Title: Vitamin D Deficiency Is a Risk Factor for Infections in Patients Affected by HCV-Related Liver Cirrhosis.

Authors: Antonio Riccardo Buonomo, Emanuela Zappulo, Riccardo Scotto, Biagio Pinchera, Giuseppe Perruolo, Pietro Formisano, Guglielmo Borgia, Ivan Gentile

PII: S1201-9712(17)30205-9
DOI: http://dx.doi.org/doi:10.1016/j.ijid.2017.07.026
Reference: IJID 3005

To appear in: *International Journal of Infectious Diseases*

Received date: 8-6-2017
Revised date: 11-7-2017
Accepted date: 31-7-2017

Please cite this article as: Buonomo Antonio Riccardo, Zappulo Emanuela, Scotto Riccardo, Pinchera Biagio, Perruolo Giuseppe, Formisano Pietro, Borgia Guglielmo, Gentile Ivan.Vitamin D Deficiency Is a Risk Factor for Infections in Patients Affected by HCV-Related Liver Cirrhosis.*International Journal of Infectious Diseases* http://dx.doi.org/10.1016/j.ijid.2017.07.026

Vitamin D Deficiency Is a Risk Factor for Infections in Patients Affected by HCV-Related Liver Cirrhosis.

Authors:

Antonio Riccardo Buonomo,[*] Emanuela Zappulo,* Riccardo Scotto,*Biagio Pinchera,*Giuseppe Perruolo,[†] Pietro Formisano[†] Guglielmo Borgia,* and Ivan Gentile*

[*]Department of Clinical Medicine and Surgery, Section of Infectious Diseases, University of Naples "Federico II", Italy

[†]Department of Medical Translational Sciences, University of Naples "Federico II", Italy

Address correspondence to: Antonio Riccardo Buonomo, Department of Clinical Medicine and Surgery, Section of Infectious Diseases, University of Naples "Federico II", via S. Pansini 5, 80131 Naples, Italy; e-mail: antonioriccardobuonomo@gmail.com; telephone number: +390817463740; Fax number: +390817463094.

Abstract

Objectives: To evaluate the prevalence of vitamin D deficiency and its impact on infections in HCV-related liver cirrhosis.

Methods: We enrolled 291 patients affected by HCV-related liver cirrhosis. Serum vitamin D levels were dosed at enrolment. The presence of infection was assessed at baseline and during follow-up based on physical examination and laboratory analyses.

Results: Vitamin D deficiency (<20 ng/mL) was diagnosed in 68.3% of patients, and a total of 102 infections were detected. Urinary tract infections were the most common infections diagnosed (41.2%). Vitamin D deficiency rates were higher in patients with decompensated cirrhosis (Child-Pugh B vs A p=0.008, and Child-Pugh C vs A p=0.024). Infection was significantly associated with vitamin D deficiency (p<0.001), MELD score >15 (p=0.003), Child-Pugh class B/C vs A (p<0.001), and active hepatocellular carcinoma (HCC) (p<0.001). At multivariate analysis, vitamin D deficiency (p<0.01), HCC (p<0.05), hospitalization (p<0.001) and exposure to immunosuppressant agents (p<0.05) were independent risk factors for infection at baseline.
Conclusions: Vitamin D may play a role in the development of infections in patients affected by liver cirrhosis and preventive strategies with vitamin D supplementation are to be evaluated in randomized controlled trials.

KeyWords: Vitamin D, infections, cirrhosis, HCV, liver disease

Introduction

Vitamin D is a secosteroid hormone involved in several processes other than bone and calcium homeostasis (Perez-Lopez, 2007). Most circulating vitamin D is photosynthesised in the skin from UVB radiation, which converts the cholesterol metabolite 7-dehydrocholesterol into vitamin D3 (Christakos et al., 2016). Vitamin D3 and Vitamin D2 are also ingested with food. These metabolites are converted into the activated form of vitamin D, 1,25(OH)(calcitriol) (Kitson and Roberts, 2012) through a two-step hydroxylation process: the first step occurs in the liver and is mediated by 25-hydroxylase (CYP2R1), while the second step occurs predominantly in the kidney and, to a lesser extent, in other tissues (lymph nodes and skin), and is mediated by 1α-hydroxylase (CYP27B1).Vitamin D exerts its effect at such sites as the cardiovascular system, muscle, pancreatic endocrine cells and adipose tissue (Caprio et al., 2016, Christakos et al., 2016, Holick

and Garabedian, 2006, Sibaii et al., 2016). Both *in vitro* and *in vivo* studies demonstrated that, among other functions, vitamin D affects the innate and adaptive immune response (Hewison, 2011, Khoo et al., 2012, Mangin et al., 2014, White, 2012). Lymphocytes T and B, macrophages and dendritic cells express CYP27B1 enzymes and can metabolize 25(OH)vitamin D to calcitriol. Calcitriol binds to its receptor in the nucleus of key innate immune cell types, particularly in antigen-presenting cells, thereby acting as a transcription factor for the antimicrobial peptides cathelicidin and beta defensins. This process leads to enhanced phagocytic, chemotactic, and antimicrobial activity (Gombart, 2009, Lin, 2016, Liu et al., 2006, Wang et al., 2004). Moreover, also the adaptive immune response may depend on vitamin D. In fact, vitamin D, promotes the switch from the Th1 (pro-inflammatory) to the T helper 2 (anti-inflammatory)–mediated immune response (Gatti et al., 2016, Hoe et al., 2016, Ragab et al., 2016). Therefore, vitamin D seems to play a key role in modulating antimicrobial-specific inflammatory responses.

