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Peripheral blood regulatory T cell measurements correlate with serum vitamin D level in patients with psoriasis

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Abstract. – OBJECTIVE: Vitamin D is the precursor of a hormone (1,25-dihydroxyvitamin D3), which has many biological effects in the skin. The immune modulator properties of vitamin D are mediated in part through effects on regulatory T cells (T-reg). Currently, in psoriasis, the relationship between vitamin D and T-reg has not well elucidated.

We assess whether vitamin D status is correlated with circulating T-reg in patients affected by psoriasis and if there is a correlation with the severity of the disease evaluated with Psoriasis Area Severity Index (PASI) score

PATIENTS AND METHODS: For each patient we have analyzed, PASI-score, serum levels vitamin D and regulatory T cell percentages. Spearmen's coefficient was used between serum vitamin D levels and the predictors. Subsequently, the independent predictive factors were assessed by Multiple Regression.

RESULTS: A total of 26 patients were included in our analysis. Using no parametric Spearman's Coefficient test between serum levels of vitamin D and the single variables, we found an association with T-reg population ($p < 0.001$) and with PASI-score ($p = 0.04$).

CONCLUSIONS: While vitamin D treatment induces a cytokine profile known to favor the differentiation of T cells with suppressive activity, at the same time, several studies showed how vitamin D can prime for tolerogenic dendritic cells able to favor the differentiation of Treg from T naïve cells. Low levels of vitamin-D may decrease the number of circulatory T-reg, disrupting the immunological homeostasis in psoriatic patients and encouraging the inflammatory activity.

Key Words:
Psoriasis, Vitamin D, T regulatory cells.

Introduction

Vitamin D is the precursor of a hormone (1,25-dihydroxyvitamin D3), which has many biological effects in the skin (e.g. the regulation of the cell growth and a pivotal role in the immune system). Normal conditions of vitamin D are due to the sun exposure, supplying about 90% of the vitamin D demand; while, the diet contributes to about 10%. As known, psoriatic patients show low levels of vitamin D[1,2] and treatment with vitamin D derivate improves clinical condition in this class of patients[3].

The immune modulator properties of vitamin D are mediated in part through effects on regulatory T cells (T-reg)[4]. In fact T-reg can be also induced in the peripheral tissues from the naïve cells following antigen presentation by tolerogenic dendritic cells due to the exposure to vitamin D[5]. Besides, experimental studies showed that the biologically active metabolite of vitamin D (1,25-dihydroxyvitamin D) is able to direct the T cell compartment into a more anti-inflammatory and regulated state, inhibiting Th1 and Th17 and promoting Th2 and T-reg[6-8]. Though previous studies have documented the *in vitro* effects of vitamin D on T-reg production, up to date, no study has examined possible links between this micronutrient and T-reg levels in psoriatic patients.

In the present report, we assess whether vitamin D status is correlated with circulating T-reg in patients affected by psoriasis and if there is a correlation with the severity of the disease according to the evaluation of the Psoriasis Area Severity Index (PASI) score.

Patients and Methods

We included in this work 26 Caucasian subjects affected by moderate to severe psoriasis. Inclusion criteria were: age > 18 years old, history of psoriasis for at least 6 months with or without articular involvement and no systemic treatment

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for at least 2 months, before the date of the blood sampling.

Exclusion criteria were: treatment with systemic or biological therapies, vitamin D supplements and other relevant medical conditions at the investigator's discretion. The characteristics of the patients are described in Table I.

A written informed consent was acquired from all subjects participating in this study, according to the declaration of Helsinki. The study was approved by Ethics Committee.

The aim of our study was to evaluate the correlation between serum levels of vitamin D and the predictors age, T-reg (expressed in percentage) and PASI-score; while, separately, in a second time, our aim was to evaluate the correlation between T-reg and PASI-score

All vitamin D samples were assayed at the same laboratory; vitamin D levels ≥ 30 ng/ml have been considered in the *normal* range, serum levels between 20 ng/ml and 29 ng/ml as *insufficiency* and levels ≤ 19 ng/ml as *deficiency*. All the analyses have been performed in the period between the month of April and September at a latitude of 41°54'39"24 North. The collected serum was immediately shielded from direct light and stored at 220 uC. At the end of the study, all samples were analyzed simultaneously. The serum values of vitamin D were measured with a commercially available radioimmuno-assay (Immunodiagnostic Systems Holdings, Boldon, UK).

