Low baseline plasma L-Glutamine concentration identifies hepatocellular carcinoma patients at high risk of developing early gastrointestinal adverse events during sorafenib treatment.

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BACKGROUND & AIM: Gastrointestinal adverse events (GIAEs) are common in patients with advanced hepatocellular carcinoma (HCC) treated with sorafenib. Diarrhea is a prevalent event responsible for treatment interruptions and dosage modifications. The mechanisms and impact of diarrhea in sorafenib treated patients is not well understood. Absorption of nutrients depends on the integrity of the intestinal barrier. When this is compromised, the gut permeability increases, and this may prime development of gastrointestinal complications. We evaluate the role of baseline blood L-Glutamine (L-Gln) levels in the prediction of GIAE development early during treatment (eGIAE).

PATIENTS & METHODS: Blood L-Gln was measured in 135 patients with advanced HCC prior to starting sorafenib. L-Gln was determined by quantification of glutamate generated by the hydrolysis of glutamine. Any adverse event developed during therapy was registered in a prospective database. We used Mann-Whitney U and Fisher's exact test to compare quantitative or categorical variables respectively, Kaplan-Meier method to analyse time to event variables, log-rank test for the survival functions and Cox regression models to estimate hazard ratios (HR).

RESULTS:

- One-hundred seventeen of the patients were male (86.7%) and the vast majority (87.4%) exhibited a good performance status (PS 0). Almost half corresponded to BCLC B (49%) following the treatment stage migration concept and 51% to BCLC C stage. Table 1 summarizes the baseline characteristics and clinical variables of the 135 patients.

Table 1: Baseline characteristics and clinical variables of patients

PATIENTS	n=135
Age (years), median [IQR]	62.48 [56.33 to 70.03]
Gender (male), n (%)	117 (86.67)
Cirrhosis (Yes), n(%)	131 (97)
Etiology, n (%)	
HCV	73 (54.07)
Alcohol	37 (27.42)
HBV	10 (7.41)
NASH	3 (2.22)
Co-infection HCV + HBV	2 (1.48)
Criptogenic	5 (3.7)
Others	5 (3.7)
Total bilirubin (mg/dL), median [IQR]	1 [0.8 to 1.6]
ALT (IU/L), median [IQR]	54 [31 to 95]
AST (UI/L), median [IQR]	65.5 [39 to 99]
Alkaline phosphatase (IU/L), median [IQR]	252 [159 - 385]
GGT (IU/L), median [IQR]	139 [82 to 289]
Albumin (g/L), median [IQR]	39 [35 to 42]
Hemoglobin (g/dL), median [IQR]	13.5 [12 - 14.5]
Prothrombin time (%), median [IQR]	81 [68 to 89]
Hematocrit (%), median [IQR]	41 [37 - 44]
Platelets (count 10^9), median [IQR]	135 [90 - 186]
Leukocytes (count 10^9), median [IQR]	53 [40.8 - 66]
L-Glutamine (mgr/mL), median [IQR] / tertiles (Low; Middle; High)	19.23 [16.21 to 22.17] / (<17.26 ; (17.32 – 21.42] ; >21.42)
Child-Pugh Score (Non-Cirrhotic** or A / B), n (%)	117 (86.67) / 18 (13.33)
Ascites (No), n (%)	111 (82.22)
Encephalopathy (No), n (%)	135 (100)
Vascular Invasion (Yes), n (%)	41 (30.37)
Extra-hepatic spread (Yes), n (%)	36 (26.67)
ECOG-PS (0 / 1), n (%)	118 (87.41) / 17 (12.59)
Alpha-fetoprotein (ng/dL), median [IQR]	24 [6 to 207]
BCLC stage (A* or B / C), n (%)	66 (48.89) / 69 (51.11)
Previous treatment, n (%)	
No treatment	42 (31.11)
Liver transplant	3 (2.22)
Ablation	20 (14.81)
Surgery	14 (10.37)
Sequential	12 (8.89)
Chemoembolization	41 (30.37)
Tamoxifen	2 (1.48)
Y90	1 (0.74)
Diabetes (Yes), n (%)	46 (34.07)
Arterial Hypertension (Yes), n (%)	56 (41.48)

