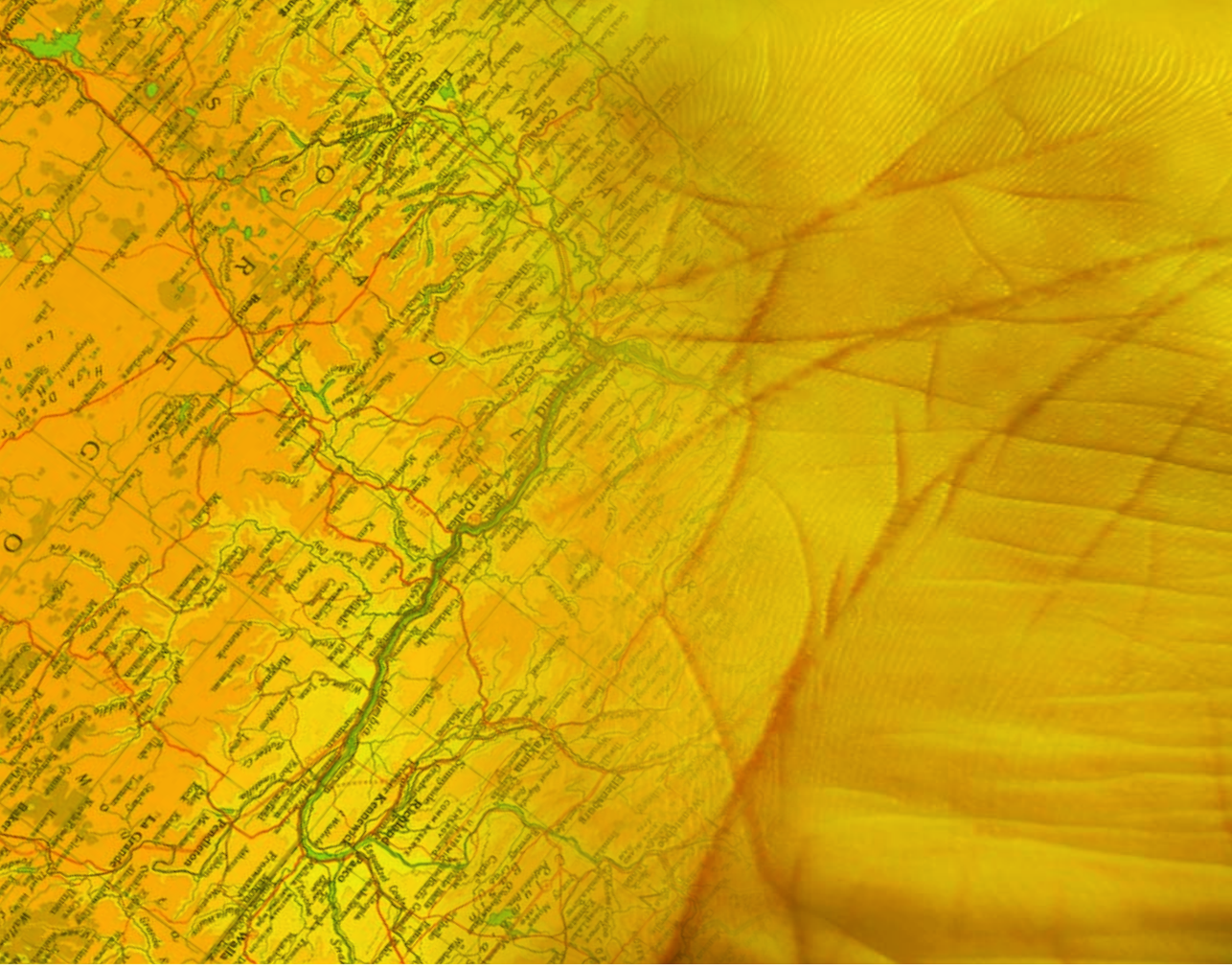

Pitfalls and opportunities in the treatment of atopic dermatitis

Sara G.A. van Velsen

De huid is mijn wereld. De huid is voor mij als het oppervlak van de aarde. Alwaar ik werk, leef en liefheb. Waar ik gebaande paden volg. Waar ik de collega's die mij voorgingen bedank voor hun baanbrekend werk.

De huid is een wereld waarin mensen leven. Waarin ik op verkenning ga, in onbekend terrein. Waar ik mijn kennis deel en ook probeer om grenzen te verleggen.

Sara G.A. van Velsen

Pitfalls and opportunities in the treatment of atopic dermatitis

Constitutioneel eczeem: systemische bijwerkingen van lokale corticosteroïden en effectiviteit van nieuwe therapieën (met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 28 juni 2011 des middags te 4.15 uur

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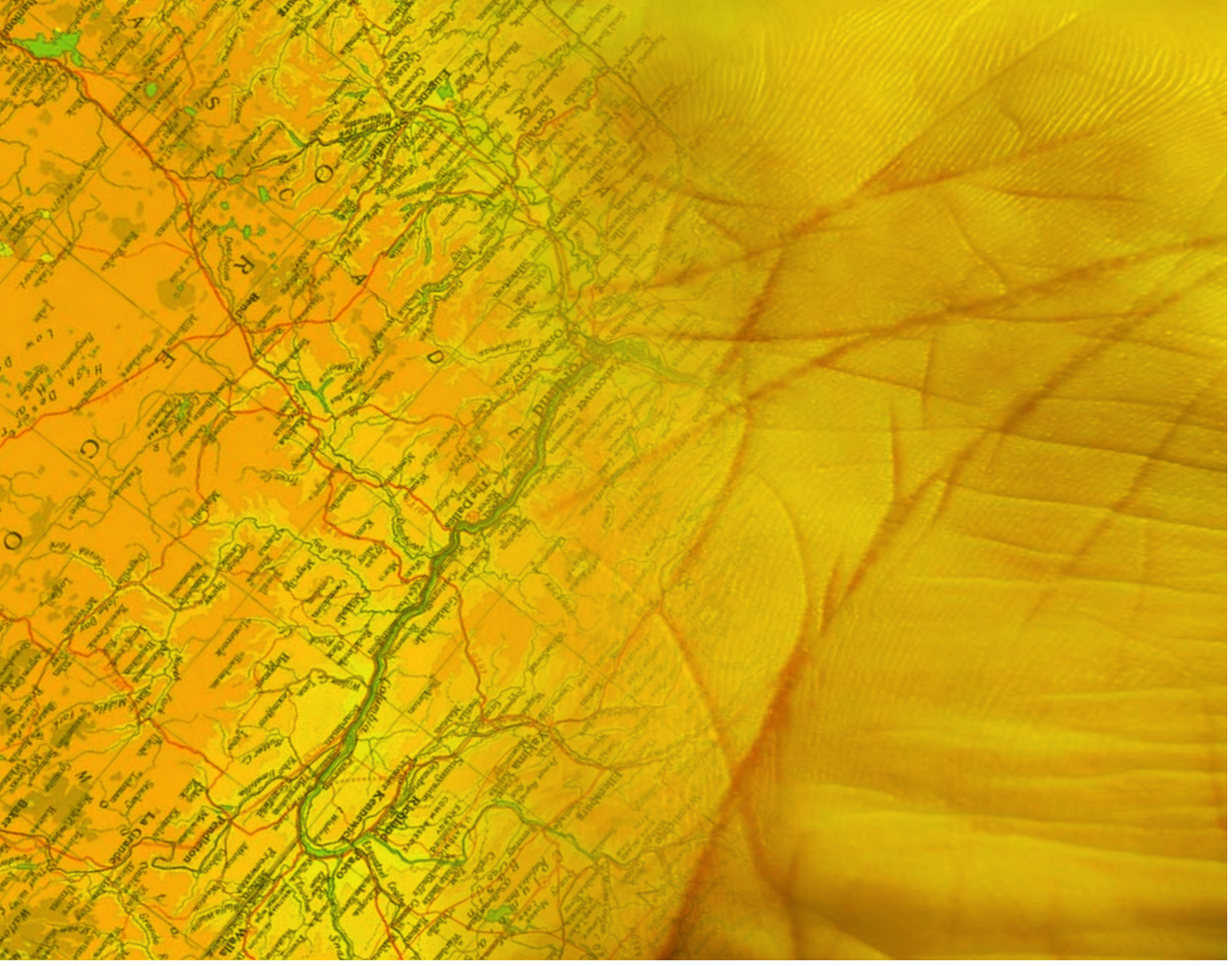

Introduction

Atopic dermatitis

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease characterized by pruritus, erythematous papules and plaques with scaling, excoriations and lichenification with a cutaneous distribution varying with age.[1;2] Furthermore, the skin of AD patients is dry (xerosis) with an impaired barrier function of the stratum corneum.[3;4] The diagnosis of AD is based on the criteria of Hanifin and Rajka (Table 1).[5] In the past decade, the prevalence of AD has been estimated to be 10-20% of children[6;7] and 2-10% of adults.[1;8] AD frequently starts in early infancy (early-onset AD) and approximately 90% of patients with AD have disease onset before the age of 5 years.[9] Spontaneous remission of AD is seen in 40-60% of patients before puberty.[1;9] A subgroup of AD patients has adult-onset, or late-onset AD and the majority of patients developed AD between 20 and 40 years of age. [10;11] The prevalence is found to be between 13-47% of adult AD patients.[10;11]
This inflammatory skin disease is often the prelude to an atopic diathesis in which other atopic diseases are frequently seen.[12-14] Symptoms of allergic rhinitis or asthma were present in 33.3% of children with AD between 2 and 17 years old[15] and symptoms of allergic rhinitis were found in 85% of AD patients between 8 and 50 years old.[16] Food allergy was found in up to 40% of children with AD between 6 months and 20 years of age.[17] Approximately one third of the patients will outgrow food allergy after 1 or 2 years of allergen avoidance. Up to 33% of patients with moderate to severe AD of all age groups have food sensitization[2], but AD is influenced by dietary factors in approximately 2% of adult AD patients.[17-19]

AD has an impact on quality of life. This has been investigated with use of specific quality of life questionnaires in both children and adults. In approximately 60% of children with AD impairment in performance at school was found and in 40-84% of children with AD attendance to sports and outdoor activities was impaired.[20]
Children with renal disease, cystic fibrosis and asthma reported a lower impact on quality of life than children with generalized AD.[21] Compared to other chronic childhood diseases, only children with cerebral palsy had higher scores on the impact on quality of life. Adult patients with AD also report a significant lower quality of life

Table 1.

Diagnostic criteria of atopic dermatitis according to Hanifin and Rajka.[5]

Major features (3 of 4 present)
Pruritus
Typical morphology and distribution of skin lesions
Chronic or chronically relapsing dermatitis
Personal or family history of atopy

Minor criteria (3 of 23 present)
Xerosis
Ichtyosis/palmar hyperlinearity/keratosis pilaris
Immediate (type I) skin test reactivity
Elevated serum IgE
Early age of onset
Tendency toward cutaneous infection/impaired cell-mediated immunity
Tendency toward non-specific hand or foot dermatitis
Nipple eczema
Cheilitis
Recurrent conjunctivitis
Dennie-Morgan infraorbital fold
Keratoconus
Anterior subcapsular cataract
Orbital darkening
Facial pallor/erythema
Pityriasis alba
Anterior neck folds
Pruritus when sweating
Intolerance to wool and lipid solvents
Perifollicular accentuation
Food intolerance
Course influenced by environmental/emotional factors
White dermographism/delayed blanch

compared to the general population with regards to vitality, social functioning and mental health.[22] In comparison with diabetes and hypertension, patients with AD have a greater impact on mental health.[22]

In the pathogenesis of AD several genes have been identified; for example genes encoding cytokines involved in the regulation of IgE synthesis (5q31-33).[1]

Furthermore, loss-of -function mutations have been found in the filaggrin gene (1q21.3) encoding a key protein in epidermal differentiation. Mutations in the filaggrin gene have first been described in patients with the autosomal dominant disorder ichthyosis vulgaris[23] and this leads to a dry, scaly skin with a decreased epidermal barrier function.[1] At this moment, more than 45 filaggrin ancestral-specific mutations in populations world-wide have been identified.[24;25] In a recent meta-analysis, a combined genotype with the two most common filaggrin polymorphisms (R501X and 2282del4) resulted in an increased AD risk with an odds ratio of 3.58 and 3.12.[26;27] Mutations in the filaggrin gene have been found in up to 50% of AD patients.[28;29] However, filaggrin deficiencies are found in 10% of healthy Europeans as well[30] suggesting that gene-environment interactions also play an important role in the pathogenesis of AD.

The immunopathogenesis of AD is complex. In the acute phase of AD Langerhans cells capture allergens via cell bound allergen specific IgE in the epidermis. For that reason the adaptive immune response will be activated stimulating proliferation and influx of Th2 lymphocytes in the skin. Allergic sensitization is enhanced by the impaired epidermal barrier function due to filaggrin polymorphisms.[24;25] Furthermore, due to mutations in the filaggrin genes the pH of the stratum corneum is raised, which may result in an increased serine protease activity and in pathogenic colonization with the Staphylococcus aureus bacteria leading to further disruption of epidermal barrier function.[25] Serine proteases activate keratinocytes to produce cytokine thymic stromal lymphopoietin (TSLP) via the protease-activated receptor-2 (PAR-2) pathway.[25] Allergens may also possess protease activity. For that reason they may also activate the innate immune system by activating the PAR-2 on keratinocytes.[25] TSLP is overexpressed in AD skin and is thought to activate dentritic cells to drive a Th2 T-cell polarization with production of IL-4, 5 and 13. Th2 cells are recruited to the skin with help of chemokines such as CCL17 (thymus and activation regulated chemokine; TARC), CCL-27 (CTACK) and CCL5 (RANTES). The increased production of IL-4 results in IgE isotype switching by B-cells. IL-5 attracts eosinophils and prolongs their survival. Eosinophils are present in acute eczema and patients with severe AD may have blood eosinophilia.[31] The course of AD is biphasic, the Th2-predominant acute phase is followed by a Th1- predominant chronic phase.[32]

Monocytes are recruited to the skin via monocyte chemotactic protein 1 (MCP-1), produced by activated Langerhans' cells in the acute phase of AD.
These monocytes differentiate into inflammatory dentritic epidermal cells (IDEC) and produce IL-12 and IL-18. This leads to a switch from Th2 to Th1 with production of interferon-gamma (INF-γ), IL-5 and IL-31.[1;33]

Treatment

Topical corticosteroids

The discovery of corticosteroids dates back to 1950 where Kendall and Hench won the Nobel Prize in Physiology and Medicine[34] and revolutionalized the treatment of rheumatic disease.[35;36] Corticosteroids have proven their effectivity as an anti-inflammatory treatment for a wide range of other inflammatory diseases such as asthma[37], inflammatory bowel disease[38;39] and inflammatory skin diseases like AD.[40]
Topical corticosteroids form the mainstay of treatment for AD. Severity signs and symptoms of AD such as pruritus, scaling, erythema, vesiculation and papulation have been shown to improve during treatment with topical corticosteroids.[40] The anti-inflammatory and immunosuppressive effects of topical corticosteroids are mediated by regulation of corticosteroid-responsive genes. The steroid binds to the glucocorticoid receptor in the cytoplasm of target cells in the epidermis and dermis. The glucocorticoid-receptor complex can inhibit transcription of proinflammatory cytokines such as IL-1 through IL-6, INF-γ and TNF-α.[41;42]
In Europe four potency classes of topical corticosteroids are known (class I-IV). Corticosteroid strength has been classified according to the vasoconstrictor assay where penetration of the steroid induces blanching of the skin via vasoconstriction.[43] A relationship has been demonstrated between the blanching of the skin and the alleviation of inflammation in the skin.[44] In Table 2 the most frequently used topical corticosteroid preparations are divided into their respective potency classes. In this thesis the European classification on topical corticosteroid potency is used and we will focus on three topical corticosteroids that are used frequently in the Netherlands: 0.005% fluticasone propionate ointment (potent; Cutivate®), 0.1% betamethasone valerate ointment (potent; Betnelan®) and 0.05% clobetasol propionate ointment (superpotent; Dermovate®).

Table 2.

Potency classes of topical corticosteroids according to the European classification.

Class	Active moiety	Brand name/Generic	Formulation
Class I, least potent	Hydrocortisone acetate	Generic	1%, cream, ointment
Class II, mid potent	Clobetasone butyrate	Emovate	0.05%, cream, ointment
	Flumethasone	Locacorten	0.02%, cream
	Hydrocortisone butyrate	Locoid	0.1%, cream, ointment, lotion, gel
	Triamcinolone acetonide	Generic	0.1%, cream, ointment
Class III, potent	Betamethasone valerate	Betnelan, generic	0.1%, cream, ointment, lotion, emulsion
	Betamethasone dipropionate	Diprosone	0.05%, cream, ointment, lotion
	Desoximethasone	Topicorte, Ibaril	0.25%, cream, emulsion
	Diflucortolone	Nerisona	0.1%, cream
	Fluticasone propionate	Cutivate	0.05%, cream; 0.005%, ointment
	Mometasone fuorate	Elocon, generic	0.1%, ointment, lotion
Class IV, superpotent	Betamethasone dipropionate in propylene glycol	Diprolene	0.05%, cream, gel
	Clobetasol propionate	Dermovate, generic	0.05%, cream, ointment, lotion, gel, foam, shampoo

Side effects of corticosteroids

The side effects of orally administered corticosteroids are well known, and in short can consist of the occurrence of diabetes mellitus, increased risk of infection, delayed wound healing, ophthalmic effects, peptic ulcer disease, skin changes, adrenal suppression and changes in bone metabolism.[45;46] Application of topical corticosteroids can also lead to side effects, both local and systemic. Local side effects are: atrophy of the skin, the occurrence of teleangiectasia in the skin, striae, steroid rosacea, acne, hypopigmentation, hypertrichosis perioral dermatitis, purpura, aggravation of cutaneous infections, delayed wound healing and alterations in skin elasticity.[47] Systemic side effects may occur after percutaneous absorption. Percutaneous absorption of topical corticosteroids may be enhanced in patients with AD because of the decreased epidermal barrier function that is associated not only with mutations in the filaggrin gene[25], but also with active inflammation.[48] In this thesis we study the effects of topical corticosteroids on the hypothalamus-pituitary-adrenal (HPA-axis) and on bone mineral density (BMD).

The Hypothalamus-Pituitary-Adrenal Axis

The secretion of cortisol is regulated by the HPA-axis. Corticotropin-releasing hormone (CRH) by the hypothalamus stimulates adrenocorticotropic hormone (ACTH) secretion by the pituitary gland. ACTH is secreted in pulses with a diurnal rhythm. The highest plasma ACTH concentrations occur in early morning (between 4 and 6 a.m.) and the lowest plasma ACTH concentrations occur at night. ACTH stimulates the adrenal gland to produce cortisol, which also follows a diurnal rhythm with the highest concentrations in early morning (basal serum cortisol level).
Presence of cortisol in the circulation inhibits both ACTH and CRH synthesis via a negative feedback loop, and this will lead to inhibition of the HPA-axis.[49] Figure 1 shows the regulation of the adrenal cortisol secretion by the HPA-axis. Thus, the presence of exogenous corticosteroids in the circulation may inhibit the production of endogenous cortisol. For that reason the endogenous basal serum cortisol level is used as an outcome parameter for percutaneous absorption of topical corticosteroids. Basal serum cortisol levels between 0.20-0.60 µmol/L are considered normal.
Chronic suppression of ACTH and CRH by exogenous corticosteroids may lead to systemic side effects such as adrenal atrophy and loss of cortisol secretory capability.[50] Furthermore, high levels of exogenous corticosteroids (hypercortisolism) can induce iatrogenic Cushing's syndrome.[51]

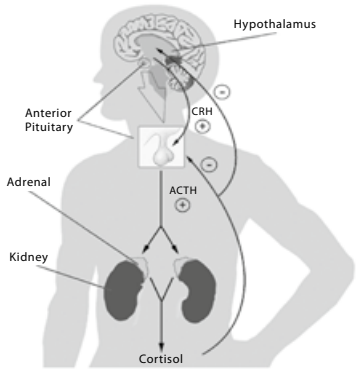

Figure 1.
Thy hypothalamus pituitary adrenal axis.

In the 1960s-1980s research has been done on percutaneous absorption of topical corticosteroids. During maintenance therapy with topical corticosteroids in patients with AD or psoriasis mainly normal serum cortisol levels are observed when amounts of < 100 g 0.1% betamethasone valerate ointment (European class III) per week[52-54] and < 50 g of 0.05% clobetasol propionate ointment (European class IV) per week[55] are used. This suggests that systemic side effects of topical corticosteroids are unlikely to occur with use of these amounts.

However, low serum cortisol levels are observed during treatment with 30 g of topical corticosteroids daily of mid potent, potent and superpotent classes (European class II, III and IV) in patients with active inflammatory skin diseases.[56;57] Thus, in these patients percutaneous absorption of topical corticosteroids led to systemic availability, which inhibited the HPA-axis. When treatment with topical corticosteroids was stopped, serum cortisol levels normalized after 3-4 days.
To prevent relapses topical corticosteroids (class II and III) are given as intermittent maintenance therapy (2-4 times/week) in patients with moderate/severe AD.[40] Sofar, there are no data on adrenal gland recovery during maintenance therapy after intensive use of potent and superpotent topical corticosteroids in patients with an exacerbation of AD. We have addressed this topic in chapter 2.
As shown, research on percutaneous absorption of topical corticosteroids has been primarily done by measurement of cortisol levels in patients with inflammatory skin diseases during and after topical corticosteroid therapy. Measurement of the actual serum concentrations of the topically applied corticosteroid could provide direct evidence of percutaneous absorption and correlates serum levels of synthetic corticosteroids with adrenal cortisol production. Only one study investigated systemic availability of clobetasol propionate after a single topically applied dose of 0.05% clobetasol propionate ointment. This was done by a radioimmunoassay. Competition for the binding site on an antigen for clobetasol propionate between a known quantity of radioactive labelled clobetasol propionate and clobetasol propionate in the serum results in a radioactive signal from which a binding curve can be made.[56] The concentrations of the serum concentrations of clobetasol propionate in serum can be read form this binding curve. Nowadays, liquid chromatography-tandem mass spectrometry (LC/MS/MS) is the most preferred analytical technique for bioanalytical steroid assays, due to its selectivity and sensitivity.[58;59] In chapter 3 a new bioanalytical assay for the detection of clobetasol propionate in serum is presented using LC/MS/MS. In chapter 4 measurement of serum clobetasol propionate concentration is combined with serum cortisol levels in adult patients with severe AD.

Bone mineral density

Bone mass and BMD increase during adolescence and puberty until peak bone mass is reached at approximately 20 years of age.[60] Bone mass acquisition during childhood and having an optimal BMD during adulthood is an important determinant of fracture risk.[61] Determinants in reaching optimal bone mass are genetic-ethnic factors, hormonal status, calcium intake, physical activity (especially weight bearing activity) and weight.[62] Vitamin D is also important for bone mineralization and vitamin D deficiency is associated with an increased risk on osteopenia and osteoporosis.[63] Use of oral corticosteroids is known to negatively influence BMD.[64]

Corticosteroids can directly influence bone by inhibiting osteoblast formation and thus a reduction in bone formation. Furthermore, malabsorption of calcium in the intestines can lead to a secondary hyperparathyreoidism leading to increased bone resorption.[64;65] This leads to a decrease in BMD.[66] Systemic availability of topically applied corticosteroids may have a negative effect on BMD in both children and adults. However, literature on BMD in patients with AD is scarce. In a study on 28 adult patients with widespread AD BMD was significantly lower in patients who had used topical corticosteroids of moderate to high potency compared to patients who had used no or low potent topical corticosteroids.[67] Furthermore, a more recent study found that 30.4% of a group of 125 adult patients with moderate to severe AD had low BMD, or a Z-score ≤ -1.[68] No evidence was found for a negative effect of topical corticosteroid use in the previous 5 years. However, through logistic regression, a (non significant) trend was found towards an increased risk of low BMD with higher use of topical corticosteroids.[68]

Information on the influence of topical corticosteroids on BMD in children with AD is even scarcer. One study on 43 children with moderate to severe AD found that patients using both topical corticosteroids and cyclosporin A (CsA) (n=6) had a lower BMD than patients using topical corticosteroids alone.[69] No other studies are available for comparison. There is need for additional studies on this subject, both in adults and children with AD. The adult study group described by Aalto-Korte[67] is small, and the study by Haeck et al.[68] is a single time point study. We performed a longitudinal study on the change of BMD during topical corticosteroid use in adult patients with AD. The results are described in chapter 5.

As for the data on the effect of topical corticosteroids on BMD in children, the study by Pedreira et al. was not designed to investigate the influence of topical corticosteroids on BMD, as topical corticosteroid use during the study period was not calculated.[69] Topical corticosteroid use was assessed using a corticosteroid index. This index was calculated on one time point by multiplying the strength of the corticosteroid applied, the frequency of application, the affected body surface area and the number of years on topical corticosteroid treatment. However, use of topical corticosteroids changes in time because disease activity of AD changes. Furthermore, many children use more than one potency class of topical corticosteroid. This may hamper the usefulness of this index. The study focused on the finding of a decreased BMD in the group of children who had used CsA. We feel that this result should be interpreted with caution because it is based on only 6 patients. In chapter 6, we measured BMD in children with moderate to severe AD and investigated if use of topical corticosteroids during the previous 5 years led to a decrease in BMD.

Measuring bone mineral density

The most widely used technique to measure BMD is dual-energy X-ray absorptiometry (DXA). It determines the amount of mineral in a specific body site (lumbar spine, hip) or in the whole body by measuring bone in two dimensions and then calculating the BMD (g/cm^2) by dividing the bone mineral content by the bone area.[60;70] The BMD calculated in a patient is compared to reference (normative) values of healthy children or adults.[61] BMD is presented as a T- or Z-score, and the diagnosis of osteoporosis or osteopenia is based on these scores. The T-score is defined as the standard deviation (SD) score of the observed BMD compared with that of a healthy adult peak bone mass. A T-score of less than -1 SD indicates osteopenia and a T-score of -2.5 SD or below indicates osteoporosis.[71] The Z-score is defined as the SD score based on healthy persons of the same age. In children only Z-scores are used to define low BMD. The International Society of Clinical Densitometry (ISCD) states that a Z-score of less than or equal to -2 SD indicates a low-for-chronological-age BMD, and a Z-score of less than or equal to -2 SD in combination with a clinically significant fracture history indicates osteoporosis (Table 3).[72] Both the T-and Z-score are specific for male or female patients, and reference values are available for both men and women.

Table 3.

Measuring bone mineral density in adults and children.

Adults	**T-score definition[71]**
Normal bone mineral density	≥ −1
Osteopenia	−2.5 < −1
Osteoporosis	≤ −2.5

Children	**Z-score definition[72]**
Normal bone mineral density	> −2
Low bone mass for chronological age	≤ −2
Osteoporosis	≤ −2 AND clinically significant fracture history*

** one long bone fracture of lower extremity, two long bone fractures of upper extremity or vertebral compression fracture.*

Oral immunosuppressive therapy

When topical corticosteroids fail to alleviate the symptoms and signs of AD in patients with severe and extensive skin disease, treatment with oral immunosuppressive drugs may be required. In this thesis we will focus on three immunosuppressive drugs in the treatment of patients with severe AD: Cyclosporin A (CsA; Neoral®), Enteric-Coated Mycophenolate Sodium (EC-MPS; Myfortic®) and everolimus (Certican®).

