

# Positive correlation of 25-hydroxyvitamin D plasma level and T helper activity in chronic hepatitis C patients

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**Abstract**—The immune modulating role of vitamin D has been extensively studied but less documented in chronic infection with hepatitis C virus (HCV). The aim of our study was to assess the vitamin D status and adaptative immunity in HCV chronic infected patients. 46 patients were selected, 25 diagnosed with chronic hepatitis C infection and 21 healthy controls. HCV patients were classified according to the degree of hepatic necroinflammatory activity recorded using Actitest. A2-A3 scores were considered relevant for active HCV hepatitis, while A0-A1 indicated inactive HCV hepatitis. We measured: a) vitamin D status using 25-hydroxyvitamin D plasma level (Elisa method) and the calcium-phosphorus equilibrium, b) the immune status according to the CD4+ T helper cells and CD8+ T helper cells detected by flow cytometry, and c) the extent of liver cytolysis disclosed by alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. All HCV patients, as well as healthy controls displayed a vitamin D deficiency (25-hydroxyvitamin D plasma level 29.571 nmol/l, and 29.271 nmol/l, respectively). A strong positive correlation between 25-hydroxyvitamin D and CD4+ T helper cells ( $R=0.703$  Pearson correlation) was found in active HCV patients. Correlations between 25-hydroxyvitamin D and the ALT and AST levels were weak and divergent: positive in patients with active HCV hepatitis and negative in patients with inactive HCV hepatitis. Serum CD4+ T helper count in active HCV patients correlated positively with serum total calcium ( $R=0.841$  Pearson correlation) and ionized calcium ( $R=0.652$ ). In conclusion, positive correlations were recorded in active HCV hepatitis between the immune response (CD4+ T helper cells count), the plasma 25-hydroxyvitamin D, total serum calcium, and ionized calcium.

**Keywords**—chronic hepatitis C, serum calcium, T helper cells, vitamin D.

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## I. INTRODUCTION

VITAMIN D is a steroid hormone with pleiotropic actions, ranging from calcium homeostasis to immune regulatory functions [1], [2]. The immune function of vitamin D has been associated with autoimmunity disorders, various cancers, allograft survival as well as with certain infectious diseases [3-6]. The discovery of vitamin D receptors (VDR) expression in most tissues and immune system cells has further highlighted the complex paracrine functions of vitamin D.

The biologically active form of vitamin D, 1,25 hydroxyvitamin D (1,25(OH)D), modulates the adaptative immune system by direct effects on T and B cells proliferation and dendritic cells (DCs) maturation. Molecular actions of vitamin D lead to a shift from T helper(Th)1 to Th2 phenotype as well as to the development of DCs with tolerogenic properties. Thus vitamin D exhibits an immunosupresor effect, promotes self tolerance and limits the inflammation [7]. However, the plasma and tissue concentration required for the immune modulating action of vitamin D remain unknown.

The optimal status of vitamin D is presently evaluated using 25-hydroxyvitamin D (25(OH)D) plasma level, an indicator related to bone biomarkers [4]. The plasma level of 25(OH)D required for normal bone and mineral homeostasis is 30 to 32 ng/ml (75 to 80 nmol/l) [4]. A level of 21-29 ng/ml (52 to 75 nmol/l) indicate a relative insufficiency of vitamin D. A level below 20 ng/ml (50 nmol/l) indicates a vitamin D deficiency with unfavorable consequences concerning the bone metabolism. The ensuing immune implications of 25(OH)D plasma levels are less understood.

The metabolism of vitamin D is closely related to the hepatic function, as this is the site for the 25-hydroxylation of vitamin D (the first step in the activation of vitamin D), bile removal of vitamin D metabolites and the synthesis of vitamin D binding protein. Consequently people with liver disorders frequently develop vitamin D deficiencies such as hepatic osteodystrophy, a multifactorial disease [8]. On the other hand, the impact of serum levels of vitamin D on the outcome of liver diseases is unclear. The immunosuppressive effect of vitamin D was experimentally confirmed in liver transplant studies, thus proving the favorable role of vitamin D or vitamin D analogs in preventing the rejection of transplanted liver tissue [9]. Recent studies suggested wider correlations

regarding the plasma level of vitamin D or genetic polymorphisms of VDR with hepatobiliary diseases including the viral hepatitis B or C [10-16].