Peripheral blood lymphocytes were isolated through Lymphoprep density gradient centrifugation (Nycomed Pharma AS, Oslo, Norway). Cells were first incubated with fluorochrome-conjugated monoclonal antibodies against surface antigens, anti-CD3-Percp, anti-CD4-FITC and anti-CD25-APC, for 20 min at 4 °C; then cells were fixed, permeabilized and intracellularly stained with anti-FOXP3-PE according to the manufacturer's protocol, using fixation and permeabilization buffers (all antibodies and reagents were purchased from BD-Biosciences, San José, CA, USA). Multicolor immunofluorescence analysis was performed using a FACSCalibur cytometer (BD Biosciences, San José, CA, USA) and Cellquest software (BD Biosciences, San José, CA, USA or alternatively FLOWJO software (Treeastar, CA, USA). The evaluation of the T-reg subpopulations by flow cytometry was determined as the frequency of CD25+brightFOXP3+ expressing cells within the CD3+CD4+ compartment.

Statistical Analysis

For the patients the following parameters have been analyzed: (≤ 50 or ≥ 51 years), PASI-score (≤ 9 and ≥ 10), serum levels vitamin D and regulatory T cell percentages.

Assuming that the effects of the predictive variables are constant over time, we used the Spearmen's coefficient between serum vitamin D levels and the predictors. Subsequently, the independent predictive factors were assessed by multiple regression. To study a correlation between T-reg and PASI score an Odds-ratio was also included in the analysis.

In all statistical methods a p value < 0.05 was considered statistically significant.

Results

A total of 26 patients were included in our analysis. They were 15 male versus 9 female. The median age was 55.5 years (ranging between 23-85). Regarding PASI-score, the median PASI was 12.3 (ranging between 5.6 and 29.8).

Median levels of vitamin D were 15.85 ng/Ml (ranging between 8 and 28 ng/mL); precisely no patient reached the minimum levels of sufficiency, 6 patients showed an *insufficiency* of vitamin-D, while the remaining 20 patients a *deficiency*. (Figure 1A).

Table I. Patients characteristics.

	Median/N (%)	Range (min-max)
Male/Female (N)	15 (57.69%)/9 (42.31%)	–
Age (years)	56.8	23-85
Duration of disease (years)	20.35	3-53
PASI score	13.7	5.6-29.8
Treg%	3.97	2.3-5.89

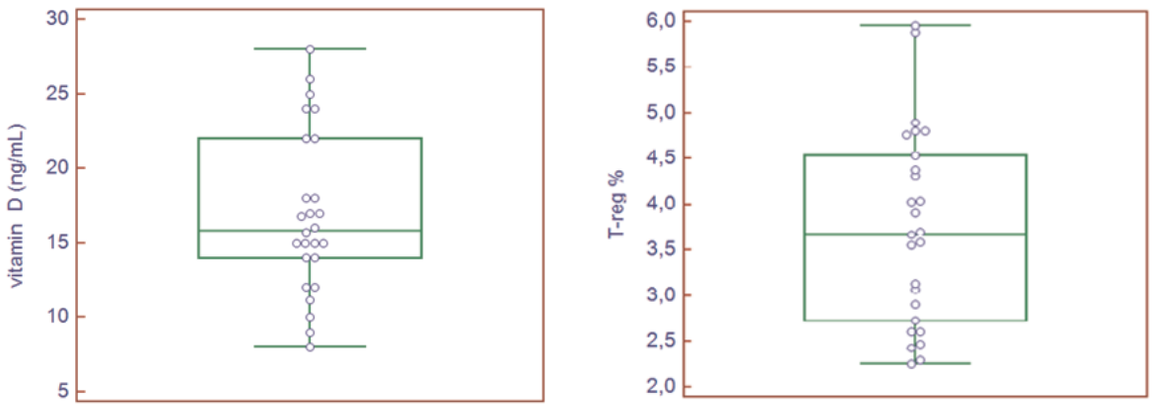

Figure 1. ***A,*** Box-Plot. Median levels of vitamin D levels. ***B,*** Box-Plot. Distribution of T-reg percentages.

Mean T-reg percentages were found to be in a low range for all the subjects analyzed. In fact, the value of T-reg was mainly localized in the range between 3.5% and 4% (minimum value 2.67% and maximum value 5.9%). (Figure 1B) Specifically, only 2 patients showed T-reg values ≥ 5%; besides, these patients were also the ones with the highest levels of vitamin D.

