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LETTER TO THE EDITOR

The serum-ascites vitamin D gradient (SADG): A novel index in spontaneous bacterial peritonitis

KEYWORDS
Vitamin D;
Cirrhosis;
SBP;
Infection;
Hypovitaminosis D;
CRP

CRP	C-reactive protein
MELD	Model for End-stage Liver Disease
PMN	polymorphonuclear leukocytes
SADG	serum-ascites vitamin D gradient
SBP	spontaneous bacterial peritonitis
VDR	vitamin D receptor

Spontaneous bacterial peritonitis (SBP), which is the most common infective complication of liver cirrhosis, is mostly due to Gram-negative bacteria [1,2]. Its incidence ranges from 10% to 30% in hospitalized patients, with a mortality as high as 46% [1]. The cornerstone of the diagnosis of SBP is a polymorphonuclear leukocyte (PMN) count in ascitic fluid exceeding 250 cells/microL. However, it has been suggested that dosage of specific molecules in ascitic fluid, namely, C-reactive protein, lactoferrin and calprotectin, may be additional prognostic or diagnostic tools in patients with SBP [3,4].

Among its various biological activities, 25-hydroxyvitamin D (25-OH vitamin D) — the first catabolite of vitamin D — is involved in the immune response. Low levels of circulating 25-OH vitamin D are associated with infections in patients with cirrhosis [5]. Serum 25-OH vitamin D levels are generally lower in these patients than in controls [6] and even lower in patients with decompensated cirrhosis [7].

The aim of our study was to investigate the role of vitamin D levels in ascitic fluid in SBP patients.

Therefore, we prospectively enrolled consecutive patients with liver cirrhosis and ascites who underwent paracentesis from 1 December 2015 to 31 March 2017 at two Italian Universities (Department of Clinical Medicine and Surgery, Section of Infectious Diseases, AOU Federico II of Naples, Area of Clinical Medicine and Hepatology, University Hospital Campus Bio-medico of Rome). The only exclusion criterion was vitamin D supplementation in the previous 12 months. All patients gave consent for anonymized data collection for scientific purposes upon hospital admission. The demographic, clinical and laboratory characteristics of the patients were recorded. We also measured the leukocyte and PMN count, albumin levels, and pH. Spontaneous bacterial peritonitis was diagnosed according to current guidelines, namely in case of a polymorphonuclear leukocyte (PMN) count in ascitic fluid exceeding 250 cells/microL [4]. We also screened patients at enrollment for the presence of infections other than SBP.

Vitamin D deficiency was defined as a 25-OH vitamin D serum concentration below 20 ng/mL, insufficiency as 25-OH vitamin D concentrations between 21—29 ng/mL, and sufficiency as 25-OH vitamin D concentrations between 30—100 ng/mL, according to guidelines [8]. A blood sample was taken for serum 25-OH vitamin D measurement on the day the patient underwent paracentesis. The serum ascites 25-OH vitamin D gradient (SADG) was calculated with the formula:

25-OH vitamin D in serum − 25-OH vitamin D in ascites

As statistical test we used the Kolmogorov—Smirnov Test to test the normality of distribution for continuous variables. Continuous variables are reported as mean ± standard deviation in case of normal distribution and median and interquartile range when variables were non-normally distributed. Categorical variables are reported as frequency (percentage), and the Chi^2 test was used for comparisons. When comparing two groups, data were analyzed using the Student-*t* test and the Mann—Whitney U Test, in normally or non-normally distributed variables, respectively. As correlation tests, we used the Pearson and Spearman test for normally and non-normally distributed variables, respectively. All data obtained were analyzed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA).

In our study, we enrolled 54 patients in the study. The clinical and key laboratory characteristics of patients with

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Table 1 Serum and ascites parameters in patients with and without SBP.