The hepatitis C virus, a flavivirus, is involved in parenteral transmitted infections with increased risk of chronic persistence and inflammatory liver damage [17]. 80% of the acute HCV infections evolve toward chronic persistence for no apparent reason. By comparison, hepatitis B virus and other acute liver infections with flaviviruses resolve spontaneously in 90% of cases. The host immunity failure in HCV infection is surprising as it occurs in immunocompetent patients with a normal response to antigen stimulation.

The cellular immune response holds a major importance in the HCV clearance or persistence since it influences both antigen recognition and Th response. A multispecific and sustained antigen-specific immune response requiring CD4+Th cells and CD8+ Th cells is essential for recovery [7]. In vitro studies on HCV replication started in 1999 using cellular specific systems [18] and had a major role in understanding the viral life cycle and the favorable conditions of HCV persistence. Vitamin D inhibiting effects on HCV replication were also recorded in these studies [19]. In addition, the low level of serum 25(OH)D observed in clinical studies on chronic HCV patients suggested a possible correlation with liver fibrosis progression or interferon-based therapy sustained virological response [15], [16]. Nevertheless, the actual role of vitamin D in the context of hepatic inflammatory process is unknown. Given the major significance of the inflammatory response mediated by Th cells in HCV clearance, as well as the anti-inflammatory actions displayed by vitamin D in vitro [7], [20], it is questionable whether vitamin D could have a positive influence on HCV infection and if so which stages of the HCV infection require higher levels of vitamin D.

The objective of our study was to assess the plasma levels of 25(OH)D in HCV infected patients compared with healthy controls. The peripheral Th cell response, phosphocalcium metabolism and liver hepatocytolysis activity were also analysed in comparison with the 25(OH)D plasma level.

## II. MATERIALS AND METHODS

### A. Patients

We performed the study on 46 Caucasian subjects aged 43.6 years (SD=16.14), 17 males and 29 women, HIV negative. Of these, 25 subjects were diagnosed with HCV infection while 21 were healthy controls. Patients presented a body mass index < 30kg/m<sup>2</sup>, no underlying renal disease, cardiovascular disease, diabetes, autoimmunity or malignancy, and were not undertaking any medications or vitamin supplements.

### B. Samples

We collected samples between January and September 2009 after we obtained the informed consent. Serum collected for 25(OH)D was placed in EDTA tubes centrifuged for 20 minutes at 1100 – 1300 rpm then stored at -80°C prior to

analysis.

### C. Tests

Subjects had baseline laboratory tests performed by standard hospital laboratory methods including: hemogram, alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and ionized serum calcium, serum phosphorous, hepatitis viral markers (Anti-HCV, HBsAg, Anti-HBs, HBeAg, Anti-HBe, Anti-HBc IgG/IgM), Anti-HIV, the CD4+Th cells and CD8+Th cells number (flow cytometric detection). The RNA HCV viral load was detected with Real-time PCR assay (Roche Cobas TaqMan, limit of detection 45ui/ml). The Fibrotest and Actitest score (BioPredictive) was used to assess the fibrosis and necroinflammatory liver activity. Plasma levels of 25(OH)D, expressed as nmol/L were assessed using Elisa (IDS 25-Hydroxy Vitamin D EIA kit, Immunodiagnostic Systems Ltd, UK -detection range 6–360 nmol/L).

All protocols followed the manufacturer's Instructions. Each Elisa test was run in duplicate, with mean absorbance computed from the average for 2 wells normalized to a zero calibrator well. Levels of vitamin D in test samples were derived by fitting a 2-parameter logistic curve to 6 standard levels. The intra-assay CV for 25 (OH)D were <8%. The inter-assay CV for 25 (OH)D was <10%.

