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Hyperammonemia during treatment with valproate in critically ill patients

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ABSTRACT

Introduction: Hyperammonemia (HA) is a potential side-effect of valproate (VPA) treatment, which has been described during long-term administration. The aim of this study was to evaluate the incidence, the impact and the risk factors of HA in critically ill patients.

Methods: We reviewed the data of all adult patients treated in our mixed 35-bed Department of Intensive Care over a 12-year period (2004–2015) who: a) were treated with VPA for more than 72 h and b) had at least one measurement of ammonium and VPA levels during the ICU stay; patients with Child-Pugh C liver cirrhosis were excluded. HA was defined as ammonium levels above 60 μg/dl.

Results: Of a total of 2640 patients treated with VPA, 319 patients met the inclusion criteria (median age 64 years; male gender 55%); 78% of them were admitted for neurological reasons and ICU mortality was 30%. Median ammonium levels were 88 [63–118] μg/dl. HA was found in 245 (77%) patients. For those patients with HA, median time from start of VPA therapy to HA was 3 [2–5] days. In a multivariable analysis, high VPA serum levels, mechanical ventilation and sepsis were independently associated with HA during VPA therapy. In 98/243 (40%) of HA patients, VPA was interrupted; VPA interruption was more frequent in patients with ammonium levels $>$ 100 μg/dl than others ($p = 0.001$). HA was not an independent predictor of ICU mortality or poor neurological outcome.

Conclusions: In this study, HA was a common finding during treatment with VPA in acutely ill patients. VPA levels, sepsis and mechanical ventilation were risk factors for HA. Hyperammonemia did not influence patients' outcome.

1. Introduction

Hyperammonemia (HA) is defined as elevated blood ammonium level, which is typically encountered in patients with acute or decompensated chronic liver diseases but also in other populations of critically ill patients [1–3]. In a recent study evaluating non-hepatic HA showed a cumulative incidence of 60% in critically ill patients [2]. Potential causes of HA are various and, amongst all, include several drugs, such as valproate (VPA) [1]. VPA is known for its anticonvulsant properties since decades and is frequently used for the treatment of seizures, migraine and for several psychiatric pathologies [4–6]. In the ICU, VPA is administrated to treat seizures or to prevent acute symptomatic seizures.

The pathogenesis of VPA-induced HA has not been fully elucidated; VPA alters the urea cycle at different levels (i.e., reduced synthesis of N-acetylglutamate, a prime metabolite activator of the urea cycle; decreased production of mitochondrial acetyl-CoA, which causes decreased urea metabolism; carnitine deficiency, which leads to beta-oxidation impairment followed by urea cycle inhibition), resulting into ammonium conversion from protein catabolism to urea [7]. HA without hepatic dysfunction may appear after a few days up to several months or years after initiation of VPA, even when normal doses or serum VPA concentrations within normal ranges are observed [6]. VPA-induced HA is asymptomatic in 51% of cases, although in some

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cases VPA can induce hyperammonemic encephalopathy (VHE), which can manifest with asterixis, alteration of consciousness, and refractory seizures [5].

Two studies [8,9] have demonstrated a correlation between VPA dose or VPA blood concentration and blood ammonium level in epileptic patients on chronic VPA therapy. Use of concomitant AED (antiepileptic drug), in particular of phenytoin (PHT) [8–10], barbiturates and topiramate, and urea cycle disorders [5] were identified as independent risk-factors for HA. Nevertheless, there were controversial findings on the influence of gender on HA (i.e., one study showed that men were at higher risk of HA while the other showed opposite data). Moreover, critically ill patients might be at higher risk of HA because they often received multiple concomitant therapies or present with severe impairment of organ function, which can lead to metabolic interferences and can impair the ammonium clearance by the kidneys and the muscles.

To our best knowledge, no studies have been carried out yet in critically ill patients to evaluate the incidence and risk factors of VPA-induced HA. Thus, the aims of this study were: (a) to evaluate the incidence of HA in critically ill patients treated with VPA; (b) to identify predictors of HA in this patients' population; (c) to assess the impact of HA on patients' outcomes.

