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psoriasis
and its effect on peripheral blood levels of
various T-lymphocyte subpopulations:
a clinical and flow cytometric immunophenotypical study**

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**The efficacy of narrow-band ultraviolet B phototherapy
in the treatment of patients with active chronic plaque psoriasis
and its effect on peripheral blood levels of
various T-lymphocyte subpopulations:
a clinical and flow cytometric immunophenotypical study**

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13 **Running title:** NB-UVB phototherapy & T-lymphocytes in psoriasis

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43 **Key words:** Flow cytometry; immunophenotypical analysis; narrow-band ultraviolet B
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45 phototherapy; psoriasis; T lymphocytes

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3 *Papagiannaki K, Kotsovilis S, Antoniou C, Dimitrakopoulou A, Arsenis G, Routsias JG,*
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5 *Tseleni-Kotsovili A, Tsakris A. The efficacy of narrow-band ultraviolet B phototherapy in*
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7 *the treatment of patients with active chronic plaque psoriasis and its effect on peripheral*
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9 *blood levels of various T-lymphocyte subpopulations: a clinical and flow cytometric*
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11 *immunophenotypical study.*

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18 19 20 **Abstract**

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25 **Background & objectives-** Psoriasis is a chronic inflammatory T-lymphocyte-mediated
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27 cutaneous disease. The main objectives of the present study were to evaluate the clinical
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29 efficacy of narrow-band ultraviolet B (NB-UVB) phototherapy in the treatment of patients
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31 with active chronic plaque psoriasis (CPP), using the Psoriasis Area and Severity Index
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33 (PASI), as well as to examine its effect on the peripheral blood levels of various T-
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35 lymphocyte subpopulations (CD3+, CD4+, CD8+ T lymphocytes, NK/T cells
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37 (CD3+CD16+, CD3+CD56+ or CD3+CD16+CD56+), NK cells (CD3-CD16+, CD3-
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39 CD56+ or CD3-CD16+CD56+), CD3+CLA+, CD3+CD8+CLA+, CD3+CD4+CD25+ and
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41 CD3+CD8+CD25+ T lymphocytes).

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46 **Methods-** Twenty patients with active CPP (test group) and 10 individuals without active
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48 or past psoriasis of any form (control group) were studied. CPP patients were treated with
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50 NB-UVB phototherapy. Inter- and intra-group statistical comparisons were performed, with
51
52 respect to the PASI score and the percentage of circulating T-lymphocyte subpopulations,
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54 determined by flow cytometric immunophenotypical analysis.

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58 **Results-** In the test group, mean PASI after NB-UVB phototherapy was highly statistically
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60 significantly lower than that before NB-UVB phototherapy. The peripheral blood levels of

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3 CD3+CD4+CD25+ T-lymphocytes were statistically significantly increased ($p=0.037$),
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6 whereas those of CD3-CD56+ and CD3-CD16+CD56+ NK cells were decreased, virtually
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8 statistically significantly ($p=0.052$ and $p=0.063$, respectively); finally, those of the
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10 remaining T-lymphocyte subpopulations remained statistically unaltered.

11
12 **Conclusions-** NB-UVB phototherapy is a clinically efficacious therapeutic modality for
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14 CPP. Alterations in the peripheral blood levels of CD4+CD25+ T lymphocytes, and
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16 possibly also CD3-CD56+ and CD3-CD16+CD56+ NK cells, after NB-UVB phototherapy
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18 of active CPP might be related to the efficacy of NB-UVB phototherapy.
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Summary Statement

Background & objectives: Psoriasis is a chronic inflammatory T-lymphocyte-mediated disease. The main objectives of this study were to evaluate the clinical efficacy of narrow-band ultraviolet B (NB-UVB) phototherapy in treating chronic plaque psoriasis (CPP) and to examine its effect on the peripheral blood levels of various T-lymphocyte subpopulations.

Results: Peripheral blood levels of CD3+CD4+CD25+ T-lymphocytes were significantly increased, whereas those of CD3-CD56+ and CD3-CD16+CD56+ NK cells were virtually significantly decreased.

Conclusions & implications: Alterations in peripheral blood levels of CD4+CD25+ T lymphocytes, and possibly CD3-CD56+ and CD3-CD16+CD56+ NK cells, might be used as efficacy parameters for NB-UVB phototherapy of CPP.

Introduction

Psoriasis (derived from the Hellenic word “psora” for itching) is a chronic inflammatory cutaneous disease affecting approximately 1-3% of the global population.¹⁻⁴ The aetiology of psoriasis is not completely understood and the disease was originally considered to be primarily a keratinization disorder of the skin, caused by keratinocyte hyperproliferation associated with abnormal epidermal differentiation.⁵ Over the latest years, the theory on the aetiopathogenesis of psoriasis has substantially changed and it is now recognized that immunity -probably autoimmunity- plays a significant role in the development of psoriasis.^{6,7} Specifically, psoriasis is a T-lymphocyte-mediated disease, histologically characterized by localized inflammation induced by the immune system.^{7,8} In the inflammatory infiltrate, cluster of differentiation 4+ (CD4+) T lymphocytes predominate within the dermis and CD8+ T lymphocytes localize mainly in the epidermis.⁹ Several T-lymphocyte subsets (CD4+, CD8+, CD45RO+, CD45RA+ and others) seem to play a primary role in the pathogenesis of psoriasis; recently, natural killer cells (NK cells) and natural killer/T cells (NK/T cells) have also been involved in the development of psoriasis.¹⁰

Ultraviolet irradiation is known to suppress the immune system and reduce inflammatory responses.¹¹ Narrow-band ultraviolet B radiation (NB-UVB) is an ultraviolet radiation with a wavelength of 311 nm \pm 2 nm and in the late 1980s the clinical use of NB-UVB was introduced as an effective method for the phototherapy of psoriasis and other cutaneous conditions, such as eczema or atopic dermatitis.¹²⁻¹⁸