Cyclosporin A

At this moment, CsA is the first choice, and only registered oral immunosuppressive therapy for patients with severe AD. CsA inhibits the phosphatase activity of calcineurin. This leads to inactivation of nuclear factor of activated T-cells (NFAT). In this way, NFAT is inhibited to regulate the transcription of genes encoding inflammatory cytokines important for T-cell proliferation. CsA especially inhibits production of interleukin-2 (IL-2).[73]

The first studies on CsA in the treatment of AD date from 1992, and describe its effectiveness in suppressing active, severe AD. Disease activity decreases within several weeks and in some studies within two weeks.[74-78] However, especially long term treatment with CsA induces side-effects such as hypertension and nephrotoxicity in 5.8% and 10.9% of patient months of active treatment.[79] This leads to dose-reduction or discontinuation of therapy with CsA in some patients with AD. Furthermore, in 4 out of 20 patients treated with CsA experienced an exacerbation of AD paralleled by an increase in serum IgE levels.[80]

Enteric-coated mycophenolate sodium

In the search for new potent anti-inflammatory drugs EC-MPS may be an effective treatment for patients with severe AD. EC-MPS contains the same active moiety (mycophenolic-acid; MPA) as mycophenolate-mofetil (MMF). MMF is now a standard component of the immunosuppressive regimen following renal transplantation.[81], but it has also been shown to be effective in the treatment of AD in various case series.[82-86]

Compared to the first choice and effective oral immunosuppressive therapy for AD, CsA[40], MPA not only inhibits the clonal expansion of T-cells, but also of B-cells.[87] MPA selectively and reversibly inhibits inosine monophosphate dehydrogenase (IM-PDH), which is a key enzyme in the *de novo* synthesis of purines. Purines, the building blocks of DNA and RNA, are required for the proliferation of both T- and B-cells. T- and B-cells rely almost solely on this *de novo* pathway, and their proliferation will be inhibited. Other cell types rely on the purine *salvage* pathway, with is catalysed by another enzyme. In this way neurons and brain cells are almost independent of IM-PDH. But enterocytes, for example, are approximately for 50% dependent on IM-PDH for their proliferation.[88] Since enterocytes partly rely on the *de novo* synthesis, it is not surprising that one of the most frequent side-effects of MPA is diarrhoea. But also other gastrointestinal side-effects such as abdominal pain and dyspepsia can occur.[87;88] Up to 70% of renal transplant patients experience gastrointestinal side effects[81], and gastrointestinal side effects were also observed in patients with AD during treatment with MMF.[82;85]

EC-MPS is designed to improve the upper gastrointestinal tolerability[89]and contains an enteric coating that delays the delivery of MPA in the stomach. Both EC-MPS and MMF, given in equimolar dosis, produce equivalent MPA exposure in renal transplant patients.[90] Compared to MMF, the incidence and severity of gastrointestinal side effects are lower during treatment with EC-MPS in renal transplant patients. [91;92]
Furthermore, conversion from MMF to EC-MPS in organ transplant patients with gastrointestinal side effects leads to an improvement of the gastrointestinal tolerability. Side effects such as nephrotoxicity and hypertension are rarely seen during treatment with either EC-MPS or MMF.[93;94] This makes EC-MPS a promising alternative treatment for patients with severe AD. No studies are available on EC-MPS for the treatment of patients with severe AD who are unresponsive or have side effects to topical corticosteroids or first choice oral immunosuppressives such as CsA. Therefore, we performed an observational study on the efficacy and safety of EC-MPS in adult patients with severe AD.

Everolimus

Everolimus is an immunosuppressive and antiproliferative macrolide derived from rapamycin (sirolimus). It shows increased oral bioavailability compared to sirolimus, but is has a similar mechanism of action.[95] Everolimus inhibits an adjacent step in T-cell activation compared to CsA. By inhibiting the kinase activity of mammalian target of rapamycin (mTOR) everolimus blocks the IL-2 and IL-15 driven cell proliferation of both T-cells and B-cells, but also of vascular smooth muscle cells.[73;96] Because the mode of action of everolimus is complementary and synergistic to that

of CsA, the use of everolimus in organ transplant patients may allow dose reduction of CsA.[96;97] Dose reduction of CsA may decrease nephrotoxicity; a main side effect of CsA.[79] Indeed, it has been shown that the combination of everolimus and CsA could maximize immunosuppression, whilst preserving renal function.[97-102] Combined treatment with everolimus and CsA has also been shown effective in controlling severe psoriasis.[103;104] Everolimus may also be a promising additive in the treatment of AD. No information on the efficacy and safety of everolimus in patients with AD is available yet. We have treated two adult patients with severe AD with everolimus either in combination with CsA or with prednisone.

Measuring disease activity of atopic dermatitis

Measurement of AD disease activity is important to determine the effect of therapy, not only in clinical trials, but also during treatment in the outpatient clinic.[105] Many objective scoring systems are available, where a trained investigator has to assess disease extent and/or severity.[106] For example, the Eczema Area and Severity Index (EASI)[107], objective SCoring Atopic Dermatitis (SCORAD) index[108] and the The Six Area Six Sign Atopic Dermatitis (SASSAD) severity score[109] are validated and widely used to measure AD disease activity.[106] However, because these scoring systems are time consuming, their use in general clinical practice is limited. Patient oriented scoring systems may bypass this. The Self-Administered Eczema Area and Severity Index (SA-EASI) is derived from the EASI, and is developed to allow the patient or patients' parent/caregiver to evaluate both the extent and severity of AD at any moment. The SA-EASI is highly correlated to the EASI, but has not been related with the objective SCORAD and the SASSAD score. To enhance the use of the SA-EASI to follow disease activity during treatment one needs to know the correlation with these frequently used investigator based scoring systems.

A laboratory marker, Thymus and Activation-Regulated Chemokine (TARC), could also be used to assess AD disease activity.[110-112] TARC is a ligand for CC chemokine receptor 4 (CCR4) that is selectively expressed on Th2 cells and has shown to induce the selective migration of Th2 cells. Serum TARC levels are selectively elevated in patients with AD.[110;111] Furthermore, levels of serum TARC correlate with disease activity measured by the Leicester Sign Score (extensive version of SASSAD score)[110;113], SCORAD[112] and parallel disease severity during treatment with CsA, EC-MPS or topical corticosteroids.[110;111;114] Serum TARC levels have not yet been correlated with the SA-EASI. In chapter 7 we investigated the correlation between the SA-EASI and investigator based scoring systems (objective SCORAD and SASSAD) and with a laboratory marker (TARC) in children with AD.

Outline of this thesis

This thesis addresses systemic side effects of topical corticosteroids (*pitfalls*) and new treatment options (*opportunities*) in patients with moderate to severe AD.

Chapters 2, 3 and **4** describe the effects of topical corticosteroids on the HPA-axis, with adrenal gland cortisol production as the main outcome parameter.
Chapter 2 focuses on differences between potency classes of topical corticosteroids on the recovery of adrenal cortisol production after a period of intensive use of topical corticosteroids in patients with severe AD.
Chapter 3 and **4** are complementary to each other. In **chapter 3** a method is described to measure systemic levels of clobetasol propionate by liquid chromatography-tandem mass spectrometry (LC/MS/MS) during use of 0.05% clobetasol propionate ointment in patients with severe AD. **Chapter 4** shows the relation between systemic availability of clobetasol propionate and adrenal gland cortisol production during treatment with 0.05% clobetasol propionate ointment in patients with severe AD.

In **chapter 5** the relation between topical corticosteroid use and bone mineral density is investigated in children with moderate to severe AD. In **chapter 6** the relation between topical corticosteroid use and the change in bone mineral density is investigated during a two-year follow-up period in adult patients with moderate to severe AD.

In **chapter 7** correlations between a patient oriented AD disease activity score, the SA-EASI, and two investigator based scoring systems (objective SCORAD and SASSAD) are investigated in children with AD. Furthermore, the correlation between the SA-EASI and a laboratory marker (TARC) is investigated in the same patient group.

Chapter 8 and **9** show the efficacy and side effects of two new oral immunosuppressive drugs, enteric-coated mycophenolate sodium (EC-MPS) and everolimus, in adult patients with severe AD.

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Chapter 2

Percutaneous absorption of topical corticosteroids in patients with severe atopic dermatitis

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Abbreviations:
AD: atopic dermatitis
ACTH: adrenocorticotropic hormone
T½ el: elimination half life time

Abstract

Objective

To study the influence of topical corticosteroids on adrenal gland function in patients with severe atopic dermatitis during and after treatment with topical corticosteroids in hospital.

Design

Observational study. Measurement of basal serum cortisol levels and the amount/potency class of topical corticosteroids used in hospital and during maintenance therapy after discharge.

Setting

Dermatology inpatient department at a tertiary care medical centre.

Patients

Fifty-seven patients with severe atopic dermatitis were included at the moment of admission to the hospital.

Main outcome measures

Basal serum cortisol levels.

Results

Baseline cortisol levels were normal in a pilot group of 5 patients (0.43 µmol/L ± 0.19).
On day 2, after the first application of 20-30 g topical corticosteroids in hospital basal cortisol levels decreased in 51 (89.5 %) patients (0.02 µmol/L ± 0.03). Cortisol levels did not differ between patients treated with 0.05% clobetasol propionate or 0.1% betamethasone valerate ointment (p=0.23). At discharge, 28 out of 51 (54.9%) had normal cortisol levels and 23 out of 51 (45.1%) had low cortisol levels. At that moment, 10-30 g topical corticosteroid daily was used. All patients treated with 0.05% clobetasol propionate prior to discharge had low cortisol levels at discharge compared to only 40% of the patients (14 out of 35) using 0.1% betamethasone valerate and none of the patients using 0.005% fluticasone propionate ointment.
The differences in cortisol levels at discharge between these three topical corticosteroids were statistically significant. After discharge, basal cortisol levels normalized in all patients after 41 ± 50 days; when 51 ± 32 g topical corticosteroids were used per week irrespective of potency class.

Conclusions

After an initial decrease, tapering the daily amount of topical corticosteroids restores cortisol levels in half of the patients at the end of hospitalization. At discharge, low basal serum cortisol levels were found in all patients using 0.05% clobetasol propionate. During maintenance therapy with topical corticosteroids (mean 51 g/week) all patients have restored cortisol levels.

Introduction

Virtually all topical corticosteroid compounds can penetrate the skin. Absorption occurs in normal individuals[1], but especially when the skin is inflamed and the integrity of the epidermal horny layer is disrupted for example in patients with atopic dermatitis (AD).[2-5] Percutaneous absorption of topical corticosteroids may result in systemic levels of the topically applied corticosteroid and inhibition of adrenal gland cortisol production. The amount of percutaneous absorption depends on many factors such as the extent of body surface affected by skin disease, the mode of application, the amount used per day or week and the molecular structure/potency of the topical corticosteroid.[6]

In this observational study, we investigated the effect of topical corticosteroids on adrenal gland function in patients with severe AD. Serum cortisol levels were monitored during hospitalization, when topical corticosteroid therapy was intensified and during maintenance therapy after discharge. Furthermore, the suppressive effect on adrenal cortisol production was compared between different classes of topical corticosteroids.

Patients and methods

Fifty-seven adult patients with severe AD were included at the moment of admission to the hospital. The diagnosis was made according to the criteria of Hanifin and Rajka.[7] If possible a basal serum cortisol and ACTH measurement was done prior to the first application of topical corticosteroids to study the effect of a single application of topical corticosteroids on adrenal cortisol production.
All patients were treated with approximately 20-30 g of potent topical corticosteroids (0.05% clobetasol propionate ointment (n=31) or 0.1% betamethasone valerate ointment (n=26) on the day of admission (day 1). The next morning (day 2) basal serum cortisol was measured in all patients. The inpatient treatment regime was not standardized but individualized by the dermatologist in charge. Also, the type of topical corticosteroid used and the duration of hospitalization were set by the dermatologist in charge. Patients were discharged when skin disease was controlled. Basal serum cortisol measurement was repeated at the day of discharge from the hospital. If the levels were low at discharge, basal serum cortisol measurement was repeated when patients returned to the outpatient clinic. During, and after hospital admission the amount of topical corticosteroids was recorded.
The study was approved by the local ethical committee of the University Medical Center Utrecht.

Basal serum cortisol and ACTH levels
The basal serum cortisol and ACTH levels were measured between 8.00 and 10.00 am. Cortisol was measured using a chemiluminescent immunoassay on the Dxi (Beckman Coulter Inc. Fullerton, CA U.S.A.). The lower limit of detection was 0.011 µmol/L and interassay variation was <8.0%. ACTH was measured using an electrochemiluminescence immunoassay on the Modular E170 (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). The lower limit of detection was 1.0 ng/L and interassay variation was <7,5%.
Basal serum cortisol levels between 0.20-0.60 µmol/L are considered normal.
And basal serum ACTH levels are considered normal between 5-70 ng/L.

Statistical analysis
Statistical analysis was performed using the program SPSS for Windows (version 15.0, 2006; SPSS inc., Chigago, IL, U.S.A.). Since skewed distribution of outcome parameters were observed, nonparametric tests were used. Differences between the mean rank for different treatment groups were compared using the Kruskal-Wallis Test.

Results

Fifty-seven patients with severe AD, 32 women and 25 men (age between 18–82 years), were included at the day of admission (day 1). Baseline characteristics of the patients are described in table 1. Baseline measurement of serum cortisol and ACTH was only done in 5 patients, because most patients arrived at the hospital after 10.00 am. In these patients a normal basal serum cortisol level (0.43 µmol/L ± 0.19) and ACTH level (19.6 ng/L ± 8.02) was measured.
Patients were treated with 20-30 g of topical corticosteroid ointment on day 1 of admission. Thirty-one patients received 0.05% clobetasol propionate ointment and 26 patients received 0.1% betamethasone valerate ointment.
Basal serum cortisol levels decreased in 51 (89.5 %) patients (Subgroup 1). Basal serum cortisol levels were 0.02 ± 0.03 µmol/L for the patients treated with 0.05% clobetasol

propionate ointment and 0.03 ± 0.03 µmol/L for the patients treated with 0.1% betamethasone valerate ointment. No statistical significant difference was found between the basal serum cortisol levels in the different treatment groups (p=0.23). Basal serum cortisol levels remained normal in 6 (10.5 %) patients (Subgroup 2; mean 0.38 µmol/L ± 0.15) on day 2 of admission (Figure 1).

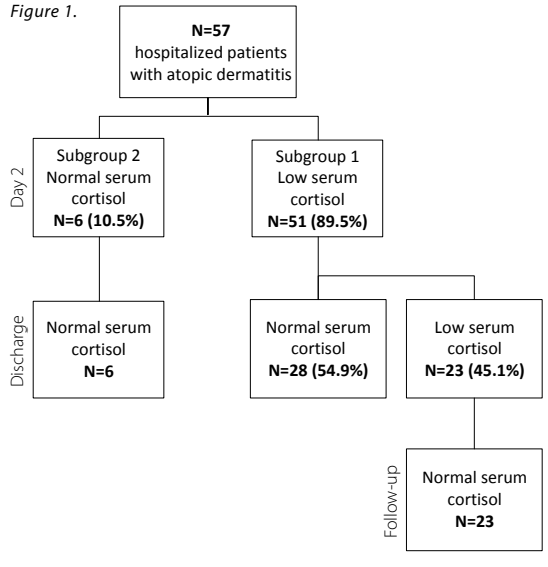
Table 1.

Baseline characteristics of AD patients.

Number of patients	57
Men (%)	25 (43.9)
Women (%)	32 (56.1)
Age (mean years ± SD)	39 ± 17
Duration hospitalization (mean no. of days ± SD)	16 ± 6

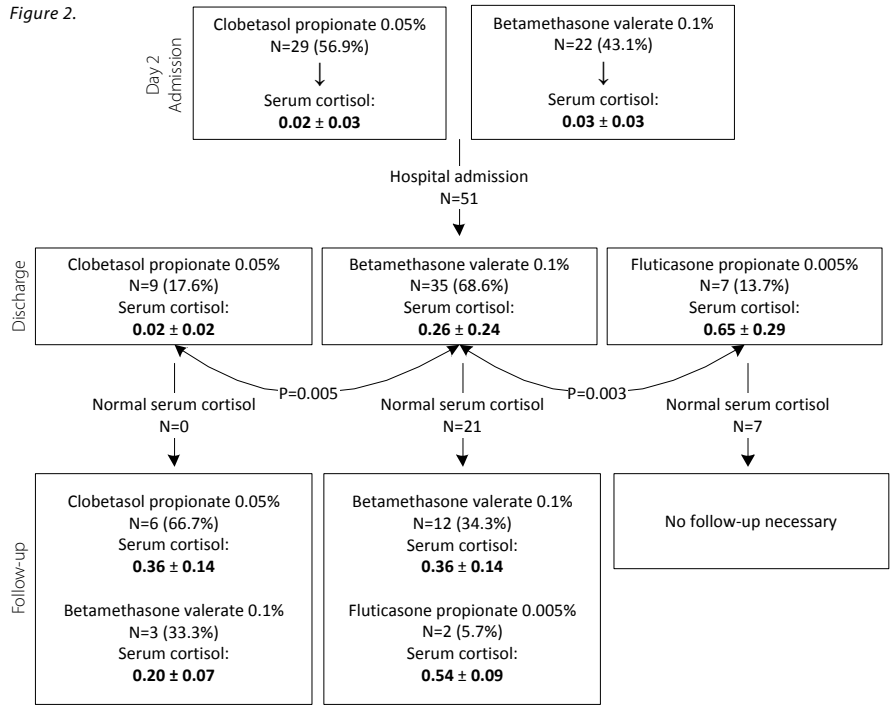
During hospital admission the frequency and amount of application of topical corticosteroids decreased from twice daily 20-30 g during the first week of hospitalization, to a maximum amount of 10-30 g once daily or every other day at discharge. Also, the type of topical corticosteroid was changed during hospitalization. Patients were discharged after a mean of 16 ± 6 days. The time point of discharge was set by the dermatologist in charge, when disease activity and body surface affected was low (approximately 10% or less).

Figure 1.



Subgroup 2 (n=6) with normal basal serum cortisol level at day 2 all had normal basal serum cortisol levels at discharge (mean 0.50 µmol/L ± 0.17). In the five days before discharge 10-30 g of 0.1% betamethasone valerate was used in 4 patients and 0.005% fluticasone propionate in 2 patients. Of subgroup 1 (n=51) with low basal serum cortisol levels at day 2 of admission, 28 (54.9 %) patients had normal basal serum cortisol levels at discharge (mean 0.48 µmol/L ± 0.22) and 23 (45.1 %) patients still had decreased levels of basal serum cortisol (mean 0.03 µmol/L ± 0.03). All patients (n=9) in subgroup 1 treated with 0.05% clobetasol propionate ointment (10-30 g daily) prior to discharge had low basal serum cortisol levels. Forty percent of the patients (14 out of 35) using 0.1% betamethasone valerate ointment (10-30 g daily) prior to discharge had low basal serum cortisol levels. In contrast, all patients using 0.005% fluticasone propionate ointment (10-30 g daily) prior to discharge had normal basal levels of serum cortisol (Figure 2).

Figure 2.



Overall in subgroup 1, basal serum cortisol levels at discharge were significantly lower in patients using 0.05% clobetasol propionate ointment than in patients using 0.1% betamethasone valerate ointment (p=0.005). On the other hand the basal serum cortisol levels were significantly lower in patients using 0.1% betamethasone valerate ointment compared to patients using 0.005% fluticasone propionate ointment (p=0.003) (Figure 2).

In all patients with low basal serum cortisol levels at discharge (n=23) serum cortisol levels had normalized to a mean of 0.35 µmol/L ± 0.15 within 41 ± 50 days after discharge. During this follow-up period the overall amount of topical corticosteroids used was decreased to a mean of 51 ± 32 g/week. The majority of the patients used 0.1% betamethasone valerate ointment (65.2%) with a mean amount of 58 ± 34 g/week, 6 patients (26.1%) still used 0.05% clobetasol propionate ointment with a mean amount of 37 ± 27 g/week, and 2 patients (8.7%) 0.005% fluticasone propionate ointment with a mean amount of 43 ± 11 g/week (Table 2).

Table 2.

Quantity of topical steroids used and repeat serum cortisol after discharge in patients with low serum cortisol at discharge (n=23).

Topical corticosteroid use (g/week)	N	%	Basal serum cortisol (µmol/L ± SD)
0 - 19	3	13.1	0.31 ± 0.08
20 - 39	8	34.8	0.36 ± 0.17
40 - 59	2	8.70	0.44 ± 0.23
60 - 79	7	30.4	0.37 ± 0.16
80 - 99	1	4.30	0.36 ± 0.00
≥ 100	2	8.70	0.23 ± 0.08

Discussion

During hospital admission all patients with severe AD were treated with high amounts of potent or superpotent (class III or IV) topical corticosteroids. In almost all patients a suppression of the adrenal gland arose within 24 hours, due to percutaneous absorption of topical corticosteroids. This was reflected by a fall in basal serum cortisol level. This observation is consistent with other studies on the effect of potent topical corticosteroids on the adrenal gland function in patients with severe inflammatory skin diseases.[4;8;9]

On the first day of admission, most patients were either treated with 0.05% clobetasol propionate ointment, which is generally considered to be the most potent topical steroid (class IV), or 0.1% betamethasone valerate ointment, which is considered to be a potent topical steroid (class III). Basal serum cortisol levels were equally low after a single application of 20-30 g/day of one of these steroids.
This is also consistent with previous studies on percutaneous absorption of topical corticosteroids in patients with severe eczema or psoriasis.[4;10-12]

On the contrary, 6 patients (subgroup 2) had normal basal serum cortisol levels on day 2. Two patients were clinically in remission and the reason for admittance to the clinic was to taper and stop treatment with either cyclosporin A or mycophenolate sodium under the application of topical corticosteroids (0.05% clobetasol propionate and 0.1% betamethasone valerate ointment) to prevent an exacerbation of their eczema. It could be speculated that epidermal barrier function was not much impaired, and hence percutaneous absorption was low compared to patients who were admitted with a clinically severe eczema. Two other patients were admitted because of severe itch and sleeplessness although their eczema was also clinically in remission. They were treated with 0.1% betamethasone valerate ointment. One patient was treated with 0.05% clobetasol propionate ointment, but also used the drug orgametril, a progestagen prescribed for early menopausal symptoms. Estrogen therapy may increase basal serum cortisol, and thus may lead to false negative results.[13] However, one patient was admitted because of an exacerbation of AD.
This patient had widespread skin disease covering his arms, hands, feet, trunk and neck. He was treated with 0.1% betamethasone valerate ointment. Although the amount of percutaneous absorption may show inter-individual differences[1], the presence of normal basal serum cortisol in this patient is difficult to explain.

Recovery of adrenal cortisol production occurred during hospital admission in the majority (54.9 %) of subgroup 1, most likely due to decreased percutaneous absorption during healing of the epidermal barrier function. This observation is consistent with earlier studies where recovery of adrenal cortisol production was seen during therapy.[4;11] But if patients were treated with the superpotent topical corticosteroid 0.05% clobetasol propionate prior to discharge, the basal serum cortisol level remained low at discharge in all cases.
During maintenance therapy with more than 50 g per week, but also during therapy in patients with severe and extensive eczema or psoriasis 0.05% clobetasol propionate most often causes adrenal gland suppression.[1;10;14] The potency of 0.05% clobetasol propionate has also been tested in healthy volunteers with an intact epidermal barrier function. If more than 90 g weekly of ointment was applied to the skin, profound suppression of the adrenal gland was seen.[1] Clobetasol propionate is one of the most potent topical corticosteroids, as tested by the vasoconstrictor assay. Even when applied to normal healthy human skin adrenal suppression is seen.
All patients from subgroup 1 who received treatment with clobetasol propionate at discharge still showed adrenal suppression. These patients used 10-30 g ointment once daily, which is up to 210 g weekly. This is well above the 50 g per week, as mentioned by Allenby et al.[10], where adrenal suppression is seen in the majority of patients.

A large number of patients was treated with 0.1% betamethasone valerate ointment (68.6%) at discharge. In contrast to the patients who were treated with 0.05% clobetasol propionate ointment only 40% of these patients had a low basal serum cortisol at discharge. None of the patients receiving 0.005% fluticasone propionate ointment had a low basal serum cortisol at discharge. Patients were only discharged when skin disease was controlled, thus differences in disease activity were small.

To evaluate the effect of 0.1% betamethasone valerate and 0.005% fluticasone propionate ointment on adrenal cortisol production, two patients with an exacerbation of AD (body surface affected 33 and 36%) were treated with 30 g 0.005% fluticasone propionate ointment on the first day of a hospital admission after a baseline serum cortisol and ACTH measurement. Both serum cortisol and ACTH levels were normal and did not change after a single application of 0.005% fluticasone propionate ointment. However, the second day of admission patients were treated with a single application of 30 g 0.1% betamethasone valerate ointment. Now, basal serum cortisol and ACTH levels decreased significantly (cortisol 0.54 to 0.03 and 0.38 to 0.05 µmol/L; ACTH 28 to <2 and 26 to 9 nmol/L).

Thus, it seems that 0.1% betamethasone valerate ointment can induce adrenal gland suppression more likely than 0.005% fluticasone propionate ointment after a single application of 30 g/day on diseased skin. This suggests higher potency for percutaneous absorption for betamethasone valerate. Furthermore, the difference in T ½ el between fluticasone propionate (3 hr) and betamethasone valerate (36-48 hr)[15] could also contribute to the differences in serum cortisol levels at discharge. Because betamethasone valerate has a higher T ½ el than fluticasone propionate, levels of betamethasone valerate will be longer systemically available.

All patients with a low basal serum cortisol level at discharge (45.1% of subgroup 1) showed a complete recovery of their adrenal cortisol production after discharge, when the amount of topical corticosteroid used was tapered. The majority of these patients received treatment with an average of 58 ± 34 g/week 0.1% betamethasone valerate ointment (65.2%), and 26.1% of the patients continued treatment with an average of 37 ± 27 g/week 0.05% clobetasol propionate ointment. This is also consistent with earlier studies in patients with eczema and psoriasis where low cortisol serum levels are hardly observed during maintenance therapy with a maximum of 100 g 0.1% betamethasone valerate weekly[5;16], and with a maximum of 50 g 0.05% clobetasol propionate weekly.[10]

In conclusion, in this cohort of patients with severe AD an initial inhibition of adrenal cortisol production was observed during therapy with either potent (0.1% betamethasone valerate) or superpotent (0.05% clobetasol propionate) topical corticosteroids. At discharge, when skin disease was controlled, adrenal cortisol production restores in part of the patients using 0.1% betamethasone valerate ointment, but in none of the patients using 0.05% clobetasol propionate ointment. All patients using 0.005% fluticasone propionate ointment at discharge had normal cortisol levels.
All patients, including patients on maintenance therapy with 0.05% clobetasol propionate, showed a complete recovery of adrenal cortisol production after admission during maintenance therapy.
When the high amounts of potent topical corticosteroids used during treatment of patients with severe AD are tapered correctly after a beneficial clinical response, the initial adrenal gland suppression is followed by recovery to normal cortisol production, even when the most potent topical corticosteroids are used. We only advice measurement of serum cortisol levels if patients fail to taper the amount of topical corticosteroids after short intensive therapy, especially when >50 g per week of 0.05% clobetasol propionate is used.

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Chapter 3

Liquid chromatography-tandem mass spectrometric assay for clobetasol propionate in human serum from patients with atopic dermatitis

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clobetasol propionate, clobetasone butyrate, topical corticosteroid, LC-MS/MS, human serum, atopic dermatitis

Abbreviations:
APCI: Atmospheric Pressure Chemical Ionization
APPI: Atmospheric Pressure PhotoIonization
CID: Collision Induced Dissociation
IS: Internal Standard
ESI: Electrospray Ionization
LC/MS/MS: Liquid Chromatography-tandem Mass Spectrometry
LLOQ: Lower Limit of Quantification
SRM: Selected Reaction Monitoring
QC: Quality Control

Abstract

A bioanalytical assay for the topical corticosteroid clobetasol propionate was developed and validated. For the quantitative assay 0.5 ml human serum samples, supplemented with clobetasone butyrate as internal standard, were extracted with hexane-ether.
Evaporated and reconstituted extracts were injected on a polar embedded octadecyl silica column with isocratic elution using formic acid in water-methanol as mobile phase.
The eluate was led into the electrospray interface with positive ionization and the analyte was detected and quantified using the selective reaction monitoring mode of a triple quadrupole mass spectrometer.
The assay was validated in the range 0.04-10 ng/ml, the lowest level of this range being the lower limit of quantification. Precisions were 5-10% and accuracies were between 102 and 109%.

The drug was stable under all relevant conditions.

Finally, the assay was successfully applied on patients suffering from severe atopic dermatitis treated topically with clobetasol propionate.

Introduction

Topical corticosteroids represent the mainstay account for the most part of treatment of atopic dermatitis. Clobetasol propionate (Figure 1) is a potent topical corticosteroid often used for the treatment of severe, active atopic dermatitis. Although clobetasol propionate is highly effective in controlling this disease, percutaneous absorption of the drug during daily application of 20-30 g Dermovate® ointment (0.05% (w/w) clobetasol propionate) can lead to suppression of the adrenal gland function with a decrease in cortisol production.[1-3] The amount of percutaneous absorption of clobetasol propionate, and the effect on the adrenal gland, probably depends on many factors such as the extent of body surface affected by atopic dermatitis, the mode of application and the amount used per day.[4]
In order to quantify the (low) systemic levels of clobetasol propionate in a pharmacokinetic study, a radioimmunoassay was used by Hehir et al.[3] As far as we know, other assays for systemic levels of clobetasol propionate have not been published hitherto.
The development and validation of a sensitive bioanalytical assay for clobetasol propionate in human serum using LC-triple quadrupole MS is reported hereafter. The topical use of clobetasol propionate leading to low systemic serum levels (approximately 1 ng/ml, 24 hr after treatment[3]) particularly demands the high sensitivity of the presented method.