* 2 BCLC-A patients; ** 4 Non-cirrhotic patients. HCV: Hepatitis C virus; HBV: Hepatitis B virus; NASH: non-alcoholic steatohepatitis; BCLC: Barcelona Clinic Liver Cancer; AST: Aspartate aminotransferase; IU: International Units; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transferase; IQR: Interquartile range

- **Adverse Events:** Fifteen per cent of patients developed eGIAE, being diarrhea the most frequent one. Table 2 depicts the number and type of GIAE at 30 and 60 days of treatment.

- **L-Gln concentration and eGIAE incidence:** Cox regression models dividing patients according to the median concentration value identified a protective role of higher L-Gln concentration in developing eGIAE at 30 and 60 days (Figure 1a & 1b), with a HR 0.77 (95% CI 0.65-0.91, p = 0.0022 when adjusted for BCLC stage and PS (Table 3).

Table 3: Cox regression models

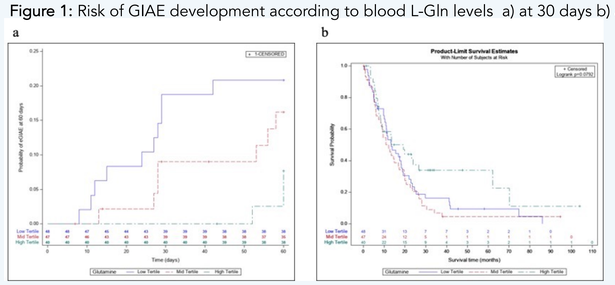
EVENT	Variable	adjusting baseline co-variables	HR (95%CI)	P-value
Gastrointestinal AE at 30 days	L-Glutamine (mgr/mL)	none	0.81 (0.7 - 0.93)	<0.01
		Child-Pugh Score	0.81 (0.7 - 0.93)	<0.01
		ECOG-PS	0.77 (0.66 - 0.91)	<0.01
		BCLC stage	0.81 (0.7 - 0.94)	<0.01
		BCLC stage + Child-Pugh Score	0.81 (0.7 - 0.93)	<0.01
		BCLC stage + ECOG-PS	0.77 (0.65 - 0.91)	<0.01
		ECOG-PS Child-Pugh Score	0.77 (0.66 - 0.91)	<0.01
		BCLC stage + Child-Pugh Score + ECOG-PS	0.77 (0.65 - 0.91)	<0.01
Gastrointestinal AE at 30 days	Low tertile vs Others	none	3.53 (1.18 - 10.53)	0.02
		Child-Pugh Score	3.56 (1.19 - 10.69)	0.02
		ECOG-PS	4.47 (1.45 - 13.78)	<0.01
		BCLC stage	3.33 (1.11 - 9.98)	0.03
		BCLC stage + Child-Pugh Score	3.43 (1.14 - 10.32)	0.03
		BCLC stage + ECOG-PS	4.98 (1.53 - 16.18)	<0.01
		ECOG-PS Child-Pugh Score	4.46 (1.45 - 13.75)	<0.01
		BCLC stage + Child-Pugh Score + ECOG-PS	5.03 (1.56 - 16.25)	<0.01
Gastrointestinal AE at 60 days	L-Glutamine (mgr/mL)	none	0.89 (0.8 - 0.99)	0.04
		Child-Pugh Score	0.89 (0.8 - 0.99)	0.03
		ECOG-PS	0.88 (0.79 - 0.99)	0.03
		BCLC stage	0.89 (0.8 - 0.99)	0.04
		BCLC stage + Child-Pugh Score	0.89 (0.8 - 0.99)	0.03
		BCLC stage + ECOG-PS	0.88 (0.78 - 0.98)	0.02
		ECOG-PS Child-Pugh Score	0.88 (0.79 - 0.98)	0.03
		BCLC stage + Child-Pugh Score + ECOG-PS	0.88 (0.79 - 0.98)	0.02
Death	High tertile vs Others	none	0.63 (0.4 - 0.97)	0.04
		Child-Pugh Score	0.63 (0.4 - 0.97)	0.04
		ECOG-PS	0.61 (0.39 - 0.96)	0.03
		BCLC stage	0.61 (0.39 - 0.94)	0.03
		BCLC stage + Child-Pugh Score	0.6 (0.39 - 0.94)	0.03
		BCLC stage + ECOG-PS	0.6 (0.38 - 0.93)	0.02
		ECOG-PS Child-Pugh Score	0.61 (0.39 - 0.96)	0.03
		BCLC stage + Child-Pugh Score + ECOG-PS	0.6 (0.38 - 0.93)	0.02