	Total ($n=54$)	Patients with SBP ($n=16$)	Patients without SBP ($n=38$)	P
Age, years	65.8 ± 9.9	66.0 ± 6.6	65.7 ± 11.2	0.936
Male gender, *n* (%)	32 (59.3)	10 (62.5)	22 (57.8)	0.067
Etiology of liver cirrhosis, *n* (%)				0.192
HCV	32 (59.3)	12 (75.0)	20 (52.6)	
HBV	4 (7.4)	0 (0)	4 (10.5)	
Alcohol-related	7 (13.0)	0 (0)	7 (18.4)	
Autoimmune	2 (3.7)	1 (6.3)	1 (2.6)	
Metabolic	2 (3.7)	0 (0)	2 (5.3)	
Cryptogenetic	7 (13.0)	3 (18.8)	4 (10.5)	
Child—Pugh class				
B, *n* (%)	36 (66.7)	6(37.5)	30 (78.9)	0.002
C, *n* (%)	18 (33.3)	10 (62.5)	8 (21.1)	
MELD score[a]	15 (10; 22)	15 (12; 23)	14 (10; 19)	0.552
History of HCC, *n* (%)	11 (20.4)	4 (25)	7 (18.4)	0.263
Esophageal varices, *n* (%)	45 (83.7)	13 (81)	32 (84.2)	0.090
Portal vein thrombosis, *n* (%)	14 (25.9)	5 (31.3)	9 (23.6)	0.742
Serum albumin[a], g/dL	2.9 (2.6; 3.4)	3.0 (2.8; 3.5)	2.9 (2.6; 3.4)	0.834
ALT, U/L	41 ± 31	42 ± 36	41 ± 38	0.964
Total bilirubin, mg/dL[a]	2.1 (1.2; 3.1)	2.1 (1.2; 3.0)	2.1 (1.1; 3.3)	0.963
Pseudocholinesterase[a], U/L	2071 ± 894	1999 ± 757	2113 ± 977	0.699
Creatinine[a], mg/dL	1.3 (1.0; 1.6)	1.6 (1.1; 2.4)	1.2 (0.9; 1.6)	0.246
CRP[a], mg/dL	2.3 (1.0; 5.7)	3.5 (2.3; 6.0)	1.5 (0.6; 3.4)	0.039
Hemoglobin, g/dL	10.2 ± 1.7	10.5 ± 1.8	10.2 ± 1.7	0.560
Platelet, $10^3/\mu L$	110.4 ± 73.3	89.7 ± 45	119.4 ± 81	0.179
Quick time, s	53 ± 17	54 ± 16	52 ± 18	0.719
α-FP[a], ng/mL	6.7 (3.0; 16)	6.3 (1.4; 14)	6.8 (3.1; 59.2)	0.802
Procalcitonin[a], ng/mL	0.7 (0.3; 1.8)	1.0 (0.3; 2.0)	0.6 (0.3; 1.9)	0.881

Data are expressed as mean ± standard deviation or median (interquartile range). Categorical data are expressed as absolute number (percentage). SBP: spontaneous bacterial peritonitis; MELD: Model for End-stage Liver Disease; RP: C-reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; Afp: alpha-fetoprotein.

[a] Non-normally distributed variables. Difference between groups tested with test Mann—Whitney test instead of Student's *t*-test.

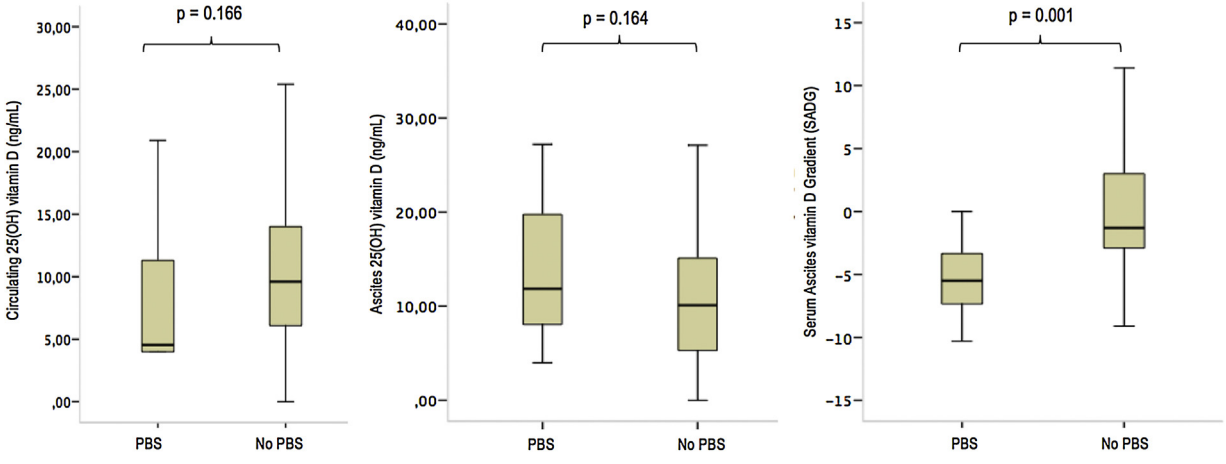

Figure 1 Serum and ascitic levels of 25(OH) vitamin D and serum ascites 25-OH vitamin D gradient (SADG) values in patients with or without SBP. Box-plots of vitamin D levels in serum (A) and ascites (B) and SADG (C) values in patients with or without SBP. SBP: spontaneous bacterial peritonitis.