### D. Diagnosis

Diagnosis of HCV infections followed CDC criteria (Guidelines For Viral Hepatitis Surveillance And Case Management) [21]. The scores for liver necro-inflammatory activity ranged from A0 to A3 (A0= no activity, A1 = minimal activity, A2 = moderate activity, A3 = severe activity). Liver fibrosis was graded from F0 to F4. Cirrhosis was defined according to the Fibrotest score (F≥4), abdominal ultrasonography and liver function tests. [22].

*Vitamin D status* was indicated by the 25(OH)D serum levels. Vitamin D deficiency/ insufficiency was defined as a 25(OH)D concentration lower than 80 nmol/L and 50 nmol/L respectively.

*The liver necroinflammatory status* was classified according to the Actitest (A0-A3) and Fibrotest (F0-F4). 13 patients presented low necroinflammatory activity (A0-A1) and 12 patients presented a high necroinflammatory activity (A2-A3). 5 patients were graded for cirrhosis (F4) and 20 for chronic hepatitis. The extension of necroinflammatory lesions was indicated by the ALT and AST level.

### E. Statistical analysis

Results were disclosed as means or median. When Bartlett's test indicated that the group comparisons had equal variances Student T or one-way ANOVA and Tukey's multiple comparison post hoc tests were performed [23], [24]. When the group data showed unequal variances, nonparametric Mann-Whitney or Wilcoxon/Kruskal-Wallis and Dunn's multiple comparison post hoc tests were used. Correlations were evaluated for statistical significance with Pearson's test.

$P < 0.05$  was considered significant. Statistical tests were performed using SPSS software (version 15).

The study was performed in accordance with the principles of the Declaration of Helsinki. Approval was obtained from the hospital's Institutional Review Board and Ethics Committee and written informed consent was obtained from all patients and controls.

### III. RESULTS

The study evaluated 25 patients with HCV infection (RNA HCV between  $22808$  and  $2770 \times 10^6$  ui/ml) and 21 healthy subjects. Subjects were classified according to the underlying necroinflammatory activity into patients with active HCV hepatitis (A2-1 patient and A3-11 patients) and patients with inactive HCV hepatitis (A0-3 patients and A1-10 patients). Relevant demographic and biological data collected from HCV patients are presented in table 1.

TABLE I

BASELINA DEMIGRAPHIC, LABORATORY AND ACTITEST/FIBROTEST RESULTS OF PATIENTS WITH HEPATITIS C INFECTIONS

Variable	HCV patients	controls	p value
	mean $\pm$ SD/median value		
Age	50.58 $\pm$ 14.87	35.61 $\pm$ 13.90	0.0012
Sex (male/female) (number cases)	8/17	9/12	0.4473
Alanine amino-transferase (ALT) ui/l	70	25	0.0000
Aspartate amino-transferase (AST) ui/l	59	23	0.0000
White blood cell count	6.328 x 10 <sup>3</sup> $\pm$ 2.1248 x 10 <sup>3</sup>	7.5143 x 10 <sup>3</sup> $\pm$ 1.7746 x 10 <sup>3</sup>	0.0483
Lymphocytes	2.2560 x 10 <sup>3</sup> $\pm$ 0.8073 x 10 <sup>3</sup>	2.2857 x 10 <sup>3</sup> $\pm$ 0.7023 x 10 <sup>3</sup>	0.8957
CD4 cells/ $\mu$ l	836.136 $\pm$ 310.5243	899.615 $\pm$ 260.1405	0.5402
CD8 cells/ $\mu$ l	429.363 $\pm$ 183.9603	590.416 $\pm$ 198.8295	0.0239
CD4/CD8	2.1005 $\pm$ 0.6911	1.7658 $\pm$ 0.8659	0.2263
Serum 25(OH)D nmol/l	29.5714 $\pm$ 7.5514	29.2701 $\pm$ 9.4078	0.9046
Serum calcium (total) mg/dL	9.6000 $\pm$ 0.4651	9.800 $\pm$ 0.3225	0.1081
Serum calcium (ionized) mg/dL	4.1400 $\pm$ 0.2062	4.1750 $\pm$ 0.1713	0.5628
Serum phosphorus mg/dL	3.7304 $\pm$ 0.6034	3.8524 $\pm$ 0.6750	0.5303

