.g., dementia or prior cerebrovascular disease); psychiatric disorders; alcohol misuse in the previous 6 months or use of sedatives (e.g., benzodiazepines, neuroleptic, antiepileptic, and opiate drugs); and heart, respiratory, or renal failure that could confound the assessment of the mental state. Seventy-six patients had had a previous bout of overt HE. Of all the patients, 98 from Padua and 88 from Rome were outpatients. They took typical drugs used in patients with cirrhosis: diuretics, propranolol in those with esophageal varices, lactulose, and rifaximin, when needed—in addition to vitamin D or other vitamin supplementation and proton pump inhibitor or other specific treatments for concurrent conditions, when needed.

A subgroup of 200 patients with cirrhosis (151 from Rome and 49 from Padua) was prospectively followed for up to 1 year in the outpatient clinic and by telephone interviews. Information was obtained from all patients. Both the first bout of HE ≥grade 2 and survival were considered. Patients who underwent liver transplantation in the follow-up were censored on the day of transplant. Seventy-six patients had a bout of HE ≥grade 2 in the follow-up (50 from Rome and 16 from Padua [33%]). The follow-up for the first bout of HE ≥grade 2 was 216 (70-365) days (median and interquartile range). Forty-six patients died (38 from Rome and 8 from Padua), and 17 were transplanted (6 from Rome and 11 from Padua). The follow-up for survival was 328 (128-365) days.

Another subgroup of 12 patients with cirrhosis who were admitted for HE ≥2 was tested upon admission using the West Haven classification, the Glasgow Coma Scale, the ANT_1, and ammonia levels. They were retested after 5-7 days to verify the dynamics of the above behavioral and biochemical markers of HE.

The protocol was approved by the hospital ethics committee. All participating subjects provided written, informed consent. The study was conducted according to the Declaration of Helsinki (Hong Kong amendment) and good clinical practice (European) guidelines.

MEASURES

The ANT

Subjects were asked to list as many animals as they could (for ANT instructions, see the Supporting Information). All repetitions and errors were excluded from the calculations. The ANT_1 was performed in patients and healthy controls.

TABLE 1. Demographic Data of Controls and Demographic, Clinical, and Biochemical Data of Patients With Cirrhosis

Healthy Controls (n = 208)			Controls With IBD (n = 40)		
Gender (males)		42%	55%		
Age (years)*		54 (37-70)	43 (36-50)		
Age classes (%)			Age classes (%)		
18-40 years		29	18-40 years		44
41-60 years		29	41-60 years		44
61-70 years		18	61-70 years		7
71-80 years		13	71-80 years		5
>80 years		11	>80 years		0
Education level (%)		Mean age ± SEM	Education level (%)		Mean age ± SEM
Elementary school	28	66 ± 2	Elementary school	7	60 ± 9
Middle school	38	49 ± 2	Middle school	12	47 ± 4
High school	19	49 ± 3	High school	61	42 ± 3
University degree	15	49 ± 3	University degree	20	44 ± 4

Patients With Cirrhosis (n = 327: Rome n = 155, Padua n = 172)

	Total	Rome	Padua		Total	Rome	Padua
Gender (males %)	69	66	72	Age (years)*	60 (48-70)	64 (53-74)	60 (51-67)
Education level (%)				Ascites (%)			
Elementary school	34	39	31	None	59	61	58
Middle school	37	33	41	Mild/moderate	29	28	30
High school	21	20	21	Severe	12	11	12
University degree	8	9	8				
Etiology of cirrhosis (%)							
				Albumin (g/L)*	35 (31-40)	34 (24-32)	36 (28-40)
Virus-related	50	56	44	Total bilirubin (μmol/L)	24 (14-58)	26 (18-53)	24 (13-58)
Alcoholic	26	22	29	INR	1.3 (1.2-1.5)	1.4 (1.2-1.6)	1.3 (1.1-1.4)
Mixed	15	14	15	Na (mmol/L)	138 (134-140)	137 134-139)	139 (135-141)
Others	9	8	11	Creatinine (μmol/L)	80 (65-100)	79 (65-103)	81 (64-100)
				Ammonia (μmol/L)	56 (30-90)	55 (30-63)	57 (31-97)
Child-Turcotte-Pugh class (%)							
A	39	41	37	MELD score	12 (9-16)	12 (9-16)	12 (8-17)
B	32	33	32				
C	29	26	31				
Previous episodes of HE ≥grade 2 (%)	35	30	40				

*Median (interquartile values)
Abbreviations: INR, international normalized ratio; SEM, standard error of the mean.

