

Soluble E-selectin and soluble ICAM-1 levels as markers of the activity of atopic dermatitis in children

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The expression of adhesion molecules is up-regulated in the skin of atopic dermatitis (AD) patients, and the levels of the soluble adhesion molecules sE-selectin and sICAM-1 have been reported to reflect the endothelial activation in the skin of AD patients. The objective of the study was to investigate the relationship between symptom score and levels of sE-selectin, sICAM-1 and sVCAM-1 before and after 2 weeks of treatment. Eighteen children with an exacerbation of AD were admitted and treated with corticosteroid dilutions under occlusive wet dressings (wet-wrap treatment). Symptom score (objective SCORAD) and levels of sE-selectin, sICAM-1, and sVCAM-1 were assessed before and after 2 weeks of treatment. A significant correlation between the objective SCORAD before treatment and the level of sE-selectin ($p < 0.05$), but not the level of sICAM-1 ($p = 0.7$) or sVCAM-1 ($p = 0.5$) was observed. The treatment resulted in a high degree of remission, which was reflected by a significant decrease in the level of sICAM-1 ($p < 0.01$), whereas there was only a trend in the level of sE-selectin to decrease ($p = 0.08$). The level of sE-selectin after 2 weeks of treatment still correlated significantly with the objective SCORAD before treatment ($p < 0.005$). Soluble E-selectin is a relative objective marker for the severity of AD. SCORAD is a treatment-sensitive symptom of AD, whereas E-selectin may be a more stable underlying systemic representation of AD.

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Atopic dermatitis (AD) is a common chronically relapsing inflammatory skin disease affecting 8.9–20.4% of those born after 1970 (1). Different serum parameters have been reported to reflect the inflammation in AD. Total serum IgE is increased in about 70% to 80% of the patients with AD, but is only loosely associated with the severity of AD (2). Eosinophil cationic protein (ECP – a marker for eosinophil activation), soluble interleukin-2 (IL-2) receptor and soluble CD14 (a marker for monocytes) reflect distinct components of the inflammation in AD and have been reported to correlate with the clinical severity of AD (3–5). Recently, soluble adhesion molecules were proposed as markers of disease activity in AD (6–12). The adhesion molecules

E-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are increased in acute and chronic skin lesions in AD (13). The serum levels of sE-selectin and sICAM-1 released from endothelial cells were observed to correlate directly with the cell surface expression *in vitro* (14). Thus, the levels of sICAM-1 and sE-selectin may reflect the intensity of the inflammation as well as the extent of skin involvement in AD. We have previously reported a correlation between the level of sE-selectin and the severity of disease as measured by objective SCORAD (15, 16). Whereas a large number of studies have established the relation between symptom score and the level of sE-selectin, conflicting results have

been reported on the applicability of sE-selectin for monitoring the effect of treatment (10–12, 17). The aim of the present study was to investigate whether the levels of sE-selectin, sICAM-1 and sVCAM-1 reflect the severity of AD and the clinical improvement of AD after 2 weeks of treatment in children with severe AD.

Methods

Patients and design

Eighteen children aged 4 months to 9 years (18, 19) were included. All patients had AD resistant to conventional therapy as outlined in guidelines (20). At the moment of inclusion, disease severity was severe in 16 of the patients and moderate in 2 of the patients as judged by objective SCORAD (21). There was one drop-out, for objective SCORAD evaluations, $n = 17$, while E-selectin was missing in another two patients ($n = 15$).

Patients were treated according to the wet-wrap protocol as previously described (22, 23). Goodyear et al. (1991) described the initial wet-wrap protocol as a treatment that results in remissions after only 3–5 days in severe generalized AD (described in (22)). Briefly, patients were admitted to the hospital and treated once daily with a diluted corticosteroid (fluticasone propionate cream 0.05%) on the whole body under occlusive wet dressings (tubifast) for 2 weeks. Other treatments for AD except antihistamines were stopped. The severity of AD was assessed by SCORAD at the beginning and at the end of the treatment.

