# DIVERSITY OF CD1A POSITIVE CELLS IN CASE OF 25-HYDROXYVITAMIN D DEFICIENCY IN PATIENTS WITH METABOLIC SYNDROME

Jana Janovska[1], Julia Voicehovska[2], Regina Kleina[3], Janis Kisis[4], Raimonds Karls[5], Olga Zubova[6], Sergejs Babikovs[7]

**Abstract:** Vitamin D has immunomodulatory properties, which influence the immune system through a number of mechanisms, including the activation of dendritic cells (DCs). Langerhans cells (LCs) are dendritic cells in epidermis and belong to the skin immune system. DCs are professional antigen-presenting cells playing a major role in the induction of immune responses by activating native T-cells. In literature, there are no reports regarding the influence of vitamin D on DCs in patients with metabolic syndrome (MS). Thus, the aim of this study is to explore potential immunomodulatory activity of vitamin D on LCs in case of metabolic syndrome.

In this study, we have conducted an analysis on a group of patients, both male and female, diagnosed with metabolic syndrome between the age of 40 and 55. Patients' clinical examinations, measurement of blood pressure, and waist circumference were conducted. Blood biochemical analyses (cholesterol, HDL, LDL, vitamin D level, etc.) were also determined. Full-thickness circular 4-mm *Punch* biopsies were taken from 49 patients. Specimens were stained with haematoxylin and eosin, as well as immunohistochemistry using a transmembrane CD1a Langerhans' cells marker was parformed by DakoCytomation EnVision method.

The average age of patients is 43 years, and mean waist circumference is 95 cm. Total cholesterol is 5.5 mmol/l, LDL is 2.3 mmol/l, and average 25-hydroxyvitamin D is 27.0 ng/ml. In the skin conditioned with MS and low vitamin D level, evidence of perivascular accumulation of LCs in papillary dermis is observed, as well as a diffusion of mild interstitial cluster of LCs in some cases. In epidermis activity, the amount and filling of Birbeck's granules is changed in cases of 25-hydroxyvitamin D deficiency. In patients with low 25-hydroxyvitamin D level, an average LC quantity in one field of vision is higher in comparison to those who have normal amount of 25-hydroxyvitamin D. Therefore, it is necessary to further investigate vitamin D activity on LCs in cases of metabolic syndrome in order to determine interactions with lymphocytes, plasma cells, and mast cells as a part of the skin immune system.

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## Introduction

The skin is the largest organ in the human body. A network composed of delicate physical, chemical, and immunological barriers in the skin makes it a perfect organ to protect the integrity of the human body. Anatomically, skin is divided into epidermis, dermis, and subcutaneous tissue, from the superficial to deep tissues. Dendritic cells (DCs) are found in epidermis and tend to migrate and drain into deeper layers of the lymphatic system.

DCs represent a heterogeneous cell population residing in most peripheral tissues, particularly at sites of interphase with the environment, e.g. skin and mucosa. They represent 1–3% of total cell numbers of these tissues (Banchereau & Steinman, 1998).

In the periphery, immature DC (iDC) can capture and process antigens. Thereafter, they migrate towards T-cell rich areas of the secondary lymphoid organs through the afferent lymphatics. Antigens are presented in such a way that antigen-specific native T-cells become activated and start to proliferate. Moreover, when DC initiates a T-cell-mediated adaptive immune response, they also play an important role in polarization of T-cell reactivity (Kissenpfennig & Malissen, 2006).

[1] Corresponding author: Jana Janovska, Riga Stradins University, Latvia, j.a.janovska@gmail.com
[2] Julia Voicehovska, Riga Stradins University, Latvia, dr.julia.v@gmail.com
[3] Regina Kleina, Riga Stradins University, Latvia, rkleina@inbox.lv
[4] Janis Kisis, Riga Stradins University, Latvia, profkisis@inbox.lv
[5] Raimonds Karls, Riga Stradins University, Latvia, raimonds@dr.lv
[6] Olga Zubova, Riga Stradins University, Latvia, doczubova@gmail.com
[7] Sergejs Babikovs, Riga Stradins University, Latvia, sergejs.babikovs@gmail.com

