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# Effect of Data Normalization for Age on the Correlations between Corneometric Values and Serum Molecule Levels in Plaque-type Psoriatic Patients

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**Corneometry has been considered useful both to evaluate disease severity and to monitor psoriatic patients during treatment. However, a limitation of this technique is that the patient's age influences the corneometric determinations, thus reducing their clinical usefulness.**

**The aim of this study was, therefore, to establish whether age normalization of the corneometric results may provide more reliable data for clinical use.**

**Corneometric levels were determined in 10 plaque-type psoriatic patients, under standard conditions. Eight serum variables, including transforming growth factor-β1 and seven soluble membrane molecules, were assayed with commercially available immune-enzyme methods in the same patients, whose age and PASI scores were also recorded.**

**The age normalization procedure improved all the correlation coefficients calculated on the lesional or non-lesional corneometric values versus the PASI scores as well as versus the other serum variables. This approach may render corneometric determinations more useful to evaluate disease status or treatment effect in patient groups with plaque-type psoriasis.** ***Key words: soluble membrane molecules; TGF-β1; IL-1Ra.***

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Recently, we presented data concerning the corneometric values assessed in patients affected with plaque-type psoriasis. The lesional corneometric values were always lower than those measured in non-lesional areas; they decreased over time in treated patients and correlated inversely with the PASI scores (1). In addition, an inverse correlation was found in the lesional corneometric levels and the serum concentrations of E-selectin, a non-specific marker of extension of psoriasis, previously shown to be PASI-related (2, 3).

In other studies, including both normal and psoriatic individuals, we confirmed that the corneometric values are age-dependent (4), and a statistically significant, increasing trend was observed from lesional to non-lesional to normal skin (unpublished data). This observation led to the need for age normalization of the corneometric data. This type of correction revealed that the corneometric values could be better correlated to the serum concentrations of several molecules and also improved some of the previously described correlations (1).

## MATERIALS AND METHODS

Ten subjects affected with plaque-type psoriasis (5 females and 5 males; median age 47 years; range 20–75; median psoriasis area and severity index (PASI) score = 15.5; range 8.1–25.5) were included in this study. The PASI scores were always evaluated as usual by the same person (5).

The patients had received neither local nor systemic treatment for at least 10 days before enrollment. In addition, they presented no evidence of other diseases.

Corneous hydration was evaluated with the 820 CM corneometer (produced by Courage and Khazaka, Köln, Germany), a device using an electrical capacitance determination as a reflection of the water content in the "stratum corneum" (6). The measurements were expressed as arbitrary corneometric units (CU). The determinations were performed at $21 \pm 1°C$ and $40 \pm 10\%$ relative humidity (RH) from 10.00 a.m. to 12.00 a.m. The patients took a shower with only water at least 2–3 h before the evaluation, and no local therapies were applied. The subjects became accustomed to the environmental conditions for 30 min before each determination. The measurements were taken on the trunk, on psoriatic plaques, 3–5 cm diameter, which had appeared some days before and on non-lesional skin areas (5–10 cm from the lesion). Six measurements, in two distinct subsequent sets, were made on each patient within a 5-min interval on both the psoriatic plaques and on the non-lesional skin areas selected.

Means were used for the statistical analysis. The variation coefficients ranged within 1.2 and 11.7 (mean CV = 4.6) for the lesional and within 0.9 and 5.6 (mean CV = 2.3) for the non-lesional skin.

Soluble intercellular adhesion molecule-1 (sICAM-1), soluble L-selectin (sL-selectin), interleukin-1 receptor antagonist (IL1-RA), soluble interleukin-2 receptor (sIL-2R), soluble interleukin-6 receptor (sIL-6R), transforming growth factor-β1 (TGF-β1), β2-microglobulin (B2M) and soluble tumour necrosis factor-receptor-I (sTNF-R-I) measurements were obtained using commercially available kits, following the manufacturers' instructions.

After starting therapy with either cyclosporine or calcipotriol, the patients were observed 2 and 4 weeks later.

### *Statistical analysis*

Since the distributions of the data used in this study were unknown, only the medians and ranges have been reported to describe the results and, consequently, only non-parametric tests were used. The Friedman one-way variance analysis was employed to compare the paired data, and the correlations were analyzed by means of the Spearman's rank correlation test.

Normalization for age was obtained by dividing the corneometric values or the molecular serum levels by the patient's age, expressed in years.

## RESULTS

### *Effect of age normalization on the correlation between the lesional and the non-lesional corneometric values*

No correlation was seen between lesional and non-lesional corneometry in the 10 untreated patients ($R = 0.17$, $p = 0.6$). However, when all time points were analyzed (30 observations), the correlation coefficient was 0.5, $p = 0.003$ (Fig. 1).