Clinical scoring system

The severity of disease was scored using the objective SCORAD (21, 24) that includes the assessment of two items in a standardized manner: (a) extent (applying the rule of nine) and (b) intensity (erythema, edema/papulation, oozing/crust, excoriation, lichenification and dryness on a scale from 0 to 3). This scoring system reflects the modified consensus of the European Task Force on Atopic Dermatitis (21).

Determination of sE-selectin, sICAM-1 and sVCAM-1

Serum was harvested from venous blood and stored at -20°C . Levels of sE-selectin (HBT, Uden, the Netherlands), sICAM-1 and sVCAM-1 were determined in sera using a commercially available ELISA kit (Bio Source Fleurus, Bruxelles, Belgium). ELISA procedures were

performed according to the manufacturer's instructions. The levels of sE-selectin, sICAM-1 and sVCAM-1 were expressed in ng/ml. The detection limits of these ELISA assays for sICAM-1, sVCAM-1 and sE-selectin were 1.0, 2.0 and 2.0 pg/ml, respectively.

Statistical analysis

Statistical analysis was performed with SPSS 7.5 (SAS Institute Inc., Cary, NC, USA) using the Wilcoxon rank sum test to assess differences in the levels of sE-selectin, sICAM-1 and sVCAM-1 at the beginning and at the end of the treatment. Spearman's rank correlation was used to test monotonic relationships between the SCORAD and the levels of sE-selectin, sICAM-1 and sVCAM-1. Statistical significance was defined as $p < 0.05$.

Results

First, we examined the efficacy of the wet-wrap treatment. For evaluation of the efficacy, the objective SCORAD at the beginning and after 2 weeks of the treatment were compared and are shown in Fig. 1. There was a significant decrease in both the intensity and the extent of AD as reflected in the objective SCORAD after 2 weeks of treatment ($p < 0.0001$). One child was lost to follow-up due to personal reasons. These results demonstrated that in our study population the wet wrap was highly effective in reducing the severity of AD.

To investigate whether the serum levels of soluble adhesion molecules reflected the improvement of AD, we compared the levels of sE-selectin, sICAM-1 and sVCAM-1 at the beginning and after 2 weeks of the treatment.

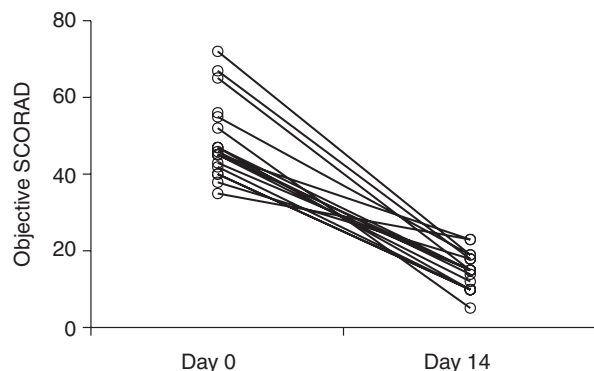


Fig. 1. Graph showing the objective SCORAD at the beginning and after 2 weeks of treatment. The change in objective SCORAD was assessed using Wilcoxon's rank sum test ($p < 0.0001$). Mild AD: objective SCORAD < 15 ; moderate AD: objective SCORAD 15–40; severe AD: objective SCORAD > 40 .

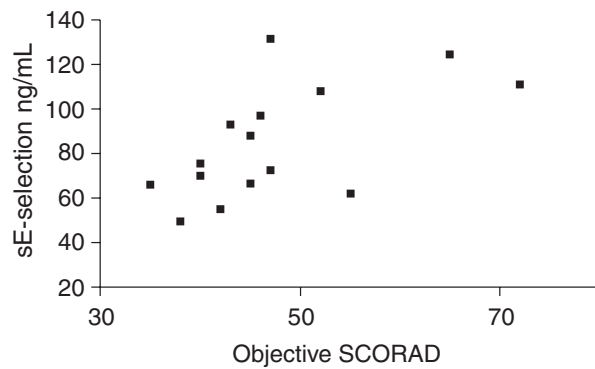


Fig. 2. Relationship between objective SCORAD and levels of sE-selectin at the beginning of the treatment (Spearman's rank correlation: $r = 0.54$, $p < 0.05$).