Vitamin D insufficiency or deficiency occurs in up to 92% of patients with chronic liver disease (Arteh et al., 2010) and the severity of deficiency correlates with the severity of liver dysfunction (Kitson and Roberts, 2012). The high prevalence of vitamin D deficiency in these patients occurs regardless of the etiology of the liver disease (Malham et al., 2011). Synthetic liver impairment was believed to be responsible for this link between vitamin D deficiency and severity of liver dysfunction. However, a direct cause-effect relationship has not been identified, and it remains to be established whether vitamin D deficiency is an effect of liver disease or a contributor to liver impairment. Notably, Rode *et al.* showed that oral vitamin D supplementation normalized 25(OH) vitamin D serum concentrations in patients with chronic liver disease, which demonstrates that liver hydroxylation was preserved in these patients (Rode et al., 2010).

It must be highlighted that vitamin D deficiency may also be due to insufficient exposure to sunlight or to an inadequate dietary intake of vitamin D,. In addition, luminal absorption of dietary sources of vitamin D in patients affected by chronic liver diseases may be impeded due to intestinal oedema, which complicates portal hypertension, or to cholestasis-induced bile salt disruption

(Kitson and Roberts, 2012). Finally, the use of steroids and jaundice-related deterioration of the synthesis of vitamin D in the skin contribute to vitamin D deficiency (Iruzubieta et al., 2014). Vitamin D deficiency has been associated with increased mortality in patients with alcoholic liver disease and in patients with cirrhosis, but the mechanism and the cause-effect relationship of this association is unknown (Putz-Bankuti et al., 2012, Trepo et al., 2013). It is conceivable that vitamin D deficiency results in higher rates of infections and, subsequently, in increased mortality rates in patients with cirrhosis. Indeed, bacterial infections reduce survival of patients with cirrhosis (Arvaniti et al., 2010, Strnad et al., 2017) and vitamin D regulates the immune response (Mangin et al., 2014).

To our knowledge, only two studies have evaluated the association between vitamin D deficiency and the occurrence of different kind of infections in patients affected by liver cirrhosis. In one study, only hospitalized patients were enrolled (Anty et al., 2014), while in both previous studies, alcohol abuse was the main cause of liver disease (Anty et al., 2014, Finkelmeier et al., 2015). In the first study, involving 88 subjects, patients with cirrhosis and severe vitamin D deficiency (<10 ng/mL) had more bacterial infections versus patients with vitamin D levels ≥10 ng/mL (54 versus 29%, p=0.02). However, vitamin D deficiency did not significantly affect mortality (Anty et al., 2014). In the second study, which enrolled 251 patients with cirrhosis, low vitamin D levels were associated with both infectious complications and mortality (Finkelmeier et al., 2015).

In the attempt to evaluate the impact of vitamin D serum levels on susceptibility to infections, we assessed the prevalence of vitamin D deficiency in a prospective cohort of inpatients and outpatients affected by HCV-related liver cirrhosis, and monitored them for three months for the presence of infections.

Materials and Methods

Patients were prospectively enrolled from 1 March 2013 to30 December 2015 in the Infectious Disease Unit of the University Hospital of Naples "Federico II". Inclusion criteria were HCV-related liver cirrhosis and age ≥18 years. Exclusion criteria were coinfection with HIV or HBV s and oral vitamin D supplementation in the previous 12 months. 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).

Cirrhosis was diagnosed based on clinical criteria, histological diagnosis, or on liver stiffness ≥13 KPa at FibroScan® (Castera et al., 2008, Gentile et al., 2014). Clinical criteria for the diagnosis of cirrhosis included laboratory (thrombocytopenia, low serum concentration of pseudocholinesterase, coagulation parameters alteration) and ultrasonographic abnormalities, and cirrhosis-related complications, namely ascites and porto-systemic encephalopathy. We collected personal, clinical and medical data (e.g.. Child-Pugh scores, MELD scores, active HCC, and HCV viral load), and other known risk factors for infections e.g.hospitalization at enrollment, malignancies, treatment with immunosuppressive agents, or diabetes mellitus.

Infections were recorded and defined as follows:

- Diagnosis of an infection in a definite site: spontaneous bacterial peritonitis (neutrophil granulocyte count ≥250 cells in the ascites) (Runyon, 2013), urinary tract infection (bacterial count $\geq 10^5$ colony forming units associated with evocative symptoms, urinary tract malformations or immunocompromission) (Grabe et al., 2015. Available from: http://uroweb.org/wp-content/uploads/19-Urological-infections_LR2.pdf [Accessed October 29, 2016]), lower and upper respiratory tract infections (affecting nose, sinuses and throat or airways and lungs, respectively), bloodstream infections (defined as growth of a microorganism from a blood culture obtained from a patient with clinical signs of infection (Laupland, 2013) and, where contamination has been ruled out, including endocarditis

(presence of 2 major criteria, 1 major criterion and 3 minor criteria or 5 minor criteria of endocarditis according to the 2015 ESC modified Duke's criteria) (Daher et al., 2012), *C. difficile* infection (evidenced by a positive test for toxins A and B) (Crobach et al., 2009), skin and soft tissue infections (changes in skin evocative of infection or US/CT/RM scan suggestive of soft tissue involvement with or without a positive culture) (Stevens et al., 2014).