Currently, a series of issues still remain unclear in the literature. It is not known whether psoriatic patients differ from non-psoriatic individuals in the peripheral blood levels of NK and NK/T cells or other T-lymphocyte subpopulations that can be present in psoriatic lesions and be involved in the disease inflammatory response, such as

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3 CD3+CLA+ cells or T-regulatory subpopulations (CD4+CD25+, CD8+CD25+). This issue
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5 could be related to disease pathogenesis or treatment. The effect of NB-UVB phototherapy
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7 on the levels of various T-lymphocyte subpopulations in the peripheral blood of psoriatic
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9 patients has also not been clarified.
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13 On the basis of these considerations, the objectives of the present study were to
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15 evaluate the efficacy of NB-UVB phototherapy in the treatment of patients with active
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17 chronic plaque psoriasis, as clinically revealed by the area (extent) and the severity of
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19 psoriatic lesions,¹⁹ as well as to examine the effect of NB-UVB phototherapy on the levels
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21 of various T-lymphocyte subpopulations (CD3+, CD4+, CD8+ T lymphocytes, NK/T cells
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23 (CD3+CD16+, CD3+CD56+ or CD3+CD16+CD56+), NK cells (CD3-CD16+, CD3-
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25 CD56+ or CD3-CD16+CD56+), CD3+CLA+, CD3+CD8+CLA+, CD3+CD4+CD25+ and
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27 CD3+CD8+CD25+ T lymphocytes) in the peripheral blood of patients with active chronic
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29 plaque psoriasis. Finally, the third study objective was to examine whether the potential
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31 alterations in the peripheral blood levels of the above-mentioned T-lymphocyte
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33 subpopulations after NB-UVB phototherapy in patients with active chronic plaque psoriasis
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35 could be used as therapeutic outcome measures and immunological parameters for
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37 assessing the efficacy of NB-UVB phototherapy.
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Materials and Methods

Selection of study population

The protocol of the study was reviewed by the Ethics and Research Committee, Medical School, University of Athens, Greece and ethical approval was obtained for the experimental procedures applied in humans. All 30 volunteers included in the study signed an informed consent form. Potential skin cancer risks were discussed with the volunteers, according to the guidelines of the British Photodermatology Group on behalf of the British Association of Dermatologists.¹⁸ The selection of study population was performed between February and June 2009.

The test group of the study included 20 untreated patients (12 males and eight females; age range 20-72 years; mean age 43.95 ± 15.46 years) with active chronic plaque psoriasis and the control group comprised 10 individuals (one male and nine females; age range 33-65 years; mean age 43.80 ± 12.96 years) without active psoriasis of any form or a history of psoriasis of any form in the past. The selection of the population of the study was performed on the basis of the following inclusion / exclusion criteria, determined before the initiation of the study.

Inclusion criteria

Study participants ought to be Caucasian volunteers aged ≥ 18 years. Patients included in the test group ought to be diagnosed with active chronic plaque psoriasis (determined solely on the basis of the clinical appearance of patients, i.e. the presence of papules or red plaques on the epidermis, covered by a silvery scale). Subjects enrolled in the control group ought not to present active psoriasis of any form or a history of psoriasis of any form in the past.

Exclusion criteria (applicable only in the test group)

Patients presenting absolute contraindications for NB-UVB phototherapy, such as xeroderma pigmentosum or lupus erythematosus,¹⁸ ought to be excluded from the study. Furthermore, patients subjected to any systemic therapy for at least four weeks or any topical therapy throughout the last two weeks of the four-week period of the treatment of active chronic plaque psoriasis also ought to be excluded from the study.

Screening examination (Tables 1 and 2)

A screening examination was carried out during the initial visit of volunteers. For each subject, a medical history was recorded (Table 1). In each patient in the test group, the area (extent) and the severity of psoriatic lesions were assessed by the degree of erythema, inflammatory infiltration and disquamation, and calculated using the Psoriasis Area and Severity Index (PASI; Table 2). PASI scores range from zero (no disease) to 72 (maximum disease area and severity).¹⁹ Patients in the test group presented PASI scores ranging from 8.2 to 25.8 before treatment. Patients with a PASI score >12 were considered to exhibit moderate to severe active chronic plaque psoriasis. In each patient in the test group, calculation of PASI was repeated after the completion of the treatment of active chronic plaque psoriasis (Table 2).

Flow cytometric immunophenotypical analysis of peripheral blood T lymphocytes

A first peripheral blood sample was collected from the antecubital vein of each patient in the test group immediately before the initiation of treatment of active chronic plaque psoriasis, and a second peripheral blood sample was obtained immediately after the completion of treatment. In the control group, only one peripheral blood sample was collected per subject. The peripheral blood specimens were collected in 2.5 ml tubes