2. Material and methods

2.1. Chemicals

Clobetasol propionate (≥98%) and clobetasone butyrate (≥98%; IS) were both obtained from Sigma (St. Louis, MO, USA). LC-MS grade water, gradient grade acetonitrile, methanol of HPLC quality and *n*-hexane (p.a.) were from Biosolve (Valkenswaard, The Netherlands). Water not used as eluent was home-purified by reversed osmosis on a multi-laboratory scale. Formic acid and diethyl ether were of analytical grade and originated from Merck (Darmstadt, Germany). Human serum was supplied by Innovative Research (Southfield, MI, USA).

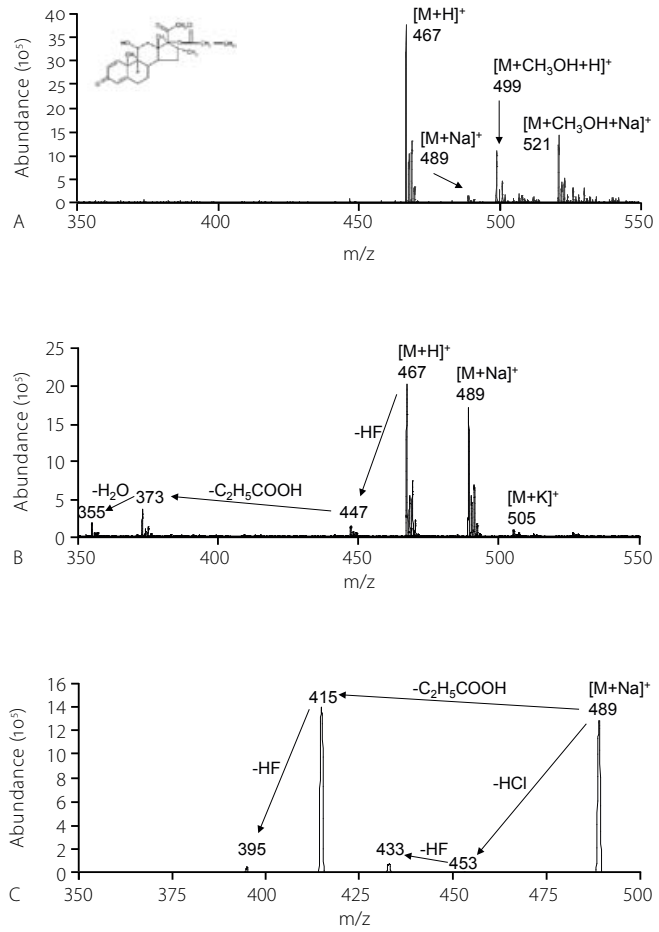

Figure 1.
Chemical structure and MS spectra of clobetasol propionate, recorded during chromatographic elution after injection of 20 µL of 1 µg/mL clobetasol propionate. (A) ESI spectrum without up-front CID (mass resolution m/z 0.2); (B) ESI spectrum with up-front CID at -22 V (mass resolution m/z 0.2); (C) product spectrum (CID = -10 V) of the sodium adduct at m/z 489.0 (mass resolution m/z 0.7).

2.2. Equipment

The LC-(UV)-MS/MS equipment consisted of a DGU-14A degasser, a CTO-10Avp column oven, a Sil-HTc autosampler, two LC10-ADvp-µ pumps, a SPD10-Avp spectrophotometric UV-Vis detector (all from Shimadzu, Kyoto, Japan) and a Finnigan TSQ Quantum Discovery Max triple quadrupole mass spectrometer (Thermo Electron, Waltham, MA, USA). For data recording and system controlling the Finnigan Xcalibur software (version 1.4, Thermo Electron) was used.

2.3. LC-MS/MS conditions

Partial-loop injections (20 µl) were made on a Polaris® 3 C18-A column (50x2 mm, dp = 3 mm, average pore diameter = 10 nm, Varian, Middelburg, The Netherlands) with a corresponding 10 mm pre-column. The column temperature was maintained at 40°C and the auto-injector sample racks were maintained at 4°C. The mobile phase (flow rate 0.5 ml/min) was composed of a mixture of water containing 1% of formic acid and methanol (30:70, v/v). The positive electrospray detection started 0.6 min after injection, ionization voltage was 4600 V, capillary temperature 268°C and nitrogen sheath, ion sweep and auxiliary gasses were 45, 16 and 17 arbitrary units, respectively. The up-front collision induced dissociation (CID) voltage was -22 V. Using unit mass resolutions, selected reaction monitoring (SRM) transitions (with collision energy, tube lens off set and dwell time) were 489.0→415.0 (-10 V; 110 V; 0.4 s) and 501.1→413.0 (-13 V; 120 V; 0.1 s) for clobetasol propionate and IS respectively.

2.4. Sample pre-treatment

To a volume of 0.5 ml human serum, pipetted into a 10-ml borate glass tube with a Teflon lined screw cap, 50 µl of 20 ng/ml IS in 50% (v/v) methanol and 4 ml diethyl ether / *n*-hexane (1/3; v/v) were added. The tube was closed and shaken by a rotary mixer at 50 rpm for 10 min. After centrifugation of the sample at 2643 *g* at ambient temperature for 5 min, the tube was placed in the freezer at -30°C for ca. 1 hr. Afterwards, the organic extract was poured off in a conical 10 ml glass tube and evaporated at 35°C under a gentle stream of nitrogen. The residue was reconstituted in 100 µl of 50% (v/v) methanol using vortex mixing. After centrifugation at 2643 *g* for 5 min the sample was pipetted into a 250 µl glass insert placed in an injection vial.

2.5. Validation

A laboratory scheme based on international guidelines[5,6] was used for the validation procedures.

2.5.1. Calibration

Stock solutions of clobetasol propionate at 1 and 2 mg/ml and IS at 1 mg/ml were prepared in methanol. The stock solutions were stored at -30°C.

The 1 mg/ml stock solution of clobetasol propionate was diluted to a 10 ng/ml calibration sample (stored at -30°C) in pooled human serum. Additional calibration samples were prepared daily at 4, 1, 0.4, 0.1 and 0.04 ng/ml by dilution with the blank matrix. All calibration samples were processed in duplicate for each daily calibration. Least-squares linear regression with the reversed square of the concentration of the analyte ($1/x^2$) was employed to define the calibration curves using the ratios of the peak area of clobetasol propionate and IS.

2.5.2. Precision and accuracy

The 2 mg/ml stock solution of clobetasol propionate was used to obtain validation (quality control (QC)) samples in pooled human serum at 8 (QC-high), 0.5 (QC-med) and, 0.05 (QC-low) ng/ml. The QC samples were stored -30°C. Precisions and accuracies, including between day variations of the accuracy, were determined by sextuple analysis of each QC in three analytical runs on three separate days for all QCs (total: n=18). Relative standard deviations were calculated for both the within day precision and the between day precision. The average concentrations found (n=18) were reported relatively to the spiked levels to represent the accuracy.

2.5.3. Lower Limit of Quantification (LLOQ)

A serum sample at the LLOQ level (0.04 ng/ml) was analyzed in six-fold. Precision and accuracy were then calculated.

2.5.4. Selectivity

Six individual serum samples were processed to test the selectivity of the assay. These samples were processed as double blanks (no clobetasol propionate, no IS) and after spiking with 0.05 ng/ml clobetasol propionate (QC-low) and addition of the IS. The spiked samples were also used to assess the inter-batch variation of the matrix effect.

2.5.5. Recovery

The extraction efficiency (recovery) was determined in quadruplicate by comparing responses of processed samples (QC-high, -med and -low) with extracts of drug-free human serum reconstituted in reconstitution solvent spiked with the analytes at the

same levels. Ionization efficiency (ion suppression or enhancement) was assessed by comparing responses of the spiked reconstitution solvent at the three QC levels with and without the presence of reconstituted blank extraction residue. The extraction and ionization efficiencies (recoveries) of the IS were assessed using identical procedures at the IS concentration used in the assay.

2.5.6. Stability

The stability of clobetasol propionate was investigated in QC-high and -low serum samples. Triplicate or quadruplicate analysis of these samples was performed after storage under relevant conditions (see table 1). The stability of clobetasol-propionate and clobetasone-butyrate was also investigated in the methanolic stock solutions using LC-UV at 240 nm.

2.6. Patient samples

Two patients suffering from severe acute atopic dermatitis were treated with 30 g ointment containing 0.05% (w/w) clobetasol propionate (Dermovate®) twice daily over the whole body during hospitalization for 7 days and once daily for 5 days (patient A: female, 19 y) and twice daily for 10 days (patient B: female, 66 y). Blood samples were collected in 10 ml Vacutainer® tubes (BD, Plymouth, UK) from the antecubital vein before and after the first day of treatment, on the last day of hospitalization and on two days in between. After coagulation for approximately 30 min. at ambient temperature, serum was separated by centrifugation at 1811 *g* for 10 min. Serum samples were stored at -30°C for not longer than 1 month and stored further at -80 °C until analysis. Sample analysis of non-blank samples (6 out of 8 were still available) was duplicated three months after the initial analysis.

Table 1.

Stability data (recovery % ± SD) of clobetasol propionate in human serum, reporting the percentage of the initial concentration.

Condition	QC-high	QC-low	N
8 h at ambient temperature	100.3 ± 4.4	80.4 ± 6.0	3
3 freeze-thaw cycles	100.8 ± 5.0	107.9 ± 11.5	3
8 weeks at -30 °C	94.9 ± 3.9	96.4 ± 14.3	4
8 weeks at -80 °C	93.6 ± 10.2	93.9 ± 9.2	4

3. Results and discussion

3.1. Method development

Because of the high sensitivity that had to be achieved, a simple protein precipitation as pre-treatment procedure was expected useless due to insufficient selectivity. Liquid-liquid extraction is a more selective procedure with the possibility to concentrate the analyte by evaporation of the extract. It has been successfully applied for several LC-MS/MS assays for corticosteroids in plasma.[7-9] The hexane-diethyl ether mixture was chosen as the extraction solvent mixture because of the possibility to optimize the ratio of both solvents. The extraction procedure reported herein was based on the method of dos Santos Pereira *et al.*[8]; 25% (v/v) diethyl ether resulted in optimal recovery of clobetasol propionate.

Steroids can be ionized by different atmospheric ionization techniques, ESI, APCI and APPI, using both, positive and negative ionization. Positive ESI has been used most frequently for endogenous steroids[10] and this combination was also shown to be suited for several synthetic glucocorticoids.[11] On the other hand, Antignac et al.[12] reported negative ionization to be the first choice for corticosteroids, due to the selective loss of the base (formate or acetate, depending on the eluent additive) and formaldehyde during CID of the negative base adduct.

For clobetasol propionate this transition of $(M + HCOO)^-$ showed a low response using ESI. Using APCI, however, a specific propanoic acid loss from the deprotonated parent molecule during CID (in the second quadrupole) was observed that could lead to a sensitive LC detection. No prominent base adduct was observed with negative APCI. In the positive mode, both, ESI and APCI, showed a prominent protonated molecule with comparable abundances. CID of the protonated clobetasol propionate showed sequential losses of hydrogen fluoride, propanoic acid and water with almost equal maximal responses for $(M\text{-}HF\text{-}HOCOC_2H_5+H)^+$ and $(M\text{-}HF\text{-}HOOCC_2H_5\text{-}H_2O+H)^+$. In addition to the protonated molecule, ESI in the positive ion mode produced sodium adducts and solvent clusters (Figure 1a). Using methanol as the organic modifier, methanol loss of the sodiated methanol cluster was the most abundant SRM transition. The use of this transition, however, did not result in sensitive detection in positive ESI because of its poor specificity. The abundance of the sodiated molecule in positive ESI could be increased by up-front CID (shown in figure 1a and b) and the CID in the second quadrupole of this stable sodium ion resulted in a specific loss of propanoic acid at a low CID voltage (Figure 1c). During the MS/MS optimization

experiments the two very promising SRM transitions with specific propanoic acid loss (from $(M-H)^-$ with APCI and from $(M+Na)^+$ with ESI) were discovered. Their use with LC-MS/MS in the SRM mode showed the highest sensitivity for positive ESI, which was then chosen for developing and validating the presented assay. As expected and based on the structural resemblance, this transition was also observed for the IS clobetasone butyrate.

3.2. Validation

SRM chromatograms of clobetasol propionate and the IS are depicted in figure 2, showing chromatograms of a double blank (no analyte, no IS), an LLOQ spiked and a patient sample.

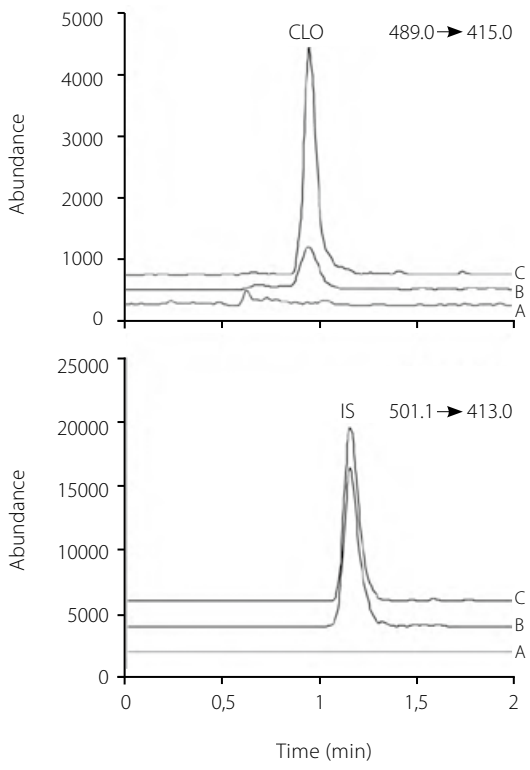

Figure 2.
SRM Chromatograms of (A) blank serum without IS, (B) Serum spiked with clobetasol propionate (CLO) at the LLOQ level (0.04 ng/ml), (C) Patient serum sample (last sample of patient B in Fig. 3) containing 0.149 ng/ml clobetasol propionate.

3.2.1. Calibration

The response function was evaluated in six analytical runs (72 calibration samples) and proved clearly to be sufficiently linear and monotone (data not shown) with average precisions for each concentration in the range 94-108%.

3.2.2. Precision and accuracy

Assay performance data from the validation samples at three concentrations are reported in table 2. Between-day variations and deviations of the accuracy ≤10% were observed for all levels. Therefore, the upper limit of the calibration range could be assigned to the upper limit of quantification and precisions, and deviations of the accuracy met the required ±15%.[5,6]

Table 2.

Assay performance data of clobetasol propionate resulting from 18 validation (QC) samples in 3 analytical runs, including between day variations of the accuracy.

Nominal concentration (ng/ml)	Within day precision (%)	Between day precision (%)	Accuracy (%)
8	5.4	8.5	101.9 ± 8.3
0.5	9.3	10.0	109.7 ± 3.8
0.05	9.7	9.9	108.7 ± 2.4

3.2.3. Lower limit of Quantification

The six-fold analysis of the 0.04 ng/ml serum sample resulted in a precision of 9.7% and an accuracy of 104.2%. This level, also being the lowest level of the calibration range, could be attributed to the LLOQ because precision and accuracy met the required ±20%.[5,6]

3.2.4. Selectivity

The analysis of six batches of blank samples showed no interfering peaks in the SRM traces for clobetasol propionate and IS in human serum. Blank responses could not be distinguished from the detector noise (signal-to-noise ratio<3) for both, clobetasol propionate and IS and were all each below 15% of the LLOQ response of clobetasol propionate (20% is required[6]) and below 0.2% of the regular signal of the IS. The absence of any interference in these experiments is a prove of the high

selectivity of the assay.[5,6] The average response of the QC-low-spiked blank samples (0.05 ng/ml; n=6, ±SD) was 0.047±0.004 ng/ml clobetasol propionate. In addition to the absence of interference this result shows no increased variation due to an inter-batch variability of the matrix effect.

3.2.5. Recovery

The recovery experiments showed only small extraction losses (<15%) for clobetasol propionate at the three QC levels and the recovery of the extraction of the IS was 76±14% (n=4). Ion suppression was below 17% for all QC levels of clobetasol propionate and was 20±7% (n=4) for the IS. These low losses of analyte and IS, during both, extraction and ionization, and the low inter-batch variability of the matrix effect all contributed to the successful validation of this assay.[5,6]

3.2.6. Stability

Recoveries of clobetasol propionate in serum after different storage procedures are shown in table 1. From the recoveries in the range of 80-108% only the 80% of the QC-low sample at ambient temperature for 8 hr needed some attention. Therefore, additional experiments at a 0.2 ng/ml level (4 hr at ambient temperature and 2 hr at 37°C) were performed. Recoveries at these conditions were 105±5% (n=4) and 106±10% (n=4), respectively. Finally, sufficient stability of the serum samples all under relevant conditions was demonstrated.

Recoveries of the analytes in methanolic stock solutions were 100.4% for clobetasol propionate and 97.1% for clobetasone butyrate (both with *n*=2 at 2 mg/ml) after being subjected for 6 h to ambient temperature. After storage at -30°C for 1 year the recovery of clobetasol propionate was 101.2% (*n*=2 at 1 and 2 mg/ml respectively) and 101.3% for clobetasone butyrate (*n*=1 at 1 mg/ml). These results facilitate the potential use of stock solutions of both corticosteroids during a whole year.

3.3. Patient samples

Pharmacokinetic plots of clobetasol propionate levels in serum *vs*. time are shown in figure 3 for two patients. The assay clearly shows its ability to quantify the clobetasol propionate serum levels of these patients during treatment with low variations of duplicate analyses. In addition, levels after termination of the clobetasol propionate treatment showed to be quantifiable for about three remaining days.
(data not shown)

4. Conclusions

The first validated assay for clobetasol propionate in human serum has now been reported. The LC-MS/MS assay meets commonly accepted criteria for precision, accuracy, recovery and stability.[5,6] The sensitivity of the method is suitable for monitoring the drug in serum in patients treated topically with the drug until three days after the last whole body treatment and meets the sensitivity of analogous assays for other corticosteroids.[7-9] The new assay showed to be a valuable tool for clinical studies with hospitalized patients suffering from atopic dermatitis during topical treatment with clobetasol propionate.

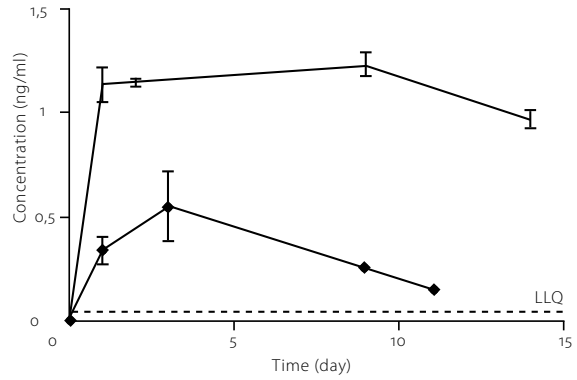

Figure 3.
Pharmacokinetic profile of two patients treated with 30 g ointment containing 0.05% (w/w) clobetasol propionate (Dermovate®) twice-daily during hospitalization for 14 days (patient A, treatment reduced to once-daily after 7 days; 4 duplicate sample analysis, only error bars are shown) and 10 days (patient B; 2 duplicate sample analysis; ◆), respectively.

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Chapter 4

The potency of clobetasol propionate: serum levels of clobetasol propionate and adrenal function during therapy with 0.05% clobetasol propionate in patients with severe atopic dermatitis

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Key words:
atopic dermatitis, topical corticosteroids, 0.05% clobetasol propionate, percutaneous absorption, cortisol, liquid chromatography-tandem mass spectrometry

Abbreviations:
AD: atopic dermatitis
BSA: Body Surface Area
LC/MS/MS: Liquid Chromatography-tandem Mass Spectrometry

Abstract

Background

Percutaneous absorption of topically applied 0.05% clobetasol propionate can be assessed indirectly by measuring cortisol levels. A direct way is to measure systemic levels of topically applied clobetasol propionate.

Methods

Serum concentrations of clobetasol propionate were measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS), and were related to serum cortisol levels in 25 patients with an exacerbation of atopic dermatitis (AD) before and after the first day of treatment with 0.05% clobetasol propionate ointment in hospital. The affected body surface area (BSA) by AD was measured.

Results

Before the start of 0.05% clobetasol propionate ointment treatment, normal cortisol levels were measured (0.47 ± 0.18 mmol/L) and clobetasol propionate concentrations could not be detected. After the first day of treatment cortisol levels decreased to 0.04 ± 0.05 mmol/L. Serum concentrations of clobetasol propionate could be detected in all patients (0.112-4.504 ng/mL). Levels did not differ between patients who had received two applications versus one application of 0.05% clobetasol propionate ointment. There was no correlation between the affected BSA and serum concentrations of clobetasol propionate.

Conclusion

Serum levels of clobetasol propionate can be measured by LC/MS/MS. When prescribing 0.05% clobetasol propionate ointment, one must bear in mind that, even after an application of 20-30 g, clobetasol propionate is systemically available and potent enough to induce adrenal gland suppression.

Introduction

Percutaneous absorption of topically applied corticosteroids may result in systemic availability and effect of the steroid[1] which is indirectly reflected by inhibition of adrenal gland cortisol production. During standardized treatment with the superpotent (European class IV) topical corticosteroid 0.05% clobetasol propionate ointment in patients with inflammatory skin diseases a decrease in cortisol levels was observed.[2-4] Depending on the amount used (25g or 7g daily) basal cortisol levels decreased respectively one or more days after the start of therapy. Measurement of the actual serum concentrations of the topically applied corticosteroid will provide direct evidence of percutaneous absorption and its effect on adrenal cortisol production.
Hehir et al. demonstrated the systemic availability of clobetasol propionate by a radioimmunoassay. After a single application of 25 g 0.05% clobetasol propionate ointment in 9 patients with atopic dermatitis (AD) a rapid rise of clobetasol propionate levels was detected in plasma. 0.05% Clobetasol propionate ointment was applied on the total body surface excluding the face, genitalia and the arm from which blood samples were drawn. Peak levels of clobetasol propionate were observed between 6 and 9 hr after application. The detection of clobetasol propionate levels was paralleled by a decrease in cortisol levels reaching a nadir 9 hr after application.[5] In this observational study we measured clobetasol propionate and cortisol levels simultaneously in patients with severe AD after the first day of clobetasol propionate treatment in hospital. Furthermore, we compared clobetasol propionate serum levels after one or two equally large applications.
The quantification of clobetasol propionate in serum was done by liquid chromatography-tandem mass spectrometry (LC/MS/MS), which is now the most preferred analytical technique for bioanalytical steroid assays, due to its selectivity and sensitivity.[6]

Patients and methods

We studied 25 patients with severe AD[7] who were referred to our clinic because of an exacerbation. Baseline measurements (day 1) of serum clobetasol propionate and serum cortisol levels were performed, if possible, prior to the first application of 0.05% clobetasol propionate ointment.
On day 1, patients were treated with 20-30 g of 0.05% clobetasol propionate ointment once (between 3-5 pm) or twice (between noon–2 and 6-7 pm). 0.05% Clobetasol propionate ointment was applied on the total body surface, excluding the face and genitalia. The next morning (day 2), serum levels of clobetasol propionate and cortisol were measured. The percentage of affected body surface area (BSA) at admission was estimated using the rule of nines.[8]

Basal serum cortisol levels

Basal serum cortisol levels were measured between 8 and 10 am using a chemiluminescent immunoassay on the Dxi (Beckman Coulter Inc. Fullerton, CA U.S.A.). The lower limit of detection was 0.011 µmol/L and inter-assay variation was <8.0%. Levels between 0.20-0.60 µmol/L were considered normal.

Serum clobetasol propionate levels

Clobetasol propionate levels were measured using a validated LC/MS/MS method in the range 0.05-10 ng/mL.9 In short, 500 µl serum, with clobetasone butyrate added as internal standard, was extracted using hexane-diethyl ether (3/1, v/v).
Evaporated and reconstituted extracts were injected on a Polaris 3 C18-A column (50x2 mm, dp = 3 mm, average pore diameter = 10 nm, Varian, Middelburg, The Netherlands), maintained at 40°C, and using formic acid-water-methanol (0.3/29.7/70, v/v/v) as the eluent. Compounds were detected using positive electrospray ionization using the selective reaction monitoring mode of a triple quadrupole mass spectrometer. Mass transitions were 489.0→415.0 and 501.1→413.0 for clobetasol propionate and clobetasone butyrate, respectively.

Statistical analysis

Statistical analysis was performed using the program SPSS for Windows (version 15.0, 2006; SPSS inc., Chigago, IL, U.S.A.). Since skewed distribution of outcome parameters were observed, nonparametric tests were used. Differences between the mean rank for different groups were compared using the Mann-Whitney Test.

Results

Twenty-five patients (15 women, 10 men) with AD (age 18-71 years) were studied. At the moment of admission 6 patients (24%) had not used topical corticosteroids for at least four weeks. Class II topical corticosteroids were used by 2 (8%) patients, i.e. 0.1% triamcinolone acetonide ointment and 0.05% clobetasone butyrate ointment. Class III topical corticosteroids were used by 17 (44%) patients i.e. 0.005% fluticasone propionate ointment (n=5), 0.1% betamethasone valerate ointment (n=10), 0.25% desoximetasone cream (n=1) and 0.1% mometasone fuorate ointment (n=1). The mean amount of topical corticosteroids used during four weeks prior to admission was 17±5 g/week (class II) and 65±53 g/week (class III). Use of these amounts is not related with the occurrence of adrenal gland suppression.[10] None of the patients had used 0.05% clobetasol propionate ointment in the four weeks prior to admission.

Day 1

Baseline serum cortisol levels before start of clobetasol propionate therapy were measured in 4 patients; cortisol levels were normal (0.47±0.18 µmol/L) and clobetasol propionate levels were not detected. Two patients had not used topical corticosteroids prior to admission, 1 patient had used 0.005% fluticasone propionate (6 g/week) and 1 patient had used 0.1% betamethasone valerate (50 g/week).
The mean affected BSA of all patients included was 59±16%. On day 1, 9 patients (36%) were treated with one application of 20-30g and 16 patients (64%) with two applications of 20-30g 0.05% clobetasol propionate ointment.

Day 2

Basal serum cortisol levels were low in 24 (96%) patients (0.04±0.05 µmol/L). Serum levels of clobetasol propionate could be detected in all patients. Serum levels of clobetasol propionate in patients who had received one application of 0.05% clobetasol propionate ointment varied between 0.173-4.504 (median: 0.41) ng/mL and in patients who had received two applications between 0.112-4.207 (median: 0.89) ng/mL (p=0.08) (Figure 1a). Basal serum cortisol levels and the affected BSA did not differ between patients with one or two applications of 0.05% clobetasol propionate ointment (p=0.13 and p=0.44 respectively) (Figure 1b and 1c). There was no correlation between the affected BSA and serum levels of clobetasol propionate neither in patients with one nor in patients with two applications of 0.05% clobetasol propionate ointment.

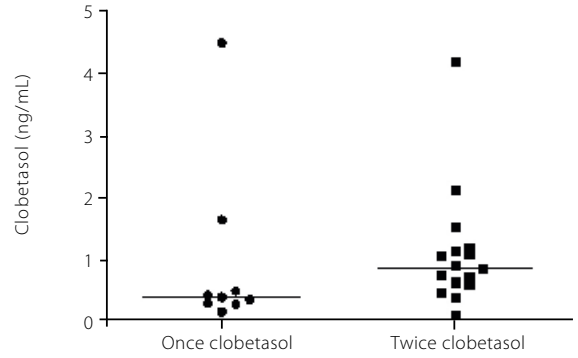

Figure 1a.
Levels of clobetasol propionate (ng/mL) on day 2 after application of 0.05% clobetasol propionate ointment once or twice on day 1 (n=25). Line at median; p=0.08.

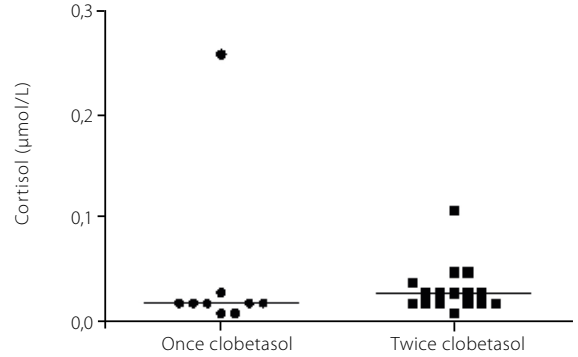

Figure 1b.
Basal cortisol levels (µmol/L) on day 2 after application on 0.05% clobetasol propionate ointment once or twice daily on day 1 (n=25). Line at median; p=0.13.