- Sepsis (presence of signs/symptoms evocative of infections plus an increase in the SOFA score ≥2) (Vincent et al., 1996).
- Presence of infection based on microbiological isolation or serology, radiological findings and related clinical symptoms.

Patients enrolled underwent a first clinical check-up in which they the provided signed informed consent to the study and agreed to promptly inform the physician in case of any infection during the three months of follow-up. At enrolment, a blood sample was collected ("baseline vitamin D level"), stored at -20°C until use. Patients were managed based on the stage of liver disease and their clinical conditions. Any infections present were recorded. Three months after enrolment, patients underwent another clinical check-up during which any interim infection was recorded.

We classified vitamin D deficiency as 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 recent, authoritative guidelines (Holick et al., 2011). 25(OH) vitamin D was measured by means of a direct, competitive chemiluminescence immunoassay using the DiaSorin LIAISON 25(OH)D TOTAL assay (DiaSorin, Inc., Stillwater, MN., USA) and analyzed with the LIAISON XL assay, which is co-specific for 25-hydroxyvitamin D_3 and 25-hydroxyvitamin D_2. Specific anti-25 (OH) vitamin D antibody was coated on magnetic particles (solid phase) and vitamin D was linked to an isoluminol derivative to form the tracer. During incubation, 25 OH vitamin D was dissociated from its binding protein, and

competed with labeled vitamin D for binding sites on the antibody. After incubation, the unbound material was removed with a wash cycle. Subsequently, the starter reagents were added and a flash chemiluminescent reaction was initiated. The light signal was measured by a photomultiplier as relative light units and was inversely proportional to the concentration of 25 OH vitamin D present in calibrators, controls, and samples. The final concentration was reported in ng/mL with a minimum of 4.0 ng/mL and a maximum of 150.0 ng/mL. Precision, as reported in the Instructions for Use of the kit, was determined by assaying six serum samples and two levels of LIAISON 25 OH Vitamin D TOTAL controls for 20 days according to the CLSI protocol EP05-A2 (Clinical et al., 2004); the total coefficient of variances ranged between 12.6–10.8% (19.8–280.0 nmol/L and 7.9–112.1 ng/mL) for serum, and 9.7–9.5% (45.0–154.5 nmol/L and 18.0–61.8 ng/mL) for kit controls. Laboratory parameters were determined at the Laboratory of Pathology, Microbiology and Laboratory Medicine of the University Hospital of Naples "Federico II".

Statistical analysis

The Kolmogorov-Smirnov test was applied to quantitative variables to check for Gaussian distribution. Data are presented as mean ± standard deviation (SD) or as median and interquartile range (IQR) in case of Gaussian and non-Gaussian distribution, respectively. For quantitative variables, Student's *t*-test for unpaired variables was used for comparison in case of Gaussian distribution, while the Mann-Whitney U-test was used for non-Gaussian distribution. For comparisons of quantitative variables among three groups, ANOVA was used. For categorical variables, the χ^2 test with Yates correction (or Fisher's exact test if appropriate) was used. We used a logistic regression model for multivariate analysis. The backward conditional stepwise method was used. The cut-off values for the stepwise method were p=0.05 for entry into the model and p=0.10 for removal from the model. For all tests, a p-value <5% at a two-sided test considered statistically significant. All statistical analyses were carried out with the Statistical Package for the Social Sciences version 18.0 (SPSS Inc. Chicago, IL, USA).

Results

We enrolled 291 patients between March 2013 and December 2015. The characteristics of the patients at the enrolment are reported in Table 1. A total of 64 patients (21.9%) presented an infection. Patients with infections showed lower serum concentrations of Vitamin D compared with patients with no evidence of infection at enrolment (8.1, IQR: 5.0-13.2 vs 16.9, IQR: 9.6-25.9, $p<0.001$). Patients with infections at the enrolment also showed a significant lower platelet count, higher PCR concentrations and a significant higher rate of HCC compared with patients without infections. Moreover, rates of patients with decompensated cirrhosis (Child-Pugh classification B or C) were higher among those with infections at baseline compared with patients with no evidence of infection (see Table 1 for details). At enrolment, 199 patients (68.4%) had vitamin D deficiency, 40 (13,7%) had vitamin D insufficiency, and 52 (17.9%) had sufficient vitamin D levels. Among the 227 patients without an infection at baseline, 37 (16.3%) developed at least one infection during follow-up. Urinary tract infections were the most common infections, followed by lower respiratory tract infections, spontaneous bacterial peritonitis and sepsis (Table 2). The etiologic agent of the infection was identified in 67(65.6%) patients, and a bacterium was isolated in 59 (88%) of them. Resistant germs were identified in 16/59 isolates (27.1%). In most cases, resistant strains were expanded spectrum beta lactamase-producing Gram-negative pathogens (Table 3).