In the skin, these cells are dedicated antigen-presenting cells (APCs) that play a key role in sensing danger and initiating both innate and adaptive responses, as well as protecting the skin from invading pathogens. The Langerhans cells have generated considerable interest since it were first described by Paul Langerhans in 1865 as aureophilic cells present within the epidermis. At first, they were falsely described as melanocytic generation cells due to their appearance. However, lately it was discovered that LCs express major histocompatibility complex (MHC) class I molecules (Tony & Ronald, 1994), which indicated LC as an important immunological antigen-presenting cells in the body (Austyn, 1987). These LCs represent approximately 1–3% of epidermal cells and form a constituent part of the skin immune system. LCs are typically characterized by the expression of CD1a and a unique cytoplasmic organelle named *Birbeck granule* (BG). BGs constitute a subdomain of the endosomal-recycling compartment, perhaps being involved in antigen-loading process (Van Wilsem, Breve, Kleijmeer, & Kraal, 1994). It has been postulated that bone-marrow-derived myeloid cutaneous lymphocyte associated antigen (CLA)-expressing LC precursors travel through peripheral blood through the dermis into the epidermis. The final differentiation of LC precursors depends on the cytokine environment of the epidermis (Van Wilsem et al., 1994). The cutaneous cytokines, such as granulocyte–macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-15 and TGF-$b_1$, contribute to the establishment of immature LCs in the epidermis. During inflammation, however, circulating precursors may play an important role in replenishing the local pool of LCs.

LCs have a different function depending on location. In the periphery, immature LCs capture and process antigens. In afferent lymphatics, LCs present antigens and acquire the capacity to present antigens to native T-cells (described as maturation of LCs). Moreover, there is the T-cell activation and proliferation (T-cell priming), as well as polarization of T-cell reactivity toward type- 1 and/or type-2 responses. In epidermis and dermis, LCs participate in the recognition of invading pathogens (viral, bacterial, etc.), toxins and harmful irradiations (Elbe et al., 1989). An over-exposure to ultraviolet radiation (UV) from the sun might potentially affect skin immunosuppression. Under UV radiation, direct (intrinsic) keratinocyte damage develops in epidermis via apoptosis with clustering of death receptors on the cell surface (extrinsic), and generation of reactive oxygen species (ROS). When apoptotic keratinocytes are processed by adjacent immature Langerhans cells, the inappropriately activated LCs could result in immunosuppression. Furthermore, UV can deplete LCs in the epidermis and impair migratory capacity (Schaerli, Willimann, Ebert, Walz, & Moser, 2005).

Vitamin D3 exerts pluripotent effects on adaptive immune functions, such as T-cell activation and maturation of dendritic cells. In addition, it has been suggested that vitamin D3 increases innate immunity in skin and to enable efficient antimicrobial defense at epithelial surfaces (Toebak & Gibbs, 2009). The surface of our skin is constantly challenged by a wide variety of microbial pathogens, but cutaneous infections are relatively rare. Within cutaneous innate immunity, the production of antimicrobial peptides (AMPs) is a primary system for protection against infection. Many AMPs can be found on the skin, and these include molecules that were discovered for their antimicrobial properties, and other peptides and proteins first known for activity as chemokines, enzymes, enzyme inhibitors, and neuropeptides. Cathelicidins were among the first families of AMPs discovered on the skin. They are now known to have two distinct functions, i.e. they possess direct antimicrobial activity and will initiate a host cellular response resulting in cytokine release, inflammation, and angiogenesis (Lee & Wu, 2013).

Synthesis of pre-vitamin D3 from 7-dehydrocholesterol occurs in skin and involves UVB radiation that penetrates the epidermis. It is known that 7-dehydrocholesterol absorbs UV light most effectively at wavelengths between 270–290 nm and, thus, the production of vitamin D3 will occur at those wavelengths. “Calciol” - the product of the transformation of 7-dehydrocholesterol, is an inactive, unhydroxylated form of vitamin D3. To form an active hormone, calciol must be hydroxylated twice

to form "calcidiol" (25-Hydroxyvitamin D3, "25-D3") and finally active "calcitriol" (1,25-Dihydroxyvitamin D3, "1,25-D3"). The two enzymes responsible for activating vitamin D3—vitamin D 25-hydroxylase (CYP27A1) and 25-Hydroxyvitamin D3 1-a- hydroxylase (CYP27B1) were initially identified in the liver and kidney (Yim, Dhawan, Ragunath, Christakos, & Diamond, 2007; Schauber, Dorschner, Yamasaki, Brouha, & Gallo, 2006). Also, keratinocytes express both enzymes and are capable of producing active 1,25-D3 independent of renal and hepatic hydroxylation steps (Prosser & Jones, 2004).