#### A. Plasma level of 25(OH)D in chronic HCV hepatitis and healthy subjects

The 25(OH)D level revealed vitamin D deficiency in all patients ( $<50$  nmol/L). No differences were recorded related to age or sex criteria. Plasma level of 25(OH)D did not differ between the two groups of subjects : 29.5714 nmol/l in HCV patients and 29.2701 nmol/l in healthy controls ( $p = 0.9046$ , Student T). (Fig 1a).

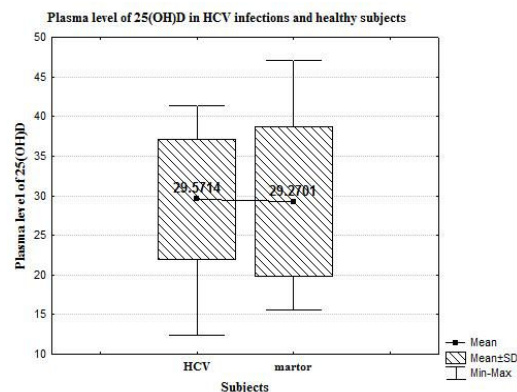


Fig 1a. Plasma level of 25(OH)D in chronic HCV hepatitis and healthy subjects

The lowest 25(OH)D plasma concentration was found in patients with cirrhosis (F4- 5 patients) but no statistic significant difference was found as opposed to chronic hepatitis (F3-11patients, F2—8 patients and F1=1 patients) and healthy subjects ( $p=0.2419$ , Student T). (Fig 1b)

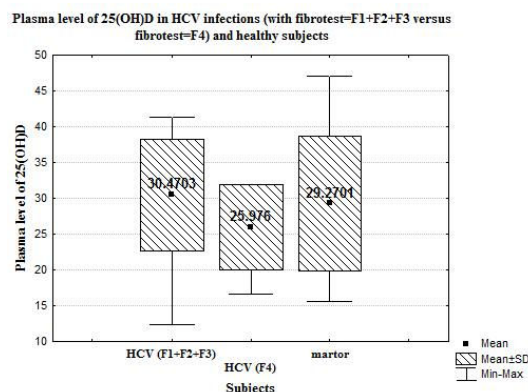


Fig 1b. Plasma level of 25(OH)D in HCV cirrhosis, chronic hepatitis and controls

#### B. Correlations between the 25(OH)D plasma level and CD4+Th cell count

A positive correlation was found for the total number of HCV patients ( $R=0.507$  Pearson correlation) and furthermore for those diagnosed with active HCV hepatitis ( $R= 0.703$  Pearson correlation). This correlation was not consistent with data recorded from inactive HCV patients ( $R=0.094$ ). (Fig. 2)

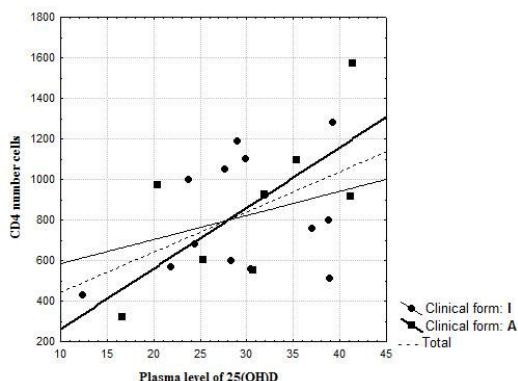


Fig. 2. Correlations between the 25(OH)D level and CD4+Th cell count in all HCV patients and the group of active (A) versus inactive (I) HCV patients

C. Correlations between the 25(OH)D plasma level and CD8+Th cell count

No significant positive correlation was obtained for either the total number of HCV patients ( $R=0.153$  Pearson correlation) or the active/inactive HCV patients ( $R=0.202$ , respectively  $R=0.094$ , Pearson correlation). (Fig3)