Clinical Assessment of HE

Patients were examined by at least one clinician with considerable expertise in HE assessment (F.C., S.M, O.R., L.R.), and those in Padua were also examined by an experienced neuropsychologist (S.S.). The assessment included a full neurological examination, in particular to detect asterixis, and grading of the neuropsychiatric abnormalities according to the West Haven criteria, using the operative criteria suggested by the American Association for the Study of Liver Diseases/European Association for the Study of the Liver practice guidelines (Supporting Table S1).[1] All patients without HE ≥grade 2 underwent the portosystemic encephalopathy battery that produces the PHES, using age-adjusted and education-adjusted norms.[3,25,26] The portosystemic encephalopathy syndrome test includes five tests that refer to the cognitive domains of attention, executive functions, and visuomotor and psychomotor abilities: the digit symbol test, trail making tests A and B, the serial dotting test, and the line trait test. The PHES is the sum of the integer scores of each test computed from the age-adjusted and education-adjusted z values as follows: score = −3 for $z \leq -3$, score = −2 for $-3 < z \leq -2$, score = −1 for $-2 < z \leq -1$, score = 0 for $-1 < z < 1$, score = 1 for $z \geq 1$.[3]

Patients with PHES ≤−4 were considered to have MHE.[25]

Neurophysiological Assessment of HE

Additionally, 146 patients from the Padua center underwent digital EEG. Spontaneous closed-eyes resting EEG activity was recorded by digital EEG equipment (Brainquick 3200; Micromed, Italy) in the morning. A standard 21-channel cap (Micromed) was used, and the electrodes were placed according to the 10-20 International System.[27] The EEG tracing was assessed by spectral analysis after visual inspection to exclude artifacts. Spectral analysis was carried out on the derivation P3-P4 in the frequency range of 1-25.5 Hz. The EEG alterations were classified into three grades according to mean dominant frequency (MDF; i.e., the mean frequency weighted by the power of each frequency band) and to the relative power of the theta and delta bands as follows: grade 1, MDF >6.8 Hz and theta relative power ≥35%; grade 2, MDF ≤6.8 Hz and delta relative power <49%; and grade 3, MDF ≤6.8 Hz and delta relative power ≥49%.[28]

STATISTICAL ANALYSIS

In the healthy group, analysis of covariance was used to evaluate if age, level of education, and gender were predictors of the ANT score and to evaluate the effect of education level and age class on the ANT score. The Newman-Keuls test was used for *post hoc* comparisons. The thresholds of normality for the ANT_1 and ANT_2 in healthy subjects were fixed at the 2.5th percentile.

In patients with cirrhosis, a general linear model was used to evaluate if a history of HE ≥grade 2 (dichotomous variable "yes" or "no") and alcohol-related etiology (dichotomous variable "yes" or "no") were predictors of ANT_1, adjusted for age (in years) and education (years of education). Receiver operating characteristic curve analysis and the Youden test were performed to determine the best threshold for the ANT_1. Correlations between the ANT score and the EEG results were assessed using the Pearson *r* correlation. Day-to-day variation was assessed by the mean of the coefficient of variation of the measures of the ANT_1 in single patients for 1 week.

Survival analysis was performed by the Kaplan-Meier method, and comparisons were performed using the log-rank test or the χ^2 test, when appropriate. A multivariate survival study was performed using the Cox model.

Survival analysis was validated by random sample splitting so that half the sample served for modeling the survival Cox regression and the other half for its validation.

Results are expressed as mean ± standard deviation, unless otherwise specified. Statistical analysis was performed using Statistica version 12 (software.dell.com) and Egret version 2.0.31 (Cytel Software Corporation, Cambridge, MA).

Results

CONTROLS

Healthy Subjects

The demographic characteristics of the healthy control subjects are summarized in Table 1. Males and females were homogeneously distributed throughout the age classes ($\chi^2 = 2.9$, $P = 0.4$); in contrast, an inverse association between age and education level ($\chi^2 = 58$, $P < 0.001$) was detected, as expected, because advanced education is rare in older individuals and limited education is rare in younger individuals, as a result of the facts that access to high school and university has increased over time (calendar effect).