2. Methods

2.1. Study population

This retrospective study was performed in the Department of Intensive Care at Erasme Hospital, Brussels (Belgium). The local Ethical Committee (Comité d'Ethique Hospitalo-Facultaire Erasme-ULB) approved the study, but waived the need for informed consent because of its retrospective nature (Protocol P2017/200). All patients admitted in ICU that underwent VPA therapy for more than 72 h, with at least one contemporary serum ammonium and VPA dosage were included in an institutional database (January 2007-December 2015) and considered eligible for the study. Assessment of ammonium levels in patients treated with VPA were decided by the attending physician in case of unexplained alteration of consciousness or prolonged therapy. Exclusion criteria were: (a) VPA therapy during ICU stay lower than 72 h; (b) Child-Pugh C liver cirrhosis.

2.2. Data collection

We collected data on demographics, ICU admission pathologies, comorbid diseases, ICU complications (i.e., acute heart failure, sepsis, syndrome of inappropriate secretion of antidiuretic hormone – SIADH, shock of any origin), intensive care unit (ICU) and hospital length of stay as well as ICU mortality. The development of infections was recorded over the ICU stay. Shock was defined as the need for vasopressor agents for more than 6 h. Glasgow Coma Scale (GCS) was computed on ICU admission and recorded daily over the ICU stay. Treatments with mechanical ventilation, continuous renal replacement therapy (CRRT), other antiepileptic drugs and vasopressors or inotropic agents were also recorded. Other concomitant therapies with potential hepatic toxicity (i.e., β-lactams, FK506, corticosteroids, amiodarone, statins, diuretics and paracetamol) were also collected. Neurological evaluation at hospital discharge was assessed using the Glasgow outcome scale (GOS) (GOS: 1 = severe injury or death without recovery of consciousness; 2 = persistent vegetative state: severe damage with prolonged state of unresponsiveness and a lack of higher mental functions; 3 = severe disability: severe injury with permanent need for help with daily living; 4 = moderate disability: no need for assistance in everyday life, employment is possible but may require special equipment; 5 = low disability: light damage with minor neurological and psychological deficits).

The reasons for VPA initiation as well as previous VPA use were collected. The number of ammonium measurements during VPA therapy, the maximum serum level of ammonium and corresponding value of serum VPA level were recorded in all patients. For the patients with HA, changes in GCS (i.e., GCS on the day of HA vs. GCS on initiation of VPA), HA treatment (i.e., oral L-carnitine, oral lactulose and/or interruption of VPA therapy) and its efficacy, were collected.

For those patients with concomitant electroencephalography (EEG) (21 EEG electrodes according to the international 10–20 system; Software: BrainRT, OSG Inc., Rumst, Belgium), either intermittent or continuous, at the moment of ammonium measurements, EEG tracing was revised by an expert neurophysiologist (LF), who was blinded to ammonium levels and the following findings were specifically reported: (a) presence of moderate (i.e., no occipital dominant rhythm, slow background, presence of variability) and/or severe (i.e., slow background, no occipital dominant rhythm, no variability) encephalopathy; (b) presence of generalized periodic discharges with triphasic morphology (triphasic waves); (c) presence of seizures.

2.3. VPA administration and definitions

The local treatment protocol recommended 25 mg/kg of VPA loading dose over 30 min, followed by a continuous infusion of 1–3 mg/kg*h over 24 h; intravenous treatment was maintained for at least 3 days and therefore shifted to oral/enteral administration (i.e., primary seizure prophylaxis) or maintained intravenously (i.e., status epilepticus or primary seizure prophylaxis or gastroparesis/gastric aspiration). Target VPA levels were 50–80 mg/l in most of patients and 90–120 mg/l in case of status epilepticus.

Based on values of local laboratory, we defined HA as ammonium level above 60 μg/dl. We arbitrarily classified HA as Grade-1 (ammonium levels 61–99 μg/dl), Grade-2 (100–150 μg/dl), Grade-3 (151–199 μg/dl), and Grade-4 ($\geq$ 200 μg/dl); patients without HA were classified as Grade-0. In patients with HA, the highest ammonium value was considered for further analysis. To assess liver injury, we collected aspartate (AST) and alanine (ALT) transaminases and total bilirubin (normal values $\leq$ 1.2 mg/dl) for all patients; in those with HA, biological data were collected on the same day of maximum ammonium level while in those without HA they were collected at the moment of the first ammonium measurement. VHE was defined as deterioration of GCS of at least 2 points, which was considered as secondary to HA according to the treating physician. Favorable neurological outcome (FO) was considered as a GOS 4–5; unfavorable outcome (UO) as a GOS 1–3. Mortality was considered at ICU discharge.