Since there is a nearly statistically significant correlation between age and both lesional and non-lesional corneometric



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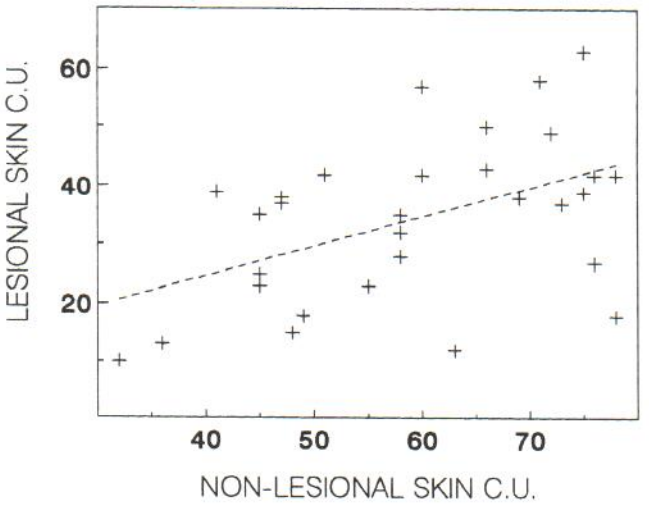


*Fig. 1.* Correlation between lesional and non-lesional corneometric units (*C.U.*) in 10 patients before and after therapy (at time 0 and after 2 and 4 weeks).

units (Fig. 2) ($R=-0.54$, $p=0.04$ and $R=-0.53$, $p=0.04$, respectively), a normalization of the corneometric data for age was made.

As reported in Fig. 3, the age normalization of the corneometric results improves the correlations between lesional and non-lesional corneometric values both at time 0 (before therapy) and at all the observation times. In particular, the statistically non-significant correlation observed at time 0 before normalization became significant ($R=0.78$, $p=0.01$), while the correlation found using all the time points improved to 0.92 ($p<0.00001$).

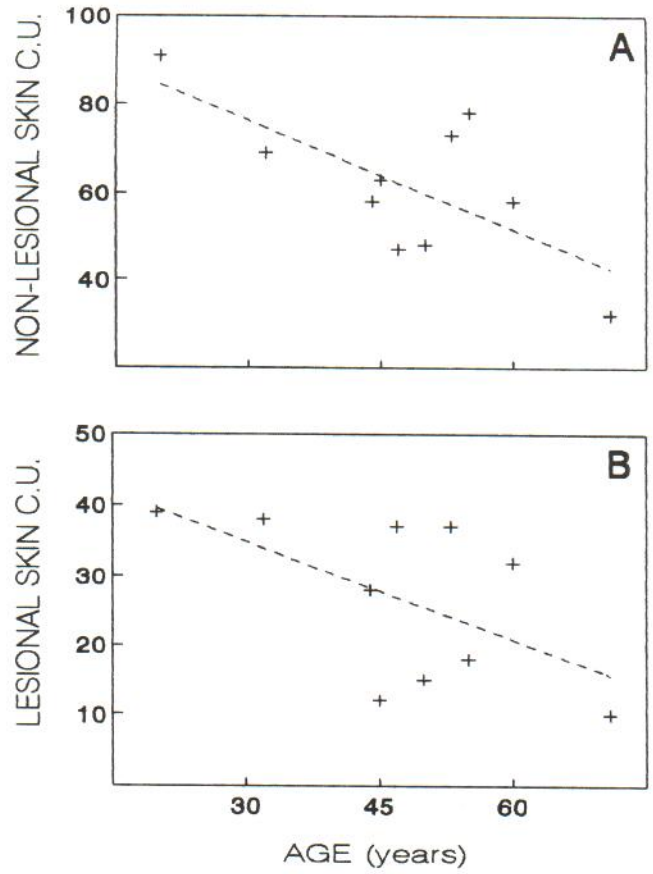


*Fig. 2.* (A) Correlation between age and non-lesional corneometric units (*C.U.*) and (B) between age and lesional corneometric units.