In healthy controls, the ANT_1 was influenced by age and education but independent of gender (Supporting Table S2). However, upon closer inspection, education produced a roof effect over 8 years so that only subjects with an education <8 years were found to list a significantly lower number of animals than subjects with education ≥8 years. Similarly, subjects with age >80 years were found to list a significantly lower number of animals than subjects ≤80 years, whereas the effect of age was negligible in subjects with age ≤80 years (Supporting Table S3).

Fixing the lower limit of reference at the 2.5th percentile, the resulting limits and the equivalent score were computed (Supporting Table S4). For ease of reference, a simplified equivalent ANT_1 (S-ANT_1) was obtained, adding three animals for individuals with fewer than 8 years of education and six animals if they were, in addition, over the age of 80 years; thus, an easy-to-remember rule is that at least 10 animals should be listed by a healthy individual, after having adjusted for age and education.

A learning effect was found (first session versus second session after 1 week: 22 ± 9 animals versus 24 ± 10 animals, $P < 0.01$), but it was limited and comprised only two animals, on average. In the patients who underwent daily repetition of the test for 1 week, the test reached a plateau on the second day and the

TABLE 2. S-ANT$_1$ in Healthy Controls, in Controls With IBD, and in Patients With Cirrhosis, Subdivided on the Basis of HE Severity

	Controls		Patients With Cirrhosis (n = 327)				
	Healthy Subjects (n = 208)	IBD (n = 40)	Unimpaired (n = 169)	Pooled MHE and Grade 1 HE (HE <Grade 2) (n = 126)	MHE (n = 76)	Grade 1 HE (n = 50)	HE ≥Grade 2 (n = 32)
S-ANT$_1$	23 ± 0.5*	25 ± 1.0	16 ± 0.7†	12 ± 0.4†,‡	13 ± 0.5†,§	11 ± 0.6†,§	4 ± 0.9†,‖

*Mean ± standard error of the mean.
†$P < 0.001$ versus healthy controls (Tukey's adjustment for multiple comparisons).
‡$P < 0.05$ versus unimpaired HE (Tukey's adjustment for multiple comparisons).
§$P < 0.01$ MHE versus grade 1 HE.
‖$P < 0.01$ versus unimpaired and versus HE <grade 2 (Tukey's adjustment for multiple comparisons).

day-to-day mean coefficient of variation was 9.1 ± 0.9%.

IBD Controls

The demographic characteristics of the IBD control subjects are summarized in Table 1. On average, in IBD control subjects, the S-ANT$_1$ was comparable to that of the healthy controls (Table 2). Notably, none of these subjects had S-ANT$_1$ <15.

PATIENTS WITH CIRRHOSIS

The clinical and demographical characteristics of the patients are reported in Table 1.

One hundred and sixty-nine (52%) patients with cirrhosis were found to be unimpaired on PHES, 32 (10%) were found to have HE ≥grade 2 upon clinical examination (n = 18 grade 2, n = 14 grade 3), 50 (15%) had grade 1 HE, and 76 (23%) had MHE. Thus, 126 (38%) were found to have HE <grade 2.

No patients had previously undergone the S-ANT$_1$. No patient refused to undergo the test, and all patients perceived the test as acceptable. The S-ANT$_1$ was found to be inversely correlated with the Model for End-Stage Liver Disease (MELD; $r = -0.16$, $P < 0.025$) and ammonia plasma level ($r = -0.35$, $P < 0.001$) and directly correlated with PHES ($r = 0.38$, $P < 0.001$) (Supporting Fig. S1). The S-ANT$_1$ was lower in patients with a history of previous HE ≥grade 2 than in those who did not have HE ≥grade 2 in the past (12 ± 0.6 versus 14 ± 0.5, $P < 0.03$).

Adjusting for the MELD score, the S-ANT$_1$ was comparable in subjects with cirrhosis of different etiology (alcoholic cirrhosis = 13.6 ± 0.6, viral = 13.1 ± 0.5, other etiologies = 13.6 ± 0.9; $F_{2,256} = 0.6$, $P = 0.5$).