2.4. Study outcomes

The primary outcome of the study was the incidence of HA in the study cohort. Secondary outcomes included: (a) to identify predictors of HA in this patients' population; (c) to evaluate EEG abnormalities associated with HA; (d) to assess the impact of HA on patients' outcomes.

2.5. Statistical analysis

Discrete variables were expressed as counts (percentage) and continuous variables as means ± standard deviation (SD) or median (25th to 75th percentiles). The Kolmogorov-Smirnov test was used, and histograms and normal-quantile plots were examined to verify the normality of distribution of continuous variables. Demographics and clinical differences between groups (no HA vs. HA; FO vs. PO; ICU survivors vs. non-survivors) were assessed using the chi-square test, Fisher's exact test, Student's *t*-test, or Mann–Whitney *U*-test, as appropriate.

Three multivariable logistic regression analysis with HA, ICU mortality or UO, respectively, as the dependent variables was performed in all patients; co-linearity between variables was excluded prior to

modeling; only variables associated with a higher risk of HA, death or UO ($p < 0.2$) on a univariate basis were introduced in the multivariate models. The different grades of HA were also included in the multivariable model, and adjusted risk ratios (RRs) with their 95% CIs were computed, using patients without HA as reference. The discriminative ability of VPA to predict HA was evaluated using receiver operating characteristic (ROC) curves with the corresponding area under the curve (AUROC). Odds ratios (OR) with 95% confidence intervals (CI) were computed. A p value < 0.05 was considered as statistically significant. Data were analyzed using IBM® SPSS® Statistics software, version 22.0, and GraphPad PRISM version 5.0 (San Diego, CA, USA).

3. Results

3.1. Study population characteristics

Over a total of 2640 patients treated with VPA therapy, 2321 were excluded (i.e., VPA therapy less than 72 h, n = 1435; <18 years of age, n = 74; Child-Pugh C liver cirrhosis, n = 50; NH4 levels not measured, n = 762) and 319 (median age 64 years; male gender 55%) patients were analyzed. Among them, 78% were admitted for neurological reasons and 24% were already on VPA treatment before ICU admission. Also, 222 (70%) patients were treated by VPA for the treatment of seizures, while the others received VPA either as seizures prophylaxis or unexplained coma. A total of 195 (61%) patients also received another antiepileptic drug. The mean length of stay in the ICU was 8 [5–7,11, 12–15,8,9] days. An UO was observed in 161 (51%) patients and ICU mortality in 96 (30%) patients. The characteristics of the study population are reported in Table 1.

3.2. Hyperammonemia and valproate

Median maximum blood ammonium levels were 88 [63–118] μg/dl; 245 (77%) patients had HA, including 129 (52%) with Grade-1, 75 (31%) with Grade-2, 23 (9%) with Grade-3 and 18 (7%) with Grade-4 (Fig. 1). The median blood ammonium level among patients with HA was 97 [83–125] μg/dl; 79 (32%) patients were considered to have a "symptomatic" HA, while 109 were asymptomatic (47%) (i.e., 57 patients were not evaluable because of concomitant sedation). The proportion of symptomatic HA progressively increased with high maximum ammonium levels (i.e., 33/97, 34% with Grade-1; 27/59, 46% with Grade-2; 11/18, 61% with Grade-3 and 8/14, 57% with Grade-4; p = 0.07). For patients with HA, the median time from VPA initiation to the HA was 3 [2–5] days. Overall, median maximum blood VPA concentrations were 79 [63–97] mg/l, and they were significantly higher in HA patients than others (79 [63–97] mg/L vs. 61 [44–80] mg/l, $p < 0.001$). Maximum blood VPA levels significantly correlated with ammonium concentrations ($r = 0.30$, $p < 0.001$ – Fig. 2).

The VPA therapy was suspended in 98 (40%) of HA patients; VPA interruption was more frequent in patients with ammonium levels > 100 μg/dl than others (72/115, 63% vs. 26/130, 20%; p = 0.001). Hyperammonemia was treated by oral L-carnitine in 58 (24%) of cases. The normalization of ammonium level was obtained in 25% of cases, although this information was available only for 118/245 (48%) of HA patients.

3.3. Predictors of hyperammonemia

Patients with HA had lower level of serum creatinine, ALT and gamma-GT when compared with others. Patients with HA were more frequently treated with mechanical ventilation than others (Table 1). Patients with HA had also significantly longer length of ICU stay, higher mortality and UO rates than patients without HA (Table 1). In the multivariable analysis (Table 2), high blood VPA level, mechanical ventilation and low serum creatinine were independently associated with HA during VPA therapy. Maximum VPA levels had an AUROC to

Table 1
Characteristics of the study population, according to occurrence of hyperammonemia (HA). Data are presented as count (%) or median (25th-75th percentiles).