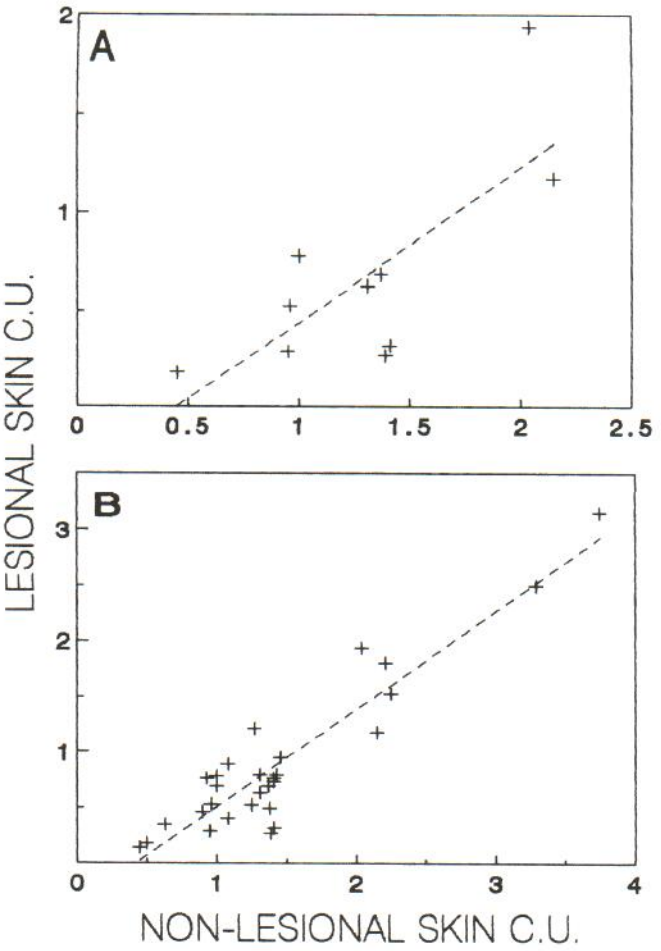


*Fig. 3.* Correlation between lesional and non-lesional corneometric units (*C.U.*) after age normalization. (A) Ten patients at time 0, and (B) the same patients evaluated at time 0 and again after 2 and 4 weeks.

*Correlations between lesional and non-lesional corneometric determinations and the PASI scores of the psoriatic patients*

No significant changes in the $p$ levels were observed when correlating the corneometric values to the PASI scores of the patients with data both normalized or not for age. In both cases, only the lesional corneometric values were PASI-related (time 0: $R=-0.46$, $p=0.046$ without age normalization and $-0.53$, $p=0.042$, with normalization; all times: $R=-0.55$, $p=0.04$, vs $-0.56$, $p=0.04$). No significant correlation was found between the PASI scores and the non-lesional corneometric values (data not shown).

*Correlations of the serum levels of eight molecules with both the lesional and non-lesional corneometric values*

Eight variables were selected among different soluble membrane molecules, released by several activated cell clones

(B2M, sIL-6R, sTNF-R-I, sL-Selectin, sICAM-1) or restricted to a few cell types (sIL-2R), already described to have variable behaviours in the sera of psoriatic patients (2, 7–10). One of the serum parameters, namely TGF-β1, belongs to the cytokine family (to our knowledge not previously described in psoriatic sera) and another, IL-1Ra, is an antagonist of the IL-1 receptor.

Age normalization of the corneometric values and serum concentrations of all the above-mentioned molecules improved from non-significance to significance the correlation coefficients for the regressions (Table II). Only sTNF-R always remained non-significant and showed no improved correlation coefficient.

As expected, correlation coefficient improvements were more evident in the non-lesional skin, where the disease manifestations were not expressed. In this case as well, all the correlation coefficients became significant with the exception of sTNF-R-I.

## DISCUSSION

Recent observations have shown that the corneometer is a valuable device for measuring the bound water content in poorly hydrated stratum corneum, such as that of psoriatic scales (6, 11).

To eliminate possible interferences due to the different duration of the plaques, we considered only recent psoriatic lesions (less than 2 weeks, as ascertained by two subsequent clinical examinations) (12).

The lesional corneometric levels showed a very poor and non-significant correlation with the non-lesional ones, when evaluated at time 0. This correlation may be improved, considering all the time points observed.

The fact that these values also depend on the subject's age, prompted us to eliminate the age interference in the correlation between lesional and non-lesional skin corneometric values. This may be achieved by age normalization of the corneometric data, using a previously described methodology (13). As shown in Fig. 3, age normalization clearly decreased the scatter between involved and non-involved psoriatic skin. The strong effect of age normalization is due to the interferences on the water content, depending on both age and disease, which cause similar effects.

A previous report also indicated that only the psoriatic lesional areas present altered corneometric data, the non-lesional skin not being significantly modified as compared to that of normal individuals (14). In this study, the interference due to the patient's age, which adds its own effect to that caused by the psoriasis, was not taken into account.

Our observations indicate that also non-lesional skin is involved in the disease. In fact, the corneometric value changes are in keeping with a skin water loss, paralleling that exhibited by the lesional skin, where the disease manifestations are well developed.