On the whole, S-ANT$_1$ was lower in patients with cirrhosis than in healthy and IBD controls (13 ± 0.3, 23 ± 0.5 and 25 ± 1.0, respectively) (Table 2).

Considering the patients with cirrhosis, the S-ANT$_1$ of patients with HE ≥grade 2 was lower than that of patients with HE <grade 2 (i.e., MHE/grade 1 pooled together, a condition labeled as covert HE by some authors), and this, in turn, was lower than that of unimpaired patients. In addition, the S-ANT$_1$ was lower in patients with grade 1 HE than in those with

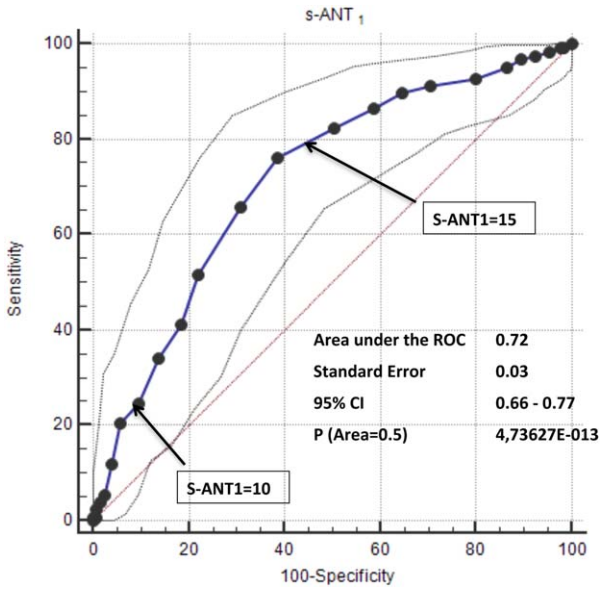

FIG. 1. ROC curves of S-ANT1 for the detection of patients with HE < grade 2 vs. unimpaired patients. The values of sensitivity/specificity, PPV/NPV, +LR/-LR for S-ANT=15 (optimal discriminating value obtained by Youden's index) and S-ANT=10 (lower limit of normality in healthy subjects) are reported in Supplementary Table S5. Abbreviations: CI, confidence interval; ROC, receiver operating characteristic.

TABLE 3. Accordance of S-ANT$_1$ Score Versus Current Accepted HE Grading Procedure

Score	S-ANT$_1$	Unimpaired	MHE	Grade 1 HE	HE grade ≥2	Total
0	≥15 animals	106 (79%)* (95% CI 72%-85%)	23 (17%) (95% CI 11%-24%)	5 (4%) (95% CI 1%-7%)	1 (1%) (95% CI 0%-4%)	135
1	≥10 and <15 animals	49 (40%)† (95% CI 32%-49%)	42 (35%) (95% CI 26%-43%)	23 (19%) (95% CI 12%-26%)	7 (6%) (95% CI 2%-10%)	121
2	<10 animals	14 (20%)‡ (95% CI 10%-29%)	11 (15%) (95% CI 7%-24%)	22 (31%) (95% CI 20%-42%)	24 (34%) (95% CI 23%-45%)	71
Total		169	76	50	32	327

*Percentages refer to the total of the row.
†Detected in only 14% of healthy controls.
‡Detected in only <0.5% of healthy controls; thus, the classification "unimpaired" based on PHES was likely incorrect, and some of them were, reasonably, false negatives on PHES examination.

MHE (Table 2). Notably, also the S-ANT$_1$ of unimpaired patients with cirrhosis was, on average, lower than that of healthy controls (Table 2), and 14 (8%) of the patients labeled as "unimpaired" (8%) had a definitely abnormal (fewer than 10 animals) S-ANT$_1$.

The EEG was found to be altered in 42% (n = 47) of the 146 patients who underwent EEG examination. There were correlations between the S-ANT$_1$ and EEG spectral parameters (MDF, $r = 0.23$, $P < 0.001$; delta relative power, $r = -0.17$, $P = 0.04$; theta relative power, $r = -0.23$, $P = 0.005$; beta relative power, $r = 0.26$, $P < 0.001$).