or without SBP are reported in Table 1. SBP was diagnosed in 16/54 (29.6%) patients. Among these, only two patients (12.5%) had culture-positive ascitic fluid. Vitamin D deficiency was observed in most patients ($n=49/54$, 90.7%) regardless of SBP diagnosis. Notably, half of the sample ($n=28$; 51.8%) had severe vitamin D deficiency. The levels of vitamin D in serum or ascites did not differ between patients with or without SBP ($P=0.166$ and $P=0,164$, respec-

tively). In the whole sample, SADG values ranged between −4 and +12 ng/mL, with a median value of −2 ng/mL (IQR −5.0 to +1.0) (Fig. 1). The SADG was significantly lower in patients with SBP than in those without SBP [−5.5 ng/mL (IQR: −7.4; −3.3) vs. −1.4 ng/mL (IQR: −3; +3.1, $P = 0.001$) (Fig. 1). On the other hand, the SADG did not differ significantly between Child B and Child C patients (SADG = −1.07 and −2.89, respectively; P = ns). As expected, CRP levels were higher in patients with SBP than in those without SBP ($P = 0.039$). No significant correlation was found between CRP and SADG values ($P = 0.071$).

We compared the SADG in patients with an infection other than SBP ($n = 12$), patients with SBP ($n = 16$) and patients without infection ($n = 26$): median SADG values were −5.6, −1.5, and −1.0, respectively. A statistically significant difference was observed between patients with SBP and the other two groups ($P = 0.005$), whereas no difference was found between patients with an infection other than SBP and those without infection.

Discussion

Fifty-four patients were enrolled in this pilot study. As expected, [9] most patients had severe vitamin D deficiency (90.7%). Interestingly, although the vitamin D levels of both serum and ascites did not differ between patients with and without SBP; the SADG was significantly lower in patients with SBP than in those without SBP (vitamin D levels were slightly higher in the ascites of patients with SBP). This prompted the question: ''What is the biological plausibility of such a finding?'' In this context, it is noteworthy that vitamin D can modulate host immune defense through the vitamin D receptor (VDR) and the 25(OH) D3 1-α-hydroxylase (CYP27B1) enzyme, which are expressed in different types of immune cells (i.e., B and T cells, and antigen presenting cells) and are responsible for the synthesis of the bioactive form of vitamin D [1.25(OH) vitamin D] [10]. This active metabolite can increase the phagocytosis of microorganisms mediated by innate immune cells that occurs consequent to the differentiation of monocytes to mature macrophages with phagocytic activity [11]. Interestingly, in the setting of SBP, the expression of both VDR and its downstream effector molecule LL-37 (or Cap-18, Cathelicidin antimicrobial peptide, a vitamin D-dependent endogenous antimicrobial peptide) is higher in the peritoneal leukocytes of patients with SBP than in those without SBP [11]. The vitamin D-VDR interaction and the subsequent hyper-expression of LL-37 may be involved in immune response against SBP and higher levels of vitamin D in the ascites of patients with SBP can be considered indirect evidence of the upregulation of this antimicrobial pathway[11]. Taken together, the evidence available indicates that, in SBP, vitamin D acts as a chemotactic and anti-bacterial agent that exerts its effects locally in ascitic fluid. Therefore, our finding of a low SADG in patients with SBP may be explained as a ''compartmentalization'' of vitamin D from serum to ascites in order to increase local, peritoneal levels of vitamin D and therefore improve antimicrobial activity in patients with SBP.

A limitation of this study is the small number of patients enrolled. This could have affected the positive predictive value of our findings, given that we found only marginal differences in vitamin D concentrations in serum and ascitic fluid. Moreover, the clinical usefulness of our finding that the SADG was significantly lower in patients with SBP than in those without SBP remains to be established. Consequently, more highly powered studies are needed to determine if the SADG has any prognostic significance in SBP patients and to evaluate modifications of the SADG in response to vitamin D supplementation.

Authors' contributions

ARB designed the study, enrolled patients and was one of the major contributor in writing the manuscript. MA performed statistical analysis and wrote the manuscript. RS performed statistical analysis and revised the manuscript. BP enrolled patients and collected samples. EZ enrolled patients, completed the database and revised the manuscript. SS enrolled patients, completed the database and collected samples. GP performed the laboratoristic analysis of vitamin D. PF performed the laboratoristic analysis of vitamin D and revised the manuscript. AC revised the manuscript. GB revised the manuscript. UVG enrolled patients and revised the manuscript.

IG enrolled patients, wrote and revised the manuscript. All authors read and approved the final manuscript.

Disclosure of interest

The study was approved by the Ethical Committee of the University of Naples ''Federico II'' approved the study (protocol number: 128/12). The privacy rights of enrolled subjects have been observed and the study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki, seventh revision).

All patients signed informed consent and gave consent for data collection in the medical records they signed at hospital admission.