To assess whether the month of enrollment (and therefore of the blood sampling) could have affected vitamin D level, we compared vitamin D levels in patients enrolled during “cloudy” months (from December to May; Group A) to those of patients enrolled during “sunny” months (from June to November; Group B). The choice of “cloudy” and “sunny” month was made in accordance with the known dyssynchrony between sun exposure and vitamin D serum concentrations (Maeda et al., 2013). Moreover, in previous studies, vitamin D concentrations were

assessed in the different months of the year and they resulted to be higher in the June-November period (Klingberg et al., 2015).

The rate of vitamin D deficiency did not differ significantly between the two groups (Group A: 67.9% *vs* Group B: 65%, p=0.647).

As shown in the Figure 1, vitamin D levels were lower in patients with more advanced cirrhosis (Child-Pugh A vs B p=0.008; Child-Pugh A vs C p=0.024; and Child-Pugh C vs B p=1.000). Such difference was also significant when it was assessed among the three Child-Pugh classes (p<0.001). We also evaluated the association between the *a priori* stated risk factors (hospitalization at enrollment, malignancies, treatment with immunosuppressive agents, or diabetes mellitus) and the presence of infection (Table 4). The presence of at least one of these risk factors for infection was significantly associated with the diagnosis of an infection (Odds Ratio, OR: 6.32; 95% Confidence Interval (CI): 2.75-14.53, p<0.001). In detail, hospitalization (OR 8.38; 95CI 4.04–17.38, p<0.001), malignancies (OR 4.13; 95CI 1.88–9.12, p<0.001), immunosuppressive therapy (OR 6.1; 95CI 2.12–17.5, p<0.01) and diabetes mellitus (OR 2.28; 95CI 1.29–4.02, p<0.01) were significantly associated with the diagnosis of an infection. Furthermore, vitamin D deficiency (OR 3.94; 95CI 1.85–8.38, p<0.001), MELD >15 (OR 3.28 95CI 1.49 –7.25 p<0.01), Child class B or C (*versus* A) (OR 4.73, 95 CI 2.58–8.67, p<0.001), active HCC (OR 7.62, 95 CI 3.04–19.12, p<0.001) were also significantly associated with infection. At multivariate analysis (Table 4), vitamin D deficiency (OR 3.09; 95 CI 1.35–7.07, p<0.01), active HCC (OR 2.86; 95 CI 1.03–7.92, p<0.05), hospitalization (OR 4.49; 95 CI 1.95–10.31, p<0.001) and immunosuppressive therapies (OR 4.4; 95CI 1.20–16.18, p<0.05) remained significantly associated with the risk of infection at enrolment, in a model that included Child class (B or C *versus* A), MELD>15 and diabetes mellitus.

In patients without signs or symptoms of an infection at enrolment (n=226), vitamin D deficiency (OR: 3.0; 95 CI 1.08–8.34, p=<0.05), Child B or C *versus* A (OR 4.1; 95 CI 1.63–10.31, p<0.01),

having at least one known risk factor for infection (OR:12.79; 95CI 2.99-54.67, p<0.01) and hospitalization (OR: 10.43; 95 CI 3.41–31.91, p<0.001) were predictive of developing an infection in the following three months (Table 5). At multivariate analysis, hospitalization was the only risk factor independently associated with the risk of infection during follow-up (OR 3.95; 95 CI 1.05-14.87–-, p<0.05) when Child-Pugh classification (B or C vs A) vitamin D deficiency and having at least one known risk factor for infection were included in the analysis (Table 5).

Discussion

We found that more than two-thirds of our sample (291 patients with HCV-related cirrhosis) had vitamin D deficiency. It is noteworthy that we found this very high rate of vitamin D deficiency in a population living in Southern Italy, which experiences sunshine throughout most of the year.

In addition, vitamin D deficiency was significantly associated with the presence of infection in our patients, regardless of the severity of liver disease or the presence of HCC. Notably, in patients without infection at baseline, vitamin D deficiency predicted the development of infection during the three-months of follow-up. Should our data be confirmed in independent large cohort trials, it is conceivable that vitamin D supplementation in patients with cirrhosis might prevent infections.

Notably, several prospective randomized trials designed to evaluate the efficacy of vitamin D supplementation in tuberculosis or in viral respiratory tract infections yielded conflicting results regarding the advantage of vitamin D supplementation (Murdoch et al., 2012, Yamshchikov et al., 2009).

New cofactors have recently been identified as possible determinants of immunological derangements and susceptibility to infections in patients with vitamin D deficiency. In particular, the human cathelicidin LL-37 has been recognized as an antimicrobial peptide involved in the protection of the epithelial barrier against infection (Vandamme et al., 2012). LL-37 is constitutively secreted in the bloodstream by immune cells: its activation is mediated by vitamin D

intracellular signaling via vitamin D receptor (VDR) (Liu et al., 2006). Its antimicrobial activity has been evaluated both in urinary tract infections and septic shock (Barbeiro et al., 2013, Nielsen et al., 2014).

Recently, Zhang et al evaluated the role of the vitamin D/LL-37 pathway in the pathogenesis and treatment of spontaneous bacterial peritonitis (SBP), a major cause of morbidity and mortality in patients with cirrhosis (Zhang et al., 2016). In fact, they hypothesized that an impaired immunological response due to inadequate levels of LL-37 caused by vitamin D deficiency could be responsible of a higher susceptibility to bacterial infections in patients with cirrhosis.