Vitamin D3 exerts pluripotent effects on adaptive immune function through:

- Adaptive T-cell immune reaction.
- Maturation and proliferation of LCs from immature dendritic cells (iDCs).
- Increasing innate immunity in skin and regulating antimicrobial defense at epithelial surfaces (cathelicidin expression).
- Regulation of keratinocyte differentiation and proliferation, as well as production of intact epidermal barrier.
- Changes in serum D vitamin level that may impact skin immunity, barrier functions, inflammatory reactions (Dixon et al., 2013; Van Etten & Mathieu, 2005).

In the skin, presence of vitamin D3 is essential for normal keratinocyte development, differentiation, and function (Schauber & Gallo, 2009). Any alteration in the local vitamin D3 concentrations and/or activation will likely affect normal cutaneous immune function, barrier function, and inflammatory responses (Yim et al., 2007; Bikle, 2004).

The minimal daily concentration of vitamin D is well known and its amount for adults, who are not in the risk group, is 1000 IU per day. Those who are in a risk group (elderly, dark-skinned, live at certain latitude, work at certain time of day, use sunscreens, have fat malabsorption, use anticonvulsant, have a chronic kidney disease, obese) need at least 2000 IU per day.

It has been reported that exposure of 6–10% of the body surface to one minimal erythemal dose, which is defined as the minimum amount of UVB radiation that induces redness in 24 hours after exposure, is equivalent to ingesting about 600–1000 IU of vitamin D (Oda et al., 2004).

Obesity is one of the risk factors for low vitamin D status that we consider investigating for patients with metabolic syndrome. According to the International Diabetes Federation [IDF] definition (Alberti & Zimmet, 2006), a person can be characterized as potentially having metabolic syndrome if he or she has central obesity (waist circumference $\geq$ 80 cm and $\geq$ 94 cm for women and for men, respectively), along with two of any of the following factors: high triglycerides, low HDL cholesterol, high blood pressure, and high plasma glucose. In the skin, metabolic syndrome causes a chronic latent inflammation that impact all skin functions, such as barrier, immune, and endocrine function. Due to obesity there are inadequate balance between HDLs and LDLs, which may reduce defensive skin immunological responses to external factors. The lipid abnormalities might facilitate and maintain the inflammatory reaction in the skin. Mainly, activity of macrophages and increased activity of matrix metalloproteinase are involved. There are currently no reports regarding the influence of vitamin D on DCs in MS patients.

## Research objectives

The aim of this research is to explore the potential immunomodulatory activity of vitamin D on LCs in the cases of metabolic syndrome.

## Materials and methods

49 patients from both genders between 40 and 55 years of age have been analyzed (I–III Fitzpatrick's skin phototype) in a dermatology clinic in Riga, Latvia. The respective study employed the group of patents with clinically and biochemistry proven diagnosis of MS which based on International Diabetes Federation criteria (IDF, 2006).

In the course of the study, several parameters were evaluated among the patients:

- Clinical examination entailing measurement of the arterial blood pressure and waist circumference;
- Instrumental examination of skin surface with "ARAMO SG" - Skin Analyzer (Aramhuvis Co.) with three different lenses (1x, 10x & 60x) for determining the moisture, sebum level, surface evenness, dyspigmentation, capillary characteristics, and skin micro-relief.
- Biochemical testing: total cholesterol level (TH), fasting plasma glucose, high density lipoproteins (HDL) levels, low density lipoproteins (LDL) levels, and 25–Hydroxyvitamin D.
- Full–thickness circular 4–mm *Punch* biopsies were obtained from the dorsal surface of the palm. Specimens were stained with haematoxylin-eosin and with Masson's trichrome.