	All Patients (n = 319)	Non - HA (n = 74)	HA (n = 245)	p Value
AGE, years	64 [51–75]	64 [49–75]	64 [52–75]	0.702
MALE GENDER, n (%)	175 (55)	45 (61)	130 (53)	0.286
NEUROLOGICAL ADMISSION, n (%)	250 (78)	55 (74)	195 (80)	0.337
PREVIOUS THERAPY WITH VPA, n (%)	76 (24)	16 (22)	60 (25)	0.756
COMORBID DISEASES				
ARTERIAL HYPERTENSION, n (%)	178 (56)	39 (53)	139 (57)	0.724
CEREBROVASCULAR DISEASE, n (%)	47 (15)	11 (15)	36 (15)	1.000
BRAIN TUMOR, n (%)	33 (10)	11 (15)	22 (9)	0.189
HEART DISEASE, n (%)	80 (25)	18 (24)	62 (25)	1.000
ARRHYTMIAS, n (%)	68 (21)	16 (22)	52 (21)	1.000
COPD/ASTHMA, n (%)	39 (12)	9 (12)	30 (12)	1.000
DIABETES MELLITUS, n (%)	52 (16)	15 (20)	37 (15)	0.287
CHRONIC KIDNEY DISEASE, n (%)	39 (12)	14 (19)	25 (10)	0.066
HEMODIALYSIS, n (%)	24 (8)	10 (14)	14 (6)	0.041
SOLID ORGAN TRANSPLANTATION, n (%)	15 (5)	5 (7)	10 (4)	0.352
LIVER CIRRHOSIS, n (%)	16 (5)	4 (5)	12 (5)	0.770
REASON FOR VPA TREATEMENT				0.513
PROPHYLAXIS AFTER NEUROSURGERY, n (%)	11 (3)	2 (3)	9 (4)	
SEIZURES, n (%)	222 (70)	49 (66)	173 (71)	
PROPHYLAXIS AFTER TBI/SAH, n (%)	77 (24)	22 (30)	55 (22)	
UNEXPLAINED COMA, n (%)	9 (3)	1 (1)	8 (3)	
AMMONIUM AND VPA				
DURATION OF VPA THERAPY, days	3 [2–5]	5 [3–13]	3 [2–5]	0.115
MAX BLOOD VPA LEVEL, mg/L	76 [57–93]	61 [44–80]	79 [63–97]	< 0.001
MAX BLOOD NH4 LEVEL, μg/dL	88 [63–118]	45 [36–53]	97 [83–125]	< 0.001
SYMPTOMS RELATED TO HA				
ABSENT, n (%)	109 (34)	–	109 (44)	
PRESENT, n (%)	79 (25)	–	79 (32)	
NOT EVALUABLE, n (%)	57 (18)	–	57 (24)	
CONCOMITANT THERAPIES				
VASOPRESSORS, n (%)	204 (64)	42 (57)	162 (66)	0.167
OTHER AED, n (%)	195 (61)	33 (45)	162 (66)	< 0.001
PHENYTOIN, n (%)	112 (35)	19 (26)	93 (38)	0.053
BARBITURATES, n (%)	11 (3)	1 (1)	10 (4)	0.468
LEVETIRACETAM, n (%)	127 (40)	17 (23)	110 (45)	0.010
TOPIRAMATE, n (%)	7 (2)	1 (1)	6 (2)	1.000
BETA-LACTAM ANTIBIOTICS, n (%)	222 (70)	47 (64)	175 (71)	0.198
TACROLIMUS, n (%)	10 (3)	3 (4)	7 (3)	0.703
CORTICOSTEROIDS, n (%)	101 (32)	17 (23)	84 (34)	0.087
STATINS, n (%)	34 (11)	6 (8)	28 (11)	0.522
DIURETICS, n (%)	102 (32)	19 (26)	83 (34)	0.203
AMIODARONE, n (%)	48 (15)	8 (11)	40 (16)	0.272
PARACETAMOL, n (%)	262 (82)	54 (73)	208 (85)	0.024
MECHANICAL VENTILATION, n (%)	215 (67)	34 (46)	181 (74)	< 0.001
PARENTERAL NUTRITION, n (%)	3 (1)	2 (3)	1 (0)	0.136
ENTERAL NUTRITION, n (%)	264 (88)	62 (84)	216 (88)	0.112
LABORATORY DATA ON THE DAY OF AMMONIUM ASSESSMENT				
CREATININE, mg/dL	0.7 [0.6–1.0]	0.8 [0.6–1.5]	0.7 [0.5–1.0]	0.027
C-REACTIVE PROTEIN, mg/dL	9 [4–16]	12 [6–18]	9 [4–16]	0.018