Table 2: Number and type of GIAE at 30 and 60 days

GIAE	30 days	60 days
Diarrhoea n (%)	7 (5.2%)	10 (7.4%)
Abdominal pain n (%)	5 (3.7%)	7 (5.2%)
Hemorrhoidal pain n (%)	2 (1.5%)	2 (1.5%)
Hemorrhoidal bleeding n (%)	-	1 (0.7%)
Candidiasis n (%)	1 (0.7%)	1 (0.7%)
Acute pancreatitis n (%)	1 (0.7%)	1 (0.7%)

GIAE: GastroIntestinal Adverse Events

Figure 1: Risk of GIAE development according to blood L-Gln levels a) at 30 days b) at 60 days

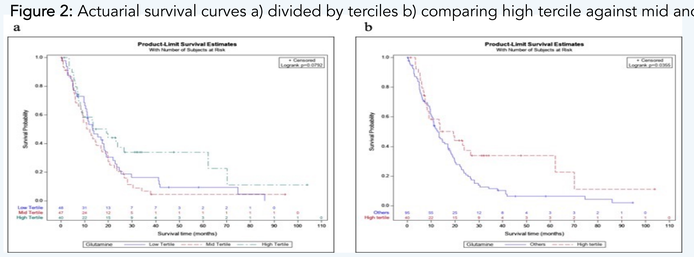


- **Risk of death:** Cox regression models identified an association with the risk of death when stratifying patients by terciles according to their L-Gln levels. Patients in the high tercile (n=40) were at a lower risk of death with a HR value of 0.59 (95%CI 0.38 – 0.93) when adjusted for BCLC staging and PS with a p value <0.01 (Table 4). Figure 2 shows the actuarial survival of the patients divided by terciles (Panel a) and the comparison between high vs mid and low terciles (Panel b). As shown, the median survival of patients with high L-Gln is significantly better than that of patients with low or median values (p-value<0.04). Table 4 displays the comparison by terciles either independently or when grouping strata.

Table 4: Death risk compared by terciles independently and grouped strata

	Patients at risk	Events	Median time (95% IC), months	p-value (Log-Rank)
All	135	111	13.31 (10.78 - 17.49)	
High tercile	40	26	19.36 (8.05 - 26.50)	0.04
Others	95	85	12.75 (9.96 - 17)	
Low tercile	48	43	13.31 (10.32 - 18.34))	0.7
Others	87	68	13.35 (9.01 - 19.63)	
High Tercile	40	26	19.36 (8.05 - 26.50)	0.08
Mid Tercile	47	42	12.23 (8.35 - 17.23)	
Low Tercile	48	43	13.31 (10.32 - 18.34)	

Figure 2: Actuarial survival curves a) divided by terciles b) comparing high tercile against mid and low groups.



CONCLUSION: Our study shows for the first time the association of baseline blood L-Gln levels with eGIAE development in HCC patients during sorafenib treatment. Low L-Gln concentrations might reflect a potentially compromised intestinal barrier that becomes clinically relevant early after treatment start. Diarrhea is the most frequent of such events and their control may prime dose adjustments or even treatment interruption that may prevent the expected survival benefits of sorafenib.