According to Youden's index, the optimal cutoff value to detect patients with HE <grade 2 versus unimpaired subjects was S-ANT$_1$ 15 < (Fig. 1). In addition, considering S-ANT$_1$ < 10 (i.e., low cutoff in healthy subjects), a three-step scoring (score 0 = S-ANT$_1$ ≥15, score 1 = 10 ≤ S-ANT$_1$ < 15, score 2 = S-ANT$_1$ <10) was implemented. This is both easy to remember and easy to use in clinical practice; the values of sensitivity, specificity, positive predictive power, negative predictive power, positive likelihood ratio, and negative likelihood ratio (+L and –L) are reported in Supporting Table S5.

Applying the above scoring scheme based on S-ANT$_1$, 22% (n = 71) of patients had a score of 2, 37% (n = 121) had a score of 1, and 41% (n = 135) had a score of 0. This score was found to be correlated with PHES ($r = -0.40$, $P < 0.0001$) and the MDF of the EEG ($r = -0.18$, $P < 0.05$). A negative correlation was found with the Child-Pugh score ($r = 0.22$, $P < 0.01$), and a trend was found for a correlation with the MELD score ($r = 0.12$, $P < 0.06$).

The S-ANT$_1$ score was well related to HE stage ($\chi^2 = 15$, $P = 0.0005$) because subjects with a score of 0 were likely normal (79%), those with a score of 1 were likely abnormal (60%), and those with a score of 2 were very likely abnormal (80%) (Table 3). Of note, the percentage of truly abnormal individuals in the latter group was likely higher because healthy controls had values of S-ANT$_1$ ≥10; therefore, it is reasonable that these patients were false negative on PHES. In the subgroup of patients who were retested after recovery from HE ≥grade 2, the dynamics of the S-ANT$_1$ closely reflected the clinical outcome (Table 4).

PROGNOSTIC STUDY

Using sample random split validation stratified by previous episodes of HE ≥grade 2, the S-ANT$_1$ was found to have a prognostic value regarding the 1-year risk of HE ≥grade 2 and death. The model fitted to the first half of the sample was found to be valid for the second half of the sample and was independent of a history of HE ≥grade 2 and the MELD score (Table 5). Of note, the β coefficients of the regressions for the

TABLE 4. Dynamics of the S-ANT$_1$ and the Other Indexes of HE in the Subjects Admitted for HE ≥Grade 2 and Retested After Recovery

Timing	West Haven Score	Glasgow Coma Scale	S-ANT$_1$	Ammonia (μmol/L)
Admission	2 (2-2.5)*	14 (12-15)	3.5 (0-7.5)	129 (98-158)
5-7 days after admission	0 (0-1)	15 (15-15)	10.5 (7.5-14.5)	70 (38-145)

*Median (interquartile range).

TABLE 5. Predictors of HE ≥Grade 2 and Death With the Cox Regression Model (Backward Procedure From the Model Comprising S-ANT$_1$, Previous Bouts of HE ≥Grade 2, and MELD) in the Study Group and the Validation Group (Random Splitting Procedure Stratified for Previous Episodes of HE): Note the Overlapping of the β Coefficient of S-ANT$_1$

	Variable	Study Group (n = 100)		Validation Group (n = 100)	
Episodes of overt HE		38		39	
Deaths		28		18	
		β coefficient ± SEM	P	β coefficient ± SEM	P
HE ≥grade 2 (1-year follow-up)	S-ANT$_1$	−0.069 ± 0.031	0.029	−0.073 ± 0.030	0.024
	Previous HE ≥2	0.539 ± 0.320	0.090	0.898 ± 0.374	0.016
Death (1-year follow-up)	S-ANT$_1$	−0.057 ± 0.038	0.130	−0.076 ± 0.037	0.041
	MELD	0.099 ± 0.038	0.009	*	
	Previous HE ≥2	*		0.631 ± 0.43	0.145

*Did not enter into the model.
Abbreviation: SEM, standard error of the mean.

variable S-ANT$_1$ were found to be very close (within the 95% confidence limits) in the study group and in the validation group.

The prognostic value of the S-ANT$_1$ score obtained from the whole series is shown in Fig. 2 (see also Supporting Table S6).