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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References

[1] Pericleous M, Sarnowski A, Moore A, Fijten R, Zaman M. The clinical management of abdominal ascites, spontaneous bacterial peritonitis and hepatorenal syndrome: a

review of current guidelines and recommendations. Eur J Gastroenterol Hepatol 2016;28:10—8, http://dx.doi.org/10.1097/MEG.0000000000000548.
[2] Gentile I, Buonomo AR, Scotto R, Zappulo E, Borgia G. Infections worsen prognosis of patients with cirrhosis irrespective of the liver disease stage. Eur J Intern Med 2017;46:e45—7, http://dx.doi.org/10.1016/j.ejim.2017.09.014.
[3] Kadam N, Acharya S, Shukla S, Gupta K. Ascitic fluid high sensitive C-reactive protein (hs-CRP). A prognostic marker in cirrhosis with spontaneous bacterial peritonitis. J Clin Diagn Res 2016;10:OC20—4, http://dx.doi.org/10.7860/JCDR/2016/17931.7610.
[4] Runyon BA. AASLD practice guideline management of adult patients with ascites due to cirrhosis: update 2012. Hepatology 2013;57:1651—3, http://dx.doi.org/10.1002/hep.00000.
[5] Anty R, Tonohouan M, Ferrari-Panaia P, Piche T, Pariente A, Anstee QM, et al. Low levels of 25-hydroxy vitamin d are independently associated with the risk of bacterial infection in cirrhotic patients. Clin Transl Gastroenterol 2014;5:e56, http://dx.doi.org/10.1038/ctg.2014.6.
[6] Putz-Bankuti C, Pilz S, Stojakovic T, Scharnagl H, Pieber TR, Trauner M, et al. Association of 25-hydroxyvitamin D levels with liver dysfunction and mortality in chronic liver disease. Liver Int 2012;32:845—51, http://dx.doi.org/10.1111/j.1478-3231.2011.02735.x.
[7] Konstantakis C, Tselekouni P, Kalafateli M, Triantos C. Vitamin D deficiency in patients with liver cirrhosis. Ann Gastroenterol Q Publ Hell Soc Gastroenterol 2016;29:297—306, http://dx.doi.org/10.20524/aog.2016.0037.
[8] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911—30, http://dx.doi.org/10.1210/jc.2011-0385.
[9] Buonomo AR, Zappulo E, Scotto R, Pinchera B, Perruolo G, Formisano P, et al. Vitamin D deficiency is a risk factor for infections in patients affected by HCV-related liver cirrhosis. Int J Infect Dis 2017;63:23—9, http://dx.doi.org/10.1016/j.ijid.2017.07.026.
[10] Szymczak I, Pawliczak R. The active metabolite of vitamin D3 as a potential immunomodulator. Scand J Immunol 2016;83:83—91, http://dx.doi.org/10.1111/sji.12403.
[11] Zhang C, Zhao L, Ding Y, Sheng Q, Bai H, An Z, et al. Enhanced LL-37 expression following vitamin D supplementation in patients with cirrhosis and spontaneous bacterial peritonitis. Liver Int 2015;36:68—75, http://dx.doi.org/10.1111/liv.12888.

Antonio Riccardo Buonomo[a,*]
Michele Arcopinto[b]
Riccardo Scotto[a]
Emanuela Zappulo[a]
Biagio Pinchera[a]
Simona Sanguedolce[c]
Giuseppe Perruolo[b]
Pietro Formisano[b]
Antonio Cittadini[b]
Umberto Vespasiani Gentilucc[c]
Guglielmo Borgia[a]
Ivan Gentile[a]

[a] *Department of Clinical Medicine and Surgery, Section of Infectious Diseases, University of Naples ''Federico II'', Naples, Italy*
[b] *Department of Translational Medical Sciences, University of Naples ''Federico II'', Naples, Italy*
[c] *Department of Medicine, Unit of Internal Medicine and Hepatology, University Campus Bio-Medico of Rome, Rome, Italy*

* Corresponding author. University of Naples ''Federico II'', Via Pansini, 5, 80131 Naples, Italy.
E-mail addresses: antonioriccardobuonomo@gmail.com (A.R. Buonomo), michele.arcopinto83@gmail.com (M. Arcopinto), ri.scotto@gmail.com (R. Scotto), e.zappulo@gmail.com (E. Zappulo), biapin89@virgilio.it (B. Pinchera), s.sanguedolce@unicampus.it (S. Sanguedolce), gperruolo@gmail.com (G. Perruolo), fpietro@unina.it (P. Formisano), antonio.cittadini@unina.it (A. Cittadini), u.vespasiani@unicampus.it (U. Vespasiani Gentilucc), borgia@unina.it (G. Borgia), ivan.gentile@unina.it (I. Gentile)
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