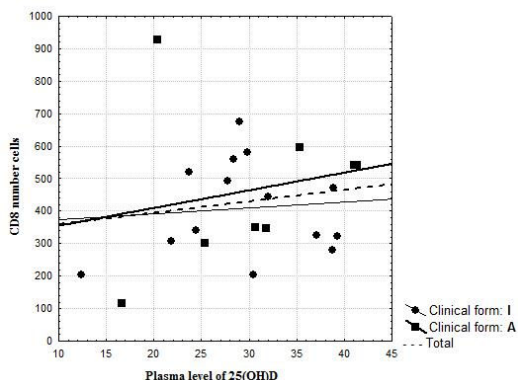


Fig 3. Correlations between the 25(OH)D plasma level and CD8+Th cell count in all HCV patients and the group of active (A) versus inactive (I) HCV patients.

D. Correlations between 25(OH)D plasma level and ALT and AST level

We have not found any significant correlation between 25(OH)D and serum aminotransferases for the total number of patients diagnosed with HCV infection. Nevertheless, a weak correlation was found in active HCV patients between plasma 25(OH)D and ALT level ( $R=0.530$  Pearson correlation) and a slightly negative one in patients with inactive HCV infection ( $R= - 0412$ ).

The correlation between plasma 25(OH)D and AST level was positive, close to the limit for active HCV patients ( $R=0.401$  Pearson correlation) and slightly negative for the inactive HCV group. ( $R= -0.553$ ). No such correlations were found in healthy controls. (Fig.4a and Fig. 4b)

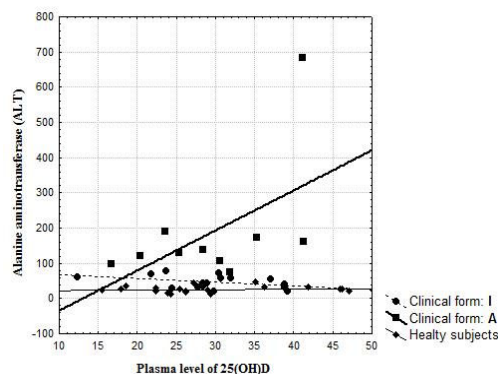


Fig. 4a. Correlations between plasma 25(OH)D and ALT level in inactive (I) versus active (A) HCV hepatitis

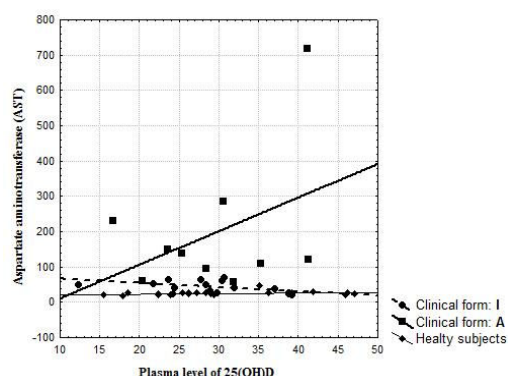


Fig. 4b. Correlations between plasma 25(OH)D and AST level in inactive (I) versus active (A) HCV hepatitis

E. Correlation between CD4+Th cell and CD8+Th cell count and calcium levels

A strong positive correlation was observed between the CD4+Th cell count and the total calcium values in patients with active HCV hepatitis ( $R=0.841$  Pearson correlation). These patients also presented a significant positive correlation between the CD4+Th cell count and the ionized calcium level ( $R=0.621$ ) as well as between CD8+Th cell and ionized calcium ( $R=0.652$  Pearson correlation) (Fig 5a and Fig 5b). No similar correlations were found in patients with inactive HCV infection.