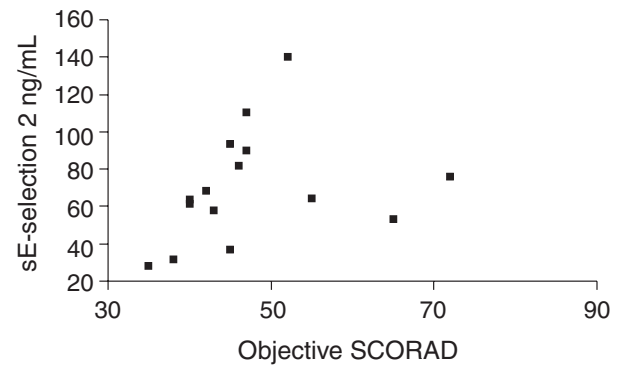


Fig. 3. Relationship between objective SCORAD at the beginning and levels of sE-selectin after 2 weeks of treatment (Spearman's rank correlation: $r = 0.53$, $p < 0.05$).

Although there was a trend for the levels of sE-selectin to decrease after treatment, statistical significance was not reached in this period ($p = 0.08$). The serum levels of sICAM-1 were significantly reduced after treatment ($p < 0.01$), whereas the levels of sVCAM-1 did not change significantly after treatment ($p = 0.5$). These results indicated that the marked improvement in AD was only reflected by the decreased level of sICAM-1, but not by the levels of sE-selectin or sVCAM-1.

Next, we investigated whether the serum levels of soluble adhesion molecules reflected the severity of AD. The levels of sE-selectin, sICAM-1, and sVCAM-1 were compared with the objective SCORAD. A significant correlation between the objective SCORAD and the level of sE-selectin was noted ($r = 0.54$; $p < 0.05$) (Fig. 2), while no correlation was observed with the levels of sICAM-1 ($r = -0.12$; $p = 0.7$) or sVCAM-1 ($r = -0.17$; $p = 0.5$). No significant Spearman rank correlations were seen at the start and the end of treatment for SCORAD ($r_s = 0.134$; $p = 0.609$) or for E-selectin ($r_s = 0.454$; $p = 0.089$), although during treatment patients seem to maintain their relative positions in the distribution for E-selectin than for SCORAD.

The level of sE-selectin after 2 weeks of treatment still correlated significantly with the objective SCORAD at the beginning ($r = 0.53$; $p < 0.05$) (Fig. 3). Objective SCORAD decreased dramatically in all 17 subjects ($p < 0.0005$). E-selectin had not decreased significantly ($p = 0.088$), although it had decreased in 10 out of 15 subjects. Furthermore, we noticed that children with high levels of sE-selectin at the beginning tended to have high levels of sE-selectin after treatment as well ($r = 0.45$; $p = 0.09$). A change in SCORAD is negatively

correlated with pre-treatment SCORAD ($r_s = -0.89$; $p < 0.0005$) and with pre-treatment E-selectin ($r_s = -0.65$; $p = 0.009$). A change in E-selectin is not correlated significantly with pre-treatment SCORAD ($r_s = -0.01$; $p = 0.980$), or with pre-treatment E-selectin ($r_s = -0.36$; $p = 0.182$), or with a change in SCORAD ($r_s = -0.07$; $p = 0.819$). A significant positive rank correlation of pre-treatment SCORAD with pre-treatment E-selectin ($r_s = 0.62$; $p = 0.013$), as well as with post-treatment E-selectin ($r_s = 0.53$; $p = 0.043$) was seen.

Six children had asthma diagnosed by a physician of whom three children had current asthma medication with inhalation corticosteroids, but none of the children had current wheezing. There was no significant difference in the levels of sE-selectin, sICAM-1 and sVCAM-1 between the children with and without a diagnosis of asthma ($p > 0.05$).