A total of 119 patient with chronic liver diseases were enrolled. Serum 25(OH)vitamin D levels of were measured in all patients. In 47 patients with cirrhosis and ascites, the levels of VDR and LL-37 were tested in peritoneal leucocytes: a comparison of their rates in patients with or without SBP was performed. Moreover, in non-infected patients, the peritoneal macrophages were cultured and activated by lipopolysaccharide (LPS) in order to evaluate VDR and LL-37 expression before and after the incubation with vitamin D.

Serum concentrations of 25OH vitamin D resulted insufficient or deficient in all patients with cirrhosis. The authors observed that when vitamin D levels were low, bacteria impaired VDR and LL-37 pathways in peritoneal macrophages so to escape antimicrobial response. Supplementation with vitamin D could enhance peritoneal macrophage VDR and LL-37 expressions, an important weapon in the fight against SBP in patients with decompensated cirrhosis.

However, only two observational studies have evaluated the association between low vitamin D levels and the risk of different kind of bacterial infections in patients with cirrhosis. Both studies showed that vitamin D deficiency was common in this population (Anty et al., 2014, Finkelmeier et al., 2015). Anty *et al*. reported that this deficiency was a predictive factor for infections irrespective of the stage of the liver disease or C reactive protein level (Anty et al., 2014). In the second study (Finkelmeier et al., 2015), low levels of vitamin D were found to be related with liver dysfunction and mortality (the relative risk within the first and third tertile of 25-OH vitamin D was 6.37 for

liver dysfunction and 4.31 for mortality). However, both studies were conducted on small samples and most patients enrolled were alcohol abusers, which may have influenced serum vitamin D levels.

Various aspects of vitamin D supplementation in patients with liver cirrhosis are still obscure. In fact, the dose and frequency of administration of vitamin D supplementation is not well defined, and it remains to be established whether daily administration may be better than weekly or monthly bolus. It is also possible that vitamin D absorption may be impaired in patients with cirrhosis, thus therapeutic drug monitoring should be more frequently performed. Notably, the most recent guidelines support the assessment of vitamin D levels and its supplementation in the management of patients with liver cirrhosis.

Together with exogenous supplementation, a new strategy to restore "vitamin D competence" could consist in the administration of immune-modulatory agents that enhance the expression and activity of VDR (Mangin et al., 2014, Marshall et al., 2006). This approach could be particularly effective in the setting of bacterial infections. In fact, it has been speculated that microbes, mainly intracellular microbes, can escape the immune response by deregulating the VDR (Coughlan et al., 2012, Xu et al., 2003, Yenamandra et al., 2009). It is becoming increasingly clear that a complex interplay exists among vitamin D, its receptor and infective agents. Therefore, a double-step (not mutually exclusive) vitamin D recovery strategy can be hypothesized in the fight against infections and their consequences: a preventive strategy, based on vitamin D supplementation in patients with vitamin deficiency at risk of infections, and an active strategy with immune-modulatory agents during infection to improve the immune response and clinical outcome. The prevention of infections will be a major concern in the future of hepatology, due to their enormous burden in terms of morbidity and mortality in patients with liver cirrhosis (Bunchorntavakul et al., 2016, Gustot et al., 2009, Hernaez et al., 2017, Moreau, 2016, Tandon and Garcia-Tsao, 2008, Wong et al., 2005).

In conclusion, vitamin D deficiency may play a role in the susceptibility to infections in patients with liver disease but it is still debated whether it is an actor or a spectator in this interaction. Little

is known about the efficacy and dosage of vitamin D supplementation or about immune-modulatory agents in this setting. Randomized controlled trials are needed to assess the role of vitamin D supplementation in preventing infections in patients with liver cirrhosis.

Conflicts of Interest and Source of Funding:

A.R.B. received a grant (*Borsa di Studio in Epatologia 2014*) from Fire Onlus for this study. All the other authors report no actual or potential conflict of interest relevant to the subject of this article. I.G. acted as a consultant for AbbVie and MSD. He received a grant from Gilead Sciencies (in the framework of Fellowship program). The other authors declare no conflict of interest.