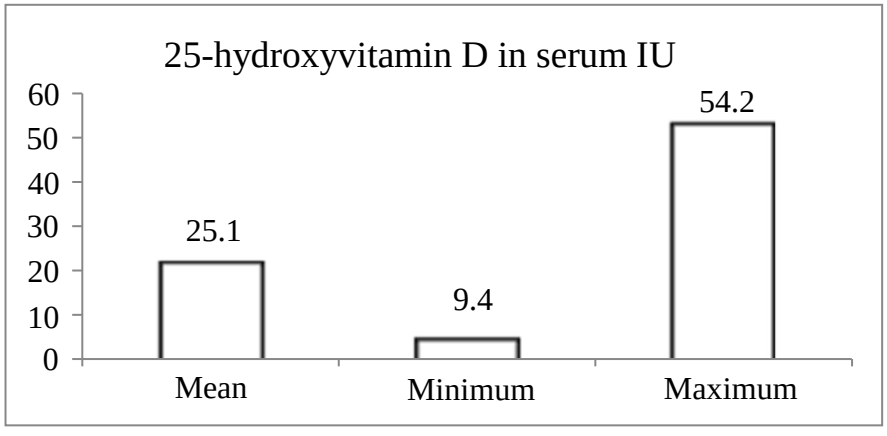
Immunohistochemical antibody staining was conducted with DakoCytomation EnVision system (Denmark) for the following antigens: CD1a for Langerhans cells. Axiostar Plus microscope with a ruler was used to measure adipocyte dimensions under 400x magnification. CD1a positive Langerhans cells were evaluated in three fields of vision. The average quantity of CD1a positive Langerhans cells was calculated under 400x magnification. All data were documented and analyzed with Microsoft Excel.

## Results

The group of 49 patients with MS has a mean waist circumference of 95 cm for both genders. An average body mass index (BMI) is 25.5 kg/m². The ratio of women-to-men is 1.6:1. The mean, minimum, and maximum levels of 25–hydroxyvitamin D are shown in Figure 1, assuming a normal vitamin D level in serum in average adults varies between 30–100 IU.

Regarding the blood biochemistry, an average cholesterol level is 6.1 mmol/l, LDL is 3.5 mmol/l, HDL is 0.5 mmol/L, and CRP is 2.1 mg/L.

Figure 1: Vitamin D level in serum




Source: Authors

Through dermatoscopic imaging, in the case of MS, facial skin dyspigmentation and pronounced telangiectasia were revealed, yet skin micro- relief has not been altered.

Average thickness of epidermis was 0.71 mm (patients with MS) and 0.46 mm (without MS). Thickness of *stratum spinosum* was 0.48 mm (patients with MS) and 0.25 mm (without MS). Thickness of *stratum corneum* was 0.18 mm (patients with MS) and 0.16 mm (without MS). Enlargement of adipocytes up to 0.13 mm and fibrosis of deep vessels have been revealed. Amount of DCs in epidermis varied from 32.6 to 13.5; quantities per field of vision are shown in Table 1.

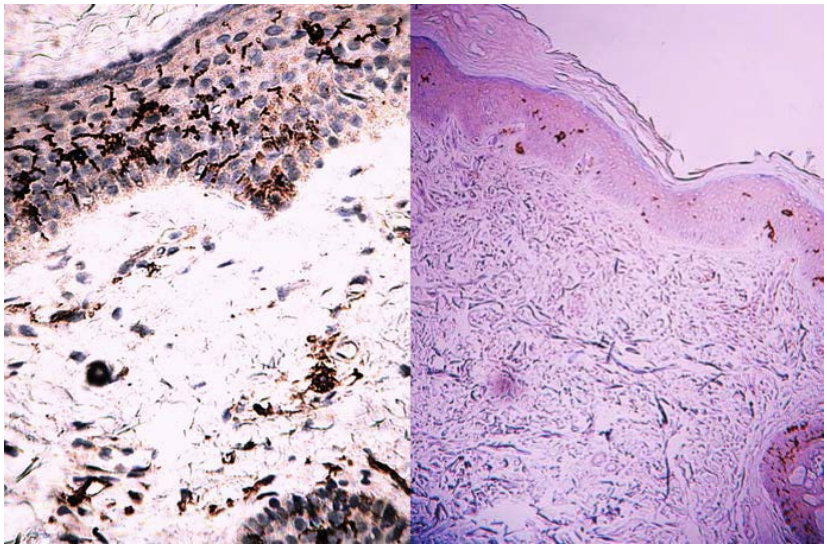
Table 1: Association of 25-hydroxyvitamin D level in serum and amount of LC in epidermis.