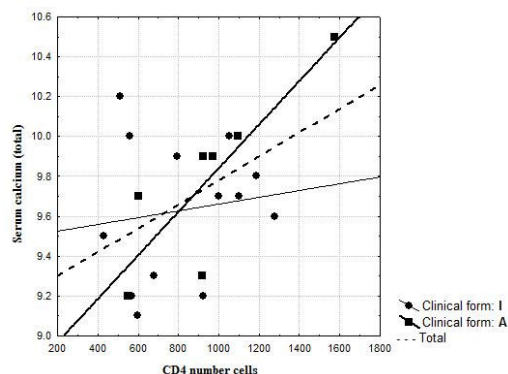


Fig 5a. Correlation between CD4+Th cell count and calcium level in inactive (I) versus active (A) HCV hepatitis

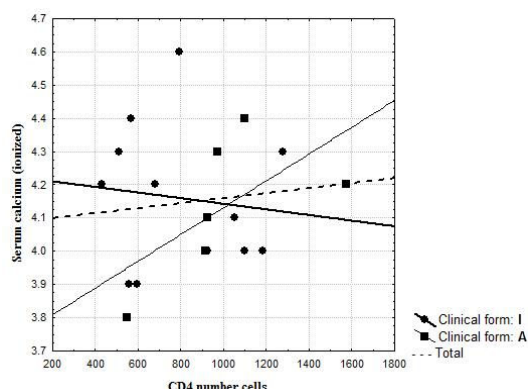


Fig 5b. Correlation between CD4+Th cell count and the ionized calcium level

#### IV. DISCUSSIONS AND CONCLUSION

Vitamin D is synthesized in the skin from 7-dehydrocholesterol following exposure to ultraviolet light or derived from dietary sources. The activation of vitamin D is the result of two subsequent hydroxylations catalyzed by P450 cytochrome. The first hydroxylation involves the liver, resulting 25(OH)D. Though inactive, this is the major circulating form of vitamin D. The second reaction leads to 1,25(OH)D, the active form of vitamin D. The latter reaction occurs primarily in the kidney, where it is regulated by the parathyroid hormone (PTH) and serum calcium. Antigen presenting cells (APC) cells (monocytes, macrophages, DCs) also produce 1,25(OH)D according to immune signals [25],[26]. The hypothesis that APC cells should produce high levels of 1,25(OH)D after immune stimulation [27] was the cornerstone for numerous studies regarding the immune regulating action of vitamin D. DCs are not only a site of extrarenal production of 1,(25)OHD, but also primary targets of the immunomodulatory activity of 1,(25)OHD resulting in the inhibition of DC's differentiation and maturation. Consequently DCs present tolerogenic properties, are less responsive to inflammatory chemokines, delay the inflammatory response induced by Th1 cells and stop the tissue damage. In addition, vitamin D promotes a simultaneous shift from Th1 to a Th2 cell response [28], [29], and induces apoptosis by caspase-independent and caspase-dependent pathways [30]. Although the importance of vitamin D in the inflammatory and immune response has already been proved in various studies the serum level required for the immune modulating function of vitamin D is still unknown and data obtained in vitro are contradictory..

Vitamin D deficiency and polymorphisms of VDR are cited in various auto-immune diseases and neoplasms [31]. Several studies have proposed vitamin D or vitamin D agonists as supplementary treatment in some diseases [32], [33]. The plasma level of 25(OH)D has also been assessed in infections. Of particular interest were tuberculosis, HIV infection and respiratory infections [34-36]. A systematic review of randomized controlled clinical trials having studied vitamin D plasma level in different infections highlighted the high

prevalence of vitamin D insufficiency in human populations worldwide, thus suggesting that normal vitamin D concentrations are needed to prevent infections [37]. Nevertheless certain results were questionable [38], [39] and further accurate studies on the immune regulatory action of vitamin D are required.