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3 (Becton-Dickinson, Mountain View, CA, USA) containing ethylenediaminetetraacetic acid.
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5 For each subject, two tubes were used for sampling. The first tube was used for blood cell
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7 count and the second one was employed for flow cytometric immunophenotypical analysis.
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9 In particular, for the first tube monoclonal antibodies against human antigens, namely
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11 PERCP-conjugated anti-CD3, PE-Cy7-conjugated anti-CD4, APC-Cy7-conjugated anti-
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13 CD8 and FITC-conjugated anti-CLA were used, whereas for the second tube PERCP-
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15 conjugated anti-CD3, PE-Cy7-conjugated anti-CD4, APC-Cy7-conjugated anti-CD8,
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17 FITC-conjugated anti-CD16, PE-conjugated anti-CD25 and APC-conjugated anti-CD56
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19 were utilized (Becton-Dickinson, Mountain View, CA, USA). Complete blood counts and
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21 differential counts of leukocytes were obtained. The preparation of blood cells for flow
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23 cytometry was performed by incubating 0.1 ml of blood in each tube with the above-
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25 mentioned monoclonal antibodies for 15 min at 0-4°C. Two milliliters of fluorescence-
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27 activated cell sorting lysing solution (Becton-Dickinson, Mountain View, CA, USA) were
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29 added to the samples for 10 min. Subsequently, centrifugation was performed at 1500
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31 revolutions per minute (rpm) for 5 min, using a commercially available centrifuge (Jouan
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33 C312 Centrifuge, Jouan, Inc., Winchester, VA, USA) and the supernatant fluid was
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35 removed. The pellet was resuspended in 2 ml of phosphate-buffered saline (PBS) and a
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37 second centrifugation was performed at 1500 rpm for 5 min. Once more, the supernatant
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39 fluid was removed and 2 ml PBS were added to the pellet. A third centrifugation was
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41 performed at 1500 rpm for 5 min and the supernatant fluid was removed. Three hundred
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43 microliters of a stabilizing fixative solution (BD-Pharmagen, BD Biosciences, Le Pont de
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45 Claix, France) were added. The samples were analysed using a six-colour flow cytometer
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47 (FACSCanto, Becton-Dickinson, Mountain View, CA, USA).

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49 The screening examination and subsequent sample collection was performed
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51 between February and June 2009 in the Photobiologic Department of the Venereal and Skin
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3 Diseases Hospital “Andreas Syggros”, Athens, Greece, while sample processing and
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5 analysis was carried out within 24 hours after blood collection in the Cytometry Laboratory
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8 of the Department of Immunology and Histocompatibility of “Laikon” General Hospital,
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10 Athens, Greece.
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12 13 14 15 **Therapeutic interventions**

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17 Patients in the test group were treated with NB-UVB phototherapy that was
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19 initiated in the Photobiologic Department of the Venereal and Skin Diseases Hospital
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21 “Andreas Syggros”, Athens, Greece during the first week after blood sampling, using a
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23 full-body ultraviolet therapy system (UV 5001 BL, Herbert Waldmann GmbH & Co. KG,
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25 Villingen-Schwenningen, Germany), equipped with 24 TL01 radiators operating at 100 W
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27 (F85/100W-01) for NB-UVB phototherapy. NB-UVB phototherapy was performed for
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29 three days weekly^{20,21} throughout four weeks, using an initial NB-UVB radiation dosage of
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31 0.1-0.4 J/cm² (depending on the skin type of each patient) that gradually increased during
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33 the four-week treatment period. During this gradual increase, each dosage was 20% higher
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35 than the preceding one, according to the instructions of the manufacturer. For each patient,
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37 12 dosages were applied in total. NB-UVB radiation was being emitted at a distance of 21
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39 cm from the patient and at a temperature of 20°C. During the operation of the full-body
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41 ultraviolet therapy system, all patients were wearing safety glasses and the sensible parts of
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43 their body (genitalia) were covered.
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51 In all patients, NB-UVB phototherapy was performed by the same specially-trained
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53 nurse, following the instructions of the manufacturer. The aforementioned full-body
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55 ultraviolet therapy system was being calibrated every week by a technician of the Venereal
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57 and Skin Diseases Hospital “Andreas Syggros” and annually by the above-mentioned
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59 manufacturer. TL01 radiators are continually being substituted every two years.
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Study outcome variables

The outcome variables of the study were the mean change in PASI scores after NB-UVB phototherapy in the test group, in comparison with the corresponding pre-treatment PASI scores; and the mean change in the levels (expressed as proportions / percentages) of various T-lymphocyte subpopulations (CD3+, CD4+, CD8+ T lymphocytes, NK/T cells (CD3+CD16+, CD3+CD56+ or CD3+CD16+CD56+), NK cells (CD3-CD16+, CD3-CD56+ or CD3-CD16+CD56+), CD3+CLA+, CD3+CD8+CLA+, CD3+CD4+CD25+ and CD3+CD8+CD25+ T lymphocytes) in the peripheral blood of patients in the test group, in comparison with the corresponding pre-treatment values.

Statistical analysis

Quantitative data were expressed as mean value \pm standard deviation (when they followed the normal distribution) or as median and interquartile range (when they did not follow the normal distribution), while categorical data were expressed using their frequency (percentage). The subject was used as the statistical unit. The Kolmogorov-Smirnov test was used to evaluate the normality of data.

Inter-group (test versus control group) statistical comparisons of variables before treatment were performed using the independent samples *t*-test (in case of data normality), the Welch's *t*-test (in case of unequal variances of samples) or the Mann-Whitney *U* test (in case of absence of data normality).

In the test group, intra-group (before versus after treatment) statistical comparisons of variables were performed using the paired samples *t*-test (in case of data normality) or the Wilcoxon test (in case of absence of data normality).

All statistical tests were two-sided and the differences were considered to be statistically significant, when $p < 0.05$. The statistical analysis was performed using

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3 commercially available software (Statistical Package for the Social Sciences, version 16.0,
4 SPSS Inc., Chicago, IL, USA).
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10 **Results**

15 **Safety of study population**

17 All 30 participants safely completed the study, without any complications / adverse
18 events.
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24 **Baseline comparability of study groups (Table 3)**

25 No statistically significant difference ($p>0.05$) in the peripheral blood levels of all
26 T-lymphocyte subpopulations examined (CD3+, CD4+, CD8+ T lymphocytes, NK/T cells
27 (CD3+CD16+, CD3+CD56+ or CD3+CD16+CD56+), NK cells (CD3-CD16+, CD3-
28 CD56+ or CD3-CD16+CD56+), CD3+CLA+, CD3+CD8+CLA+, CD3+CD4+CD25+ and
29 CD3+CD8+CD25+ T lymphocytes) was revealed between the test and control group
30 (Table 3).
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43 **Effect of NB-UVB phototherapy on peripheral blood levels of various T-lymphocyte** 44 **subpopulations (Table 4 and Fig. 1)**

