**Materials and Methods**

We enrolled 14 patients affected by generalized vitiligo followed up at the Dermatologic Clinic of Brescia (Italy) from January to August 2018. Patients affected by segmental vitiligo or younger than 18 years, pregnant or breastfeeding women, patients reporting current use of potentially photosensitizing drugs and a history of photosensitizing dermatoses were excluded.

All patients gave informed consent. The study followed the principles outlined in the Declaration of Helsinki and was approved by the Local Ethics Committee.

All patients with vitiligo were examined by the same physicians (E.M., M.R.) at the time of enrolment with the assessment of complete disease history, phototype and concomitant diseases and medications.

*NB-UVB Phototherapy*

NB-UVB irradiation (311 ± 2 nm) was administered using a 7001K stand-up cubicle (Waldmann Medizintechnik, Villigen-Schwenningen, Germany) equipped with 48 NB-UVB fluorescent tubes (TL-01 lamps, Philips, Eindhoeven, the Netherlands). Irradiance was measured with an SR 9910 spectroradiometer (Macam Photometrics Ltd., Livingston, UK) and found to be 4.82 mW/cm2 at skin level. NB-UVB phototherapy was administered twice weekly on non-consecutive days for 12 weeks. According to current guidelines [9] and presuming vitiligo to be equivalent to Fitzpatrick skin phototype I or II, the initial dose was 0.15 J/cm2. Dose increments were delivered at each session. The increment dose was 10% in the absence of any erythematous reaction and 5% if a faint and transitory erythema was seen. No increment was delivered if the erythema was well defined and persistent. The treatment sessions were skipped until complete recovery if a severe burn developed.

*Phototesting and Spectrophotometric Procedures*

Before phototherapy treatment, a phototesting procedure was administered at baseline by exposing a series of 6 square areas (1 × 1 cm) of a vitiligo patch and adjacent healthy skin of the same area of the upper limb to increasing doses of solar simulated radiation (SSR, Multiport Solar Simulator model 601, Solar Light, Glenside, PA, USA). The doses had a 25% geometrical increase: 0.02, 0.025, 0.031, 0.039, 0.048 and 0.061 J/cm2.

Visual assessment of the minimal erythema dose (MED) took place after 24 h. The MED was defined as the minimal SSR dose that is followed by a just perceptible erythema with at least one border from unexposed skin [10]. If the test was negative, higher dosages were subsequently administered (0.038, 0.048, 0.063, 0.078, 0.098, 0.122 J/cm2).

A second phototest, performed with the same parameters, was assessed after the phototherapy cycle in the same skin areas analysed at baseline.

For quantitative measurement of skin erythema and pigmentation, a portable spectrophotometer (CM-700d, Konica Minolta Sensing Inc., Osaka, Japan) and an analysis program (CM-SA, Konica Minolta Sensing Inc.) were used. The wavelength range of the measured spectrum is 400–700 nm.

The device uses the spectral reflectance data to calculate L\*a\*b\* values of the CIE system of colour quantification [11]. For the aims of the present investigation, two colorimetric parameters, the a\* and the melanin index (MI\*), were used to assess the degree of erythema and pigmentation, respectively [12, 13]. The a\* colour coordinate stands for red or green chroma: a high +a\* value means an intense red chroma and a high absolute value of –a\* means an intense green chroma [11, 13]. The MI\* was calculated according to the following formula: MI = 100 × log(1/reflectance of red light) [12].

To minimize the variations of visual and colorimetric assessments, patients lay down in the prone position for at least 10 min under controlled standard environmental conditions of air temperature (22–22.5°C) and air humidity (43–45% relative air humidity) before optical measurements and mean values were recorded after measuring 5 times in the centre of the irradiated sites. The measurements were performed by a doctor with experience in spectrophotometers (M.A.), in order to mime the variation of the pressure applied on the skin, as this might be the main confounding variable when using spectrophotometers. Spectrophotometric evaluation after NB-UVB treatment was performed on baseline MED skin areas.

To investigate the presence of photoadaptation, the following calculation was used to determine each patient’s percentage change in MED from before to after treatments, defined as photoadaptation factor (MED-PF):

MED-PF = [(MEDfinal – MEDinitial)/MEDinitial] × 100.

The same calculation was assessed using the spectrophotometric parameter a\* and identifying the a\* photoadaptation factor (a\*-PF).

Patients who had a positive mean percentage change in MED value from before to after treatment were considered as photoadapters. While negative mean percentage changes in a\* values, as a consequence of skin erythema reduction, were considered a sign of spectrophotometric photoadaptation.

*Statistical Analysis*

Collected data were analysed using the IBM SPSS™ Statistics 25.0 and IBM Sample Power™ 3.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6 software (GraphPad Software Inc., La Jolla, CA, USA).

According to previous data [14], expecting a standard deviation of 3.5 and assuming a minimal clinical difference of 3.2, a power of test (1 – β) of 83% and an α-value of 0.05, the sample size was to be 13 patients.

Continuous variables were summarized calculating medians, minimum and maximum values as appropriate.

Normal distribution of collected data was analysed by the Kolmogorov-Smirnov test.

The comparison of baseline MED, skin erythema (a\*) and pigmentation (MI\*) and photoadaptation factors (MED-PF and a\*-PF) between affected and healthy skin was assessed by the Mann-Whitney non-parametric test. The same parameters were compared with those assessed after phototherapy, both for healthy and vitiligo skin, by the non-parametric Wilcoxon test. All results were considered statistically significant for *p* values ≤0.05.

MED and a\* variation of affected skin according to skin phototype was determined by the non-parametric Kruskal-Wallis test.

Pearson correlation was used to analyse any correlation between baseline MED and skin phototype and baseline MED and MED-PF and a\*-PF both in affected and unaffected skin.

Any association between disease duration (years) and MED, a\* value and MI\* variation was evaluated by linear regression.