Vitamin D acts via specific nuclear receptors localized in most tissues and cells, including immunologically relevant cells. The recent discovery of VDR expression in hepatocytes, Kupffer cells and hepatic stellate cells [40-43] was correlated with bile acid synthesis, autoimmune activity, oncogenesis, liver cholestatic or noncholestatic lesions [44-48] and fibrinogenesis suppression [49]. Clinical studies suggested a correlation between 25(OH)D levels and progression of liver diseases [50]; others did not find a difference between 25(OH)D levels in cirrhotic and noncirrhotic patients [51] or between various Child-Pugh groups [52]. Research on 25(OH)D level and the therapeutic benefits was also initiated in hepatitis with B virus or C virus [11], [15], [16], [53], [54]. HCV chronic patients with certain VDR polymorphisms treated with interferon and ribavirin combination and supplemented with vitamin D, displayed an increased and sustained virological response. This favorable effect of vitamin D [16], [55-57] adds to the documented correlations observed between the low concentration of vitamin D and chronic necroinflammation or liver fibrosis [15]. To conclude with, most clinical studies recorded low vitamin D levels in chronic hepatic diseases. These also emphasized the vitamin D favorable effect in the HCV evolution and treatment.

Interestingly, in vitro studies reported strikingly different effects in the action of vitamin D, which ultimately appear to disprove the clinical observation. Thus, despite the clinical results, the experimental data, suggest a favorable effect of vitamin D on HCV progression.

Vitamin D immunomodulatory activity appears to synchronize with HCV activity in the initial stages of infection by following mechanisms:

- Vitamin D inhibits the development of CD4+Th1 cells which are essential for the HCV clearance [57-60];
- Vitamin D limits the inflammation through its negative effects on DCs [25] therefore enhancing HCV persistence [61], [62];
- Vitamin D as well as HCV promotes liver necroinflammation by enhancing cell apoptosis [63];
- Vitamin D as well as HC, enhances DCs immunotolerance [61];

Taking into account the aforementioned data, we studied the vitamin D status in HCV patients with chronic hepatitis and its correlation with the hepatic necroinflammatory response and the immune response.

We assessed the vitamin D status using the serum 25(OH)D value and observed an extremely low value of 25(OH)D (<50 nmol/l) in all patients. The 25(OH)D low plasma level could be regarded as a population deficiency rather than a consequence of the HCV infection. Numerous population

studies reported low serum levels of 25(OH)D in Romania as well as in other countries [64-66]. Although the above serum levels of vitamin D should classify it as a deficiency [67], the impact on the immune modulating action of vitamin D is puzzling and likely to be influenced by multiple factors. Therefore the influence of VDR polymorphisms has been recorded independently of the plasma vitamin D level in the evolution of hepatitis C or regarding treatment benefit [55]. Recent research advocated the key role of osteopontin, a vitamin D induced cytokine which controls the hepatic necroinflammation and hepatocarcinoma genesis [68]. Numerous other circumstances could influence the outcome of liver diseases irrespective of the 25(OH)D level. This clause could partly explain the discordant results of several studies regarding the plasma level of vitamin D in patients with chronic liver diseases [48], [52], [69-71], including those with hepatic osteodystrophy [8]. For this reason, the treatment with vitamin D in hepatic osteodystrophy, as well as in other hepatic illnesses is open to debate.

The present study found no correlation between 25(OH)D and hepatic fibrosis score as it was indicated by Fibrotest. Patients with cirrhosis (F4) expressed a slightly lower 25(OH)D value but no statistic significant difference was found compared to patients with low fibrosis (F0-F3) (25.9760 nmol/l versus 30.4703 nmol/ml). The study also assessed the serum ALT and AST values and their correlation with the 25(OH)D level. Patients with active HCV infection (A2-A3) presented higher plasma concentrations of ALT or AST, while lower values were recorded in patients with inactive HCV infection (A0-A1). No significant correlation was found between the serum 25(OH)D and the level of serum aminotransferases. Nonetheless active HCV patients displayed a weak positive correlation between 25(OH)D and ALT ( $R=0.530$ ). A weak negative correlation with AST ( $R= -0.553$ ) was observed in patients with inactive HCV. The vitamin D level appears to exhibit two different effects in the HCV infection. In patients with necroinflammation (active hepatitis in this study) the effect would be that of further exacerbating the hepatocytolysis. The opposite action could be observed in patients without necroinflammation (inactive hepatitis). However the number of patients included in this study was small and the correlations were subsequently weak. The aforementioned observations need to be reevaluated on a larger scale.