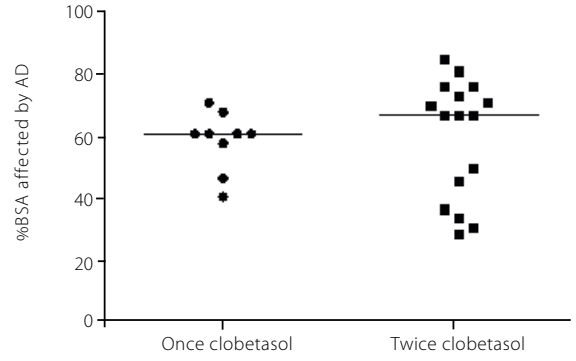

Figure 1c.
Percentage body surface area (BSA) affected by AD at admission; once versus twice daily 0.05% clobetasol propionate ointment on day 1 (n=25). Line at median; p=0.44.

Discussion

Use of 0.05% clobetasol propionate ointment in patients with severe AD resulted in detectable serum concentrations of clobetasol propionate in all patients; either after a single (20-30g) or after two applications (20-30g twice daily). The next morning, adrenal gland suppression was seen in all but one patient.
Median clobetasol propionate levels after two applications were higher compared to clobetasol propionate levels after a single application; but this difference was not statistically significant. Most likely this is due to the large variation in clobetasol propionate levels between patients and the small study population.
Hehir et al. used a radioimmunoassay to detect levels of clobetasol propionate in plasma in patients with AD.[5] Levels varied between 0.4-3.2 ng/mL (mean 1.5±1.1) 13-15 hr after one application of 25 g 0.05% clobetasol propionate ointment. The same range of variation was found in our patients 13-15 hr after a single application (20-30g) of 0.05% clobetasol propionate ointment (0.173-4.504 ng/mL; mean 0.96±1.4).
This suggests that plasma clobetasol propionate levels measured by a radioimmunoassay are comparable to serum levels measured by LC/MS/MS.

A drawback of this study is that baseline serum cortisol levels were only measured in 4 patients before the start of 0.05% clobetasol propionate ointment therapy. This was due to the observational setting of this study. However, the weekly amount of topical steroids, even those of the more potent classes, used during the 4 weeks before admission is most likely too low to induce low serum cortisol levels. Wilson et al. has shown that use of class III topical corticosteroids of 100 g/week resulted in normal plasma cortisol concentrations in patients with eczema and psoriasis.[11] In our study a normal baseline serum cortisol level was measured in a patient who had used 50 g/week of class III topical corticosteroid in the four weeks prior to admission.
Sixteen out of the 19 patients who had used topical corticosteroids in the four weeks prior to admission had used less than 100 g topical corticosteroids (class II and III) per week. Two patients had used 100 g/week and one patient 150 g/week, all of class III topical corticosteroids. None of the patients had used 0.05% clobetasol propionate ointment in the 4 weeks prior to investigation.
Application of 12.5 g 0.05% clobetasol propionate ointment on skin of healthy subjects also resulted in decreased cortisol levels within 24 hours after application.[12] This suggests that clobetasol propionate is absorbed through normal skin as well.
It may be hypothesized that percutaneous absorption of clobetasol propionate is enhanced through lesional skin of AD patients because of a decreased epidermal barrier function. For that reason, a correlation between clobetasol propionate levels and the BSA affected may be expected. The absence of a significant correlation in this study may be due to inter-individual differences in AD severity and consequently in epidermal barrier function in lesional AD lesion. A decreased epidermal barrier function of non-lesional skin in patients with AD may be another explanation for the lack of correlation between clobetasol propionate levels and BSA.[13]
The degree of adrenal gland suppression after a single application of 0.05% clobetasol propionate ointment may vary between individuals, but in the majority of patients cortisol levels were low when clobetasol propionate levels were detected. After a single application of 20-30 g 0.05% clobetasol propionate ointment cortisol levels were already under the detection limit of 0.011 µmol/L in all but one patient. This may explain why cortisol levels did not differ between patients with one or two applications of 0.05% clobetasol propionate ointment.

Topical corticosteroids are preferred to oral corticosteroids in the treatment of AD because of fear for systemic side effects such as adrenal gland suppression.[14] However, highly potent topical corticosteroids such as 0.05% clobetasol propionate ointment may behave differently since a single application of 20-30g 0.05% clobetasol propionate ointment induces low serum cortisol levels. The biological half-life of clobetasol propionate is unknown, but it has been shown that cortisol levels remain low until 96 hr after a single application of 25 g of 0.05% clobetasol propionate ointment in patients with eczema or psoriasis[5]. The biological half-life of prednisolone is 12-36 hr.[10] In contrast to a single application of 20-30 g 0.05% clobetasol propionate ointment, cortisol levels were normal 24 hours after a single orally administered dose of 25 mg prednisolone in healthy volunteers.[15] This suggests that the biological half-life of clobetasol propionate is longer than the biological half-life of prednisolone.
Due to its long biological half-life the therapeutic potency of clobetasol propionate may even equal that of high dose oral corticosteroids. Twenty-four hours after a single dose of 40 mg prednisone in patients with pulmonary disease levels of cortisol varied between 0.07-0.33 (median: 0.20) µmol/L.[16] In our study, a single application of 20-30 g 0.05% clobetasol propionate ointment resulted in levels of cortisol between 0.01-0.26 (median: 0.02) µmol/L. Recently, Joly et al. reported that clobetasol propionate therapy (20 g twice daily) is equally effective as prednisone (up to 1 mg/kg) in the treatment of bullous pemphigoid.[17] Data on cortisol levels were not given, but it may be assumed that the therapeutic effect of 0.05% clobetasol propionate ointment was due to systemic absorption.

Although this is an observational study, this report shows that clobetasol propionate serum levels can be measured by liquid chromatography-tandem mass spectrometry (LC/MS/MS). Furthermore, it emphasizes on the systemic availability and systemic potency of 0.05% clobetasol propionate ointment, even after an application of 20-30 g.

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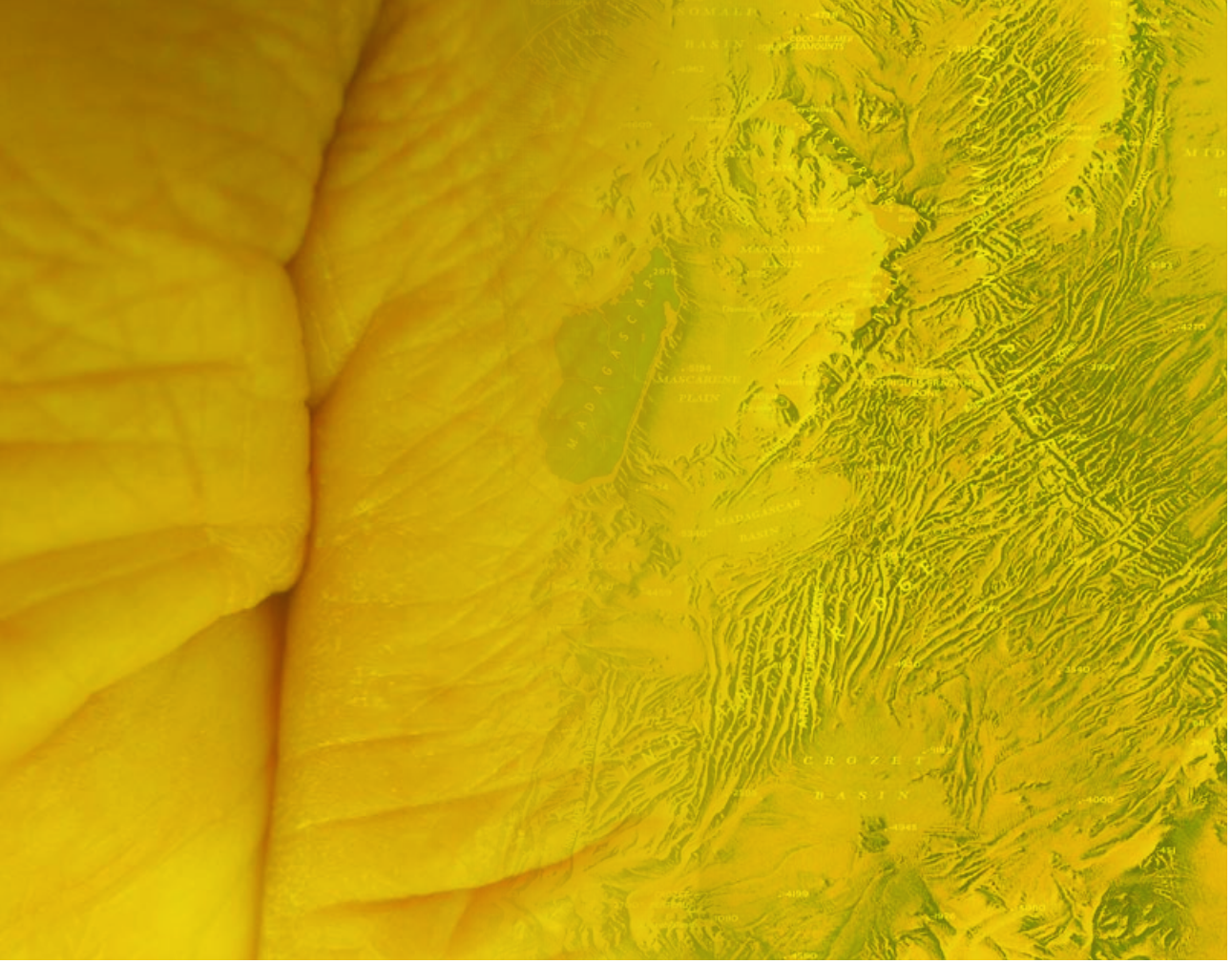

Chapter 5

Two-year follow-up of bone mineral density in adults with moderate to severe atopic dermatitis: no effect of topical corticosteroids

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Submitted

Key words:
atopic dermatitis, bone mineral density, follow-up, topical corticosteroids

Abbreviations:
25-OH-vitamin D: 25-hydroxyvitamin D
AD: atopic dermatitis
β-CTx: C-terminal telopeptide of type I collagen
BMD: Bone Mineral Density
BMI: Body Mass Index
BSAP: bone-specific alkaline phosphatase
DXA: Dual-energy X-ray Absorptiometry
CV: Coefficient of Variation
PTH: parathyroid hormone
WHO: World Health Organization

Abstract

Background

Previously, low bone mineral density (BMD) was found in approximately one third of adult patients with atopic dermatitis (AD), not related to the use of topical corticosteroids. Objectives: To perform a follow-up study to re-evaluate BMD after two years and relate this to the use of topical corticosteroids.

Patients and methods

70 of the 125 patients of the initial study, and 5 additional patients with moderate to severe AD participated in this study. Using Dual-Energy X-ray Absorptiometry, bone mineral density was measured at lumbar spine and hips. Cumulative dose of topical and oral corticosteroids was calculated from pharmacy prescription records. Life style parameters were collected by a questionnaire. Biochemical parameters of bone metabolism and disease activity (serum thymus and activation-regulated chemokine) were measured.

Results

Absolute baseline BMD in the lumbar spine and total hip did not differ significantly from the follow-up absolute BMD. The change of BMD and number of patients with a T-score <-1 at baseline did not differ from the change of BMD and number of patients with a T-score <-1 at follow-up. Dividing the patients in those using < or ≥ 75 g topical corticosteroids per month (resp. group 0 and 1), BMD in the lumbar spine and hip increased in group 0 with 0.07% and 0.17% respectively. In group 1 BMD increased with 0.14% in the lumbar spine and decreased with 0.72% in the hip. These differences were clinically and statistically not significant, also when corrected for possible confounders.

Conclusions

In this 2-year follow-up study no difference in BMD between baseline and follow-up was seen and, more importantly, the change in BMD did not differ between patients who had used low or high amounts of topical corticosteroids during follow-up.
Further investigation is needed to asses which patients are at risk for developing a clinically relevant decrease in BMD.

Introduction

A high risk for bone loss has been described in patients with inflammatory diseases such as asthma and rheumatoid arthritis. This may be due to the inflammatory nature of these diseases, the chronic use of oral (or inhalation) corticosteroids or a combination of both.[1-5] Recently it has been reported that patients with atopic dermatitis (AD) may also be at risk for bone loss.[6,7] Since application of large amounts of potent topical corticosteroids may result in significant percutaneous absorption in patients[8-10] the cumulative use of large amounts of topical corticosteroids may be a risk factor for decreased bone mineral density in patients with AD.

Haeck et al. evaluated BMD at a single time point in 125 patients with moderate to severe AD.[7] A total of 38 patients (30.4%; 28 men and 10 women) had a Z-score ≤−1 (low BMD). Osteoporosis (T-score ≤−2.5) was diagnosed in six patients (4.8%; three men and three women) and osteopenia (T-score −2.5 < −1) in 41 patients (32.8%; 28 men and 13 women). There was no difference in cumulative dose of topical or oral corticosteroids in a period of 5 years prior to the study between patients with a low and those with a normal BMD. Because only a single BMD measurement was done in this study, no information is available on the influence of topical corticosteroids on the change in BMD.

The aim of the current study was to evaluate the effect of topical corticosteroid use on the change in BMD in patients with AD during a two-year follow-up period. Additionally, risk factors known to influence osteoporosis and biochemical markers of cumulative bone resorption and formation at baseline and after two years were compared.

Patients and methods

Patients

Haeck et al. has previously described the prevalence of osteopenia and osteoporosis in 125 AD patients.[7] For the current study, we have asked all 125 patients to participate in a second BMD measurement after 2 years. Fifty-five patients were lost to follow-up; mainly because they did not respond to a written invitation or had to travel too long. The current study population describes the changes in BMD in 70 of the 125 patients with the addition of 5 patients not previously described, resulting in a total of 75 adult patients with moderate to severe AD in this longitudinal study. The diagnosis of AD was made according to the criteria of Hanifin and Rajka.[11] At the moment of inclusion, all patients were treated with potent topical corticosteroids (European class III) at our outpatient department. Patients were asked to return to the outpatient clinic for the follow-up BMD measurement 2 years after baseline. The local medical ethical committee approved this study and patients gave their written informed consent.

Bone mineral density measurements

Bone mineral density of all patients was measured at baseline and at follow-up by dual-energy X-ray absorptiometry (DXA), using the Hologic QDR-4500 Discovery A (software version 12.3, Hologic inc. Bedford, MA, U.S.A.) X-ray Bone Densitometer. The manufacturer specified that the coefficient of variation (CV) is less than 1% (calculated: 100 x SD/mean). Daily quality assurance was performed, and all patients were assessed by the same analyst. Prior to the BMD measurement height and weight of all patients were measured. BMD was measured in the lumbar vertebrae L1-L4 and in both hips. The absolute BMD (expressed in g per square centimeter) and T-score (the number of standard deviations above or below the mean value of a sex matched reference population) of each patient was calculated. Because Dutch BMD reference data are not available, the reference database incorporated in the Hologic software was used. This reference database consists of age-and gender specific BMD reference values of healthy adults in Europe and the United States. The World Health Organization (WHO) definition of low BMD or osteopenia is a T-score between -1 and -2.5. Osteoporosis is defined as a T-score ≤-2.5.[12] The DXA-scans were assessed by nuclear medicine physicians who were aware of the patients' diagnosis, but not of the treatment history. When a T-score of <-1 was found in the lumbar spine or the hips at baseline, patients received a dietary supplementation with 500 mg calcium/440 IE vitamin D once daily.

Clinical data

Clinical data were obtained from a structured questionnaire and from the hospital record. Age, sex, amount of daily dairy intake (cow's milk, yoghurt or cheese) and level of general physical activity (i.e. participation to sports) of every patient was recorded at baseline and at follow-up.

Corticosteroid use

Cumulative dose of topical corticosteroids, oral corticosteroids and inhalation/nasal/ocular corticosteroids was calculated between the baseline and follow-up BMD measurement with use of pharmacy records of each patient. Topical corticosteroid use was expressed in total grams of all potency classes (European class I-IV).
The amount of topical corticosteroids used between baseline and follow-up was categorized into two groups according to the median use of all potency classes: use of < 75 g per month or use of ≥ 75 g per month. Oral corticosteroids and inhalation/nasal/ocular corticosteroid use was expressed in total milligrams (use of other oral corticosteroids than prednisone were converted to milligrams of prednisone equivalents).

Calcium use and use of other oral immunosuppressive drugs

Use of calcium supplements and total number of months of use was recorded between baseline and follow-up with use of pharmacy records. Use of other oral immunosuppressive drugs including cyclosporin A, mycophenolate sodium, azathioprine and tacrolimus were recorded in the same way.

Biochemical parameters

Plasma concentrations of parathyroid hormone (PTH) were measured in respectively 68 (90.7%) and 53 (70.7%) of the patients at baseline and follow-up. Serum concentrations of 25-hydroxyvitamin D (25-OH-vitamin D) were measured in respectively 67 (89.3%) and 53 (70.7%) of the patients at baseline and at follow-up. Vitamin D deficiency was defined as a serum 25-OH-vitamin D level of <50 nmol/L and vitamin D insufficiency was defined when levels were between 51 and 74 nmol/L.[13] In addition, serum levels of bone-specific alkaline phosphatase (BSAP) were measured in respectively 67 (89.3%) and 48 (64%) of the patients at baseline and follow-up. C-terminal telopeptide of type I collagen (ß-CTx) were measured in respectively 68 (90.7%) and 53 (70.7%) of the patients at baseline and follow-up.

Statistical analysis

Statistical analysis was performed using the program SPSS for Windows (version 15.0, 2006; SPSS inc., Chicago, IL, U.S.A.). Data were expressed as frequencies (number of patients and percentages) for categorical variables and as mean ± SD for continuous variables. Body mass index (BMI) was calculated as weight divided by squared length (kg/m2). First, paired t-tests, and the McNemar test were done to investigate the difference in absolute BMD and T-scores (lumbar spine and total hip) at baseline and at follow-up.
The mean percentage change in absolute BMD between baseline and follow-up was calculated for both the lumbar spine and the total hip. A preliminary analysis showed no significant difference in BMD change between patients with calcium/vitamin D supplementation and patients without supplementation. Furthermore, an interaction between calcium/vitamin D supplementation and topical corticosteroid use on change in BMD was not present (lumbar spine p=0.32; total hip p=0.67). Therefore, patients with and without calcium/vitamin D supplementation were analyzed as one group.
To investigate if use of topical corticosteroids was associated with a decrease in BMD, first a t-test was done to compare percentage change in BMD in patients who had used <75 g topical corticosteroids per month (Group 0) with patients who had used ≥75 g topical corticosteroids per month (Group 1). Furthermore, ANCOVA was done to adjust for age, sex, BMI, dairy intake, physical activity and other corticosteroid use. In this way the mean percentage change in BMD between group 0 and group 1 was calculated after adjustment for potential confounders. The interaction between use of oral immunosuppressive drugs and topical corticosteroid use was not significant. Furthermore, the relation between topical corticosteroid use and change in BMD did not change when including use of oral immunosuppressive drugs. Finally, baseline levels of 25-OH-vitamin D, PTH, BSAP and ß-CTx were compared between group 0 and group 1 with a t-test or a Mann-Whitney test in case of non-normality and differences between baseline and follow-up levels were investigated with either a t-test or the Wilcoxon Signed Rank test in case of non-normality.

Results

Seventy-five patients, 39 men and 36 women with moderate to severe AD were included. At the moment of inclusion, all patients used topical corticosteroids for the treatment of their AD. The duration between the baseline BMD measurement and the follow-up measurement was 2.4 ± 0.5 years. Baseline characteristics of the study population are described in Table 1.

Changes in bone mineral density between baseline and follow-up

Overall, baseline absolute BMD in the lumbar spine and total hip did not differ significantly from the follow-up absolute BMD (Table 2). Furthermore, the number of patients with a T-score <-1 at baseline did not differ from the number of patients with a T-score <-1 at follow-up. Additionally, the change in BMD during follow-up did not differ between patients with a T-score <-1 (osteopenia or osteoporosis; lumbar spine 0.99% ± 4.24 and total hip 0.02% ± 3.88) at baseline compared to patients with a T-score ≥-1 (normal; lumbar spine -0.47% ± 3.98 and total hip -0.31% ± 2.99) at baseline. The corresponding p-values are 0.43 for the lumbar spine and 0.87 for the total hip.

Table 1.

Baseline characteristics of patients with AD.

Number of AD patients	75
Sex (no. of patients):	
- Men	39 (52%)
- Women	36 (48%)
Age (mean years ± SD)	40.2 ± 14.8
Body mass index (mean kg/m² ± SD)	24.1 ± 3.6
Number of years between baseline and follow-up BMD measurement (mean ± SD)	2.4 ± 0.5
Dairy intake (mean units daily ± SD)	3.0 ± 1.8
Physical exercise (no. of patients):	
- > 2 times weekly (high)	17 (23.6 %)
- 1 - 2 times weekly (normal)	32 (44.4 %)
- < 1 time weekly (low)	23 (31.9%)
Biochemical parameters (mean levels ± SD)	
- PTH (pmol/L)	3.01 ± 1.82
- 25-hydroxyvitamin D (nmol/L)	65.12 ± 30.27
- BSAP (µg/L)	11.27 ± 5.03
- ß-CTx (ng/ml)	0.30 ± 0.22

Table 2.

Bone mineral density measurements at baseline and during follow-up in patients with AD.

Bone mineral density changes (n=75)	Baseline	Follow-up	p-value
Lumbar spine T-score (no. of patients):			
- Normal	44 (58.7%)	41 (54.7%)	0.61
- Osteopenia	28 (37.3%)	31 (41.3%)	0.61
- Osteoporosis	3 (4.0%)	3 (4.0%)	0.61
Total hip T-score (no. of patients):			
- Normal	58 (77.3%)	60 (80.0%)	0.69
- Osteopenia	17 (22.7%)	15 (20.0%)	0.69
- Osteoporosis	0 (0.0%)	0 (0.0%)	0.69
BMD lumbar spine (mean g/cm² ± SD)	1.04 ± 0.15	1.04 ± 0.13	0.47
BMD total hip (mean g/cm² ± SD)	0.97 ± 0.13	0.95 ± 0.14	0.06

Medication use between baseline and follow-up

Between baseline and follow-up the majority of AD patients (93.2%) were treated with European class III topical corticosteroids. Use of topical corticosteroids (European class I-IV) varied between 1-480 g (mean 86.8 ± 80.5 g; median 74.3 g) per month. Only one (1.4%; 480 g per month) patient had used more than the recommended amount for safe use of topical corticosteroids according to the Dutch guidelines being maximally 240 g per month.[14]

Twenty-seven (36%) patients were treated with oral corticosteroid courses. Forty-five (60%) patients also regularly used inhalation/ocular or nasal corticosteroids because of allergic asthma, allergic rhinitis or eczema of the eyelids. Twenty-nine (38%) patients had used an oral immunosuppressive drug between baseline and follow-up. Mean duration of use was 12 ± 9 months. Calcium-vitamin D supplementation was used in 46.7%, but continuous use was registered in only 25.7% of these patients (Table 3).

Table 3.

Medication use between baseline and follow-up BMD measurement.

Use of topical corticosteroids (no. of patients)	75 (100%)
Cumulative dose of topical corticosteroids used (mean g ± SD)*:	
- Class I	8 ± 23
- Class II	241 ± 383
- Class III	1921 ± 1868
- Class IV	302 ± 630
- Total all classes	2472 ± 2150
Amount of topical corticosteroids used per month**:	
- Mean g ± SD	86.8 ± 80.5
- < 75 g/month	50.0%
- 75-199 g/month	44.6%
- 200-239 g/month	4.0%
- > 240 g/month	1.4%
Use of oral corticosteroids (no. of patients)	27 (36%)
Cumulative dose of oral corticosteroids (mean mg ± SD)*	669 ± 1647
Use of inhalation/nasal/ocular corticosteroids (no. of patients)	45 (60.0%)
Cumulative dose of inhalation corticosteroids (mean mg ± SD)*	154 ± 281
Use of oral immunosuppressive drugs (no. of patients)	29 (38%)
- Cyclosporine	12 (16.2%)
- Mycophenolate sodium	11 (14.9%)
- Azathioprine	2 (2.7%)
- Tacrolimus	3 (4.1%)
Use of calcium/vitamin D supplementation (no. of patients)	35 (46.7%)

** Cumulative dose calculated between baseline and follow-up.*
*** All classes of topical corticosteroids.*

Topical corticosteroids and change in bone mineral density

The percentage change in BMD in the two topical corticosteroid use groups is presented in figure 1a (lumbar spine) and 1b (total hip). Table 4 presents the unadjusted and adjusted percentage change in BMD for patients using < or ≥ 75 g topical corticosteroid per month (resp. group 0 and group 1). The BMD in the lumbar spine increased with 0.07% in group 0 and increased with 0.14% in group 1. This difference was clinically and statistically not significant (p=0.81). Adjustment for age, sex, BMI, dairy intake, physical activity and other corticosteroid use did not change this result. BMD in the hip increased with 0.17% in group 0 and decreased with 0.72% in group 1. This difference was also clinically and statistically not significant (p=0.27) and adjustment for possible confounders did not change this result either.

Table 4.

Mean bone mineral density changes in patients using < or ≥ 75 g topical corticosteroid per month.

Bone mineral density changes (mean % ± SD)	Unadjusted BMD Lumbar spine	Adjusted BMD Lumbar spine*	UnadjustedBMD Total Hip	Adjusted BMD Total Hip*
< 75 g topical corticosteroid use/ month (Group 0; n=37)	0.073 ± 3.913	0.657 ± 4.307	0.170 ± 3.296	0.164 ± 3.223
≥ 75 g topical corticosteroid use/ month (Group 1; n=34)	0.138 ± 4.399	0.204 ± 4.431	−0.716 ± 3.197	−0.484 ± 3.18
p-value	0.806	0.571	0.273	0.100

** Adjusted for: age, sex, dairy intake, physical activity, BMI and other steroid use.*

Biochemical parameters

None of the baseline levels of 25-OH-vitamin D, PTH, BSAP and ß-CTx differed between patients using <75 g topical corticosteroids per month and patients using ≥75 g topical corticosteroids per month during follow-up. An insufficient level of 25-hydroxyvitamin D was seen in 30% of the patients with and in 27.5% of the patients without calcium/vitamin D supplementation. A deficient level of 25-hydroxyvitamin D was also seen in 30% of the patients with and 28.6% of the patients without calcium/vitamin D supplementation. The number of patients with a 25-hydroxyvitamin D insufficiency or deficiency did not differ between baseline and follow-up (p=0.25 without calcium/vitamin D supplementation; 0.50 with calcium/vitamin D supplementation). Two patients (3%) had PTH levels slightly above the upper reference of 7 pmol/L at baseline versus three patients (5.7%) at follow-up. None of the patients had PTH levels below the lower reference of 1 pmol/L. Levels of BSAP were above the upper reference limit in 6 patients (8%) at baseline and in none of the patients at follow-up. This difference was not statistically significant (p=0.08). Levels of ß-CTx were above the upper reference limit in 3 patients (4.4%) at baseline and in 1 patient (1.9%) at follow-up. This difference was not statistically significant (p=0.32).

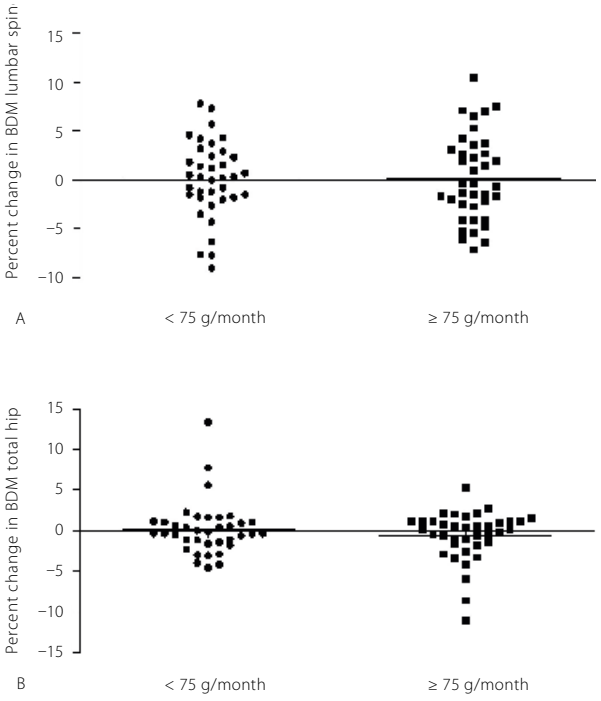

Figure 1.
The percentage change in BMD in the two topical corticosteroid use groups (<75 g/month and ≥75 g/month) of the lumbar spine (A) and total hip (B).