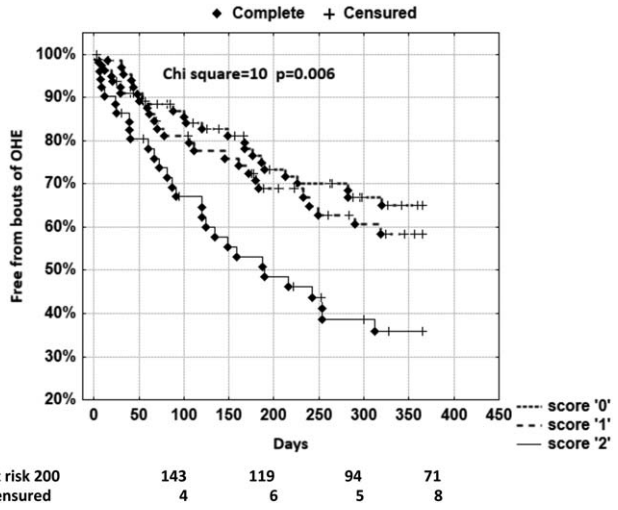

FIG. 2. Prognostic value of the S-ANT$_1$ score on the risk of the occurrence of HE≥ grade 2 in the follow up. Subjects at risk: score 0 = 81, score 2 = 66, score 3 = 53. A score of 2, corresponding to S-ANT$_1$<10, is clearly related to a high risk of HE≥ grade 2 in the follow-up. Of note, using the continuous values of S-ANT$_1$ and the occurrence of at least a previous bout of HE≥2, a risk index for future bouts of HE results from the equation 100*EXP(-0.071*S-ANT$_1$+0.34*previous bout of HE -yes = 1, no = 0-), which was derived by multivariate Cox regression. Abbreviation: OHE, overt HE.

Discussion

This study (1) provided the standardization of the ANT$_1$ in healthy controls, the resulting thresholds of normality, and the equivalent values for a simplified ANT$_1$ (S-ANT$_1$); (2) investigated the use of the S-ANT$_1$ in patients with cirrhosis in comparison to HE that has been ascertained with currently accepted criteria; and (3) verified the predictive value of the S-ANT$_1$ on the 1-year probability of HE ≥grade 2 and death. The ANT$_1$, which was never used for the assessment of HE in other laboratories, was found to be easy to administer, well accepted by patients, simple to score, and associated with the degree of HE and the risk of bouts of HE ≥grade 2 in the follow-up. Learning in retesting was detectable, but this was limited, as was day-to-day variability. All of these features support the routine use of this easy question as a tool for rapid assessment of mental state.

The reference values for ANT$_1$ in healthy controls in our study closely corresponded to those derived from standardization samples of thousands of individuals from the Italian population,[29-31] and despite the fact that the ANT$_1$ depends on age and education, simplified equivalent values were easily obtainable by adding three animals for subjects with a low level of education (less than 8 years of formal education) and six animals for those who were both aged and poorly educated. This simplification was possible because of the relevant ceiling effects concerning education (which significantly influenced the results only in very poorly educated subjects) and age (which significantly influenced the results only in significantly aged people). This simplified adjustment of the

ANT_1 (S-ANT_1) avoids the complex standardization systems that are usually required for psychometric testing.[25,32,33] This procedure produced a very simple classification system in which the person using the test is required to remember only the easy values of 10 and 15 to score the test, as well as a simple procedure to produce the equivalent score (add three animals for individuals with less than 8 years of education and six animals if they were, in addition, over the age of 80 years). At any rate, the use of a three-step score (0, 1, 2) for the S-ANT has clear ceiling effects toward low and high values, so it is only a simplification that does not summarize all the information of the S-ANT_1. In fact, the higher the value of the S-ANT_1, the lower the likelihood of cognitive impairment and of HE. Of note, the test was shown to have a limited learning effect and a day-to-day variability of about 10% so that a variation of the S-ANT_1> 20% is unlike to occur by chance. Similar information has not been obtained before from tools to monitor HE.

The reference values for the ANT found in our study are perfectly in line with epidemiological studies performed in many western countries: The Netherlands,[34] Spain,[35] Portugal,[36] France,[37] Sweden,[38] Canada,[39,40] and the United States[41] (Supporting Table S7). This agreement strengthens the rationale of the proposal of two thresholds of 10 and 15 for the S-ANT_1. Of note, the value of 15 animals or fewer was considered a valuable index of mild cognitive impairment or, at any rate, a value requiring further investigation into cognitive decline in any individual in previous studies,[41-43] and values around 10 are strong evidence of poor cognition.