(continued on next page)

Table 1 (*continued*)

	All Patients (n = 319)	Non - HA (n = 74)	HA (n = 245)	p Value
TOTAL BILIRUBIN, mg/dL	0.5 [0.3–0.7]	0.5 [0.3–0.7]	0.5 [0.3–0.7]	0.904
ɣGT, IU/L	32 [19–79]	57 [26–110]	28 [18–64]	< 0.001
ALT, IU/L	22 [15–38]	27 [19–51]	21 [14–35]	0.005
AST, IU/L	31 [18–58]	38 [20–60]	29 [18–57]	0.231
TOTAL PROTEINS, g/dL	5.9 [5.3–6.4]	6.0 [5.5–6.4]	5.9 [5.2–6.4]	0.118
LACTATE, mmol/L	1.4 [1.0–2.0]	1.4 [0.9–2.1]	1.4 [1.1–2.0]	0.154
UREA, mg/dL	32 [20–55]	40 [22–80]	32 [20–52]	0.08
THERAPY OF HYPERAMMONEMIA				
VPA DISCONTINUATION, n (%)	98 (34)	–	98 (40)	
LACTULOSE, n (%)	30 (11)	–	30 (12)	
CARNITINE, n (%)	58 (21)	–	58 (24)	
LACTULOSE + CARNITINE, n (%)	3 (1)	–	3 (1)	
NORMALIZATION HA, days	5 [3–7]	–	5 [3–7]	
EFFICACY OF THERAPY, n (%)	61 (19)	–	61 (25)	
COMPLICATIONS				
SIADH, n (%)	40 (13)	10 (14)	30 (12)	0.841
HEART FAILURE, n (%)	42 (13)	8 (11)	34 (14)	0.562
SEPSIS, n (%)	80 (25)	13 (18)	67 (27)	0.095
SHOCK, n (%)	95 (30)	24 (32)	71 (29)	0.565
OUTCOME				
ICU LOS, days	8 [5–14]	6 [5–10]	9 [6–15]	< 0.001
ICU MORTALITY, n (%)	96 [30]	14 [19]	82 [34]	0.020
GOS AT HOSPITAL DISCHARGE	3 [1–5]	4 [2–5]	3 [1–4]	0.027
UNFAVORABLE NEUROLOGICAL OUTCOME, n (%)	161 (51)	29 (39)	132 (54)	0.034

VPA = Valproic Acid, COPD = chronic obstructive pulmonary disease, HA = hyperammonemia, TBI = trauma brain injury, HSA = subarachnoid hemorrhage, AED = antiepileptic drugs; ɣGT = γ-glutamyltransferase; ALT = alanine transaminases; AST = aspartate transaminases; SIADH = syndrome of inappropriate antidiuretic hormone secretion; EEG = electroencephalography; ICU = intensive care unit; GOS = Glasgow outcome score; LOS = Length of stay.

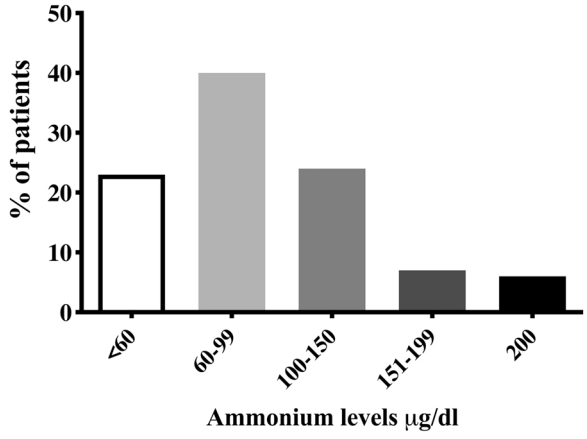

Fig. 1. Proportion of patients with different levels of ammonium.

predict HA of 0.68 (95% CIs 0.62–0.75; p < 0.001); maximum VPA levels > 79 mg/l had 54% (95% CIs: 47–60%) sensitivity and 74% (95% CIs: 63–83%) specificity to predict HA.