Discussion

In this 2-year follow-up study no difference in BMD between baseline and follow-up was seen and, more importantly, the change in BMD did not differ between patients who had used low or high amounts of topical corticosteroids during follow-up. Additionally, the number of patients with a T-score <-1 at baseline did not differ from the number of patients with a T-score <-1 at follow-up.
From our data we cannot conclude that topical corticosteroids decrease BMD in patients with moderate to severe AD. A possible explanation is that the amount of topical corticosteroids used by this study population is low since all but one patient had used amounts below the threshold for safe use of topical corticosteroids.[14] Furthermore, the majority of patients with AD use topical corticosteroids only during a flare up of their eczema. After improvement, topical corticosteroids are frequently stopped till the next flare occurs. It has been shown that bone loss is reversible after a short course of oral corticosteroids in patients with rheumatoid arthritis and pulmonary sarcoidosis.[15,16] For patients with AD this could mean that the effect of short, intensive use of even potent topical corticosteroids on BMD will be reversed thereafter. In patients receiving oral corticosteroids the greatest decrease in BMD is seen during the first 12 months of treatment.[17,18] After this period bone loss continues, but at a lower rate, in spite of continuing oral corticosteroid therapy.[18,19] The mean age of our study population is 40 years (17-80 years) suggesting that these patients were already suffering from AD and therefore using topical corticosteroids for a long period of time. This may also be an explanation for the lack of association between changes in BMD and topical corticosteroid use after two years of follow-up.

Besides corticosteroids, BMD may also be influenced by chronic inflammation as has been described in patients with inflammatory bowel disease[20] and rheumatoid arthritis.[21,22] Patients belonging to group 1 did use more topical corticosteroids compared to patients from group 0. This suggests that patients from group 1 had more activity of their eczema during the follow-up period compared to patients from group 0. For that reason the decrease in BMD in group 1, although being non-significant, may also be due to increased cutaneous inflammation during the follow-up period. However, since the use of topical corticosteroids in patients with AD will always be related to the severity of the eczema it will be difficult to discriminate between the influence of both factors on the change in BMD over time.

We observed no significant difference in the change of BMD between patients with a T-score <-1 and a T-score ≥ -1 at baseline. There was a tendency that patients with a T-score ≥ -1 (being normal) at baseline even showed a negative change in BMD both for total hip (-0.31%) and lumbar spine (-0.47%) compared to patients with a T-score <-1. Although the numbers of AD patients in this study are low the data may suggest that the decrease in BMD in AD patients with a T-score <-1 is not progressive during the two-year follow-up period. A change between two BMD measurements only reflects a 'real' change if the random error of the DXA system is exceeded.[23,24]
The random error or least significant change (LSC) has been described to be between 3.3-4.7% for the total hip.[23] In the current study the decrease in BMD after 2 years in patients using high (≥75 g per month) amounts of topical corticosteroids was only 0.484% for the total hip, a very small decrease. Further follow-up studies are necessary to evaluate if this small decrease continues over time and after which time period this decrease becomes clinically relevant.

The aforementioned aspects can only be properly addressed by long term follow-up studies starting soon after the diagnosis of AD has been made, and even more importantly, directly after corticosteroid therapy has been started. This gives the opportunity to compare the change in BMD over time between patients with mild and severe AD, and assess the influence of topical corticosteroid use more accurately both at the start and during long-term use of topical corticosteroids. Furthermore it will give information if and how fast BMD further decreases as soon as the T-score is <-1.

Markers of bone formation (BSAP) and bone resorption (ß-CTx) also show attenuation over time, and are more useful in assessing the acute effect of corticosteroids on bone (within 12 months) than the chronic effect.[1,25] This may explain why our study did not show significant differences between baseline and two year follow-up.

Due to a high association of AD with asthma and allergic rhinitis[26], thirty-six percent of the patients had used oral, and sixty percent inhalation, nasal or ocular corticosteroids. Although adjustment for the use other corticosteroids was performed in the data analysis it still is a limitation of this study. A strong point of this study is additional adjustment for other possible confounders (e.g. dairy intake).

In conclusion, overall BMD at follow-up did not differ from baseline. Moreover, use of ≥75 g topical corticosteroids per month in patients with moderate to severe AD did not result in a significant decrease in BMD compared to use of <75 g per month. Additional long-term follow-up studies are necessary to investigate which patients with AD are at risk for developing a clinically relevant decrease in BMD.

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Chapter 6

Bone mineral density in children with moderate to severe atopic dermatitis

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Key words:
atopic dermatitis, children, bone mineral density, topical corticosteroids, CsA

Abbreviations:

AD: atopic dermatitis
BMAD: Bone Mineral Apparent Density
BMD: Bone Mineral Density
CsA: Cyclosporin A
CV: Coefficient of Variation
DXA: Dual-energy X-ray Absorptiometry
ISCD: International Society for Clinical Densitometry
IQR: Inter Quartile Range
25-OH-vitamin D: 25-hydroxyvitamin D
PTH: parathyroid hormone
SCORAD index: SCOring Atopic Dermatitis index
SD: Standard Deviation
SE: Standard Error
TARC: Thymus and Activation-Regulated Cytokine

Abstract

Background

Recently, low bone mineral density was reported in 30.4% of adult patients with atopic dermatitis (AD).

Objective

The aim of this study was to determine the prevalence of low bone mineral density (BMD) in children with moderate to severe AD and to investigate the relation between BMD and corticosteroid and cyclosporin A (CsA) therapy.

Methods

Lumbar spine BMD was measured by dual-energy X-ray absorptiometry in 60 children (age 5-16 years) with moderate to severe AD. BMD (in g/cm^2) was expressed in Z-scores; the number of standard deviations above or below the mean value of an age- and sex matched reference population. In children, low BMD was defined as a Z-score ≤-2. Information on lifestyle parameters and bone fractures were collected by use of a standardized questionnaire. The cumulative dose of corticosteroids and CsA therapy was calculated for the previous 5-year period.

Results

Three patients (5%) had low BMD; one patient (1.7%) had osteoporosis.
The observed prevalence of low BMD in this study (6.7%; 95% confidence interval: 1.8-16.2%) does not differ from the expected prevalence of low BMD in the general population (p=0.06). Overall, use of topical corticosteroids in the previous five years was not associated with a decrease in BMD (Z-score). When children received additional systemic treatment (oral corticosteroids and/or CsA) in the previous five years BMD decreased, although not statistically significant. Correction for life style parameters did not change these associations.

Limitations

Limited number of patients. The cumulative dose of corticosteroids and CsA therapy was only registered for the previous 5 years, and relatively low amounts of topical corticosteroids were used. The definition of low BMD differs between adults (Z-score ≤-1) and children (Z-score ≤-2). Because there is no Dutch BMD reference population for children, normative BMD references were obtained from a different population (US children).

Conclusions

Low BMD did not occur more frequently in this population of children with moderate to severe AD compared to the general population. Use of topical corticosteroids in the previous 5 years was not associated with a decrease in BMD.

Introduction

Atopic dermatitis (AD) is a common inflammatory skin disease in children worldwide, with a variable prevalence of 12-37%.[1] Topical corticosteroids represent the mainstay of treatment, but there is widespread concern about adverse effects. Systemic use of corticosteroids in children is associated with a decrease in bone mineral density (BMD) and bone turnover.[2, 3] Topically applied corticosteroids can also exert a systemic effect through percutaneous absorption.[4, 5] Recently, a high incidence (30.4%) of low BMD (Z-score ≤-1) was described in adult patients with moderate to severe AD.[6]

During childhood and adolescence, BMD increases until peak bone mass is reached at approximately 21 years of age.[7] Achieving a normal peak bone mass is important in the prevention of low BMD and osteoporosis later in life. Pedreira et al. did not find an association between the use of topical corticosteroids and a decreased BMD in children with moderate to severe AD although decreased BMD in children using cyclosporin A (CsA) was observed.[8] These authors did not define low BMD by Z-scores and presented the absolute decrease in BMD. Since the definition of low BMD in children is based on Z-scores[9], it cannot be concluded that an absolute decrease in BMD is of clinical relevance.

Other factors than topical or oral medication may put children with moderate to severe AD at risk for low BMD. Treatment of co-existing atopic diseases must be considered since up to 33% of the children with AD have asthma or allergic rhinitis[10] and may use inhalation/nasal corticosteroids. Up to 30% of children with AD can have intolerance or allergy to cow's milk at some point in their lives[11] and calcium intake could be insufficient. Furthermore, atopic dermatitis has the potential to interfere with physical activity.[12] The aim of this study was to investigate the frequency of low BMD (Z-score ≤-2) and its association with AD therapy (topical corticosteroids, oral corticosteroids, CsA), AD associated therapy (inhalation corticosteroids) and life style parameters (dairy intake, physical activity) in children with moderate to severe AD.

Table 1.

Definition of risk groups.

	Risk (+)
Group 1	Medium/high topical corticosteroid use + inhalation corticosteroid use
Group 2	Medium/high topical corticosteroid use + inhalation corticosteroid use + oral corticosteroid use
Group 3	Medium/high topical corticosteroid use + inhalation corticosteroid use + cyclosporin use
Group 4	Medium/high topical corticosteroid use + inhalation corticosteroid use + oral corticosteroid use + cyclosporin use

Children were assigned to a risk group if all of the above criteria were present.

Patients and methods

Patients

In this cross-sectional study 60 children with moderate to severe AD were included between January 2008 and February 2009. All patients were aged between 5 and 16 years. The diagnosis of AD was made according to the criteria of Hanifin and Rajka.[13] All children were currently treated at the outpatient department of Pediatric Dermatology/Allergology our institution. Children were included if one of the following criteria was present: objective SCORAD index >20; >4 visits to the outpatient department in one year; history of a hospital admission for the treatment of AD; current use of potent topical corticosteroids (class III) or history or current use of systemic immunosuppressive treatment (prednisone and/or CsA). Children with co-morbidity such as renal diseases, rheumatoid arthritis, inflammatory bowel disease, cystic fibrosis, endocrinological disorders or bone diseases were excluded. The local medical ethical committee approved this study and patients and/or their parents or caregivers gave their written informed consent.

Questionnaire

All patients were interviewed with the help of their parents by use of a structured questionnaire. Participants were asked if they suffered or had suffered from allergic asthma, allergic rhinitis or cow's milk allergy. The amount of daily dairy intake (cow's milk, yoghurt or cheese), the level of general physical activity (i.e. sports attendance) and daily exposure to sunlight was determined. Subsequently, the total number of years patients were treated for AD was recorded.

Corticosteroid and CsA use

Cumulative dose of topical, inhaled and systemic corticosteroids and topical tacrolimus and pimecrolimus was calculated for 5 years prior to the visit to our clinic. This was carried out using the pharmacy records of each patient as previously described by Haeck et al.[6] Use of CsA was recorded for the last 5 years. The total number of years of AD therapy was determined from the patients' history. Cumulative topical and inhalation/nasal corticosteroids were divided into three groups according to the amount used in the previous 5 years. Topical corticosteroid use was graded as 'low' (<30 g per month), 'medium' (30-50 g per month) or 'high' (>50 g per month). Use of inhalation/nasal, oral corticosteroids and CsA was categorized into two groups: use or no use in the previous 5 years. In this way 4 different risk groups were determined (Table 1).

Bone mineral density measurement; DXA-scan

Bone mineral density was measured by dual-energy X-ray absorptiometry (DXA), using the Hologic QDR-4500 Discovery A (software version 12.3, Hologic inc. Bedford, MA, U.S.A.) X-ray Bone Densitometer. The manufacturer specified coefficient of variation (CV) is 1% (calculated: 100 x SD/mean). The standard deviation for the lumbar spine BMD is 0.00806 g/cm^2. Daily quality assurance was performed, and all children were assessed by the same analyst. Prior to the BMD measurement, height and weight of the children was measured. Then, BMD was measured in the lumbar vertebrae L1-L4. Data are expressed as Z-scores; the number of standard deviations above or below the mean value of an age- and sex matched reference population. Because BMD in DXA is dependent of bone size, and a larger bone size may artificially inflate BMD[14], correcting Z-score for body height is necessary to prevent incorrect diagnosis of osteoporosis in children with a small body size for their age.[15] Children with AD can have small body height due to chronic inflammation[16,17] and we corrected all analysis for body height (age adjusted). Reference data for healthy Dutch children are not available for the Hologic Bone Densitometer.[18] The pediatric reference database incorporated in the Hologic software was used. This reference population consists of age-and gender specific BMD reference values from more than 1400 Caucasian children in the United States.[19] According to the International Society for Clinical Densitometry (ISCD) recommendations[9] osteoporosis was defined as a Z-score ≤-2 with a fracture history. Low BMD was defined as a Z-score ≤-2 without a significant fracture history. The DXA-scans were reported by nuclear medicine physicians who were aware of the patients' diagnosis, but not of the treatment history.

Disease activity

Disease activity was recorded on the same day as the BMD measurement was performed, and was scored using the objective SCoring Atopic Dermatitis (SCORAD) index.[20] In addition the level of serum Thymus and Activation-Regulated Cytokine (TARC) was measured as an objective marker for disease activity.[21,22] TARC reference levels are 0-100 pmol/L.

Biochemical parameters

Plasma concentrations of parathyroid hormone (PTH) and serum concentrations of calcium, albumin, phosphate and 25-hydroxyvitamin D (25-OH-vitamin D) were measured. Vitamin D deficiency was defined as a serum 25-OH-vitamin D level of <50 nmol/L and vitamin D insufficiency was defined when levels were between 51 and 74 nmol/L.[23]

Statistical analysis

Statistical analysis was performed using the program SPSS for Windows (version 15.0, 2006; SPSS inc., Chicago, IL, U.S.A.). Data were expressed as frequencies (number of patients or percentages) for categorical variables and as a mean ± SD for continuous variables. The children's length and weight were expressed by age-and sex adjusted Z-scores. To investigate whether the expected versus observed percentage of children with a low BMD differed significantly the binominal test was done. (test proportion set on 2.5%)

The association between topical corticosteroid use and lumbar spine Z-score was investigated by use of univariate and multivariate linear regression. Topical corticosteroid use was expressed as a continuous variable and as a categorical variable. The association between the four risk groups and lumbar spine Z-score was determined. Multivariate linear regression was done to adjust for length Z-score, weight Z-score, dairy intake and physical exercise as these are potential confounders.
The correlation between the number of fractures and lumbar spine Z-score was determined using the Spearman rho test, since the number of fractures had a skewed distribution. Differences in the number of fractures in risk group 1-4 were determined by use of the Mann-Whitney test. Finally, we compared Z-scores between normal, deficient and insufficient levels of 25-OH-vitamin D.

Results

Baseline characteristics of study population

Baseline characteristics are presented in Table 2. Sixty children, 24 boys and 36 girls; age 5-16 year, with moderate to severe AD were included. At the moment of inclusion patients had active skin disease, indicated by a mean objective SCORAD of 31 ± 14 (± SD) and a median serum TARC of 945 (IQR: 457-1895) pmol/L. Patients had received treatment for 1.5-16.2 (median 7.7) years. All patients used topical corticosteroids to control their skin disease at the moment of inclusion; most frequently class III topical corticosteroids (class IV being the most potent) were used. Total cumulative use (class I-IV) of topical corticosteroids in the previous 5 years (± SD) was 2044 ± 1838 g. Additionally, 25% of the patients required treatment with one or more courses of oral corticosteroids and 13.3% were treated with CsA in the previous 5 years. Furthermore, 53.3% were diagnosed with asthma/allergic rhinitis and used inhalation/nasal corticosteroids and 53.4 % had cow's milk allergy (currently or in the past). Dairy intake and physical activity were low in 71.1% and 13.3% respectively.

Bone mineral density

Three patients (5%) were diagnosed with low BMD (Z-score ≤-2 in lumbar spine), and one patient (1.7%) was diagnosed with osteoporosis (Z-score ≤-2 and a significant fracture history) (Table 2). All 3 patients with low BMD had used topical corticosteroids for at least 10 years. None of these patients had previously used oral corticosteroids or CsA. One patient also used inhalation corticosteroids and his dairy intake and physical activity were inadequate. Topical corticosteroid use was low in 2 patients with a cumulative use of 555 g and 575 g respectively and high in one patient with use of 2990 g in 5 years. Disease activity at the moment of inclusion was high with an objective SCORAD of 24, 33 and 45. One patient reported a bone fracture after trauma. No significant family history of fractures was present.
The patient with osteoporosis (age 6 years) had not previously used inhaled/oral corticosteroids or CsA. Topical corticosteroids were applied since 3 years with a cumulative amount of 1230 g. The objective SCORAD at inclusion was 30. Dairy intake and physical activity were within normal limits. The patient reported two arm fractures and one digital fracture after trauma. The younger sister of this patient reported two fractures after trauma; she had no history of AD. This patient was referred to an endocrinologist where no abnormalities in bone-related laboratory parameters were found.

Table 2.

Baseline characteristics of AD patients (n=60).

Number of AD patients	60
Gender (no. of patients):	
- Boys	24 (40%)
- Girls	36 (60%)
Age (mean years ± SD)	10.5 ± 3.4
Length Z-score (mean ± SD)	-0.17 ± 1.14
BMD lumbar spine Z-score (no. of patients)*:	
- Normal	56 (93.3%)
- Low BMD	3 (5 %)
- Osteoporosis	1 (1.7%)
Cumulative dose of topical corticosteroids (mean g ± SD)**:	
- Class I	47 ± 96
- Class II	385 ± 806
- Class III	1558 ± 1405
- Class IV	56 ± 134
- Total all classes	2044 ± 1838
Cumulative dose of topical tacrolimus or pimecrolimus (median g and IQR)	120 (30-330)
Use of oral corticosteroids (no. of patients)	15 (25%)
Cumulative dose of oral corticosteroids (mean mg ± SD) **	559 ± 406
Use of inhalation corticosteroids (no. of patients)	32 (53.3%)
Cumulative dose of inhalation corticosteroids (mean mg ± SD) **	326 ± 294
Use of cyclosporin (no. of patients)	8 (13.3%)
Duration of cyclosporin therapy (mean months of use ± SD) **	11 ± 5
Duration of AD treatment (mean years of treatment ± SD)	8.1 ± 3.8
Disease activity:	
- Objective SCORAD (mean score ± SD)	30 ± 12
- SA-EASI (mean score ± SD)	15 ± 12
- TARC (median pmol/L (IQR))	945 (459-1895)
Dairy intake (no. of patients):	
- < 4 U/day*** (low)	43 (71.7 %)
- > 4 U/day (normal)	17 (29.3 %)
Physical exercise (no. of patients):	
- > 2 times weekly (high)	25 (41.7 %)
- 1-2 times weekly (normal)	27 (45.0 %)
- < 1 time weekly (low)	8 (13.3 %)
Fractures (no. of patients):	
- no fractures	45 (75 %)
- one or more fractures	15 (25%)

AD, atopic dermatitis; BMD, bone mineral density; IQR, Inter Quartile Range;
SA-EASI, Self–administered Eczema Area and Severity Index; TARC, thymus and activation-regulated cytokine

** ISCD criteria: low BMD if Z-score ≤-2.0; osteoporosis if Z-score ≤-2.0 and a clinically significant fracture history;*
*** use in 5 years prior to inclusion; *** U=one unit of dairy, i.e. one glass.*

Expected versus observed numbers of patients with low BMD

In a normally distributed population 2.5% (2 out of 60 in this study) are expected to have a low BMD (Z-score ≤-2). The observed percentage of children with a low BMD is 6.7% (4 out of 60; 95% confidence interval: 1.8-16.2%) in the study population. The difference between the expected and observed percentage of children with a low BMD is not significant (p=0.06).

Cumulative dose of topical corticosteroids and bone mineral density

Use of topical corticosteroids was not associated with a lower lumbar spine Z-score (adjusted for length, inhalation/oral corticosteroids and CsA; ß=0.064, SE=0.077, p=0.408). When topical corticosteroid use was divided into groups with low, medium and high cumulative use, higher use of topical corticosteroids was not associated with a decrease in lumbar spine Z-score (Table 3 and Figure 1). Additional adjustment for weight, physical activity and dairy intake did not change the association.

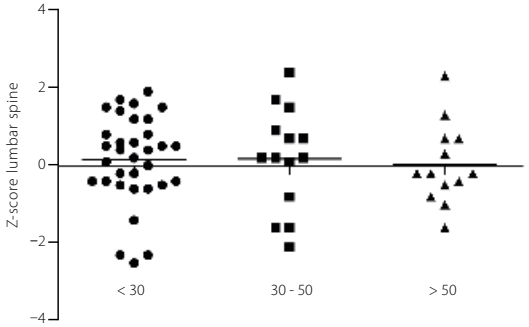

Figure 1.
Lumbar spine Z-scores in children with AD (n=60) using different amounts of topical corticosteroids. Topical corticosteroids is defined as mean dose in grams per month of all classes of topical corticosteroids used in the previous 5 years; p = 0.935.

Topical corticosteroids, inhalation corticosteroids, oral corticosteroids, CsA and bone mineral density

Patients using medium or high amounts of topical and inhalation/nasal corticosteroids (risk group 1; n=20) did not have a lower lumbar spine Z-score than patients using low (n= 40) amounts of topical and no inhalation corticosteroids (ß=0.294, SE=0.291, p=0.316), when adjusted for length.

Table 3.

Association between lumbar spine Z-score and topical corticosteroid use/risk groups.

	Number of patients per group	Univariate ß (p-value)	Multivariate ß (p-value)*
Cumulative topical corticosteroid use all classes in previous 5 years (per kg)	60	0.015 (0.856)	0.105 (0.260)**
Cumulative topical corticosteroid use all classes in previous 5 years (per group of use)	low 33 medium 14 high 13	medium vs low 0.024 (0.948) high vs low −0.124 (0.746)	medium vs low 0.200 (0.582)** high vs low 0.248 (0.567)**
Group 1 (risk + vs −)	− 40 + 20	0.220 (0.486)	0.294 (0.316)
Group 2 (risk + vs −)	− 50 + 10	−0.112 (0.780)	−0.082 (0.825)
Group 3 (risk + vs −)	− 55 + 5	−0.516 (0.337)	−0.303 (0.549)
Group 4 (risk + vs −)	− 57 + 3	−0.912 (0.179)	−0.573 (0.373)

** Adjusted for Z-score height, ** adjusted for Z-score height, inhalation and oral corticosteroid use, cyclosporin use.*

However, lumbar Z-score decreased with 0.082 when patients had additionally used oral corticosteroids (risk group 2; n=10; ß = -0.082, SE=0.37, p=0.825) and lumbar spine Z-score decreased with 0.303 when patients had additionally used CsA (risk group 3; n=5; ß=-0.303, SE=0.50, p=0.549). Finally, children with additional use of both oral corticosteroids and CsA in the previous 5 years (risk group 4; n=3) had the most prominent decrease in lumbar spine Z-score (ß=-0.573, SE=0.64, p=0.373).
(Table 1 and 3)
Additional adjustment for weight, physical activity and dairy intake did not change the association.

Topical tacrolimus and pimecrolimus use

46.7% of the patients had used either topical tacrolimus or pimecrolimus in the previous 5 years. Median use was 120 g in the previous 5 years (Table 2), and use was often restricted to several weeks or months. No child had used topical tacrolimus or pimecrolimus continuously.

Fractures

25% of the patients had a history of one or more bone fractures. No fragility fractures were reported. Lumbar spine Z-score was weakly correlated with the occurrence of fractures (r=0.27, p=0.037). Of the 3 children with a lumbar spine Z-score <-2 only one child reported a bone fracture. Overall, patients in risk groups 1-4 did not have significantly more fractures (data not shown).

Biochemical parameters and bone mineral density

Levels of serum calcium, albumin, phosphate and PTH were normal in all patients. A deficiency of 25-OH-vitamin D (<50 nmol/L) was found in 5 out of 60 patients. In this group, all patients had a normal lumbar spine Z-score. Insufficiency of 25-OH-vitamin D (51-74 nmol/L) was found in 25 out of 60 patients; 3 patients in this group had a low lumbar spine Z-score. Normal 25-OH-vitamin D (>75 nmol/L) was found in 30 patients, and in this group 1 patient had a low lumbar spine Z-score (Table 4). All patients received more than 10 minutes of sunlight daily. There was no statistical significant difference in lumbar spine Z-score and 25-OH-vitamin D levels (p=0.858).

Table 4.

Laboratory parameters of n=60 children with atopic dermatitis.

	Mean ± SD	Reference values*
Calcium (mmol/L)	2.41 ± 0.06	2.17 – 2.64
Albumine (g/L)	40.67 ± 2.52	34 - 50
Phosphate (mmol/L)	1.45 ± 0.18	0.90 – 1.80
PTH (pmol/L)	3.21± 1.04	1 - 7
25-OH-vitamine D (nmol/L)	77.12 ± 27.08	> 75

PTH, Parathyroid hormone; 25-OH-vitamin D, 25-hydroxyvitamin D.

** Age group specific reference values are included in the reference values presented here.*

Discussion

Children with moderate to severe AD may be at risk for having low BMD. We assessed several risk factors such as corticosteroid and CsA use and life style parameters for their potential to influence BMD in 60 children with moderate to severe AD. In this study we found that 4 out of 60 (6.7%) children with AD have a low BMD (Z-score ≤-2) in the lumbar spine. The difference between the expected (2.5%) and observed (6.7%) percentage of children with a low BMD is not statistically significant. This means that we could not find evidence that low BMD is measured more frequently in this population of children with moderate to severe AD compared to the general population.

The amount of topical corticosteroids used in the previous 5 years by the patients in this study did not seem to be related with a decrease in BMD. This result is consistent with the outcome of the study done by Pedreira et al. in spite of methodological differences in calculating topical corticosteroids use.[8] None of the 3 children with a low BMD had used >50 g of topical corticosteroids per month. The child with osteoporosis had even used <30 g of topical corticosteroids per month. Overall, the use of topical corticosteroids in our study population was low according to the practical guideline on topical corticosteroid use in children.[24] Furthermore, quantification of topical corticosteroids by pharmacy records can lead to an overestimation of the actual use, as these only represent the amount of topical corticosteroids prescribed by the dermatologist. Finally, the limited sample size of this study may have contributed to this finding and future studies should be performed in larger patient groups.

We did not find a negative association between additional use of inhalation/nasal corticosteroids and BMD (Group 1). Data on the association between inhaled corticosteroids and low BMD is contradictory. Kelly et al. reported reduced bone mineral accretion in male children with asthma through puberty.[25] Other studies found that long term (2-6 years) inhaled corticosteroids had no significant effect on BMD.[26,27] In the current study the number of patients using inhalation/nasal corticosteroids may be too small to detect any effect on BMD. Furthermore, the lifelong cumulative amount of inhalation/nasal corticosteroids was not available.
BMD tended to decrease when children had used systemic treatment in addition to topical/inhalation/nasal corticosteroids, especially when children were treated with both oral corticosteroids and CsA in the previous 5 years (group 4). Pedreira et al. also found a decreased BMD in children with moderate to severe AD, associated with the use of CsA (n=6).[8] Comparison with our data is difficult since children using inhalation

or oral corticosteroids at inclusion were excluded and data on the use of these drugs in the past were not given in the study of Pedreira et al. Furthermore, the authors did not express BMD in Z-scores. A limitation in our study is that the number of children using additional systemic treatment is low.
A role of CsA in inducing bone loss is identified in organ transplant recipients[28] and also in patients with rheumatoid arthritis[29] but the exact mechanism involved in CsA-induced bone loss is not well defined. Since the pharmacological mechanism of topical tacrolimus and pimecrolimus is similar to that of CsA, we also calculated the cumulative amount of these drugs. Use of topical tacrolimus and pimecrolimus was, however, limited and statistical analysis on the influence of these topical immunomodulators on BMD was not done. Our data suggests that the use of both oral corticosteroids and CsA in combination with topical/inhalation corticosteroids may be associated with a trend towards a decrease in BMD. However, larger numbers of patients are needed to verify these results.