References

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. Clinical and translational gastroenterology 2014;5:e56.
Arteh J, Narra S, Nair S. Prevalence of vitamin D deficiency in chronic liver disease. Digestive diseases and sciences 2010;55(9):2624-8.
Arvaniti V, D'Amico G, Fede G, Manousou P, Tsochatzis E, Pleguezuelo M, et al. Infections in patients with cirrhosis increase mortality four-fold and should be used in determining prognosis. Gastroenterology 2010;139(4):1246-56, 56 e1-5.
Barbeiro DF, Barbeiro HV, Zampieri FG, Cesar Machado MC, Torggler Filho F, Gomes Cunha DM, et al. Cathelicidin LL-37 bloodstream surveillance is down regulated during septic shock. Microbes and infection 2013;15(5):342-6.
Bunchorntavakul C, Chamroonkul N, Chavalitdhamrong D. Bacterial infections in cirrhosis: A critical review and practical guidance. World journal of hepatology 2016;8(6):307-21.
Caprio M, Infante M, Calanchini M, Mammi C, Fabbri A. Vitamin D: not just the bone. Evidence for beneficial pleiotropic extraskeletal effects. Eating and weight disorders : EWD 2016.
Castera L, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. Journal of hepatology 2008;48(5):835-47.
Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. Physiological reviews 2016;96(1):365-408.
Clinical, and, Laboratory, Standards, Institute. Evaluation of precision performance of quantitative measurement methods. Wayne, PA, USA: CLSI document, 2004.
Coughlan CA, Chotirmall SH, Renwick J, Hassan T, Low TB, Bergsson G, et al. The effect of Aspergillus fumigatus infection on vitamin D receptor expression in cystic fibrosis. American journal of respiratory and critical care medicine 2012;186(10):999-1007.
Crobach MJ, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing Clostridium difficile-infection (CDI). Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2009;15(12):1053-66.
Daher EF, Silva Junior GB, Santos SQ, CC RB, Diniz EJ, Lima RS, et al. Differences in community, hospital and intensive care unit-acquired acute kidney injury: observational study in a nephrology service of a developing country. Clinical nephrology 2012;78(6):449-55.
Finkelmeier F, Kronenberger B, Zeuzem S, Piiper A, Waidmann O. Low 25-Hydroxyvitamin D Levels Are Associated with Infections and Mortality in Patients with Cirrhosis. PloS one 2015;10(6):e0132119.
Gatti D, Idolazzi L, Fassio A. Vitamin D: not just bone, but also immunity. Minerva medica 2016;107(6):452-60.
Gentile I, Buonomo AR, Zappulo E, Borgia G. Is it possible to predict HCV-related liver cirrhosis non-invasively through routine laboratory parameters? Le infezioni in medicina : rivista periodica di eziologia, epidemiologia, diagnostica, clinica e terapia delle patologie infettive 2014;22(1):11-8.
Gombart AF. The vitamin D-antimicrobial peptide pathway and its role in protection against infection. Future microbiology 2009;4(9):1151-65.
Grabe M, Bartoletti R, Bjerklund Johansen TE, Cai T, Çek T, Köves B, et al. Guidelines on urological infections. European Association of Urology 2015. Available from: http://uroweb.org/wp-content/uploads/19-Urological-infections_LR2.pdf [Accessed October 29, 2016].
Gustot T, Durand F, Lebrec D, Vincent JL, Moreau R. Severe sepsis in cirrhosis. Hepatology (Baltimore, Md) 2009;50(6):2022-33.
Hernaez R, Sola E, Moreau R, Gines P. Acute-on-chronic liver failure: an update. Gut 2017.
Hewison M. Antibacterial effects of vitamin D. Nature reviews Endocrinology 2011;7(6):337-45.

Hoe E, Nathanielsz J, Toh ZQ, Spry L, Marimla R, Balloch A, et al. Anti-Inflammatory Effects of Vitamin D on Human Immune Cells in the Context of Bacterial Infection. Nutrients 2016;8(12).
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. The Journal of clinical endocrinology and metabolism 2011;96(7):1911-30.
Holick MF, Garabedian M. Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In: Favus M, editor. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 6th edition ed. Washington, DC: American Society for Bone and Mineral Research; 2006. p. 129-37.
Iruzubieta P, Teran A, Crespo J, Fabrega E. Vitamin D deficiency in chronic liver disease. World journal of hepatology 2014;6(12):901-15.
Khoo AL, Chai L, Koenen H, Joosten I, Netea M, van der Ven A. Translating the role of vitamin D3 in infectious diseases. Critical reviews in microbiology 2012;38(2):122-35.
Kitson MT, Roberts SK. D-livering the message: the importance of vitamin D status in chronic liver disease. Journal of hepatology 2012;57(4):897-909.
Klingberg E, Olerod G, Konar J, Petzold M, Hammarsten O. Seasonal variations in serum 25-hydroxy vitamin D levels in a Swedish cohort. Endocrine 2015;49(3):800-8.
Laupland KB. Incidence of bloodstream infection: a review of population-based studies. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2013;19(6):492-500.
Lin R. Crosstalk between Vitamin D Metabolism, VDR Signalling, and Innate Immunity. BioMed research international 2016;2016:1375858.
Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 2006;311(5768):1770-3.
Maeda SS, Saraiva GL, Hayashi LF, Cendoroglo MS, Ramos LR, Correa Mde P, et al. Seasonal variation in the serum 25-hydroxyvitamin D levels of young and elderly active and inactive adults in Sao Paulo, Brazil: The Sao PAulo Vitamin D Evaluation Study (SPADES). Dermato-endocrinology 2013;5(1):211-7.
Malham M, Jorgensen SP, Ott P, Agnholt J, Vilstrup H, Borre M, et al. Vitamin D deficiency in cirrhosis relates to liver dysfunction rather than aetiology. World journal of gastroenterology : WJG 2011;17(7):922-5.
Mangin M, Sinha R, Fincher K. Inflammation and vitamin D: the infection connection. Inflammation research : official journal of the European Histamine Research Society [et al] 2014;63(10):803-19.
Marshall TG, Lee RE, Marshall FE. Common angiotensin receptor blockers may directly modulate the immune system via VDR, PPAR and CCR2b. Theoretical biology & medical modelling 2006;3:1.
Moreau R. Acute-on-chronic liver failure: a new syndrome in cirrhosis. Clinical and molecular hepatology 2016;22(1):1-6.
Murdoch DR, Slow S, Chambers ST, Jennings LC, Stewart AW, Priest PC, et al. Effect of vitamin D3 supplementation on upper respiratory tract infections in healthy adults: the VIDARIS randomized controlled trial. JAMA : the journal of the American Medical Association 2012;308(13):1333-9.
Nielsen KL, Dynesen P, Larsen P, Jakobsen L, Andersen PS, Frimodt-Moller N. Role of urinary cathelicidin LL-37 and human beta-defensin 1 in uncomplicated Escherichia coli urinary tract infections. Infection and immunity 2014;82(4):1572-8.
Perez-Lopez FR. Vitamin D: the secosteroid hormone and human reproduction. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2007;23(1):13-24.
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 international : official journal of the International Association for the Study of the Liver 2012;32(5):845-51.
Ragab D, Soliman D, Samaha D, Yassin A. Vitamin D status and its modulatory effect on interferon gamma and interleukin-10 production by peripheral blood mononuclear cells in culture. Cytokine 2016;85:5-10.
Rode A, Fourlanos S, Nicoll A. Oral vitamin D replacement is effective in chronic liver disease. Gastroenterologie clinique et biologique 2010;34(11):618-20.