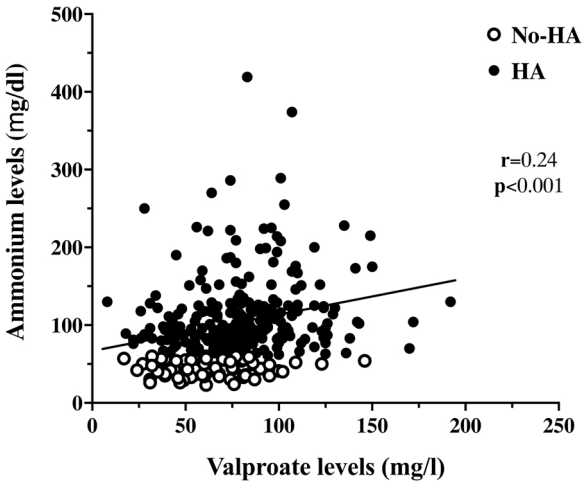

Fig. 2. Correlation between valproate and ammonium levels (HA = hyperammonemia).

Table 2
Multivariable analysis to identify independent predictors of hyperammonemia during therapy with valproate (VPA).

Variable	p value	OR	95% CI for OR	
			Lower	Upper
Max VPA level (mg/L)	0.001	1.021	1.008	1.034
Mechanical Ventilation	0.023	1.277	1.151	1.324
Creatinine, mg/dL	0.003	0.724	0.586	0.895

3.4. Association of hyperammonemia with EEG abnormalities

In the EEG analysis (n = 264, 83% of the study cohort), patients with HA more often had moderate or severe encephalopathy (MSE) than the others, although the difference was not statistically significant (158/216, 73% vs. 29/48, 60%; p = 0.11). The occurrence of MSE progressively increased on EEG progressively increased from Grade-0 to Grade-4 HA (from 60% to 83%; p = 0.057) (Fig. 2). The occurrence of triphasic waves (21/216 vs. 3/48, p = 0.778) or seizures (26/216 vs. 4/48, p = 0.619) on EEG was similar among patients with HA when compared to others and across different grades of HA. Fig. 3.

3.5. Neurological outcome

Differences between patients with FO and UO are reported in Supplemental Table 1. The highest blood ammonium level was greater in

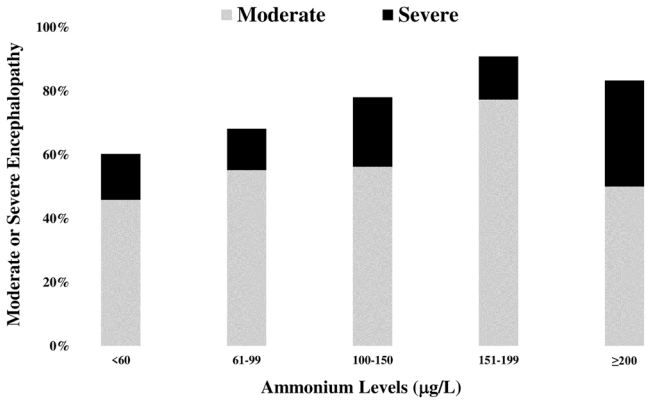

Fig. 3. Proportion of patients with moderate or severe encephalopathy (MSE) on EEG, according to ammonium levels.

patients with UO than in others (97 68−124] vs. 83 [57–105] μg/dl; p = 0.001); however, no differences were found between the maximum serum level of VPA of the two groups (78 [57–94] vs. 75 [57–91] mg/l; p = 0.42). In the multivariable analysis, older age, concomitant use of other AED, high lactate blood levels and the use of mechanical ventilation, but not HA, were independently associated with UO (Table 3). Moreover, after adjustment for these factors, the severity of HA was not associated with neurological outcome (Supplemental Table 2).

3.6. ICU mortality

Differences between survivors and non-survivors are reported in Supplemental Table 1. Non-survivors more frequently developed HA than survivors (85% and 73%; p = 0.020). Moreover, we observed differences between the highest blood ammonium levels (97 [71–119] vs. 84 [58–116] μg/dl; p = 0.03) and the maximum VPA levels (84 [65–98] vs. 74 [53–90]mg/L; p = 0.001) between the two groups. In the multivariable analysis, male gender, concomitant use of other AED, the use of mechanical ventilation, chronic heart disease and high lactate blood levels, but not HA, were independently associated with ICU mortality (Table 3). Moreover, after adjustment for these factors, the severity of HA was not associated with mortality (Supplemental Table 3).