45 In the test group, mean PASI score after NB-UVB phototherapy of active chronic
46 plaque psoriasis was highly statistically significantly lower than that before NB-UVB
47 phototherapy ($p<0.0005$; Table 4). It is noteworthy that the highest PASI score after NB-
48 UVB phototherapy was 8.9 in a patient and therefore in all 20 patients in the test group the
49 PASI scores were <12 after NB-UVB phototherapy, in contrast to the PASI scores before
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3 NB-UVB phototherapy, which additionally ranged from 12 to 20 or even >20 in certain
4 patients (Table 2).
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8 No statistically significant difference ($p>0.05$) in peripheral blood levels of the
9 majority of T-lymphocyte subpopulations examined (CD3+, CD4+, CD8+ T lymphocytes,
10 NK/T cells (CD3+CD16+, CD3+CD56+ or CD3+CD16+CD56+), certain NK cells (CD3-
11 CD16+), CD3+CLA+, CD3+CD8+CLA+ and CD3+CD8+CD25+ T lymphocytes) was
12 revealed between the time-points before and after NB-UVB phototherapy of active chronic
13 plaque psoriasis in the test group (Table 4). It could be noted that the peripheral blood
14 levels of some NK cells (CD3-CD56+ and CD3-CD16+CD56+) tended to be lower after
15 than before NB-UVB phototherapy in the test group, but this difference, although not
16 statistically significant, virtually reached statistical significance ($p=0.052>0.05$ and
17 $p=0.063>0.05$, respectively; Table 4).
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32 In contrast, the peripheral blood levels of CD3+CD4+CD25+ T lymphocytes were
33 statistically significantly higher after than before NB-UVB phototherapy in the test group
34 ($p=0.037<0.05$; Table 4 and Fig. 1).
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41 **Results of statistical analysis for patients with moderate to severe active chronic** 42 **plaque psoriasis (PASI score >12)** 43 44

45 The statistical analysis was repeated specifically for patients with moderate to
46 severe active chronic plaque psoriasis (PASI score >12) and the obtained results as a rule
47 remained unaltered, with only sporadic minor exceptions.
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52 In particular, for patients with moderate to severe active chronic plaque psoriasis
53 (PASI score >12) in the test group, the peripheral blood levels of CD3-CD56+ and CD3-
54 CD16+CD56+ NK cells were clearly not statistically significantly decreased after than
55 before NB-UVB phototherapy ($p=0.396$ and $p=0.518$, respectively). In contrast, for the
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3 entire pool of patients in the test group (i.e. with active chronic plaque psoriasis of any
4 severity and any PASI score), the peripheral blood levels of CD3-CD56+ and CD3-
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6 CD16+CD56+ NK cells were not statistically significantly decreased after than before NB-
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8 UVB phototherapy, but differences for both NK-cell subpopulations virtually reached
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10 statistical significance ($p=0.052$ and $p=0.063$, respectively; Table 4).
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15 In another case, for patients with moderate to severe active chronic plaque psoriasis
16 (PASI score >12) in the test group, the peripheral blood levels of CD3+CLA+ cells were
17 marginally statistically significantly lower than the corresponding levels in the control
18 group, when a parametrical test (independent samples t -test) was used ($p=0.043$). In
19 contrast, for the entire pool of patients in the test group (i.e. with active chronic plaque
20 psoriasis of any severity and any PASI score), the peripheral blood levels of CD3+CLA+
21 cells were marginally not statistically significantly different from the corresponding levels
22 in the control group, when the same parametrical test (independent samples t -test) was used
23 ($p=0.056$; Table 4), whereas the peripheral blood levels of the same cells were marginally
24 statistically significantly lower in the test group before NB-UVB phototherapy than in the
25 control group, when a non-parametrical test (Mann-Whitney U test) was used ($p=0.042$;
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27 Table 4).
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46 Discussion