Using no parametric Spearman's coefficient test between serum levels of vitamin D and the single variables, we found an association with T-reg population ($p < 0.001$; Spearmen's coefficient: 1), with PASI-score ($p = 0.04$; Spearmen's coefficient: - 0. 37), but not with the age ($p = 0.4$; Spearmen's coefficient: 0.2) (Table II). Performing multiple regression, T-reg population remained the only statistically significant, predictive variable ($p < 0.001$), while PASI-score ($p = 0.08$) and age ($p = 0.3$) did not reach the statistical significance (Table II).

Finally, we found that patients with a PASI-score ≥ 10 showed lower percentage of T-reg levels (around 3.5%), than patients with a PASI-score ≤ 9 (around 4.5%). These values were statistically significant in both no parametric Spearman's coefficient ($p < 0.001$; Spearmen's coefficient: -0.8) and in Odds-ratio ($p < 0.003$; OR: 4) (Table III).

Table II.

	p^a	p^b
T-reg%	*< 0.001*	*< 0.001*
Age	0.4	0.3
PASI-score	0.04	0.08

Vitamin-D levels in correlation with T-reg%, age and PASI-score p^a indicates no parametric Spearman's coefficient test between serum levels of vitamin D and the single variables; p^b indicates multiple regression between serum levels of vitamin D and the single variables. In *Italic* significant values.

Discussion

Vitamin D status has been linked with multiple sclerosis (MS) either in large epidemiological studies, as well as in experimental *in vitro* and animals studies[9,10]. This is the first *in vivo* study, which has investigated the correlation between vitamin D status and T-reg function in psoriatic patients.

Our data showed in both univariate and multivariate analyses that serum vitamin D levels correlate positively with serum levels of T-reg; as well as a positive correlation between serum vitamin D levels and the severity of psoriasis (PASI) was observed (Table II). In a second time, the same correlation was maintained between T-reg and PASI-score (Table III).

In our report, the serological analysis have been performed during a period with usual high production of vitamin D due to environmental (between

Table III.

T-reg%	PASI	p^a	p^b
↓	↑	< 0.001	< 0.003

Variations of T-reg% population and PASI-score evaluated with no parametric Spearman's coefficient test (p^a) and Odds-Ratio (p^b).

the month of May and September) and latitudinal aspects (41°54'39"24 latitude North). In fact, the intensity of UV radiations is crucial in the production of vitamin D, since it has been defined a seasonal variability, with increased production in summer and decreased production in winter. Although all these aspects, the median serum levels of vitamin D was generally low in our patients, as reported in the Box-plot (Figure 1B).

Our findings emphasize the role of vitamin D in the pathogenesis of psoriasis and its immune regulatory role. Low levels of this micronutrient may decrease the number of circulatory T-reg, disrupting the immunological homeostasis in psoriatic patients and encouraging the activity of Th1, Th17 and Th22. Besides, while Fururahshi et al[11] showed a relation between reduction of PASI and restoring of T-reg population (after a photo-chemotherapy treatment and without focusing on the serological levels of vitamin D), we have found the same relationship with respect to the vitamin D serological levels.

However, currently, the relationship between vitamin D and T-reg has not well elucidated in psoriasis. In this regard, while some authors[12] reported that topical vitamind D enhances the suppressive capacity of CD4+CD25+ (Foxp3+) cells residing in the skin-draining lymph nodes (SDLN) of mice, not[13] otherwise exposed to antigen, other authors showed how chronic treatments of mice with calcipotriol and ovoalbumin (OVA), applied to the same site, can result in the expansion of OVA-specific regulatory T cells in the draining lymph nodes. Finally, RANKL expression has been linked with the expansion of regulatory T cells following UV irradiation[14], as well as with chronic topical calcipotriol, in a VDR-dependent manner[13]. All these aspects could indicate significant correlations between vitamin D and T-reg, in psoriatic patients. In fact, while vitamin D treatment induces a cytokine profile known to favor the differentiation of T cells with suppressive activity[15,16], at the same time, numerous studies have also shown how vitamin D can prime for tolerogenic dendritic cells able to favor the differentiation of Treg from T naïve cells[17,18].

Conclusions

According to our finding, vitamin D is an immune modulator, but if it can be considered as a disease modulator in psoriasis remains uncertain. Before that the conclusions can be drawn about the potential role of vitamin D as an immune modulator in psoriasis through T-reg, further studies investigating the immune modulating mechanism will be necessary.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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