4. Discussion

In the present study, we showed that, in a selected population of critically ill patients in whom ammonium levels were monitored during VPA therapy, HA was observed in 77% of patients; in 17% of these patients, ammonium levels exceeded 150 μg/dl. High VPA levels, the use of mechanical ventilation and low serum creatinine were independently predictors of HA during VPA therapy. However, HA was not independently associated with poor outcome in these patients.

Data concerning the occurrence of HA in critically ill patients are controversial. In a review of the literature, Chicharro et al. [10] reported that the incidence of HA in non-critically ill patients treated with VPA ranged from 16% to 100% (median value of 47%), after the analysis of 14 cross-sectional studies, and from 71% to 100% (median of 90%) in 3 prospective studies. More recently, two studies evaluating VPA in epileptic patients showed a prevalence of HA of 43% [8] and 28% [9], respectively. These differences may be related to the different definitions of cut-off values for HA [9], different methods used to measure ammonium levels and blood sampling conditions [10]. In our cohort, the incidence of HA was higher than the previously reported in the most of the studies, probably because critically ill patients have more frequently had an altered protein metabolism, liver disease, and total parenteral nutrition than non-critically ill patients [1], which are all risk-factors for hyperammonemia.

Table 3
Multivariable analysis with unfavorable outcome (UO) or ICU mortality as the dependent variables performed in all patients.

Unfavorable neurological outcome			
Variable	Odds ratio	95% Confidence Interval	P value
Age, years	1.018	1.001–1.035	**0.038**
Mechanical ventilation	9.428	4.837–18.376	**< 0.001**
Other AED	2.769	1.564–4.904	**< 0.001**
Lactate, mmol/L	1.504	1.096–2.063	**0.011**
ICU mortality			
Variable	Odds ratio	95% Confidence Interval	P value
Male gender	0.548	0.316–0.952	**0.033**
Mechanical ventilation	8.524	3.487–20.837	**< 0.001**
Other AED	2.875	1.524–5.423	**0.001**
Heart disease	1.979	1.081–3.625	**0.027**
Lactate, mmol/L	1.368	1.022–1.830	**0.035**

ICU = intensive care unit; AED = antiepileptic drug

We found that high plasma levels of VPA were independently associated to HA in our cohort of critically ill patients. In the literature, data concerning the correlation between dose or plasma levels of VPA and HA are controversial. Although emerging evidence showed that the increase in ammonium concentration induced by VPA is dose/plasma levels dependent [8,9,16,17], some authors argued that neither the VPA dose or its concentrations was related with the development of HA [10]. In particular, total VPA levels do not directly correlate with free levels, which would better reflect the amount of drug active at the receptor site [18]; VPA free fraction remains around 10% (i.e., VPA is highly bound to proteins) for total VPA concentrations between 20 and 60 mg/l, with subsequent linear increases for higher concentration [19]. Our finding underlines the need for daily monitoring of VPA total levels not only to optimize its anti-epileptic properties but also to identify some patients at risk to develop adverse events, such as HA, although the assessment of free VPA levels might be more relevant and the consequences of HA on patients' outcome remain limited. In addition, we found that the use of mechanical ventilation and low creatinine values were independently associated with HA. Probably, the use of mechanical ventilation appears as a factor related to the severity of the disease of the patient, rather than the direct cause to the development of HA. Thus, the most severe patients, as those receiving aggressive and invasive therapies, are also at risk to develop metabolic disturbances or adverse events due to different drugs, such as VPA therapy during critically illness.

The association between high ammonium levels and low creatinine levels remains more difficult to explain. When ammonium increases, accumulation of urea in the blood, as observed in patients with renal dysfunction and elevated creatinine levels, may be expected. Nevertheless, in patients with chronic renal dysfunction (n = 58), the concentrations of plasma ammonium were within the normal reference range and there was no correlation between ammonium and the severity of renal dysfunction or the need for dialysis [20]. We may hypothesize that in patients with low creatinine levels, which are in general those with elevated renal filtration [21], the renal uptake of some amino-acids (i.e., phenylalanine, citrulline or glutamine) may increase [21], thus resulting in increased ammonium production (i.e., augmented metabolites for the urea cycle) or reduced elimination. However, it is also important to acknowledge that collecting blood samples only once over the entire ICU stay would not be representative of the renal function of critically ill patients. As such, future prospective studies on this topic should more accurately evaluate renal function over time and better characterize any potential relationship with the occurrence of HA in this setting.