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48 The present study documented the clinical efficacy of NB-UVB phototherapy in the
49 treatment of patients with active chronic plaque psoriasis. This principal finding is in
50 agreement with previous results reported in the literature. In a comparative study, in which
51 psoriatic lesions were irradiated daily with UVB of 254 nm, 280 nm, 290 nm, 296 nm, 300
52 nm, 304 nm and 313 nm wavelengths, no clearance of psoriasis occurred by the use of
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54 wavelengths ≤ 290 nm, whereas clearance was achieved using wavelengths of 296-313 nm
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3 and the best therapeutic outcome was obtained at 313 nm.²² A meta-analysis of randomized
4 and non-randomized controlled studies concluded that NB-UVB is significantly more
5 efficacious than broad-band UVB (wavelength <290 nm) in the phototherapy of psoriasis.²³
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10 It is known that CD4+CD25+ T lymphocytes are involved in the maintenance of
11 natural self-tolerance by reducing immune responses against self- and non-self-antigens,
12 possibly during the stage of T-lymphocyte activation.²⁴ In contrast, the reduction or
13 eradication of CD4+CD25+ T lymphocytes enhances immune responses against non-self
14 antigens, but could also induce autoimmune responses against specific self-antigens and
15 therefore be a potential aetiological agent of autoimmune diseases, such as rheumatoid
16 arthritis, thyroiditis, gastritis and others.²⁴⁻²⁶ Overall, these studies revealed that
17 CD4+CD25+ T lymphocytes are regulatory T lymphocytes.²⁷ Nowadays, extensive and
18 robust evidence has demonstrated that CD4+CD25+ regulatory T lymphocytes are highly
19 important in the prevention of autoimmune responses and that the absence of regulatory T
20 lymphocytes expressing CD4, CD25 and the transcription factor forkhead box protein 3
21 (FOXP3) results in severe autoimmune response in both mice and humans.²⁷⁻²⁹
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23 Consequently, it appears reasonable to anticipate that an efficacious treatment of active
24 chronic plaque psoriasis would result in a reduction of T-lymphocyte-mediated
25 autoimmune responses, which are compatible with an increase in the peripheral blood
26 levels of CD3+CD4+CD25+ T lymphocytes induced by treatment. This plausible
27 hypothesis was confirmed in the present study, in which the peripheral blood levels of
28 CD3+CD4+CD25+ T lymphocytes were statistically significantly higher after than before
29 NB-UVB phototherapy.
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55 However, conflicting findings have been reported in the literature. In an *ex vivo*
56 study, the exposure of T lymphocytes to low-dose (10 mJ/cm²) UVB resulted in a reduction
57 in the expression of CD25.³⁰ Another previous study demonstrated that peripheral blood
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3 mononuclear cells (i.e. B- or T-lymphocytes, monocytes / macrophages etc.) expressing
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5 CD25 were elevated (both in percentage and absolute number) in psoriatic patients,
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7 compared with non-psoriatic subjects, and were not altered after topical therapy alone.³¹ As
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9 mentioned by the authors, this finding could merely indicate that the topical therapy
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11 applied was not efficacious.³¹ Another study revealed that the serum levels of the soluble
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13 T-lymphocyte product interleukin-2 receptor (CD25) were statistically significantly
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15 positively correlated with the activity of psoriasis and were reduced after clinically
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17 efficacious treatment using orally administered cyclosporin A or FK506 as
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19 immunosuppressive drugs.³² Therefore, soluble T-lymphocyte product interleukin-2
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21 receptor (CD25) was considered to be a marker for the activity of psoriasis.³² A subsequent
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23 study revealed that in patients with moderate to severe psoriasis (defined as the presence of
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25 PASI score >10), the peripheral blood levels of CD8+CD25+ T lymphocytes, but not
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27 CD4+CD25+ T lymphocytes, were highly statistically significantly correlated with the
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29 PASI score and therefore with the severity of psoriasis.³³ However, this statistically
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31 significant correlation was not demonstrated in patients with psoriasis of any severity (any
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33 PASI score).³³ No difference in the peripheral blood levels of CD4+CD25+ and
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35 CD8+CD25+ T lymphocytes was revealed between the psoriatic patients and the non-
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37 psoriatic individuals.³³ According to the interpretation proposed by the authors, the severity
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39 of psoriasis could be dependent on continuous recruitment of CD8+ or CD8+CD25+ T
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41 lymphocytes from the bloodstream to the skin.³³ However, this interpretation seems not to
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43 sufficiently account either for the lack of difference in the peripheral blood levels of
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45 CD4+CD25+ and CD8+CD25+ T lymphocytes between the psoriatic patients and the non-
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47 psoriatic individuals or for the lack of a statistically significant correlation between the
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49 peripheral blood levels of CD8+CD25+ or CD4+CD25+ T lymphocytes and the severity of
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51 psoriasis (as indicated by the PASI score) in patients with psoriasis of any severity (any
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3 PASI score). Finally, in another study³⁴ no statistically significant difference in
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5 CD4+CD25+ T regulatory lymphocyte numbers occurred after NB-UVB phototherapy,
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7 compared with pre-treatment values, despite the substantial clinical efficacy of NB-UVB
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9 phototherapy, as revealed by the significant reduction in PASI score.
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13 A limitation of the present study was that the levels of T-lymphocyte
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15 subpopulations were examined only in the peripheral blood of patients with active chronic
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17 plaque psoriasis. It has been documented that the levels of T-lymphocyte subpopulations in
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19 the peripheral blood, i.e. at a systemic level, are not necessarily analogous to the levels of
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21 infiltrating T-lymphocyte subpopulations in psoriatic cutaneous lesions, i.e. at a topical
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23 level. For example, cells expressing the NK-cell marker CD16 were reported to be
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25 statistically significantly more in psoriatic cutaneous lesions than in cutaneous sites not
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27 affected by psoriasis,³⁵ but statistically significantly fewer in the peripheral blood of
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29 psoriatic patients, compared with non-psoriatic subjects.⁹
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35 An issue of clinical interest is to compare the effect of NB-UVB phototherapy of
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37 psoriasis to the combination of bath psoralen and ultraviolet A phototherapy (PUVA)
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39 clinically and immunologically. A recent study demonstrated that PUVA phototherapy
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41 resulted in a significantly higher reduction in PASI score, compared with NB-UVB
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43 phototherapy (85.44% vs. 58.72%, respectively); PUVA phototherapy significantly
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45 reduced the mean peripheral blood levels of CD4+ T lymphocytes (36.8% post-treatment vs.
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47 42.06% pre-treatment), whereas NB-UVB phototherapy had no such significant impact.³⁶
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50 Another study revealed comparable clinical efficacy for both treatment modalities, using
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52 the PASI score as the outcome variable.³⁷ At the highest level of evidence (systematic
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54 reviews), a systematic review reported a similar clinical efficacy of both therapeutic
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56 modalities,³⁸ whereas another systematic review concluded that NB-UVB phototherapy is a
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58 less efficacious therapeutic method than PUVA phototherapy.⁹ Because of the relatively
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3 limited amount of information available on this issue, it is clear that additional research will
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6 be required in the future.