| 25-hydroxyvitamin D level in serum (IU) | Average amount of LCs per field of vision |
|---|---|
| 8.3 | 18 |
| 10.3 | 25 |
| 20.8 | 13.1 |
| Up to 30 | 7.7 |

Source: Authors

In cases of low vitamin D level, the number of CD1a+ cells was 16.2 and there was a tendency in an increase of LCs in epidermis, which is shown in Figure 2.

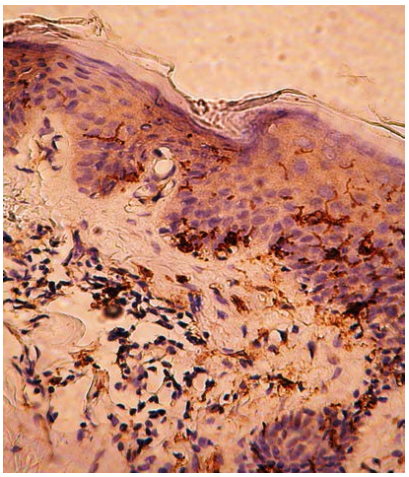
Figure 2: Increased number of LC in case of vitamin D deficiency (left) (400x, DakoCytomation EnVision), adequate amount of LC (right) (100x).



Source: Authors

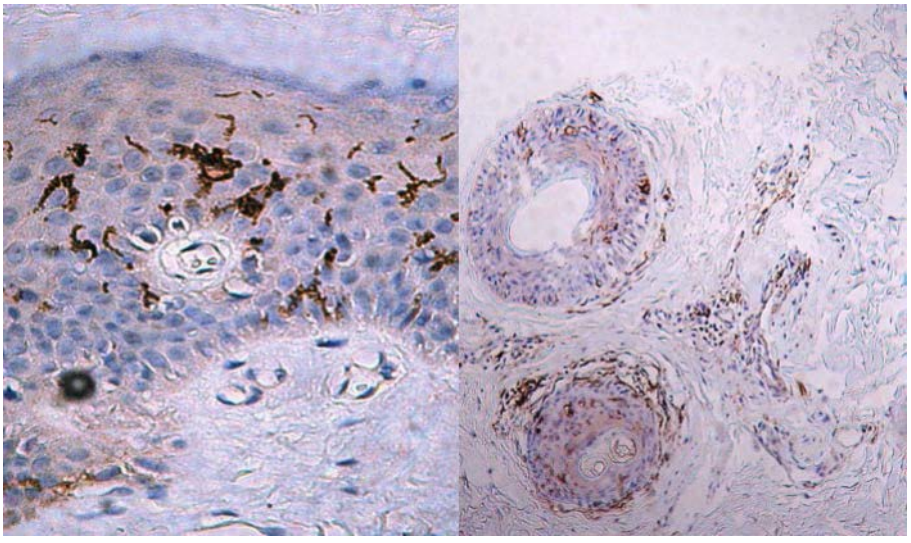
Significant accumulation of DCs around the cluster of inflammatory cells, acanthosis and hyperkeratosis were observed. Variable content of Birbeck's granules in patients with MS was evaluated, as well as in the cases of CD1a+ cells migration into papillary dermis (Figure 3). CD1a+ cells were found around apoptotic lesion in epidermis, as well as a mild inflammatory reaction around two skin appendages (Figure 4).

Figure 3: Migration of Langerhans cells into dermis (400x, DakoCytomation EnVision).



Source: Authors

Figure 4: Concentration of CD1a+ cells around apoptotic keratinocytes (left) (400x), mild inflammatory reaction and CD1a+ cells around appendages (100x, DakoCytomation EnVision).