Haeck et al. found a high prevalence (30.4%) of low BMD in adult patients with AD.[6] No relation was found with cumulative topical corticosteroid use, even though the mean cumulative use was higher in the adult population than in the current child population (2687 vs 2044 g). Use of CsA was not determined in the adult population. No difference in treatment duration was found between adults with a normal or low BMD. The definition of low BMD differs between adults and children, which hinders comparison. Haeck et al. described low BMD in adults as a Z-score ≤-1 in lumbar spine, or in one of the hips, whereas for children low BMD is described as a Z-score is ≤-2 in the lumbar spine. Furthermore, children have not yet reached their peak bone mass and catch up bone mass acquisition can occur.[30] It has been suggested that juvenile bone mineral acquisition has little effect on adult bone density because the juvenile bone is largely replaced through growth.[30] Continued bone modeling and growth may make children less prone to low BMD. This may explain the difference in prevalence of low BMD between children and adults with AD. Serial BMD measurement, at least until peak bone mass is reached, in children with moderate to severe AD is necessary to investigate the difference in prevalence of low BMD with the results in adults.[6]

The influence of corticosteroids or CsA on BMD was not confounded by other risk factors for low BMD, such as dairy intake and physical activity. Low dairy intake, especially of cow's milk is associated with low BMD.[31-33] In this population no association between the amount of dairy intake and BMD was found. However, we recorded the number of units of daily dairy intake, which is a rather crude technique. Use of the food frequency questionnaire and calculation of the daily calcium and vitamin D intake in milligrams is more accurate.[34] Physical activity can increase BMD, but it is not a major determinant of bone health.[35] In this population low physical activity was not associated with a decrease in BMD. In addition, biochemical parameters were assessed to exclude underlying bone disease. Levels of PTH, calcium, phosphate and albumin were normal in all patients. Five patients had a 25-OH-vitamin D deficiency, but with normal PTH levels, and a normal BMD.

Adequate treatment of children with low BMD can prevent fractures later in life.[36] All four children with low BMD were supplemented with calcium/vitamin D. Therapeutic intervention with bisphosphonates should not be started on the basis of a single DXA measurement.[37] For this reason BMD measurement in the children with low BMD will be repeated.

This study shows that low BMD in children with moderate to severe AD does not occur more frequently compared with the general population. The cumulative amount of topical corticosteroids used in the previous five years was not related with a decrease in BMD in children with moderate to severe AD. Children with AD who were treated with oral corticosteroids and/or CsA in addition to topical/inhalation/nasal corticosteroids in the previous five years tended to have a lower BMD, although this was not statistically significant.

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Chapter 7

The Self-Administered Eczema Area and Severity Index in children with moderate to severe atopic dermatitis: better estimation of AD body surface area than severity

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Key words:
atopic dermatitis, objective SCORAD, SA-EASI, SASSAD severity score, TARC

Abbreviations:

AD: atopic dermatitis
BSA: Body Surface Area
EASI: Eczema Area and Severity Index
Objective SCORAD index: objective SCOring Atopic Dermatitis index (also objective SCORAD)
SA-EASI: Self-Administered Eczema Area and Severity Index
SASSAD severity score: Six Area Six Sign Atopic Dermatitis severity score
TARC: Thymus and Activated-Regulated Cytokine
VAS: Visual Analogue Scale

Abstract

Background

The Self-Administered Eczema Area and Severity Index (SA-EASI) is one of the few patient based atopic dermatitis (AD) disease activity scores, and was found to be highly correlated with the EASI. Correlation with other frequently used scoring methods has not been investigated.

Objectives

The aim of this study was to evaluate the relation of the SA-EASI with two physician based disease activity scores (objective SCORAD and SASSAD score) and with a serum marker for AD (Thymus and Activation-Regulated Cytokine (TARC)) in children with AD.

Methods

Sixty children with moderate to severe AD were included. The SA-EASI was completed by caregivers and the objective SCORAD and SASSAD score were measured successively on the same day by a trained investigator. Blood for serum TARC measurement was drawn.

Results

The correlation between the SA-EASI and the objective SCORAD was high (ρ=0.61, p=<0.001), mainly based on high correlation between the body surface area (BSA) measurements of both scores (ρ=0.50, p=<0.001). The correlation with the SASSAD score (only severity measurement) was 0.43 (p=<0.001). The correlation with serum TARC levels was 0.46; p=<0.001, mainly based on the BSA score of the SA-EASI (ρ=0.42, p=<0.001).

Conclusions

Parents may have more difficulty in scoring severity of AD than scoring BSA involved. Educating parents in severity scoring of AD may improve agreement of the SA-EASI and the objective SCORAD, TARC and SASSAD score. Additional use of the SA-EASI in routine clinical practice or in trials may then facilitate more frequent but still accurate assessment of AD.

Introduction

Measuring disease activity of atopic dermatitis (AD) is important to determine the effect of treatment. Not only in clinical trials, but also in routine clinical practice dermatologists should be able to assess disease activity.[1] Many different clinical scoring systems have been developed to visually evaluate the severity of AD. Both objective (disease extent and severity) and subjective (pruritis and sleep loss) symptoms can be measured.[1,2]
Most of the objective skin scoring systems require trained investigators to administer the instrument correctly, and some scoring systems are too complicated and time consuming for routine clinical use. Self-assessment of AD can facilitate scoring of AD in a cost effective manner[3] and will enable AD disease activity assessments at home between visits to the clinic. In this way disease course can be followed more precisely during treatment. The Self-Administered Eczema Area and Severity Index (SA-EASI) is developed to allow the patient or patients' parent/caregiver to evaluate both the extent and severity of AD at any moment.[3]
The SA-EASI is derived from the Eczema Area and Severity Index (EASI), which is a thoroughly validated scoring system for AD [4]. The main difference between the EASI and the SA-EASI is that severity characteristics of AD are scored 0-3 by the physician in the EASI, and 100 mm visual analogue scales (VAS) are marked by the patient in the SA-EASI.[3,5] The SA-EASI is highly correlated to the EASI and can be used in grading the severity of AD and track changes over time.[3] The correlation between the EASI and the total SCORAD index is also high (ρ =0.88, n=42).[6] The SA-EASI has not been related with other frequently used and most widely validated AD scoring systems such as the objective SCORAD and the SASSAD score.[4] This will be useful in comparing results of studies using different AD scoring systems.
The objective SCoring Atopic Dermatitis (SCORAD) combines both disease extent and severity, is validated adequately on construct validity, interobserver reliability and sensitivity to change and is developed both for children and adult patients with AD.[4,7]
The Six Area Six Sign Atopic Dermatitis (SASSAD) severity score[8] measures six different severity signs on six different body parts, has been shown to have adequate interobserver reliability[4] and has proved to be equally applicable to children and adults.[9,10] Both scoring systems have been used widely in randomized controlled trials assessing therapeutic interventions in AD.[11,12]

Several laboratory markers such as sE-selectin[13], interleukin-16 (IL-16)[14], and Thymus and Activation-Regulated Chemokine (TARC)[15,16] have been described to play a role in the pathogenesis of AD and to correlate with clinical disease activity of AD. TARC is a ligand for CC chemokine receptor 4 (CCR4) that is selectively expressed on Th2 cells and has shown to induce the selective migration of Th2 cells. Endothelial cells and keratinocytes have been shown to produce TARC[17] and infiltration of Th2 cells in the skin is an important feature in the pathogenesis of AD. Serum TARC levels are selectively elevated in patients with AD compared to healthy controls, patients with allergic respiratory diseases or patients with psoriasis.[15,17] Furthermore, levels of serum TARC correlate with disease activity measured by the Leicester Sign Score (extensive version of SASSAD score)[15,18], SCORAD index[17] and parallel disease severity during treatment with cyclosporin, mycophenolate sodium or topical corticosteroids.[15,16] This makes serum TARC an attractive biomarker for disease activity measurement in AD.
This study investigates the relation of the SA-EASI with two physician based objective disease activity scores (the objective SCORAD and SASSAD score) and with a serum marker for AD (TARC) in children with moderate to severe AD.

Patients and methods

Patients

Sixty children (age between 5-16 years) with moderate to severe AD were studied. The diagnosis of AD was made according to the criteria of Hanifin and Rajka.[19] Evaluation of disease activity by the SA-EASI, objective SCORAD, SASSAD score and serum TARC was done successively on the same day. Patients were not asked to stop topical corticosteroid use or other therapy for AD before evaluation of disease activity. The local medical ethical committee approved this study and patients and/or their parents or caregivers gave their written informed consent.

Self-Administered Eczema Area and Severity Index (SA-EASI)

The SA-EASI was completed by the parents/caregivers at the outpatient clinic (sometimes with help of the child). The parents/caregivers were told to assess the current severity and extent of AD. Furthermore, a short explanation (approximately 5 minutes) was given on how to shade the areas currently affected by AD and on how to use a VAS scale. The SA-EASI has been described previously by Housman et al.[3] In short the first part of the SA-EASI consists of an estimation of the amount of body surface affected by AD of four different areas: head, upper extremities, trunk and

lower extremities. Each area score is multiplied by a figure assigned to the corresponding body area which differs between children ≤ 7 and children >7 years old. The second part of the SA-EASI consists of five 100 mm visual analogue scales (VAS) each representing an AD severity characteristic (redness, thickness, dryness and scratches of an AD lesion). By multiplying the total area score by the VAS scores of all severity characteristics a total score (maximum of 96) for the SA-EASI is calculated.[3] The score for itchiness is not part of the total SA-EASI score.

Objective SCoring Atopic Dermatitis (SCORAD)
The objective SCORAD was completed by a trained physician. In the objective SCORAD the subjective items (pruritis and sleep loss) have been eliminated.[7] The first part of the objective SCORAD consists of grading the extent of BSA affected by AD by the rule of nines. The second part consists of scoring the intensity of six AD severity characteristics (erythema, edema/papulation, oozing/crusts, excoriations, lichenification and dryness). Total objective SCORAD (maximum of 83) was obtained by the sum of (BSA divided by 5) and (total severity score multiplied by 3.5).[7]

Six Area, Six Sign Atopic Dermatitis severity score (SASSAD score)
The SASSAD score was completed by the same investigator as the objective SCORAD. The SASSAD score comprises assignment of six signs (erythema, exsudation, dryness, cracking and lichenification) on six sites (arms, hands, legs, feet, head and neck, trunk); each on a scale of 0 (absent); 1 (mild); 2 (moderate) and 3 (severe). Total SASSAD score (maximum of 108) is calculated by adding all severity scores on each site.[8]

Thymus and Activation-Regulated Cytokine (TARC)
TARC was measured using a quantitative sandwich enzyme immunoassay technique via pre-coated microplates with a TARC specific monoclonal antibody (Quantikine Human TARC immunoassay; R&D Systems inc., Minneapolis, MN, U.S.A.). Blood for TARC measurement was drawn on the same day disease severity was scored.

Statistical analysis
Statistical analysis was performed using the program SPSS for Windows (version 15.0, 2006; SPSS inc., Chicago, IL, U.S.A.). Data were expressed as frequencies (number of patients or percentages) for categorical variables and as a mean ± SD for continuous variables. To analyze the relationship between SA-EASI and other severity scoring indexes the correlation between the total SA-EASI score and the objective SCORAD, the SASSAD score and serum TARC levels was calculated. Furthermore, the total area score and severity score from the SA-EASI were correlated separately with the BSA and severity score from the objective SCORAD and with serum TARC levels to investigate the level of agreement between segments of these different scoring methods. Linear regression analysis was done to investigate if the objective SCORAD could be predicted by the total SA-EASI score. The levels of serum TARC were log transformed (Ln) to obtain a normally distributed variable. Most variables were normally distributed and correlations were investigated using Pearson correlation coefficient; otherwise Spearman correlation coefficient was used. A correlation coefficient (ρ) of 0.10 to 0.29 indicates a small correlation, 0.30 to 0.49 a moderate correlation and 0.50 to 1.0 a high correlation.[20]

Results

Baseline characteristics of the study population are presented in Table 1.
Sixty children, 24 boys and 36 girls; age 5-16 year, with AD were studied.

Table 1.

Baseline characteristics of AD patients.

Number of AD patients	60
Gender (no. of patients):	
- Boys	24 (40%)
- Girls	36 (60%)
Age (years ± SD)	10.5 ± 3.4
Disease activity:	
- SA-EASI (score ± SD)	15 ± 12
- Objective SCORAD (score ± SD)	30 ± 12
- Objective SCORAD < 15*	4 (6.7%)
- Objective SCORAD 15-40	45 (75%)
- Objective SCORAD > 40	11 (18.3%)
- SASSAD score (score ± SD)	27 ± 11
- TARC (pg/ml ± SEM)	2018 ± 393

** Objective SCORAD <15 indicates mild, 15-40 moderate and >40 severe disease activity.[7]*

Relation between total SA-EASI and objective SCORAD

There was a strong positive correlation between the total SA-EASI and the objective SCORAD (ρ=0.61, n=60, p=<0.001) (Figure 1.). An increase in total SA-EASI score of 1 point indicated an increase in objective SCORAD of 0.64 points (objective SCORAD = 20.5 + total SA-EASI score x 0.64; p=<0.001, R^2=0.37). Addition of other variables such as sex and age did not improve this model.

The correlation between the BSA calculated from the SA-EASI and the BSA calculated from the objective SCORAD was 0.50 (n=60, p=<0.001) (Table 2.). When the total severity score of the SA-EASI was correlated with the severity score of the objective SCORAD only a moderate correlation was found (ρ=0.37, n=60, p=0.003) (Table 2.).

Correlation between total SA-EASI and SASSAD score

The correlation between the total SA-EASI and the SASSAD score was moderate (ρ=0.43, n=60, p=<0.001) (Table 2.).

Correlation between total SA-EASI and serum TARC levels

The correlation coefficient between the total SA-EASI and log TARC was 0.46, suggesting a moderate correlation (n=60, p=<0.001) (Table 2.). The correlation coefficient between the BSA of the SA-EASI and log TARC was 0.42 (n=60; p=0.001), and the correlation between the severity score of the SA-EASI and log TARC was 0.36 (n=60; p=0.005).

Additionally, the correlation between log TARC and objective SCORAD was 0.51 (n=60; p=0.001), the correlation between log TARC and the BSA /severity score of the objective SCORAD were respectively 0.44 and 0.49 (n=60; p=0.001).

The correlation between log TARC and SASSAD was 0.42 (n=60; p=0.001).

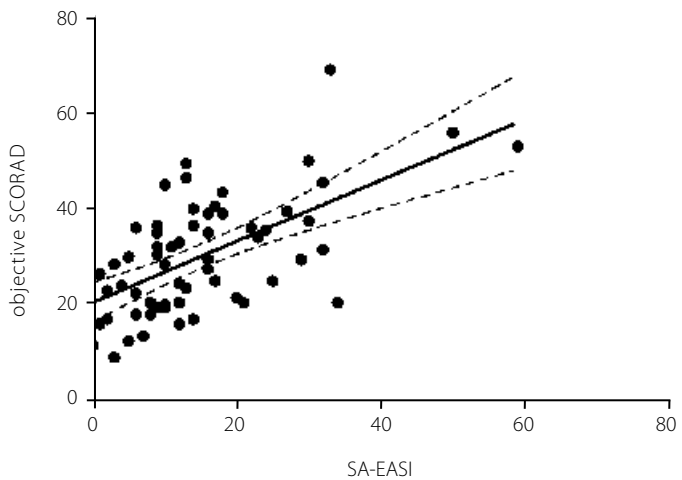

Figure 1.
Correlation between SA-EASI and objective SCORAD. Scatter plot shows linear regression line and 95% confidence band; R2=0.37; r=0.61; p=<0.0001.

Table 2.

Correlations between SA-EASI and: objective SCORAD, SASSAD and TARC.

	Correlation coefficient (ρ)	p
Total SA-EASI vs Objective SCORAD	0.61	<0.001
BSA SA-EASI vs BSA objective SCORAD	0.50	<0.001
Severity score SA-EASI vs Severity score objective SCORAD	0.37	0.003
Total SA-EASI vs SASSAD score	0.43	<0.001
Total SA-EASI vs logTARC	0.46	<0.001
BSA SA-EASI vs logTARC	0.42	0.001
Severity score SA-EASI vs logTARC	0.36	0.005

Discussion

We investigated whether the SA-EASI was related with validated and well used investigators' assessment scores for AD (objective SCORAD and SASSAD score) and with a laboratory marker for AD (TARC). We found a high correlation (ρ=0.61) between the total SA-EASI and the objective SCORAD. Moderate correlations were found between the SA-EASI and the SASSAD score and the SA-EASI and serum TARC levels (ρ=0.43 and ρ=0.46). The BSA score of the SA-EASI correlated best with the BSA score of the objective SCORAD and TARC. Correlations between the severity scores of the disease activity scores were lower.

Both the EASI and the objective SCORAD are composite indexes including assessment of both disease severity and extent without subjective symptoms.[21,22] A high correlation between the EASI and SCORAD index has been found.[6] Housman et al. found a high correlation between the total SA-EASI and the EASI.[3] When individual severity scores (e.g. erythema) of the SA-EASI were correlated with the corresponding scores of the EASI correlations were small to moderate. Only with the addition of the BSA score next to the severity scores in the SA-EASI, the correlations between the SA-EASI and the EASI increased. In accordance, we found that the high correlation between the SA-EASI and the objective SCORAD is mainly based on a high correlation

between the BSA scores. The correlation between the severity scores of both skin scoring systems was lower.

The SASSAD score is only based on a severity measurement of several body sites and assessment of BSA is not included.[8] The lack of a BSA measurement may explain why a moderate correlation with the SA-EASI was found.

Apparently, parents have more difficulty in assessing the severity of their child's AD than the BSA involved. The assessment of severity in the SA-EASI is done by a 100 mm VAS whereas in the objective SCORAD and SASSAD only a 4-point scale is used. It may be more difficult to assess severity in the SA-EASI because a VAS gives a wider range to choose from, compared to a 0-3 score of the SCORAD or SASSAD. This could explain why a lower correlation between the severity scores of the SA-EASI and the SCORAD/SASSAD was found in this study.

TARC levels correlated well with AD disease activity in adults as scored by the LSS, a skin scoring system related to the SASSAD (ρ=0.56; n=177)[15], and also with the objective SCORAD (ρ=0.60; n=40).[17] In accordance with Kakinuma et al.[17], we found a high correlation between the objective SCORAD and TARC levels in children. Additionally, both the BSA score and the severity score of the objective SCORAD correlated equally well with serum TARC levels (ρ=0.44 and ρ=0.49; n=60). We found a moderate correlation of 0.46 between SA-EASI and TARC, mainly based on BSA estimate of the SA-EASI. This also suggests that parents can more accurately assess the extent of their child's AD. Possibly, better education/training for the parents or patients' caregivers on how to assess the severity of AD can enhance the correlation between the SA-EASI and objective SCORAD, but also the correlation between SA-EASI and TARC. For example, use of example photographs of severity characteristics of AD can be of help in training parents or patient's caregivers in grading AD severity. Use of reference photographs of severity characteristics has been used for education and training on the SCORAD.[21]

A limitation of this study is that the majority of the patients in this study had moderate or severe AD at the moment of scoring according to the objective SCORAD. Active AD might be differently assessed by the patient or patients' caregiver than mild AD, and one has to be careful to extrapolate the results from this study to patients with mild AD. In addition, parents of children with mild AD may have more difficulty in assessing severity as they may have not had exposure to severe AD to act as a comparator in the way that a clinician would.

Furthermore, this is a single time-point study. When multiple SA-EASI measurements are done to reflect disease course during treatment patients and patients' caregivers may become more experiences in performing the SA-EASI. This could lead to higher correlations between both the BSA and severity characteristics of the SA-EASI and objective SCORAD.
Comparison of the SA-EASI with other tools in measuring AD from the patients' perspective such as the Patient-Oriented Eczema Measure (POEM)[23] and the Patient-Oriented SCORAD (PO-SCORAD)[24] was not done in this study, but could be useful to investigate in future studies.

In conclusion, the SA-EASI has a good correlation with the objective SCORAD in assessing disease activity in children with AD. This correlation is mainly based on good agreement between the BSA scores of the SA-EASI and the objective SCORAD. Less agreement was found between the SA-EASI and serum TARC levels; BSA score of the SA-EASI correlated better with TARC than the severity score of the SA-EASI. Also, less agreement was found between the SA-EASI and the SASSAD score where assessment of BSA is not included. The lower correlations between the severity scores of the SA-EASI and objective SCORAD/TARC suggests that parents may have more difficulty in scoring severity of AD than scoring BSA involved. Training parents in assessing the severity of AD may improve agreement between the SA-EASI and other AD scoring systems. Additional use of the SA-EASI in routine clinical practice or in trials may then facilitate more frequent but still accurate assessment of AD disease activity.

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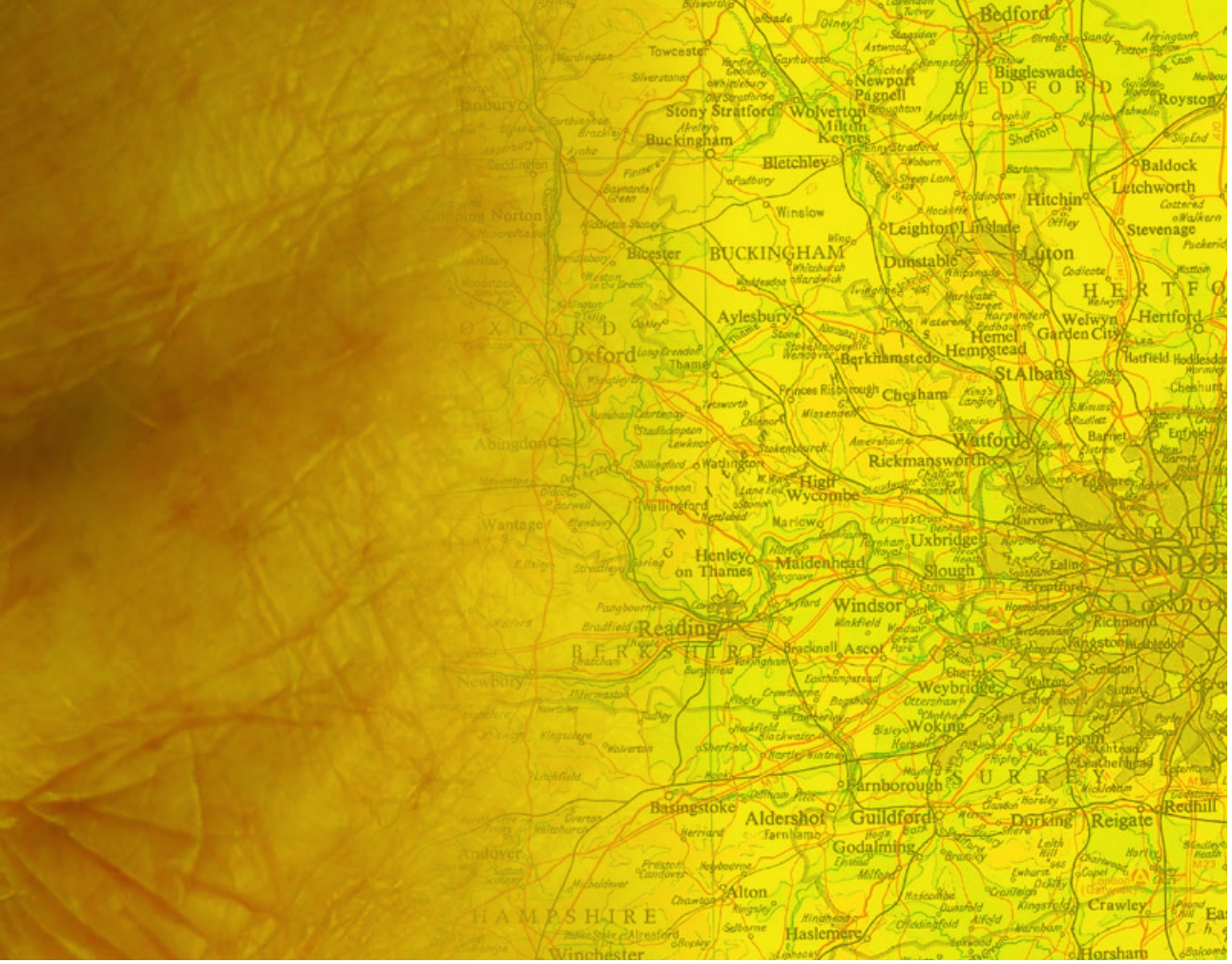

Chapter 8

First experience with enteric-coated mycophenolate sodium (Myfortic®) in severe recalcitrant adult atopic dermatitis. An open label study.

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Keywords:
atopic dermatitis, enteric-coated mycophenolate sodium

Abbreviations:
AD: atopic dermatitis
CsA: Cyclosporin A
EC-MPS: Enteric-Coated Mycophenolate Sodium
IgE: Immunoglobulin E
IMPDH: inosine monophosphate dehydrogenase
LSS: Leicester Sign Score
MMF: Mycophenolate Mofetil
Objective SCORAD index: objective SCOring Atopic Dermatitis index (also objective SCORAD)
MPA: mycophenolic acid
UV: ultraviolet
TARC: Thymus and Activation-Regulated Cytokine

Abstract

Background

Severe atopic dermatitis (AD) is often succesfully treated with oral immunosuppressive drugs such as cyclosporin A (CsA) or oral corticosteroids. However, some patients develop adverse effects or are unresponsive to these first-choice oral immunosuppressive drugs.

Objectives

To evaluate whether enteric-coated mycophenolate sodium (EC-MPS) is an effective treatment in patients with severe, recalcitrant AD.

Methods

Ten patients with severe, recalcitrant AD were treated with EC-MPS 720 mg twice daily for six months. All patients had to discontinue other oral immunosuppressive drugs due to adverse effects (n=9) or non-responsiveness (n=1). Disease activity was monitored using the objective Scoring of Atopic Dermatitis (objective SCORAD) index and the Leicester Sign Score (LSS). Additionally, the level of serum thymus and activation-regulated cytokine (TARC) was measured. During treatment, safety laboratory examination was performed. Total serum immunoglobulin E (IgE) was followed during treatment. Use of topical corticosteroids was recorded before and during treatment.

Results

Compared with baseline, the mean scores for disease activity significantly decreased during treatment with EC-MPS (objective SCORAD (p=0.04), LSS severity (p=0.01), LSS extent (p=0.01)). In addition, serum TARC levels and total serum IgE levels significantly decreased after treatment compared to before (p=0.03; p=0.05). Disease activity decreased after approximately two months of treatment and stabilized during the six-month treatment period. No differences in the amount of topical corticosteroids used in the six months prior to treatment compared to the six-month treatment period were found (p=0.39). None of the patients discontinued use of EC-MPS and only mild adverse effects were seen.

Conclusions

In this study EC-MPS in a dose of 720 mg twice daily for 6 months has proven to be an effective and well-tolerated treatment for severe, recalcitrant AD patients.

Introduction

Atopic Dermatitis (AD) is a common inflammatory skin disease worldwide, with a lifetime prevalence of 10-20% in children and 2-10% in adults.[1] Clinically, AD is characterized by chronically persistent, as well as recurrent, pruritic, erythematous skin lesions and has a profound effect on the quality of life. Although most patients with AD can be treated succesfully with topical corticosteroids, topical immunomodulators or ultraviolet (UV) phototherapy, patients with widespread and severe disease often require oral immunosuppressive treatment.

Oral corticosteroids can rapidly decrease disease activity, but long term use is limited because of side effects, such as osteoporosis or suppression of adrenal gland function.[2] Cyclosporin (CsA) has proven to be clinically effective and safe for short- and long-term treatment[3-5]. However, some patients develop side effects such as nephrotoxicity or hypertension, which requires dose reduction or discontinuation of therapy. In addition, there is a small group of patients with AD who experience increased disease activity during treatment with CsA, sometimes accompanied by an increase in total serum immunoglobulin E (IgE)[6].

Mycophenolate mofetil (MMF) has proven to be effective in the treatment of severe AD.[7;8] In the study of Neuber et al. clinical improvement was paralleled by a decrease in total serum IgE.[9] However, the use of MMF can be limited by gastrointestinal side effects such as diarrhoea and abdominal pain.[10] The incidence of gastrointestinal side effects with MMF in patients with a renal transplantation can be up to 45.5%.[10] Literature on gastrointestinal side effects during MMF therapy for AD patients is scarce. Neuber et al. described transient nausea in the early weeks of treatment in 2 out of 10 AD patients[9].

Enteric-coated mycophenolate sodium (EC-MPS) is designed to reduce the incidence of gastro-intestinal side effects of MMF.[11;12] Both substances contain the same active moiety, mycophenolic acid (MPA), and have the same therapeutic equivalence in de novo renal transplant patients.[13] MPA is a potent, selective and reversible inosine monophosphate dehydrogenase (IMPDH) inhibitor. It interferes with the de novo synthesis of purines necessary for the production of precursors for the synthesis of RNA and DNA. This pathway is essential for the clonal expansion of T- and B-cells.[14;15] EC-MPS could be an alternative treatment in patients with severe AD who are unresponsive to first-choice oral immunosuppressive drugs, or in patients who have to discontinue treatment due to side effects. Finally, EC-MPS could also be of use in AD patients with pre-existent high levels of total serum IgE.

This study describes the results of treatment with EC-MPS in 10 patients with severe AD during a six-month treatment period.