Furthermore, in our healthy controls, the ANT_1 was found to be independent of gender, in agreement with the majority of studies on verbal fluency,[29,30,34-36] with the exception of those by Rosselli et al.[44] and Raoux et al.[37] This is an advantage compared to the semantic verbal fluency for other categories of objects. This has been found to be influenced by gender,[29,34] as is reasonable given that males and females are expected to be more or less familiar with objects such as vehicles, fruits, or tools.

Obviously, the S-ANT_1 cannot be thought of as a specific tool for the detection of the sole mild cognitive impairment related to HE because other kinds of mild cognitive impairment impair verbal fluency[42,43]; however, our data show that it provides an easily obtainable measure of cognition, similar to the questions composing the Glasgow Coma Scale in comatose patients or the questions concerning orientation in time/space/identity.

A strength of the S-ANT_1 was the lack of any complaints from patients, the rapidity and ease of administration, and the correlation with HE classification obtained by cumbersome neuropsychological and electrophysiological techniques that may require trained and specialized personnel. Further, internal criteria of plausibility for the S-ANT_1 are the relationship to previous occurrence of bouts of HE ≥grade 2, to ammonia, to MELD, as well as to the risk of HE ≥grade 2 and death in the follow-up period. These elements underline that it reflects the severity of—and the risk for—HE and death.

This is not surprising because the S-ANT_1 depends, at least in part, on neuropsychological domains (executive functions, working memory, sustained attention) that are explored by neuropsychological tools recommended to diagnose MHE, i.e., the portosystemic encephalopathy syndrome test battery, the inhibitory control test,[15,45] the scan battery,[46] and the Stroop test.[20,47]

The finding that the S-ANT_1 produced lower values in the so-called unimpaired patients with cirrhosis than in healthy controls and was altered in 8% of patients who had PHES within the reference range supports the idea of a continuous spectrum of cognitive decline in patients with cirrhosis.[4] This is in agreement with previous studies showing some cognitive impairment in patients with cirrhosis, even before they reached the threshold for MHE on PHES criteria.[48,49]

Notably, the lower performance on the S-ANT_1 in patients with grade 1 HE compared to those with MHE (1) demonstrated the heterogeneity of patients labeled as having so-called covert HE (i.e., HE <grade 2) and (2) provided evidence that the S-ANT_1 is a valuable measure for the rather wide spectrum of cognitive conditions of these patients in which the use of a tool to quantify mental impairment was advocated.[4] The S-ANT_1 produced two thresholds (10 animals and 15 animals) that can provide an easy-to-memorize tool for clinical practice. Despite its simplicity of implementation, the S-ANT_1 was shown to provide useful information. In fact, the probability that a patient who has S-ANT_1 ≥15 does not have HE is at least 80% and further increases with the increase of S-ANT_1. In contrast, the probability that a patient has HE <grade 2 is at least 60% for S-ANT_1 <15 and even higher for values <10 so that HE should be accurately investigated in these individuals.

In addition, the S-ANT$_1$ provides prognostic information regarding the 1-year risk of bouts of HE ≥grade 2 and even death risk. Further, the course of S-ANT$_1$ over time is an easy and rapid tool to assess changes in mental performance, provided that the S-ANT$_1$ changes are higher than 20% of previous values.

A limitation of the study might be the enrollment of patients with prior alcohol consumption. Although alcohol misuse might impair cognition or cause brain atrophy *per se*, the enrollment of patients with alcoholic cirrhosis is representative of clinical practice and the alcoholic etiology of cirrhosis was not found to be a significant predictor of S-ANT$_1$ performance in our study. Another limit might be the enrollment of patients who had had previous bouts of HE because these subjects have increased risk of HE in their follow-up. However, exclusion of these patients would have greatly reduced the widespread practical applicability of the test. At any rate, inclusion of previous bouts of HE in the statistical prognostic model showed that the S-ANT$_1$ maintains an independent additional value with respect to a history of previous HE.

In conclusion, the present study provides evidence that the S-ANT$_1$ is an easy test that has all the required characteristics of simplicity, speed, no cost, and relationship with clinical events to be used routinely for rapid investigation of HE in patients with cirrhosis at the office and at the bedside.

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