In agreement with this hypothesis, there are different, already published observations, describing increases of some pro-inflammatory cytokines (such as IL-6, TNF-alpha and GM-

Table I. *ELISA kits used to measure the serum values of eight molecules*

| Molecules | Kit name | Producer | Normal values | Sensitivity | Unit/ml |
|---|---|---|---|---|---|
| sL-Selectin | hsL-selectin | R&D | 747–1567 | 10 | ng |
| IL-1Ra | hIL-1ra | R&D | 25–325 | 6.5 | pg |
| sIL-2R | IL-2R test kit | T-cell | 145–913 | 24 | U |
| sIL-6R | sIL-6R ELISA | Bender | 402–1894 | 5 | pg |
| TGF-β1 | TGF-β1 kit | Genzyme | 24–104 | 5 | pg |
| β2-M | β2-microglobulina | Abbott | 900–1900 | 5 | μg |
| sICAM-1 | ICAM-1 Test Kit | Bender | 183–585 | 5 | ng |
| sTNF-R-I | sTNF-R (60kDa) | Bender | 1470–4160 | 80 | pg |

Table II. *Correlation between corneometric values and the serum levels of eight molecules before and after age normalization*

Data evaluated at time 0.

| Molecules | Correlation coefficients and significance | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Lesional | | Lesional/age | | Non-lesional | | Non-lesional/age | |
| | $R=$ | $p=$ | $R=$ | $p=$ | $R=$ | $p=$ | $R=$ | $p=$ |
| sL-Selectin | 0.65 | 0.03 | 0.86 | <0.001 | 0.44 | 0.15 | 0.91 | <0.001 |
| IL-1Ra | 0.45 | 0.14 | 0.60 | 0.045 | 0.41 | 0.12 | 0.59 | 0.05 |
| sIL-2R | 0.54 | 0.07 | 0.67 | 0.025 | 0.49 | 0.10 | 0.70 | 0.015 |
| sIL-6R | 0.30 | 0.21 | 0.69 | 0.02 | 0.10 | 0.39 | 0.73 | 0.01 |
| TGF-β1 | 0.29 | 0.22 | 0.62 | 0.04 | 0.24 | 0.28 | 0.69 | 0.02 |
| β2-M | 0.45 | 0.14 | 0.75 | 0.01 | 0.49 | 0.10 | 0.84 | 0.001 |
| sICAM-1 | 0.41 | 0.12 | 0.73 | 0.01 | 0.40 | 0.12 | 0.79 | 0.004 |
| sTNF-R-I | 0.59 | 0.05 | 0.45 | 0.12 | 0.24 | 0.27 | 0.10 | 0.40 |

CSF) in uninvolved psoriatic skin (15, 16). These findings are in accordance with the concept that psoriasis, clinically expressed as a psoriatic plaque, involves all the skin and, at times, the articulations as well.

A second point analyzed was the effect of age normalization on the correlation between the corneometric values and the individual PASI score. The latter, described by Fredrikson & Patterson (5), including the local disease activity and its general extension, has been considered the most powerful clinical index to gauge the disease severity, although some criticisms have been raised (17).

A significant correlation between the corneometric units and the PASI scores was observed in a previously published report (1) and again confirmed in the present study only with the lesional levels. In contrast, the same significant correlation was not found with the non-lesional levels. Age normalization did not modify these results, probably because the effect of the patient's age on the water loss of the psoriatic lesion was quite irrelevant.

In other studies, the correlations between psoriatic disease activity and serum concentrations of different soluble molecules (mainly pro-inflammatory cytokines or soluble cytokine receptors or adhesion molecules) have already been reported (2, 3, 7, 10, 16–18). We therefore examined whether age normalization could also influence the correlations between the corneometric units and the serum levels of a series of molecules, listed in Table II.

These molecules were selected on the basis of our recent studies on the serum values, on correlations with the PASI score and on the behaviour of these parameters, after therapy, in psoriatic patients. All these evaluations cannot be reported in this study, and information concerning the pathogenetic aspect of these parameters in psoriasis may be found elsewhere (2, 10, 18–20).

From a general point of view, it is not surprising that the pathomechanisms driving psoriasis, through the effects of different biological modulators, can modify both the serum levels of various soluble membrane molecules and the characteristics of the "stratum corneum", leading to corneometric changes.

With the exception of sTNF-alpha-R-I, all the other correlation coefficients were improved by age normalization, indicating that age is important for interferences on both corneometry and the serum concentrations of some soluble molecules such as those reported in this study. In agreement with our observations, some literature data indicate that at least IL-6, TNF-alpha and IL-1β increase in elderly subjects, possibly because of the phlogistic processes that are more frequent in elderly patients (21).

In conclusion, age normalization seems to represent a valid correction of the corneometric values to stress the potentialities of these data concerning their use in the clinical evaluation of plaque-type psoriatic patients.

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