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8 Perspectives on future research might also include the examination of the effect of
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10 NB-UVB phototherapy on the levels of other T-lymphocyte subpopulations in the
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12 peripheral blood of patients with active chronic plaque psoriasis (for example, naive T
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14 lymphocytes) or the evaluation of the correlation between the levels of various T-
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16 lymphocyte subpopulations with potentially associated factors, such as patient age, the
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18 severity and the area (extent) of psoriatic lesions or the duration of psoriasis (from the date
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20 of onset to the date of examination).
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25 In conclusion, the present study confirms that NB-UVB phototherapy is a clinically
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27 efficacious therapeutic modality for active chronic plaque psoriasis. Although NB-UVB
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29 phototherapy has no significant effect on the levels of most T-lymphocyte subpopulations,
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31 it is associated with a significant increase in the peripheral blood levels of
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33 CD3+CD4+CD25+ T lymphocytes. The reported alterations in the peripheral blood levels
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35 of CD4+CD25+ T lymphocytes, and possibly also CD3-CD56+ and CD3-CD16+CD56+
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37 NK cells, after NB-UVB phototherapy of active chronic plaque psoriasis could be
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39 potentially used as therapeutic outcome measures for assessing the efficacy of NB-UVB
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41 phototherapy.
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Table 1. The main medical history data by study group at the baseline of the study

Characteristics of study participants	Test group (N=20)	Control group (N=10)
Age (mean ± SD; years)	43.95 ± 15.46	43.80 ± 12.96
Gender (males/females; N)	12/8	1/9
Duration of psoriasis >5 years (N)	11	Not applicable
Heredity of psoriasis (N)	6	Not applicable
Hyperlipidaemia (N)	7	1
Arterial hypertension (N)	7	1
Medications (N)	8	1
Smoking (N; frequency and duration)	12 active smokers; all smoked >10 cigarettes/day; in 3 smokers: duration <5 years and in 9 smokers: duration >5 years	4 active smokers; all smoked >10 cigarettes/day; in all smokers: duration >5 years
Alcohol consumption >5 times/week (N)	6	1
Psoriasis previously treated with topical therapy* (N)	18	Not applicable
Psoriasis previously	1	Not applicable

treated with biological factors [†] (N)		
Psoriasis previously treated with immunosuppressive drugs [‡] (N)	1	Not applicable

Abbreviations / footnotes:

N=Number

SD=Standard deviation

*Topical therapy had been performed using emollients and ointments, such as the calcipotriol / betamethasone dipropionate ointment (Dovobet[®], Leo Pharma A/S, Ballerup, Denmark)

[†]The biological factor used in this single patient had been etanercept (Enbrel[®], Immunex Corporation, Thousand Oaks, CA, USA)

[‡]The immunosuppressive drug used in this single patient had been methotrexate (Methoblastin[®] Tablets, Pfizer Inc., NY, USA)

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Table 2. Number of patients before and after treatment of psoriasis in relation to PASI category

PASI	Number of patients (N)	
	before treatment	after treatment
<12	8	20
12-20	8	0
>20	4	0

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Table 3. Baseline comparability of study groups, as evidenced by inter-group (test group before NB-UVB phototherapy versus control group) statistical comparisons of variables, using the independent samples *t*-test

Variable	Variable value in the test group before NB-UVB phototherapy (%; mean value \pm SD; N=20)	Variable value in the control group (%; mean value \pm SD; N=10)	Mean difference in variable value (%; test-control group)	Statistical significance (<i>p</i> value)
All lymphocytes' (CD3+ T lymphocytes, NK cells and B lymphocytes) blood levels (%)	30.43% \pm 9.19%	32.41% \pm 5.97%	-1.99%	NS (<i>p</i> =0.542)
CD3+ T-lymphocyte blood levels (%)	67.58% \pm 11.10%	66.82% \pm 9.06%	+0.76%	NS (<i>p</i> =0.854)
CD4+ T-lymphocyte blood levels (%)	42.98% \pm 9.27%	45.99% \pm 8.68%	-3.01%	NS (<i>p</i> =0.399)
CD8+ T-lymphocyte blood levels (%)	20.43% \pm 10.10%	17.17% \pm 3.63%	+3.26%	NS (<i>p</i> =0.335)
CD3+CD16+ NK/T-cell blood levels (%)	0.63% \pm 0.69%	0.31% \pm 0.28%	+0.32%	NS (<i>p</i> =0.177)
CD3+CD56+ NK/T-cell	13.70% \pm 8.58%	8.72% \pm 4.39%	+4.98%	NS

blood levels (%)				(<i>p</i> =0.098)
CD3+CD16+CD56+ NK/T-cell blood levels (%)	0.29% ± 0.47%	0.12% ± 0.06%	+0.17%	NS (<i>p</i> =0.267)
CD3-CD16+ NK-cell blood levels (%)	12.77% ± 7.35%	10.33% ± 5.84%	+2.44%	NS (<i>p</i> =0.369)
CD3-CD56+ NK-cell blood levels (%)	12.75% ± 7.06%	10.69% ± 6.21%	+2.06%	NS (<i>p</i> =0.440)
CD3-CD16+CD56+ NK-cell blood levels (%)	11.42% ± 6.84%	9.11% ± 5.63%	+2.31%	NS (<i>p</i> =0.366)
CD3+CLA+ T-lymphocyte blood levels (%)	0.66% ± 0.71%	1.31% ± 1.06%	-0.65%	NS; marginally close to * (<i>p</i> =0.056) Non- parametrical test (Mann- Whitney <i>U</i> test) revealed marginal * (<i>p</i> =0.042)
CD3+CD8+CLA+ T-lymphocyte blood levels (%)	0.16% ± 0.22%	0.18% ± 0.15%	-0.02%	NS (<i>p</i> =0.802)
CD3+CD4+CD25+ T-	13.50% ± 12.27%	17.09% ± 14.60%	-3.59%	NS

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lymphocyte blood levels (%)				($p=0.484$)
CD3+CD8+CD25+ T- lymphocyte blood levels (%)	1.01% ± 1.39%	0.56% ± 0.37%	+0.45%	NS ($p=0.333$)

Abbreviations / footnotes:

CD=Cluster of differentiation

CLA=Cutaneous lymphocyte-associated antigen

N=Number

NB-UVB=Narrow-band ultraviolet B

NK=Natural killer

NK/T=Natural killer/T

NS=No statistically significant difference ($p>0.05$)

SD=Standard deviation

*Statistically significant difference ($p<0.05$)

Table 4. Intra-group (after versus before NB-UVB phototherapy) statistical comparisons of variables in the test group ($N=20$ in all cases), using the paired samples t -test

Variable	Variable value before NB-UVB phototherapy (%; mean value \pm SD)	Variable value after NB-UVB phototherapy (%; mean value \pm SD)	Mean change in variable value (%; after-before NB-UVB phototherapy)	Statistical significance (p value)
PASI	14.72% \pm 5.52%	4.07% \pm 2.37%	-10.65%	* ($p<0.0005$)
All lymphocytes' (CD3+ T lymphocytes, NK cells and B-lymphocytes) blood levels (%)	30.43% \pm 9.19%	29.49% \pm 7.65%	-0.94%	NS ($p=0.513$)
CD3+ T-lymphocyte blood levels (%)	67.58% \pm 11.10%	67.30% \pm 11.13%	-0.28%	NS ($p=0.840$)
CD4+ T-lymphocyte blood levels (%)	42.98% \pm 9.27%	43.91% \pm 8.68%	+0.93%	NS ($p=0.456$)
CD8+ T-lymphocyte blood levels (%)	20.43% \pm 10.10%	19.97% \pm 9.28%	-0.46%	NS ($p=0.329$)
CD3+CD16+ NK/T-cell blood levels (%)	0.63% \pm 0.69%	0.55% \pm 0.72%	-0.08%	NS ($p=0.667$)

CD3+CD56+ NK/T-cell blood levels (%)	13.70% ± 8.58%	11.87% ± 11.24%	-1.83%	NS (<i>p</i> =0.594)
CD3+CD16+CD56+ NK/T-cell blood levels (%)	0.29% ± 0.47%	0.34% ± 0.66%	+0.05%	NS (<i>p</i> =0.739)
CD3-CD16+ NK-cell blood levels (%)	12.77% ± 7.35%	10.96% ± 6.12%	-1.81%	NS (<i>p</i> =0.084)
CD3-CD56+ NK-cell blood levels (%)	12.75% ± 7.06%	10.97% ± 5.93%	-1.79%	NS; marginally close to † (<i>p</i> =0.052)
CD3-CD16+CD56+ NK-cell blood levels (%)	11.42% ± 6.84%	9.71% ± 5.81%	-1.71%	NS; marginally close to † (<i>p</i> =0.063)
CD3+CLA+ T-lymphocyte blood levels (%)	0.66% ± 0.71%	0.70% ± 0.75%	+0.04%	NS (<i>p</i> =0.699)
CD3+CD8+CLA+ T-lymphocyte blood levels (%)	0.16% ± 0.22%	0.12% ± 0.12%	-0.04%	NS (<i>p</i> =0.297)
CD3+CD4+CD25+ T-lymphocyte blood levels (%)	13.50% ± 12.27%	18.68% ± 12.35%	+5.18%	† (<i>p</i> =0.037)
CD3+CD8+CD25+ T-lymphocyte blood levels (%)	1.01% ± 1.39%	0.84% ± 0.88%	-0.17%	NS (<i>p</i> =0.430)

Abbreviations / footnotes:

CD=Cluster of differentiation

CLA=Cutaneous lymphocyte-associated antigen

NB-UVB=Narrow-band ultraviolet B

NK=Natural killer

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3 NK/T=Natural killer/T
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5 NS=No statistically significant difference ($p>0.05$)
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8 PASI=Psoriasis area and severity index¹⁹
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10 SD=Standard deviation
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12 *Statistically significant difference ($p<0.001$)
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15 †Statistically significant difference ($p<0.05$)
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3 **Figure 1.** Bar chart graphically demonstrating the intra-group (after versus before NB-
4 UVB phototherapy) statistical comparisons of peripheral blood levels (%) of
5 CD3+CD4+CD25+ T lymphocytes and CD3-CD16+CD56+ and CD3-CD56+ NK cells in
6 the test group ($N=20$ in all cases), using the paired samples t -test
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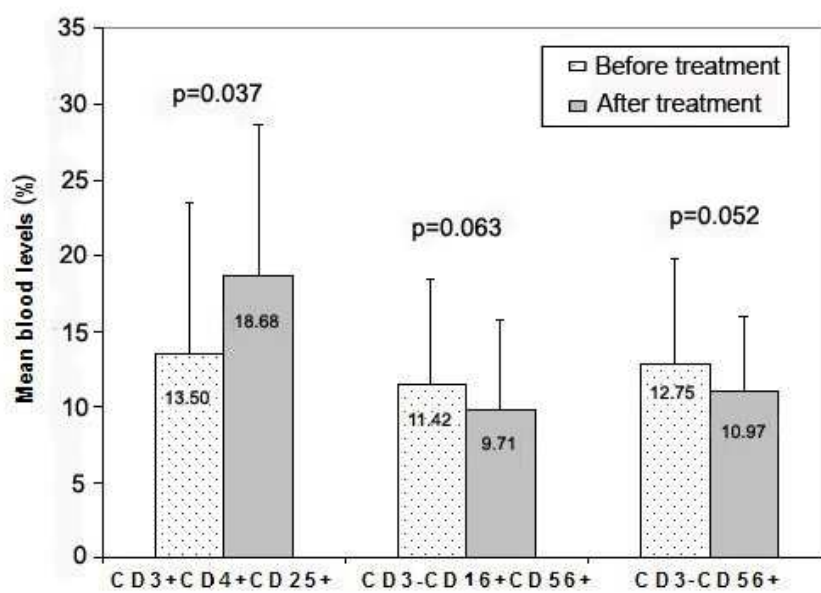