Runyon BA. Introduction to the revised American Association for the Study of Liver Diseases Practice Guideline management of adult patients with ascites due to cirrhosis 2012. Hepatology (Baltimore, Md) 2013;57(4):1651-3.
Sibaii H, El-Zayat SR, El-Shaheed AA, Mahfouz NN, Sallam SF, El Azma MH. The Hidden Function of Vitamin D. Open access Macedonian journal of medical sciences 2016;4(4):591-5.
Stevens DL, Bisno AL, Chambers HF, Dellinger EP, Goldstein EJ, Gorbach SL, et al. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2014;59(2):e10-52.
Strnad P, Tacke F, Koch A, Trautwein C. Liver - guardian, modifier and target of sepsis. Nature reviews Gastroenterology & hepatology 2017;14(1):55-66.
Tandon P, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. Seminars in liver disease 2008;28(1):26-42.
Trepo E, Ouziel R, Pradat P, Momozawa Y, Quertinmont E, Gervy C, et al. Marked 25-hydroxyvitamin D deficiency is associated with poor prognosis in patients with alcoholic liver disease. Journal of hepatology 2013;59(2):344-50.
Vandamme D, Landuyt B, Luyten W, Schoofs L. A comprehensive summary of LL-37, the factotum human cathelicidin peptide. Cellular immunology 2012;280(1):22-35.
Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. Intensive care medicine 1996;22(7):707-10.
Wang TT, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, et al. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. Journal of immunology (Baltimore, Md : 1950) 2004;173(5):2909-12.
White JH. Vitamin D metabolism and signaling in the immune system. Reviews in endocrine & metabolic disorders 2012;13(1):21-9.
Wong F, Bernardi M, Balk R, Christman B, Moreau R, Garcia-Tsao G, et al. Sepsis in cirrhosis: report on the 7th meeting of the International Ascites Club. Gut 2005;54(5):718-25.
Xu Y, Xie J, Li Y, Yue J, Chen J, Chunyu L, et al. Using a cDNA microarray to study cellular gene expression altered by Mycobacterium tuberculosis. Chinese medical journal 2003;116(7):1070-3.
Yamshchikov AV, Desai NS, Blumberg HM, Ziegler TR, Tangpricha V. Vitamin D for treatment and prevention of infectious diseases: a systematic review of randomized controlled trials. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists 2009;15(5):438-49.
Yenamandra SP, Lundin A, Arulampalam V, Yurchenko M, Pettersson S, Klein G, et al. Expression profile of nuclear receptors upon Epstein -- Barr virus induced B cell transformation. Experimental oncology 2009;31(2):92-6.
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 international : official journal of the International Association for the Study of the Liver 2016;36(1):68-75.

Table 1. Characteristics of patients at the enrollment

	All patients (N=291)	Patients with infection (N=64)	Patients without infection (N=227)	*p**
Male gender (n, %)	166 (57)	29 (46)	137 (60.4)	<0.05
Age (years; median, IQR)	68 (61-73)	69.7 (61.9-72.9)	68 (60.6-74)	0.495
Platelets (elements/µL; median, IQR)	111,000 (75,000 – 156,000)	93,000 (58,000 – 134,000)	114,500 (80.000 – 163,250)	<0.01
Vitamin D (ng/mL; median, IQR)	14.1 (8.0 – 23.9)	8.1 (5.0 – 13.2)	16.9 (9.6 – 25.9)	<0.001
MELD score (median, IQR)	8.8 (7.0 – 11.1)	10.6 (9.0 – 14.7)	8.2 (7.0-11.0)	<0.001
PCR (mg/dl; median, IQR)	0.49 (0.3 – 1.9)	2.7 (0.8-6.0)	0.33 (0.33-0.47)	<0.001
HCC (active and cured; n, %	43 (14.6)	18 (28.1)	25 (11)	<0.01
Active HCC (n, %)	22 (7.5)	14 (21.9)	8 (3.5)	<0.001
PVT (n, %)	24 (8.1)	13 (22.4)	11 (8.5)	<0.05
Cryoglobulinemia (n, %)	40 (13.6)	14 (25.5)	26 (21.47)	0.355
Esophageal varices (n, %)	59 (20.3)	25 (67.6)	34 (38.6)	<0.05
Child-Pugh class (n, %)	A: 224 (77) B: 48 (16.5) C: 19 (6.5)	A: 34 (53.1) B: 21 (32.8) C: 9 (14.1)	A: 190 (83.7 B: 27 (11.9) C: 10 (4.4)	<0.001
Patients with known risk factor of infection (n, %) i.e., with at least one of the following:	167 (57.4%)	55 (88.7)	112 (55.7)	<0.001
Hospitalization at enrolment (n, %)	135 (46.4%)	54 (84.4)	81 (39.7)	<0.001
Diabetes (n, %)	116 (39.9%)	38 (59.4)	78 (38.2)	<0.01
Immunosuppressive therapy (n, %)	15 (5.1%)	9 (14.1)	6 (2.9)	<0.01
Malignancies (n, %)	28 (9.6%)	14 (21.9)	14 (6.8)	<0.01