Patients and methods

Patients

Ten patients, five men and five women (age between 23 and 72 years), with severe AD according to the criteria of Hanifin and Rajka, were included between August 2006 and April 2007. Baseline characteristics of the patients are described in Table 1. Intensive treatment with potent topical corticosteroids and/or UV phototherapy was insufficient in controlling disease activity. All patients had previously been treated with oral immunosuppressive drugs, but had to discontinue this treatment due to side effects or nonresponsiveness (Table 2). None of the patients received oral immunosuppressive treatment or UV therapy in the 3 months prior to starting on EC-MPS. None of the patients were pregnant at the start of therapy. The study was approved by the local medical ethical committee.

Table 1.

Baseline clinical characteristics of AD patients.

Patient characteristics	Mean ± SD at baseline
Age (years)	47 ± 15
Sex (men/women)**	5/5
LSS severity score	32 ± 7
LSS extent score	47 ± 17
Objective SCORAD index	39 ± 9
TARC level (pg/ml)	3955 ± 6702
Total serum IgE (kU/L)	12250 ± 9665*
History of atopy**	9

** SEM.*

*** Expressed in number of patients.*

Treatment

EC-MPS (Myfortic®, Novartis, Basel, Switzerland) was given in a standard dose of 720 mg twice daily during 6 months. This dose contains equimolar amounts of MPA when compared to the standard dose of 1000 mg twice daily of MMF[16]. Use of bland emollients and topical corticosteroids (European class III) was permitted. The use of topical corticosteroids was monitored for the 6-month period prior to treatment and during the treatment period. The amount of topical corticosteroids used was quantified using pharmacy records.

Parameters of disease activity and total serum IgE

Before and after 2, 4, and 6 months of treatment disease activity was scored using the objective SCORAD[17] index and the LSS score.[18]
In addition, the level of serum TARC was measured as an objective marker for disease activity.[19] Furthermore, the total serum IgE level was measured before starting and after a treatment period of 3 and 6 months.

Safety

Safety laboratory examination was performed before the start of treatment, after 2 and 4 weeks of treatment and then every 4 weeks during the six-month treatment period. Blood examination included a full blood count, lymphocyte subsets, total bilirubin, alkaline phosphatase, aminotransferases (ASAT and ALAT), electrolytes, ureum, creatinine, total cholesterol, HDL-and LDL-cholesterol and triglycerides. Furthermore, at each visit patients were asked if they had experienced any subjective adverse effects.

Statistical analysis

Statistical analysis was performed using the program SPSS for Windows (version 12, 2003; SPSS Inc., Chicago, IL, U.S.A.). Because the data were not normally distributed, nonparametric tests were used. Changes in objective SCORAD, LSS, serum TARC and total serum IgE within the treatment period were compared to baseline using the Wilcoxon signed rank test. Probability levels of 0.05 and below were considered significant.

Table 2.

Use of systemic immunosuppressive therapy before start of EC-MPS.

Patient	Duration of CsA treatment	Reason of discontinuation CsA treatment	Other therapies
1	4 years	hypertension*	prednisone
			UV-therapy
2	9 months	hypertension*	n.a.
		hypercholesterolemia	
3	9 months	nephrotoxicity	n.a.
		hypertension*	
4	4 months	nephrotoxicity	n.a.
		failure of treatment	
5	2 months	hypomagnesemia	prednisone
		nausea	
		muscle ache	
		hypertension*	
6	n.a.**	n.a.	prednisone
			UV-therapy
7	2 months	failure of treatment	prednisone
			UV-therapy
8	3 months	hypertension*	prednisone
		hypercholesterolemia	
9	2 months	nausea	
		abdominal discomfort	prednisone
10	1 month	nausea	n.a.
		headache	

** Hypertension is defined as unresponsive to antihypertensive drugs.*
*** No previous treatment with CsA due to pre-existent hypertension.*
n.a. Not applicable.

Results

Parameters of disease activity and total serum IgE

All patients noted gradual improvement in the course of 4-8 weeks of treatment. This improvement remained stable over the six-months treatment period.
The mean objective SCORAD (± SD) decreased from 39 ± 9 to 27 ± 9 (p = 0.042) during treatment (Fig. 1a). Mean LSS extent decreased from 47 ± 17 to 24 ± 13 (± SD) (p = 0.012), and mean LSS severity decreased from 33 ±7 to 20 ± 6 (p = 0.012).
Clinical improvement was paralleled by a gradual decrease in serum TARC from 3955 ± 6702 pg/mL to 1744 ± 2122 pg/mL (p = 0.028) (Fig. 1b).
A decrease in total serum IgE was seen during treatment from 12250 ± 9665 kU/L to 10244 ± 6843 kU/l (± SEM) (p = 0.05). There was no significant difference (651 ± 463 versus 1019 ± 813 grams; p=0.39) in the amount of topical corticosteroids used in the six months before start of treatment and during treatment.

Safety laboratory examination

During the six-month treatment period no significant changes were found in white blood cell count, red blood cell count, level of serum creatinine and level of total serum cholesterol. Also, no changes were seen in alkaline phosphatase, aminotransferases (ALAT, ASAT) and electrolytes.

Adverse reactions

Only mild adverse reactions were seen. During the first two weeks of treatment two patients reported diarrhoea, one patient reported mild nausea, and one patient reported flatulence. Furthermore, five patients reported mild headache, which was consistent during treatment. The frequency varied from once a week to once a month. Two patients reported tiredness, and one patient noted concentration problems during his work (Table 3). In none of the patients a dose reduction or discontinuation of therapy was needed due to side effects.

Figure 1.
Mean objective SCORAD (A) and TARC (B) levels in patients with AD at baseline and during treatment with EC-MPS.

Scatter plots show mean and distribution of data.

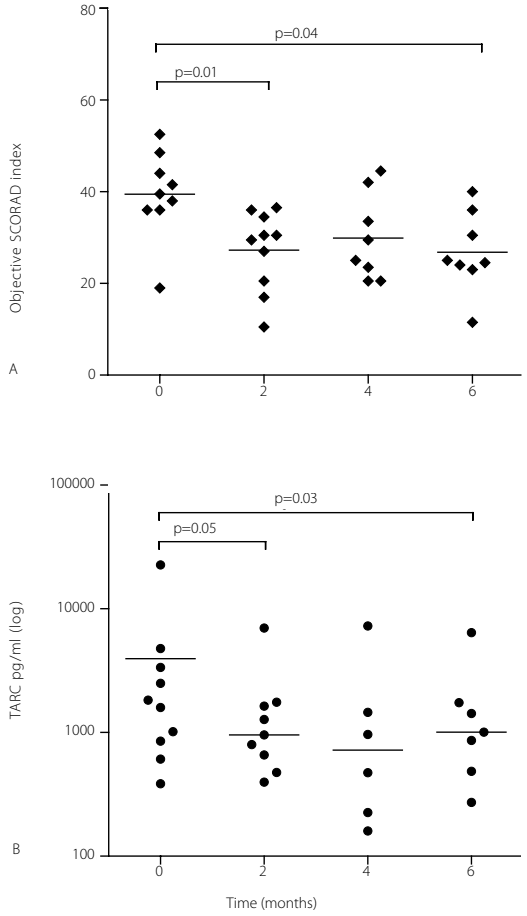

Table 3.

Adverse events during use of EC-MPS.

Organ system	Side-effect	Severity	Number of patients
Gastro-intestinal	diarrhoea	mild	2/10
	nausea	mild	1/10
	flatulence	mild	1/10
Neurological	tiredness	mild	2/10
	headache	mild	5/10
	concentration problems	mild	1/10

Discussion

In this study, treatment with EC-MPS resulted in a significant decrease of disease activity in patients with severe, recalcitrant AD. The mean improvement in disease activity scores was 40% in the six-month treatment period. After 8 weeks of treatment a significant clinical improvement was observed which remained stable till the end of the six-month treatment period. No difference in the amount of topical corticosteroids was found during treatment with EC-MPS compared to the six months prior to treatment. Thus, clinical improvement could not be attributed to increased use of topical corticosteroids.
Neuber et al. described a median improvement of SCORAD of 68% during a 12-week treatment period with MMF.[9] However, the mean disease activity at the moment of inclusion was more severe compared to our study (objective SCORAD 68.3 versus 39.4). End of study scores are comparable.
In severe AD, not responsive to topical steroids and/or UV phototherapy, CsA is the first choice of treatment. After treatment with CsA, a clinical improvement of 55% (95% CI 48-62%) can be reached after 6-8 weeks of treatment.[20] Although the results of our study show that EC-MPS is also effective in decreasing the disease activity in patients with severe AD, the clinical efficacy seems to be less potent compared to CsA. However, this may also be explained by differences in patient selection: we studied patients with recalcitrant AD in which previous treatment with CsA and/or oral corticosteroids was unsuccesfull due to therapy resistence or side effects.
A limitation, especially of long-term treatment with CsA is the occurrence of nephrotoxicity in up to 10.9% per month of treatment, and hypertension in 5.8% per month of treatment in AD patients.[20] These side effects often require dose reduction or discontinuation of therapy. In our six-months study, none of the patients developed nephrotoxicity or hypertension during treatment with EC-MPS. These findings are in accordance with earlier studies using EC-MPS/MMF.[7;9]
EC-MPS has been developed with the objective to reduce gastrointestinal side effects which are observed during treatment with MMF.[11] Data from organ transplantation studies suggest that the overall incidence of gastrointestinal side effects are comparable between renal transplant patients receiving either EC-MPS or MMF. However, the increase in severity of gastrointestinal side effects during a 12-month treatment period tended to be lower in patients receiving EC-MPS.[12] Furthermore, conversion of MMF to EC-MPS in transplantation patients with gastrointestinal side effects lead to an improvement of gastrointestinal tolerability of 84.2%.[21]

The gastrointestinal side effects in our study were mild, transient and did not lead to a dose reduction or discontinuation of therapy. The incidence of gastrointestinal side effects, however, is comparable to the study of Neuber et al. with MMF. Literature on MMF in the treatment of severe AD is scarce, and only small patient groups are described. This could be an explanation for the low incidence of gastrointestinal side effects, and for not detecting a difference between treatment with EC-MPS and MMF. The number of patients in the MMF study[9] and our EC-MPS study is too low to draw any conclusions on the incidence of gastrointestinal side effects.
No bacterial, or viral infections occurred during the six-month treatment period with EC-MPS; this is in accordance with earlier studies investigating treatment with MMF in patients with severe AD.[7-9;22] However, organ transplant patients have an increased incidence of bacterial or viral infections during treatment with EC-MPS.[23] This might be explained by the fact that, in contrast to patients with AD, organ transplant recipients are treated with multiple immunosuppressive agents.

In our study a small, but significant decrease in total serum IgE was found, in accordance with earlier studies using MMF in AD.[9] Patients with AD who have been succesfully treated with CsA did not show changes in total serum IgE levels.[4] Some patients may even show an increase of total serum IgE levels.[6] The decrease in total serum IgE in our study can be explained by the inhibitory effect of EC-MPS on B-cells. However, the clinical relevance of this small decrease in total serum IgE is unknown.

Treatment with EC-MPS in this group of patients with severe, recalcitrant AD leads to a significant decrease in disease activity after 8 weeks treatment, which was stable during a six-month treatment period. The decrease in disease activity was accompanied by a significant decrease in serum TARC and total serum IgE.
These are the first results of treatment with EC-MPS in patients with severe AD. Larger randomized controlled trials are needed to compare the effect of long-term treatment with EC-MPS to CsA and to identify subgroups of patients with severe AD who may especially benefit from treatment with EC-MPS.

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Chapter 9

Severe atopic dermatitis treated with everolimus

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Key words:
atopic dermatitis, everolimus

Abbreviations:
AD: atopic dermatitis
CsA: Cyclosporin A
IL: Interleukin
Objective SCORAD index: objective SCOring Atopic Dermatitis index (also objective SCORAD)

Abstract

Background

Patients with severe atopic dermatitis (AD) often require treatment with oral immunosuppressive drugs. Everolimus is a rapamycin-derived macrolide with immunosuppressive and antiproliferative effects. Everolimus demonstrated efficacy not only in the prophylaxis of organ rejection in kidney transplant patients, but also in decreasing disease activity in psoriasis patients.

Objective

To evaluate whether everolimus is an effective treatment in patients with severe AD.

Methods

Two patients with severe AD were treated with everolimus in combination with low dose cyclosporin A (CsA) or prednisone. During treatment, a disease activity and safety laboratory examination was performed.

Results

Everolimus either in combination with prednisone or with CsA did not result in improvement of disease activity in two patients with severe AD.

Conclusion

Everolimus does not seem to be an effective treatment in these two AD patients, either in combination with prednisone or with CsA.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease.[1,2] Treatment of AD is focused on downregulation of inflammation in the skin with topical corticosteroids or topical immune modulators. Patients with severe AD require treatment with oral immunosuppressive drugs of which the calcineurin-inhibitor cyclosporin A (CsA) has proven clinical efficacy and safety on short- and long-term treatment.[3-5] Everolimus (Certican®; Novartis, Basel, Switzerland) is an immunosuppressive and antiproliferative macrolide derived from rapamycin (sirolimus). It shows increased oral bioavailability compared to sirolimus, but the mechanism of action is the same. The effect of everolimus on T-cells is different from that of CsA. CsA inhibits the mechanism leading to the synthesis of IL-2 (arresting the cell in the Go/G1 phase) whereas everolimus blocks the synthesis of the IL-2 receptor induced by IL-2 and IL-15; it inhibits therefore cell cycle progression from the G1 into the S phase.
Since everolimus and CsA inhibit adjacent steps in T-cell activation combination of these drugs results in a synergistic immunosuppressive activity, allowing CsA dose reduction.[6,7] Sirolimus in combination with low-dose CsA is effective in the treatment of psoriasis, also a T-cell mediated disease.[8] Everolimus (1.5 mg twice daily) in combination with CsA (1 mg/kg) was effective in one patient with psoriasis.[9]
We describe two patients with severe AD who were treated with everolimus, one in combination with prednisone and one in combination with CsA.

Case reports

Case 1, everolimus in combination with prednisone
A 51-year old woman with severe AD had to discontinue CsA due to therapy-resistent hypertension. Treatment with prednisone 30 mg daily induced only a moderate response. At this time she presented with active disease with a objective SCORAD index[10] of 29 and an involved body surface of 40%. For that reason treatment with everolimus 0.75 mg twice daily was combined with prednisone 20 mg daily. Every two weeks a full blood count, hepatic and renal function, cholesterol and blood levels of everolimus were measured.
After two weeks, the blood everolimus level was 1.3 µg/L; this is below the transplantation reference therapeutic range of 3-8 µg/L. For that reason the dosage of everolimus was doubled and prednisone was tapered to 10 mg daily within two weeks. In the following 10 weeks her eczema remained active, despite a temporary dose increase of prednisone to 30 mg daily for 7 days in week 6 when objective SCORAD index was 45.5 and involved body surface was 63%. Everolimus blood levels continued to be low. Laboratory tests during treatment showed a slight decrease in haemoglobin (7.9 to 7.4 mmol/L after 12 weeks), which returned to baseline values after discontinuation of everolimus. Also, a transient lymphocytopenia (1.62 to 0.78 x 10^9/L) was observed after 6 weeks, which normalized after 10 weeks. Other laboratory tests were within normal limits. Patient reported no side effects. Everolimus was discontinued after 12 weeks because of lack of clinical response.

Case 2, everolimus in combination with CsA
A 62-year old woman with severe AD was initially treated with CsA 5 mg/kg with a good clinical response, however, the dosage had to be tapered because of hypertension.
At that moment she presented with mild disease activity, a objective SCORAD index of 9 and an involved body surface of 10%. The CsA dosage was decreased to 3 mg/kg in combination with everolimus 0.75 mg twice daily to prevent an exacerbation. Laboratory tests were done every two weeks as previously described.
In spite of having a serum everolimus level within the therapeutic range (3.1 µg/L) her eczema exacerbated after 2 weeks with a objective SCORAD index of 39 and an involved body surface of 39%. The dosage of everolimus was doubled to 1.5 mg twice daily. However, her eczema did not improve and everolimus was stopped after 9 weeks. Laboratory tests showed a decrease in haemoglobin (8.6 to 7.6 mmol/L after 9 weeks), which returned to baseline values after discontinuation of everolimus. A transient leucocytopenia (8.2 to 3.7 x 10^9/L) was observed after 6 weeks, which normalized after 9 weeks. Other laboratory tests were within normal limits. Patient reported no side effects.

Discussion

Treatment with everolimus either in combination with prednisone or with CsA did not result in improvement of disease activity in two patients with severe AD. Patient 1 had blood everolimus levels below the therapeutic range, despite a dosage of 3.0 mg daily. This may be explained by poor therapy adherence which was, however, denied by the patient. Patient 2 had blood everolimus levels within the therapeutic range in combination with low-dose CsA, but in spite of this a severe exacerbation of her eczema occurred.

Treatment with the macrolides sirolimus and everolimus seems to be effective in patients with psoriasis.[8,9]
Nonresponsiveness in our patients may be due to the fact that in contrast to AD, psoriasis is a disease characterized not only by a cutaneous influx of activated T-cells, but also by hyperproliferation of keratinocytes.[11,12] Therefore, the therapeutic success of everolimus in psoriasis may be explained by the presence of two therapeutic targets for everolimus (T-cells and keratinocyte hyperproliferation) instead of one (T-cells), as is the case in AD.
Another explanation for the nonresponsiveness in our patients may be that the dosage of everolimus (1.5 mg twice daily) is below the therapeutic range for AD. However, higher doses increase the incidence of serious adverse events.[13]
Furthermore, blocking the synthesis of the IL-2 receptor (everolimus) may be less effective in downregulating T-cell activation in AD than blocking IL-2 production (calcineurin inhibitors).
In spite of encouraging results of everolimus in psoriasis, this new immunosuppressive drug does not seem to be effective in AD, either in combination with prednisone or in combination with CsA.

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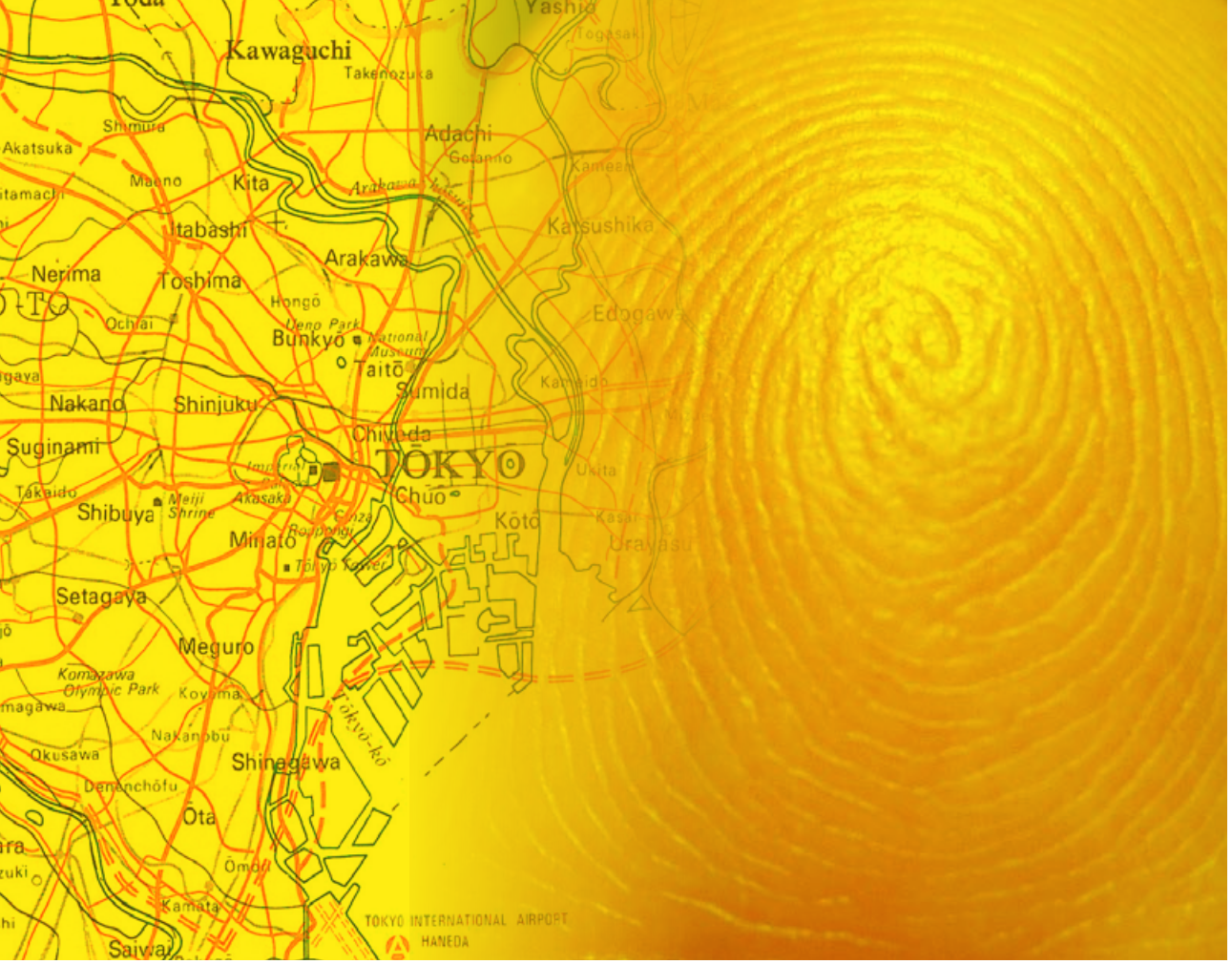


General discussion

I. Pitfalls during topical corticosteroid therapy in patients with severe AD; effects on the adrenal gland

During the treatment of AD with topical corticosteroids a balance has to be achieved between clinical efficacy and the risk of side effects. Percutaneous absorption of topical corticosteroids may lead to systemic levels of the topically applied steroid, which can inhibit the hypothalamus-pituitary-adrenal (HPA)-axis by suppression of adrenal cortisol production.[1-6] Chronic suppression of the HPA-axis may lead to adrenal atrophy[7] and iatrogenic Cushing's syndrome.[8] For that reason, clinicians should use treatment schedules with topical corticosteroids that have the lowest risk of long-term percutaneous absorption.
In chapters 2, 3 and 4 we measured the systemic effect of topical corticosteroids of different potency classes on adrenal cortisol production in patients with AD, both during an exacerbation of their eczema and on maintenance therapy. We used serum cortisol levels as a parameter for systemic availability of the topically applied corticosteroid. We have focused on three topical corticosteroids that are used frequently in the Netherlands: 0.005% fluticasone propionate ointment (potent; Cutivate®), 0.1% betamethasone valerate ointment (potent; Betnelan®) and 0.05% clobetasol propionate ointment (superpotent; Dermovate®).

Percutaneous absorption of topical corticosteroids

Many variables may influence the percutaneous absorption of topical corticosteroids. These variables can be divided in skin related and steroid related factors.

Skin related factors affecting percutaneous absorption are:

- The age of the patient. Systemic availability of topically applied corticosteroids may occur more frequently in children. Children have a higher ratio of total body surface area to body weight (2.5-3-fold of adults). The thickness of the stratum corneum and the structural components such as lipids do not differ between children and adults.[9]
- The extent of the body surface area involved.[10]
- The epidermal barrier function: The presence or absence of mutations in the filaggrin gene[11] and the presence or absence and the degree of cutaneous inflammation.[5;12]

- The duration of application.[10]
- The use of occlusive dressings (for example wet wrap treatment).[10]
- The skin region involved. There is a large variety in percutaneous absorption between different skin regions. Percutaneous absorption increases where the stratum corneum is thin.[13] Thus, percutaneous absorption is less likely to occur through the soles of the foot, and most likely to occur through the skin of the face, scrotum and eyelids (Table 1).[14] For example, the degree of percutaneous absorption through the eyelids is 300-times larger than through the soles of the foot.

Table 1.

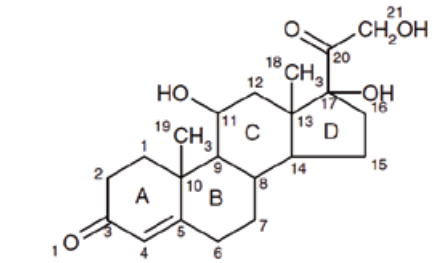
Regional variation in the percutaneous penetration.

Eyelid	High penetration
Scrotum	↓
Jaw angle	
Forehead	
Axilla	
Scalp	
Back	
Forearm (dorsal)	
Forearm (ventral)	
Palm of the hands	
Ankle (lateral)	
Sole of the foot	Low penetration

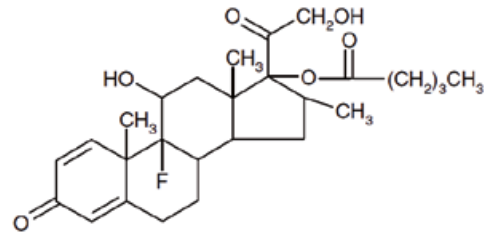
Steroid related factors affecting percutaneous absorption are:

- The vehicle.[10;15] To reach the intracellular glucocorticoid receptor in the epidermis or dermis the corticosteroid has to penetrate the stratum corneum. Each corneocyte is surrounded by intercellular lamellar lipids that regulate the passive flux of water through the stratum corneum[16;17] and this is considered very important for skin barrier function.[18] Penetration through the stratum corneum is assumed to be the rate-limiting step for percutaneous absorption.[19] Ointment formulations are more potent than creams due to their occlusive effect on the skin, which may increase hydration of the stratum corneum and enhance drug transport. Furthermore, percutaneous absorption increases by adding a chemical penetration enhancer such as propylene glycol.[18]
By altering the vehicle, 0.05% betamethasone dipropionate can either be a superpotent

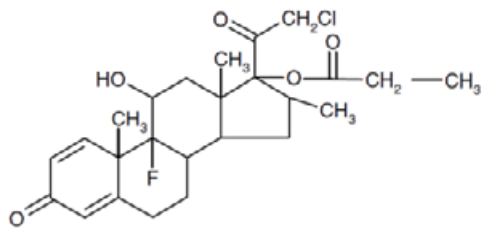
Figure 1.



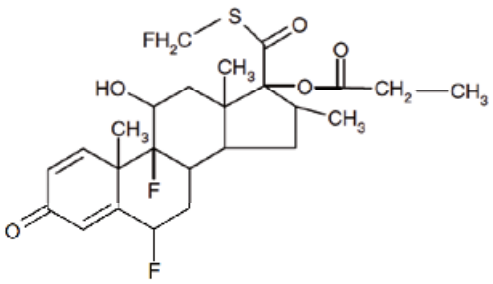
Hydrocortisone



Betametasone valerate



Clobetasol propionate



Fluticasone propionate

topical corticosteroid due to the addition of propylene glycol, or a potent topical corticosteroid when an ointment vehicle is used (See Table 2 Introduction).

- The molecular structure (potency) of the topical corticosteroid. The majority of topical corticosteroids are derived from hydrocortisone (Figure 1). By altering the chemical structure of the steroid, the properties change. To optimize topical corticosteroid activity increased affinity for the glucocorticoid receptor can be achieved by introduction of an additional double bond at C-1. The lipophilicity, metabolic resistance and activation of the glucocorticoid receptor are enhanced by fluorination of the B-ring at the C-9 or C-6 position (e.g. betamethasone valerate and clobetasol propionate).[18;20] Furthermore, increased metabolic resistance can also be achieved by adding an ester to the D-ring (e.g. betamethasone valerate).

The so-called 'soft corticosteroids' are characterized by esterification at the C-17 and 20 positions. This enhances the lipophilicity and affinity for the glucocorticoid receptor but when this drug becomes systemically available it is rapidly metabolized in an inactive derivative in the liver resulting in a low systemic bioavailability.[21] An example of a 'soft corticosteroid' is fluticasone propionate.[18] Thus, the biological half-life of fluticasone propionate is only 3 hours; the biological half-life of betamethasone valerate is 36-48 hours.[22]

Analyzing case reports describing severe side effects of topically applied corticosteroids on the adrenal gland many of the variables that increase percutaneous absorption are present. Cushing's syndrome due to long-term systemic levels of topically applied corticosteroids has only been described in either adult patients with AD or psoriasis who had applied large amounts (up to 100 g/week) of potent or superpotent topical corticosteroids for one year[23] or in infants with diaper dermatitis where potent or superpotent topical corticosteroids were applied in the diaper area (thin stratum corneum).[24-27] Furthermore, one case report describes a fatal case of iatrogenic Cushing's syndrome in an adult patient with submammary intertrigo (thin stratum corneum) who had used more than 200 g of superpotent topical corticosteroids per week for approximately 8 years.[28]

Guideline on safe use of topical corticosteroids

To prevent systemic side effects of topical corticosteroids in patients with inflammatory skin diseases guidelines on the safe use of topical corticosteroids have been developed. Expert opinions and observations in both healthy persons and patients with inflammatory skin diseases have led to an indication as to which amounts of topical corticosteroids can be used with a minimum risk of percutaneous absorption and systemic effects of the topical corticosteroid. The Dutch guideline recommends a maximum of 60-100 g per week for class II and III and a maximum of 30-50 g per week of class IV topical corticosteroids for adult patients with inflammatory skin diseases (Table 2).[22;29] However, the guideline does not differentiate between use of topical corticosteroids in patients with mild or severe inflammatory skin disease. Application of the same amount of a topical corticosteroid in a patient with severe AD may lead to increased percutaneous absorption compared to application in a patient with mild AD due to differences in impairment of the epidermal barrier function.