Discussion

The results of the present study indicate that sE-selectin is a marker of severity of AD, whereas sICAM-1 reflects short-term changes in the activity of AD. In agreement with the results of previous studies from our group (15, 16) and others (6, 10–12, 25), we observed a significant correlation between the severity of AD and the levels of sE-selectin before treatment. Unexpectedly we did not observe a significant decrease in the levels of sE-selectin by a treatment, which dramatically improved the symptom score (objective SCORAD) up to 90% after 2 weeks.

However, we observed that the levels of sE-selectin after 2 weeks of successful treatment still correlated with the objective SCORAD before treatment. Taken together, these results suggest that the serum levels of sE-selectin reflect

the severity of AD over a longer period than 2 weeks and are not so much influenced by acute fluctuations in the severity of disease. According to this assumption, the seemingly conflicting results from previous longitudinal studies on sE-selectin may be explained. Kagi et al. (10) Czech et al. (11) and Furue et al. (12) reported a significant decrease in sE-selectin levels after 16, 2–5, and 4 weeks of treatment, respectively. In contrast, there was no significant decrease in sE-selectin after 2 weeks of treatment in our study or 4 days of treatment in the study by Halmerbauer et al. who used systemic corticosteroids (17). Noteworthy, even conventional treatment with topical steroids significantly decreased sE-selectin levels if measured after more than 2 weeks (11), whereas more effective treatment associated with a major improvement in AD (systemic corticosteroids, potent corticosteroids under occlusion), did not significantly decrease sE-selectin levels during the first 2 weeks of treatment.

Why sE-selectin as a marker of severity of AD after 2 weeks of successful treatment still reflected the original symptom score, is not understood. We can only speculate on the underlying mechanisms of this finding, as little is known about the mechanisms of clearance and half-life of soluble adhesion molecules.

It is known that sE-selectin is exclusively found in lesional skin while ICAM-1 and VCAM-1 are constitutively expressed in normal skin and healthy appearing skin in patients with AD (13). Furthermore, sE-selectin expression is restricted to endothelial cells, whereas sICAM-1 and sVCAM-1 are expressed on other cell types as well. This indicates a higher specificity of E-selectin for affected skin and may explain why sE-selectin is a better indicator of inflammation in AD. However, E-selectin is not specific for AD and increased expression has been reported in psoriasis as well (11).

Adhesion molecules are glycoproteins, which mediate the attachment of leukocytes to endothelial cells thereby controlling migration. E-selectin seems to have a critical role in allergic skin inflammation as it promotes the selective migration of both CD4⁺ and CD8⁺ T cells expressing the cutaneous lymphocyte antigen (CLA), which is a skin homing factor for CD45⁺ RO⁺ memory-type T cells (26, 27).

Adhesion molecules are rapidly up-regulated on both endothelial cells and leukocytes upon exposure to cytokines such as interleukin-1 (IL-1), tumor necrosis factor- α (TNF- α) and interleukin-4 (IL-4) (28). Increased expression of ICAM-1, VCAM-1 and E-selectin has been demonstrated

in skin biopsies from chronic and acute AD lesions (13,29), and 6 h after intradermal allergen injection of sensitized individuals (30). The soluble form of the adhesion molecules, sE-selectin, sP-selectin, sICAM-1 and sVCAM-1, originates from membrane bound molecules due to shedding or proteolytic cleavage (31).

Little is known about the function of soluble adhesion molecules. It has been suggested that soluble adhesion molecules may have physiological implications, such as inhibition of adhesion by competitive binding. In contrast, it was also suggested that they may act in a pro-inflammatory manner by chemoattraction and by activating leukocytes (32). However, the levels of sE-selectin correlate directly with the cell surface expression of E-selectin in lesional endothelial cells (14) and seem to reflect the inflammation in AD.

In conclusion, we advocate the use of both a clinically based scoring system, such as the objective SCORAD, and soluble E-selectin as an objective marker of the inflammation in AD in studies on the pathogenetic mechanisms of AD.

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