. HCC, hepatocellular carcinoma. PVT, portal vein thrombosis.

* Comparisons were made between patients with and without a diagnosis of infection at the enrolment

Table 2: Sites of infection

Sites of infection	**Events (N=102)**
Urinary tract infection (n, %)	42 (41.2)
Low respiratory tract infection (n, %)	14 (13.7)
Spontaneous bacterial peritonitis (n, %)	12 (11.8)
Sepsis (n, %)	10 (9.8)
Upper respiratory tract infection (n, %)	7 (6.8)
Skin structure infection (n, %)	7 (6.8)
Clostridium difficile infection (n, %)	2 (2)
Bloodstream infection including endocarditis (n, %)	2 (2)
Visceral leishmaniasis (n, %)	1 (1)
Herpes Zoster infection (n, %)	1 (1)
Unknown (n, %)	4 (3.9)

Table 3. Isolated germs and resistance patterns

Germs	**N (%)**		
Gram-positive bacteria	9/67 (13.4%)		
		MR CoNS	2/9 (22.2%)
		MS CoNS	3/9 (33.3%)
		MSSA	4/9 (44.4%)
Gram-negative bacteria	50/67 (74.6%)		
		Escherichia coli	24/50 (48%)
		Escherichia coli ESBL +	12/50 (24%)
		Klebsiella pneumoniae	9/50 (18%)
		Klebsiella pneumoniae ESBL +	2/50 (4%)
		Clostridium difficile	2/50 (4%)
		Proteus spp.	1/50 (2%)
Fungi	6/67 (9%)		
		Aspergillus fumigatus	1/6 (16.6%)
		Candida albicans	2/6 (33.3%)
		Candida non albicans	3/6 (50%)
Protozoa	1/67 (1.5%)		

Table 4. Univariate and multivariate analysis of risk factors for infection

	Univariate Analysis			**Multivariate Analysis**		
	OR	*95CI*	*p*	*OR*	*95CI*	*p*
At least 1 risk factor for infection	**6.32**	**2.75-14.53**	**<0.001**	1.55	0.32-7.39	0.582
Hospitalization	**8.38**	**4.04-17.38**	**<0.001**	**4.49**	**1.95-10.31**	**<0.001**
Malignancies	**4.13**	**1.88-9.12**	**<0.001**	1.10	0.29-4.13	0.886
Immunosuppressive therapy	**6.1**	**2.12-17.5**	**<0.01**	**4.4**	**1.20-16.18**	**<0.05**
Diabetes mellitus	**2.28**	**1.29-4.02**	**<0.01**	1.28	0.59-2.79	0.535
Vitamin D deficiency	**3.94**	**1.85-8.38**	**<0.001**	**3.09**	**1.35-7.07**	**<0.01**
MELD > 15	**3.28**	**1.49-7.25**	**<0.01**	1.81	0.65-5.06	0.260
Child B or C (versus A)	**4.73**	**2.58-8.67**	**<0.001**	1.29	0.57-2.92	0.539
Active HCC	**7.62**	**3.04-19.12**	**<0.001**	**2.86**	**1.03-7.92**	**<0.05**

OR: Odds Ratio. 95CI: 95% Confident Intervals. *p*: p-value.

Table 5. Univariate and multivariate analysis of developing infection within 3 months follow-up

	Univariate Analysis			**Multivariate Analysis**		
	OR	*95CI*	*p*	*OR*	*95CI*	*p*
At least 1 risk factor for infection	**12.79**	**2.99-54.67**	**<0.01**	2.86	0.39-20.72	0.299
Hospitalization	**4.49**	**1.95-10.31**	**<0.001**	**3.95**	**1.05-14.87**	**<0.05**
Malignancies	1.61	0.33-7.94	0.556	2.97	0.47-18.76	0.246
Immunosuppressive therapy	1.87	0.20-17.46	0.583	1.79	0.44-7.32	0.417
Diabetes mellitus	1.79	0.76-4.23	0.182	1.25	0.52-3.03	0.619
Vitamin D deficiency	**3.0**	**1.08-8.34**	**<0.05**	2.43	0.92-6.43	0.074
MELD > 15	1.45	0.30-6.98	0.639	1.10	0.27	4.48
Child B or C (versus A)	**4.1**	**1.63-10.31**	**<0.01**	1.64	0.66-4.10	0.268
Active HCC	3.36	0.62-18.30	0.162	1.88	0.27-13.06	0.523

OR: Odds Ratio. 95CI: 95% Confident Intervals. *p*: p-value.