Finally, we did not find an association between HA and poor outcome. This could be explained by the definition of HA, which included also "mild" increase in ammonium levels, which did not affect the clinical status of the patients (44% of patients were asymptomatic). However, an additional analysis evaluating different ammonium ranges provided the same results. In addition, if HA is secondary to elevated VPA levels, it could be sufficient to withdraw therapy or add some effective therapies (i.e., oral carnitine) to quickly normalize ammonium levels in a very short period so that this complication is highly reversible and it is unlikely that hyperammonemia could impact on patients' outcome. Few studies have reported that some patients with advanced HA developed encephalopathy and seizures [22–24]. EEG monitoring can help to identify eventually seizures or disturbances associated with ammoniac encephalopathy. In our study we were not able to find correlation between HA and specific EEG altered patterns, but we found out that for increasing ammonium levels there was a growing percentage of MSE and that the high ammonium levels are risk factors associated with the development of severe encephalopathy.

Our study presents several limitations. First, we only collected biological data on the day of ammonium assessment, while liver and renal function may show several fluctuations over time and could not be accurately evaluated with a single measurement. Therefore, the data collected are not fully representative of liver and kidney function, but

since the data were collected on the day of HA, they are representative of the possible metabolic interference on ammonium cycle. Moreover, ammonium levels are also related to protein intake; however, it was impossible to exactly quantify the total amount of enteral or parenteral nutrition given to our patients, as well as calculate the total protein intake. Second, we analyzed a population of critically ill patients, who had a blood assessment of ammonium. Thus, as in patients who were asymptomatic ammonium levels were probably neglected by the attending physician (i.e., 28% of eligible patients never had ammonium measurements), the occurrence of HA during VPA therapy may be largely overestimated. Third, due to the design of the study, we could not conclude to a causal relationship between the occurrence of HA and VPA concentrations, which may just reflect the exposure to the drug (i.e., prolonged therapy) rather the intensity of therapy. Fourth, we did not routinely perform EEG examinations in all these patients, so that additional findings on EEG abnormalities which are induced by HA could not be evaluated and EEG findings could not be integrated into the multivariable model. Fifth, the study cohort included patients until 2015; the progressive introduction of other antiepileptic drugs, such as levetiracetam, has significantly decreased the use of VPA in our ICU and adding more patients would not have substantially changed the overall results of the study. Sixth, the NH4 grades were decided arbitrarily, in the absence of established criteria to categorize NH4 levels into different ranges of severity. Seventh, in the absence of a control group of non-ICU patients, it remains difficult to assess whether the occurrence of HA would be more frequent and occur earlier in critically ill patients when compared to less severe ones. Finally, we could not adequately assess clinical evolution of patients according to the decision to suspend VPA therapy and/or the introduction of some therapies aiming to reduce ammonium levels. Future prospective studies should consider repeated clinical examination of these patients in order to better understand the clinical impact of HA in this setting.

5. Conclusions

The present study showed that HA is a common finding at ICU and high blood level of VPA is a risk factor of HA. Given the importance and the frequency of the problem there is a need to pursue this line of research in larger clinical prospective study, perhaps further exploring the problem of pharmacologic interactions to which the critical patient is largely exposed. Results from these studies would undoubtedly constitute a major contribution to the clinical approach to the management of ICU patients.

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CRediT authorship contribution statement

Conceptualization, C.D.F., M.G. and F.S.T.; methodology, M.G., F.F, L.F, C.O.; formal analysis, L.P; F.S.T.; data curation, S.S, A.B, B.L.; writing—original draft preparation, C.D.F, M.G, F.S.T.; writing—review and editing, N.G, E.A, L.K, I.R, J.C. B.L; visualization, M.G;. All authors have read and agreed to the published version of the manuscript.

Data Availability

Due to ethical reasons raw data can be available from the corresponding author upon reasonable request. All generated and analyzed data are available in the main article and in its supplementary electronic files.

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Not applicable.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Erasme Hospital (Protocol P2017/200).

Informed Consent Statement

Patient consent was waived due to the retrospective and observational nature of the study.

Conflicts of Interest

The authors declare no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.clineuro.2021.107092.

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