Source: Authors

## Discussion

The immune system exists in a delicate equilibrium between inflammatory responses and tolerance. DCs are the primary professional antigen-presenting cells (APCs) initiating adaptive immune response. In this review, we summarize accumulating evidence about the role of regulatory DCs in situations where the balance between tolerance and immunogenicity has been altered leading to pathologic conditions, such as diffused chronic inflammation (Bikle et al., 2004).

There are numerous articles describing LCs peculiarities in case of local skin lesion, such as melanoma, basal cell carcinoma, etc. The role and characteristics of LCs in basal cell carcinoma (BCC) is revealed. Thus, the lower density and fewer morphological changes of LCs in the epidermis overlying BCC may give rise to alterations in the immune response to BCC (Zhang & Naughton, 2010). Another article, (Santos, Mello, Santos, & Santos, 2010), has described significant increase in the number of LCs in the group with lower potential of local aggressiveness, as compared with the

number of cells in the epidermis superposed to the basal cell carcinoma, thus limiting the aggressiveness of the neoplasm. Potapova, Luzgina, & Shkurupiy (2008) and Prignano et al., (2001) underlined the role of LCs in skin chronic inflammatory diseases such as psoriasis and atopic dermatitis, where LC participated in the reaction of allergic contact dermatitis and recognized them as antigen-presenting cells.

As considered in the cases of MS, chronic latent inflammation and metabolic disturbances persist in the skin due to oxidative stress, which may affect skin immune system (Janovska et al., 2012). Changes in CD3, CD20, CD8, accumulation of pro-apoptotic protein bcl-2, thickness of basal cell membrane of epidermis, and mild inflammatory reaction around capillaries were revealed.

Our study shows that 25-Hydroxyvitamin D deficiency in MS patients is associated with higher amount of dendritic cells and increase of their activity in epidermis. In patients with low 25-hydroxyvitamin D level in serum, average LCs quantity in one field of vision is higher (up to 25) compared with those, who have a normal amount of 25-Hydroxyvitamin D less than 10 CD1a positive cells. These peculiarities may be associated with skin immunomodulatory process and characterize LCs as antigen-presenting cells. Different amount of Birbeck's granules filling is revealed in patients, whose skin is affected by metabolic syndrome. In the cases of MS, expansion of adipocytes and dermal fibrosis may point to hypoxia in tissue, which could be directly associated with the presence of oxidative stress under metabolic syndrome.

## Conclusion

In patients with low serum vitamin D level, an average amount of Langerhans cells quantity in one field of vision is higher compared with those who have sufficient rates of vitamin D. In patients with metabolic syndrome and vitamin D deficiency, Langerhans cell activity is higher and filling of Birbeck's granules is more pronounced in comparison with those who have sufficient vitamin D status. Due to an accumulation of free radicals and defective antioxidant system, in cases of metabolic syndrome, detoxification, protection functions, barrier functions, and elimination functions of organism as a whole are reduced. Skin transmission is changed and latent chronic inflammation persists due to oxidative stress.

Patients with metabolic syndrome suffer from organ dysfunction as well as skin dysfunction. These defects occur due to inflammation and oxidative stress that worsen basic function from keeping the cells in a stressful state, which is ready for immune responses due to expression if cytokines and pro-inflammatory factors. Deregulation of T-cell system and cytokines leads to hyper-proliferation of keratinocytes and activation of neutrophils in epidermis. Reduced detoxification and elimination process in organism with metabolic syndrome occurs due to alteration of free radical accumulation and defects in antioxidative system, and may as well impact the functioning of Langerhans cells—major immune-presenting cell in the skin. Thus, it is crucial to prevent and/or optimize the therapeutic management of MS associated with dermatosis, premature skin aging, and quality of life.

It is also necessary to further conduct an investigation into Langerhans cells and vitamin D effect in the cases of metabolic syndrome in order to reveal more interaction with lymphocytes, macrophages, plasma cells, and mast cells, as a part of skin immune system. High incidence and prevalence of MS should raise the awareness of dermatologists, estheticians, and other specialists of the patient group with special needs in order to prevent early manifestations of skin dysfunction and immune system.

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