We also assessed the correlation between vitamin D plasma level and immune response in HCV and healthy patients. The clinical effect of vitamin D in the immune HCV-triggered liver injury is less known. In vitro studies revealed that 1,25(OH)D is a potent inhibitor of human CD4+ and CD8+ Th lymphocyte proliferation. In our study we evaluated the absolute count of CD4+Th cells and CD8+ Th cells in the blood of HCV patients and controls and its correlation with the 25(OH)D plasma level. The mean CD4+Th cells count was slightly decreased in HCV patients compared with controls (836 cells/ $\mu$ l compared with 899 cells/ $\mu$ l ( $p=0.54$ ). Concerning sex,

it was lower in men than in women (771 cells/ $\mu$ l and 911 cells/ $\mu$ l respectively) but without statistically significant differences. A weak positive correlation was found between 25(OH)D and the CD4+Th cells count ( $R= 0.507$ ) for the total number of HCV patients. The same correlation for patients with active hepatitis proved stronger ( $R= 0.703$ ). The mean CD8+Th cells count was considerably decreased in HCV patients compared with controls (429 cells/ $\mu$ l compared with 590 cells/ $\mu$ l, ( $p=0.02$ ) with a weak positive correlation towards the CD4+Th cells count ( $R= 0.573$ ). Correlations between the number for CD4+ Th cells and CD8+Th cells were not recorded for the total number of patients. Likewise, no significant correlation was found between the 25(OH)D level and the CD8+Th cell count neither for the total number of patients, nor for the active or inactive lot. One could hypothesize that the transitions concerning the total number of CD4+Th cell and CD8+Th cell are a characteristic feature in the chronic evolution of HCV infection and inadequate for suggesting an immune deficiency [72]. Still the positive correlation between 25(OH)D and the CD4+Th cell count (only in patients with active HCV) is strong enough to infer that a connection could exist between these values. Moreover, a positive correlation between 25(OH)D and the CD4+Th helper cells count was also documented in other chronic infections, such as the HIV infection [35], [73]. Unfortunately, the present study did not assess the major subtypes of CD4+Th cells in order to establish which population is responsible for the positive correlation with the 25(OH)D concentration.

This positive correlation contradicts the experimental data [74] as the latter describes a distinctive inhibitory effect of 1,25(OH)D on Th cells proliferation, even in inflammatory foci cytokine-rich environment, as should be the case in chronic hepatitis [75], [76]. Nevertheless, it is to be noted that in vitro studies regard the sole action of 1,25(OH)D, while the present study, as well as other clinical trials [15] addressed only the 25(OH)D concentration, a stable compound easy to assess. Prospective clinical studies should also approach the direct implications of 1,25(OH)D on cellular immunity in the HCV infection.

The calcium level was normal in all patients with no alterations of the phosphocalcium equilibrium. We recorded strong positive correlations between the concentration of ionized calcium and the CD4+Th cell and CD8+Th cell count respectively. That is, active HCV patients exhibited a strong positive correlation between CD4+Th cell count and total calcium values ( $R=0.841$ ) and a positive correlation between CD8+Th cell and ionized calcium ( $R=0.652$ ) (Fig 5a and Fig 5b). These findings indicate the possibility that serum calcium holds a role in stimulating the cellular immunity in chronic HCV hepatitis. The importance of calcium in the proliferation of Th cells and its correlation with the level of 1,25(OH)D should be further investigated.

## V. CONCLUSION

Low levels of 25(OH)D were recorded in both HCV patients and controls. The role of vitamin D in the outcome of HCV infection could differ depending on the stage of necroinflammation; active hepatitis forms with underlying necroinflammation (A2-A3) displayed positive correlations between CD4+ Th cell count, the 25(OH)D level and the total serum calcium; no such findings were present in hepatitis forms without necroinflammation. Further studies are required regarding the effects of vitamin D and the inflammatory response in the HCV infection in view of the wide spread implications of VDRs in inflammatory, auto-immune and malignant processes.

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