Figure 1

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3 **Figure 2.** Flow cytometer photographs in a 59 year-old male smoking patient with diabetes
4 and hyperlipidaemia before (Fig. 2a) and after (Fig. 2b) NB-UVB phototherapy. The
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6 peripheral blood levels (%) of CD3+CD4+CD25+ T lymphocytes (depicted in yellow
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8 colour) were significantly increased after than before NB-UVB phototherapy (mean values
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10 of 18.68% versus 13.50%, respectively). In contrast, the peripheral blood levels (%) of
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12 CD3+CD8+CD25+ T lymphocytes (depicted in pink colour) were not significantly altered
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14 after NB-UVB phototherapy, compared with pre-treatment values (mean values of 0.84%
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16 versus 1.01%, respectively).
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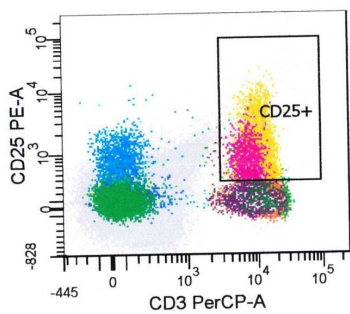


Figure 2a

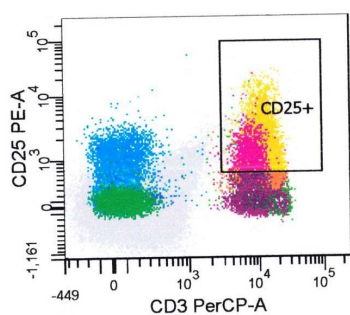


Figure 2b

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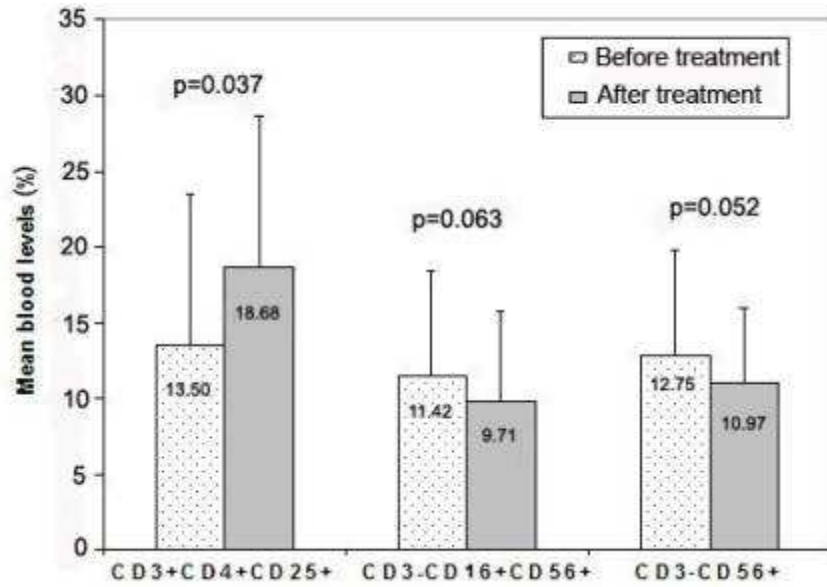


Figure 1. Bar chart graphically demonstrating the intra-group (after versus before NB-UVB phototherapy) statistical comparisons of peripheral blood levels (%) of CD3+CD4+CD25+ T lymphocytes and CD3-CD16+CD56+ and CD3-CD56+ NK cells in the test group (N=20 in all cases), using the paired samples t-test
120x87mm (96 x 96 DPI)

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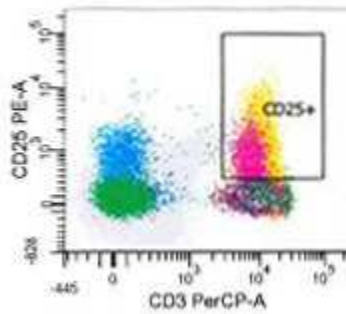


Figure 2a.

Flow cytometer photographs in a 59 year-old male smoking patient with diabetes and hyperlipidaemia before (Fig. 2a) and after (Fig. 2b) NB-UVB phototherapy. The peripheral blood levels (%) of CD3+CD4+CD25+ T lymphocytes (depicted in yellow colour) were significantly increased after than before NB-UVB phototherapy (mean values of 18.68% versus 13.50%, respectively). In contrast, the peripheral blood levels (%) of CD3+CD8+CD25+ T lymphocytes (depicted in pink colour) were not significantly altered after NB-UVB phototherapy, compared with pre-treatment values (mean values of 0.84% versus 1.01%, respectively).

48x45mm (97 x 97 DPI)

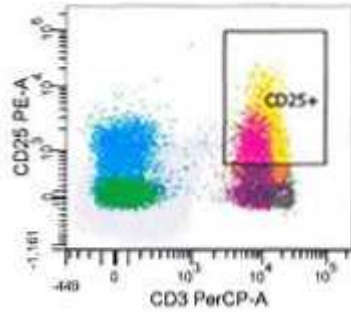


Figure 2b.

Flow cytometer photographs in a 59 year-old male smoking patient with diabetes and hyperlipidaemia before (Fig. 2a) and after (Fig. 2b) NB-UVB phototherapy. The peripheral blood levels (%) of CD3+CD4+CD25+ T lymphocytes (depicted in yellow colour) were significantly increased after than before NB-UVB phototherapy (mean values of 18.68% versus 13.50%, respectively). In contrast, the peripheral blood levels (%) of CD3+CD8+CD25+ T lymphocytes (depicted in pink colour) were not significantly altered after NB-UVB phototherapy, compared with pre-treatment values (mean values of 0.84% versus 1.01%, respectively).

56x50mm (90 x 90 DPI)

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