First, we discuss findings on systemic availability of topically applied corticosteroids in healthy adult volunteers with an intact epidermal barrier function. Subsequently, we discuss the findings on systemic availability of topically applied corticosteroids in patients with AD (chapters 2, 3 and 4). Basal cortisol levels are used as a marker for systemic availability of topically applied corticosteroids as high levels of exogenous corticosteroids inhibit endogenous cortisol production. At last, we propose some additions to the guideline on the safe use of topical corticosteroids to minimize the occurrence of long-term high systemic levels of topically applied corticosteroids and thereby minimizing the chance on systemic side effects of topical corticosteroids.

Table 2.

Dutch guideline on safe use of topical corticosteroids.

Maximum amounts in grams per week per potency class	
Class II	60 - 100
Class III	60 - 100
Class IV	30 - 50

Percutaneous absorption of topical corticosteroids in healthy volunteers

Because data on percutaneous absorption of topical corticosteroids in healthy volunteers is scarce, we performed a pilot study in which each person applied either 0.005% fluticasone propionate ointment, 0.1% betamethasone valerate ointment or 0.05% clobetasol propionate ointment. Basal serum cortisol levels were measured as an outcome parameter for systemic availability of the topically applied corticosteroid.

Fluticasone propionate ointment
Two healthy volunteers applied 30 g 0.005% fluticasone propionate ointment on the total body except the face. Baseline basal serum cortisol levels were normal (0.25 and 0.38 µmol/L). The next day basal serum cortisol levels remained normal (0.34 and 0.21 µmol/L). Upon another application of 30 g in one volunteer the next day, the basal serum cortisol level also remained normal (0.26 µmol/L) (Data not published).

Betamethasone valerate ointment
Two other healthy volunteers applied 30 g of 0.1% betamethason valerate ointment on the total body except the face. Baseline serum cortisol levels were normal: 0.64 and 0.35 µmol/L. The next morning basal serum cortisol levels remained normal: 0.28 and 0.38 µmol/L respectively.
Upon another application with 30 g or even 45 g the next day, serum cortisol levels remained normal in both volunteers; 0.45 and 0.37 µmol/L (Data not published).

Clobetasol propionate ointment
Three other healthy volunteers applied 30 g of 0.05% clobetasol propionate ointment on the total body except the face (Data not published). Baseline cortisol levels were normal: 0.40, 0.42 and 0.33 µmol/L. Within 24 hours after the first application of 0.05% clobetasol propionate ointment basal serum cortisol levels decreased to 0.23, 0.09 and <0.02 µmol/L respectively. Upon a second application of 0.05% clobetasol propionate the basal serum cortisol level in volunteer 1 decreased to 0.03 µmol/L as well. On day 8, 5 and 4 (respectively) after the last application of 0.05% clobetasol propionate basal serum levels of cortisol had normalized (Data not published).
Carruthers et al. also found a decrease of cortisol levels within 24 hours after a single application of 12.5 g of 0.05% clobetasol propionate ointment on the total body in healthy volunteers.[4]
In conclusion, 0.05% clobetasol propionate ointment is absorbed through normal skin and results in systemic levels of the topically applied steroid inhibiting adrenal cortisol production. Application of the same amount (30 g) of both 0.005% fluticasone propionate ointment and 0.1% betamethasone valerate ointment in healthy volunteers does not result in systemic levels of the steroid high enough to interfere with adrenal cortisol production.

Percutaneous absorption of topical corticosteroids in adult patients with controlled AD
Several studies showed that percutaneous absorption seems negligible when up to 100 g/week of 0.1% betamethasone valerate ointment or up to 50 g/week of 0.05% clobetasol propionate ointment is used: a decrease in cortisol levels was hardly observed in patients with controlled AD who were treated at an outpatient clinic.[12;30;31] In addition, no differences in serum cortisol levels were observed between AD patients during maintenance therapy with 0.005% fluticasone propionate ointment or placebo (twice weekly applications for 16 weeks). However, the amount of 0.005% fluticasone propionate ointment used was not calculated in this study.[32] In an almost identical study design 2 out of 44 patients with controlled AD had reduced serum cortisol levels after having been in the study for 40 and 48 weeks. These patients had applied 0.005% fluticasone propionate twice weekly, but amounts of ointment used were not described.[33]

In chapter 2 we also found normal serum cortisol levels in patients with controlled AD at the outpatient clinic who used 0.005% fluticasone propionate ointment (43 ± 11 g/week), 0.1% betamethasone valerate ointment (58 ± 34 g/week) or 0.05% clobetasol propionate ointment (37 ± 27 g/week). The amounts of topical corticosteroids are within the limits of the guideline on the safe use of topical corticosteroids (Table 2).

In conclusion, when patients with controlled AD are treated with amounts of 0.005% fluticasone propionate ointment, 0.1% betamethasone valerate ointment or 0.05% clobetasol propionate ointment in accordance with the guideline on the safe use of topical corticosteroids, a systemic effect on adrenal cortisol production is hardly seen.

Percutaneous absorption of topical corticosteroids in adult patients with an exacerbation of AD
Previous studies showed that when much higher amounts (25-30 g/day) of mid potent, potent or superpotent topical corticosteroids are used (0.1% hydrocortisone butyrate; European class II, 0.1% betamethasone valerate; European class III, 0.05% clobetasol propionate, European class IV) in patients with an exacerbation of AD or psoriasis (during an inpatient treatment), a rapid decrease in serum cortisol production is seen within 24 hours after the first application.[5;34;35] In chapter 2 and 4 we have showed that a single application of 20-30 g of both 0.1% betamethasone valerate and 0.05% clobetasol propionate ointment resulted in a rapid decrease of

serum cortisol levels in the majority of patients with an exacerbation of AD. In these patients percutaneous absorption of topical corticosteroids probably occurs because of application of large amounts of topical corticosteroids on a large body surface with a decreased epidermal barrier function due to inflammation. Indeed, in chapter 4, serum levels of clobetasol propionate could be detected within 24 hours after the application of 0.05% clobetasol propionate ointment as direct evidence of systemic availability of the topically applied steroid.
Only one study addresses the effect of fluticasone propionate cream in patients with an exacerbation of AD or psoriasis. Application with 30 g of 0.05% fluticasone propionate cream daily for seven days resulted in a decrease of serum cortisol in two out of six patients. In one patient serum cortisol levels decreased (but remained within normal ranges) and increased within the same treatment period. In the other patient the serum cortisol level was below the normal range on the sixth treatment day.[36] However, the authors concluded that the serum cortisol levels in the latter patient might have been influenced by the intake of alcohol and not by the percutaneous absorption of fluticasone propionate cream. Because data on the percutaneous absorption of fluticasone propionate in patients with an exacerbation of AD is scarce, we measured serum cortisol levels in two patients with an exacerbation of AD (affected body surface 33 and 36%). Each patient was treated with a single application of 30 g of 0.005% fluticasone propionate ointment on the first day of a hospital admission after a baseline serum cortisol measurement. Baseline serum cortisol levels were normal (0.37 and 0.43 µmol/L respectively) and remained normal after the application of 0.005% fluticasone propionate ointment (day 2; 0.38 and 0.54 µmol/L respectively). Directly after the serum cortisol measurement the same patients received a single application of 30 g 0.1% betamethasone valerate ointment. The next morning serum cortisol levels had decreased to 0.05 and 0.03 µmol/L respectively. This suggests that betamethasone valerate has a higher potency to exert a systemic effect on adrenal cortisol production than fluticasone propionate.

In conclusion, a single application of 30 g of either 0.1% betamethasone valerate ointment or 0.05% clobetasol propionate ointment results in a systemic effect on adrenal cortisol production with a decrease in serum cortisol levels within 24 hours in patients with an exacerbation of AD. A single application of 0.005%/0.05% fluticasone propionate ointment/cream in patients with an exacerbation of AD may less likely exert a systemic effect, as a decrease in serum cortisol levels is hardly observed.

Recovery of serum cortisol levels during treatment with topical corticosteroids in patients with AD

In chapter 2 we found evidence that time to recovery of serum cortisol production at the end of a hospital admission (after approximately two weeks) in patients with an exacerbation of AD differs between use of equal amounts (10-30 g daily) of 0.005% fluticasone propionate ointment, 0.1% betamethasone valerate ointment and 0.05% clobetasol propionate ointment. Recovery occurred in all patients when 0.005% fluticasone propionate ointment was used prior to discharge. Recovery of serum cortisol levels occurred in 60% when 0.1% betamethasone valerate ointment was used, but recovery was not observed when 0.05% clobetasol propionate ointment was used prior to discharge. All patients were discharged when skin disease was controlled, thus inter-individual differences in disease activity were small.
When recovery of serum cortisol production does not occur, this means that the systemic levels of topically applied corticosteroids are high enough to inhibit the adrenal gland. Again, clobetasol propionate is most potent to exert this systemic effect on adrenal cortisol production, in contrast to fluticasone propionate.

Overall conclusions

When 0.005% fluticasone propionate ointment, 0.1% betamethasone valerate ointment or 0.05% clobetasol propionate ointment are used in amounts below the thresholds advised in the guideline on the safe use of topical corticosteroids the chance on percutaneous absorption leading to high levels of systemic exogenous corticosteroids is negligible since serum cortisol levels remain within the normal range.

The differences between the potency of fluticasone propionate, betamethasone valerate and clobetasol propionate to inhibit adrenal cortisol production appear when large quantities (10-30 g daily) are applied on a large body surface.
- Fluticasone propionate is one of the 'soft' corticosteroids that is metabolized rapidly into an inactive derivative in the liver upon systemic availability.[18] Consequently, its biologic half-life is very low (3 hours).[22] The application of 0.005% fluticasone propionate ointment (30g/day) does not lead to high systemic levels, because mainly normal cortisol levels are found either in healthy volunteers or in patients with exacerbated AD.
- Application of 0.1% betamethasone valerate ointment may lead to high systemic

levels as is shown by a decrease in cortisol levels, but only when applied on the skin of AD patients and especially during an exacerbation of AD when amounts between 20-30 g per day are used. Since its biologic-half life is much longer (36-48 hours) compared to fluticasone propionate[22] systemic levels may be reached if large quantities are applied on inflamed skin. However, recovery of serum cortisol occurred in the majority of AD patients when the dose was tapered to 10-30 g/day at the moment when skin disease was less severe.

- Application of 20-30 g/day of 0.05% clobetasol propionate ointment leads to high systemic clobetasol levels both when applied on healthy skin of volunteers and in patients with excacerbated AD. Application of 10-30 g/day also resulted in high systemic clobetasol levels and consecutively in low serum cortisol levels in patients with controlled AD. The biologic half-life is not known but we speculate that it may be longer than that of betamethasone valerate, as serum cortisol levels remain low until 96 hr after a single application of 25 g of 0.05% clobetasol propionate ointment in patients with eczema or psoriasis.[34] Also, in our pilot study, cortisol levels normalized at least 4 days after a single application of 30 g 0.05% clobetasol propionate ointment in healthy volunteers.

We hypothesize that systemic side effects may therefore occur most likely during long-term application of 0.05% clobetasol propionate ointment when amounts >10 g/day are used.

Subsequently, data from literature suggest that one application of 0.05% clobetasol propionate oinment may induce systemic steroid levels comparable to those induced by 25-40 mg orally administered prednisolone. Cortisol levels were found to be normal 24 hours after a single orally administered dose of 25 mg prednisolone in healthy volunteers[37] and cortisol levels varied between 0.07-0.33 (median: 0.20) µmol/L after a single dose of 40 mg prednisone in patients with pulmonary disease.[38] In contrast, in chapter 4 we showed that cortisol levels were below the limit of detection in all but one patient (0.01-0.26 (median: 0.02) µmol/L) after a single application of 20-30 g 0.05% clobetasol propionate ointment in patients with AD. Percutaneous absorption and increased systemic steroid levels may explain the data of Joly et al. These authors compared clinical effectivity of 0.05% clobetasol propionate ointment with orally administered prednisone in patients with bullous pemphigoid.[39] Twice daily application up to 20 g of 0.05% clobetasol propionate ointment was found to be equally effective as oral prednisone in a dose up to 1 mg/kg. Although serum levels of cortisol were not reported, the application of 0.05% clobetasol propionate ointment must have resulted in systemic levels of clobetasol propionate that could have contributed to a systemic anti-inflammatory effect.

Additions to the guideline on safe use of topical corticosteroids for adults

With respect to the potential systemic effects on adrenal cortisol production, we propose some additions to the guideline on the safe use of topical corticosteroids[29] for patients with AD.

- Preferentially 0.005% fluticasone propionate instead of 0.1% betamethasone valerate or 0.05% clobetasol propionate ointment should be used when treating patients with AD to minimize the chance on high systemic levels of the topically applied corticosteroids and to minimize the chance on chronic suppression of the HPA-axis.
- When 0.005% fluticasone propionate ointment (once daily) fails to alleviate symptoms of AD switch to 0.1% betamethasone valerate ointment. When >20 g/day is used one should be aware of percutaneous absorption potentiating systemic side effects. Serum cortisol measurement is not necessary when the amount of this steroid is tapered according to the guideline on the safe use of topical corticosteroids. Switch back to 0.005% fluticasone propionate ointment when remission of AD is achieved.
- Treatment with large amounts (>10 g daily) of 0.05% clobetasol propionate ointment should be limited to AD patients who do not respond to treatment with 0.1% betamethasone valerate ointment. Because recovery of adrenal gland cortisol production takes several days after therapy with clobetasol propionate ointment is stopped, we suggest switching to 0.1% betamethasone valerate ointment instead of directly to 0.005% fluticasone propionate ointment when remission of AD is achieved. Since 0.1% betamethasone valerate ointment also induces systemic steroid levels this may temporarily compensate for a decreased cortisol production during the recovery phase of the adrenal gland.

Future studies

Intermittent versus alternate day application of topical corticosteroids

The guideline on the safe use of topical corticosteroids recommends intermittent use of topical corticosteroids in the long-term treatment of AD.[29] This means that the topical corticosteroids should be applied once daily several days in a row followed by several topical corticosteroid free days. For example: application of the steroid only from Monday-Thursday. The intermittent application scheme is thought to reduce the chance on a relapse of AD activity and to minimize the chance on systemic (and local) side effects.[29;32] This treatment scheme may be effective when topical

corticosteroids are used that have a long biologic half-life such as 0.1% betamethasone valerate or 0.05% clobetasol propionate. Because of the long biologic half-life an anti-inflammatory effect of the steroids may be expected in the 'steroid free' days as well, but the graduate decrease in the possible systemic levels could reduce the chance on adrenal gland suppression.
With regard to 0.005% fluticasone propionate ointment, which has a very short biological half-life, the intermittent treatment scheme may be less effective. In the 'steroid free' days no effect of the steroid is expected and this may result in a relapse of AD in some patients. It has been shown that alternate day application with 0.005% fluticasone propionate ointment is also effective in controlling AD.[33] A future study could compare therapeutic effect of intermittent (once daily application, four days in a row) versus alternate day (once daily application, every other day) application of 0.005% fluticasone propionate ointment in patients with controlled AD.

Clobetasol propionate versus oral corticosteroids

As Joly et al. found that topical therapy with 0.05% clobetasol propionate ointment (twice daily 20 g) is as effective as therapy with oral corticosteroids (upt to 1 mg/kg) in the treatment for bullous pemphigoid[39] we propose to investigate this in patients with an exacerbation of AD. Differences in clinical efficacy and metabolic/local side effects and time to relapse could be compared between a two-week treatment period with either 0.05% clobetasol propionate (20-30 g daily) or oral prednisone (for example 0.5 mg/kg). This could have implications on the way in which we treat a patient with an exacerbation of AD.

II. Pitfalls during topical corticosteroid therapy in patients with severe AD; effects on bone mineral density

The influence of topical corticosteroids on bone mineral density (chapter 5 and 6) is investigated in adult patients and in children with moderate to severe AD.

Bone mineral density in adult patients with atopic dermatitis

Since we have shown in chapter 2, 3 and 4 that application of large amounts of potent topical corticosteroids in patients with AD may result in systemic availability through percutaneous absorption, we hypothesized that the cumulative dose of topical corticosteroids may result in decreased bone mineral density (BMD).
In chapter 5 we performed a longitudinal study in adult patients with moderate to severe AD in which BMD measurements were repeated after two years in a subgroup of the initial single time-point study population described by Haeck et al.[40] Haeck et al. found that 32.8% of the patients had T-scores between -1 and -2.5 (osteopenia) and 4.8% of the patients had T-scores ≤ -2.5 (osteoporosis). In our study, at baseline, osteopenia was seen in 28 out of 75 patients (37.3%) and osteoporosis in 3 out of 75 patients (4.0%). In the general population 15.3% are expected to have osteopenia and 0.62% are expected to have osteoporosis. The observed percentage of patients with osteopenia and osteoporosis in our study is significantly higher compared to the general population (95% confidence interval: 26.4-49.3% for osteopenia and 0.8-11.2% for osteoporosis) (Data not published).
We did not find a difference in BMD between baseline and follow-up. Also, the number of patients with a T-score <-1 at baseline did not differ from the number of patients with a T-score <-1 at follow-up. To investigate whether use of topical corticosteroids influenced the change in BMD we calculated the change in BMD in patients using 'low' (<75 g per month) and 'high' (≥75 g per month) amounts of topical corticosteroids. We found no significant difference in the change of BMD between patients who had used 'low' or 'high' amounts of topical corticosteroids.

There may be several reasons why we could not find a relation between cumulative topical corticosteroid use and a decrease in BMD. Modulation of BMD by percutaneously absorbed topical corticosteroids may have occurred in the first years after start of topical corticosteroid therapy. In patients using oral corticosteroids for various inflammatory diseases (rheumatoid arthritis, sarcoidosis, systemic lupus erythematosus) bone loss is most prominent in the first 6-12 months of treatment. After this period bone loss continues, but at a lower rate.[41-43] This is a possible

explanation why we only found a slight decrease in BMD in the total hip over time. The relatively low use of topical corticosteroids may also explain our observation. The patients described in chapter 5 have used a median amount of 75 g per month during follow-up. This amount is within the guideline-advised safe amounts of topical corticosteroids.[22] Patients with AD may use topical corticosteroids intermittently with short courses of more intensive use during a flare of AD. At that moment the use of topical corticosteroids is most probably higher than the advised safe amounts and the epidermal barrier function is most probably affected by inflammation. Serum levels of percutaneously absorbed corticosteroids could therefore fluctuate in time. In patients with rheumatoid arthritis it has been shown that bone loss is reversible after a short course of oral corticosteroids.[44;45] This suggests that that the potential negative effect on BMD during higher use of topical corticosteroids could be reversed during the period when topical corticosteroids are used in lower amounts. The inflammatory nature of AD could also exert a negative effect on BMD. One of the inflammatory cytokines in AD, IFN-γ, has been found to have inhibitory effects on bone metabolism.[46;47]

In patients with inflammatory bowel disease and rheumatoid arthritis inflammation (in serial measurements) was shown to be an independent factor for bone loss.[44;48-51] Our study was not designed to investigate the influence of inflammation on BMD. At this moment there is no validated tool available to assess the severity of AD over time. For now, only serial measurements of disease activity by clinical scoring systems for AD or serum TARC levels could provide information on disease activity over time. Furthermore it is difficult to separate the influence of disease activity from topical corticosteroids on BMD since the use of topical corticosteroids will parallel disease activity. Additional long-term follow-up studies are necessary to investigate which patients are at risk for developing a clinically decrease in BMD.

Bone mineral density in children with atopic dermatitis

In chapter 6 we investigated the frequency of low BMD in children with moderate to severe AD and its association with the use of topical, oral and inhalation corticosteroids and CsA. In this study the difference between the observed and expected number of children with a low BMD was not significant. The incidence of low BMD was 6.7%, and this did not significantly differ from the incidence of low BMD in the general population (2.5%). The difference with the incidence of low BMD (T-score <-1) in the previously described adult population with AD by Haeck et al. (37.6%) and our adult population in chapter 5 (41.3%) is large. However, the definition of low BMD differs between adults and children: it is a mistake to assume that, with respect to BMD, children are small adults. Where low BMD in adults was defined as a Z-score of ≤-1[40] or a T-score <-1[52]; low BMD in children was defined as a Z-score of ≤-2.[53] Because catch up bone mass acquisition can occur in children[54;55], since they have not yet reached their peak bone mass[56;57], data on adults and children cannot be compared.

The amount of topical corticosteroids used by the children in the five years prior to investigation was not related with a decrease in BMD. We found a non-significant decrease in BMD when children had used systemic treatment, especially when both oral corticosteroids and CsA in addition to topical/inhalation/nasal corticosteroids were used (n=3). Pedreira et al. found a significant relation between CsA use and lower BMD in children with AD (n=6).[58] The use of CsA has been associated with a decrease in BMD in organ transplant patients and patients with rheumatoid arthritis.[59-63] Bone loss during CsA therapy is characterized by increased bone turnover and increased bone resorption[64], but its precise mechanism inducing bone loss is not well defined. Since we found a non-significant decrease in BMD in a group of 3 children who had been treated with oral corticosteroids and CsA in addition to topical/inhalation/nasal corticosteroids larger numbers of patients are needed to verify these results.

A reason why we did not find a higher number of children with AD with low BMD may be because of ongoing bone remodeling which probably neutralizes the potentially negative influence of corticosteroids or CsA on bone mass.

As previously discussed in the follow-up study on BMD in adults with AD (chapter 5), the inflammatory nature of AD could in theory also exert a negative influence on BMD in children. Again, this study was primarily designed to investigate the influence of corticosteroids/CsA and not the influence of disease activity on BMD. However, in other chronic inflammatory childhood diseases the incidence of low BMD seems to be higher.[65;66] For example, in children and adolescents with chronic inflammatory bowel disease 8% of the girls and 20% of the boys were found to have osteoporosis.[67] In juvenile rheumatoid arthritis, systemic lupus erythematosus and chronic inflammatory bowel disease an association with low BMD or a decrease in BMD and inflammation has been found.[65;68-72] But in these chronic inflammatory childhood diseases other factors are also found to be associated with low BMD, for example, weight bearing activity[70], oral corticosteroid use[71;73;74] and calcium intake.[75] Because of this multifactorial pathogenesis of bone loss it is difficult to distinguish the impact of inflammation alone.[68]

Conclusions

In chapter 5, the observed prevalence of a T-score <-1 is significantly higher than the expected prevalence (41.3% versus 15.9%) but overall BMD at follow-up after two years did not differ from baseline in adult patients with moderate to severe AD. Moreover, 'high' use of topical corticosteroids did not result in a significant decrease in BMD compared to 'low' use of topical corticosteroids. In chapter 6, the observed prevalence of low BMD (Z-score ≤ -2) in children with moderate to severe AD did not differ from the expected prevalence of low BMD in the normal population (6.7% versus 2.5%). The amount of topical corticosteroids used in the previous five years was not associated with a decrease in BMD. A non-significant decrease in BMD was found when children had additionally been treated with oral corticosteroids and CsA. The role of inflammation on BMD in patients with AD needs to be investigated. Long-term follow-up studies are necessary to investigate which patients are at risk for developing low BMD.

Treatment and prevention of low BMD in adult patients with AD

Although no differences in BMD between baseline and follow-up were seen in our 2-year longitudinal study in adult patients with AD (chapter 5), the prevalence of osteopenia was 37.3% and the prevalence of osteoporosis was 4%. The Dutch guideline on osteoporosis gives the following advice for patients with osteoporosis[76]:
-calcium supplementation with 500-1000 mg when the daily calcium intake is less than 1200 mg and calcium supplementation with 1000 mg when the daily calcium intake is negligible
-vitamin D supplementation of 800 IE daily
-bisphosphonate, for example alendronate 70 mg/week or risedronate 35 mg/week.
At this moment a treatment duration of 5 years is advised[76]
With this treatment regime the chance on osteoporotic fractures decreases significantly.[76]

In the Dutch guideline on osteoporosis, osteopenia is not mentioned. In the guideline on osteoporosis for general practitioners in The Netherlands it is stated that osteopenia does not require treatment.[77] However, in our population of adults with moderate to severe AD the high incidence of osteopenia may indicate that this population has an increased risk for low BMD. At this moment we don't know which patients with osteopenia might progress to osteoporosis and what factors are associated. In chapter 5 we only found a very small non-significant decrease in BMD in the hips during two-years of follow-up and not related to the use of 'high' amounts of topical corticosteroids, but no information is available beyond two years of follow-up in patients with AD.
At this moment life-style advices are given for all adult patients who use oral corticosteroids (>7.5 mg prednisolone equivalents/day for > 3 months) to prevent osteoporosis: physical activity should be encouraged as it has been shown that this increases BMD[78], calcium supplementation (500 mg/day) when patients have a low dietary calcium intake (less than 1200 mg/day) because daily calcium intake of less than 400 mg is associated with risk on a decrease in BMD and fractures, vitamin D supplementation is only deemed useful in the prevention of fractures if patients have no daily exposure to sunlight at all.[77;78] Next to these life-style advices, a bisphosphonate is added in postmenopausal women, or in premenopausal women and men only when BMD is low at the beginning of oral corticosteroid therapy. When >15 mg of prednisolone equivalents/day is given for > 3 months a bisphosphonate is added in all patients.[78]

Life-style advices on bone health in adult patients with moderate to severe AD

Although we could not demonstrate a correlation between topical corticosteroid use and a decrease in BMD in adult patients with moderate to severe AD, these patients may be at risk for developing low BMD because we found a high incidence of osteopenia and osteoporosis. At this moment we don't advice routine BMD measurement by DXA scans in adult patients with moderate to severe AD because we cannot identify patients who may be at risk for osteoporosis. However, we advise to optimalize bone health in adult patients with moderate to severe AD by giving life-style advices from the Dutch guidelines on the prevention of glucocorticoid

Table 3.

Life-style advices on bone health for adult patients with moderate to severe AD.

Encourage attendance to sports:	Weight bearing activity
	Sports with a high duration and a high intensity
	(e.g. running, walking)
Encourage dairy intake of 4 portions per day:	One portion of milk/yoghurt/pudding: 150 mg
	One portion of cheese: 20 g
Daily exposure to sunlight:	At least 15 minutes (at least hands and face)

induced osteoporosis to all adult patients with moderate to severe AD (Table 3):
Attendance to sports should be encouraged. Especially weight-bearing activity and sports with a short duration and a high intensity (running).
Ideally, dairy intake should consist of 4 consumptions (glasses/portions) per day. The equivalent calcium intake is 1000-1200 mg/day. If calcium intake is less than this, intake should be encouraged, when necessary with help of a dietary specialist.
Vitamin D supplementation is not necessary when patients have daily exposure to sunlight (15 minutes/day). When patients have no daily exposure to sunlight, vitamin D should be supplemented with 400 IE daily.

Treatment and prevention of low BMD children with AD
In our study population of children with moderate to severe AD the observed prevalence of low BMD did not differ from the expected prevalence of low BMD in the general population. Therefore, there is no reason to emphasize on life-style advice in children with moderate to severe AD.

Future studies

Two topics could be interesting for future research:

Role of cutaneous inflammation of AD on BMD
As inflammation has been associated with low BMD in other chronic inflammatory diseases like inflammatory bowel disease and rheumatoid arthritis, it would be interesting to investigate the effects of cutaneous inflammation on BMD in patients with AD. Since many patients with AD use topical corticosteroids, it is difficult to discriminate between the influence of disease activity and that of topical corticosteroid use on BMD. Since a marker for chronic cutaneous inflammation is not available a study answering this question is not feasible at this moment.

Follow-up of BMD in adult AD patients with osteopenia
The cohort of adult AD patients described in chapter 5 will be followed to measure BMD over a longer period of time to answer the question if patients with osteopenia do have an increased risk to progress to osteoporosis.

III. Opportunities in the treatment of adult patients with severe atopic dermatitis

Although most patients with AD can be successfully treated with topical corticosteroids, patients with severe and widespread disease may require therapy with oral immunosuppressive drugs. At this moment Cyclosporin A (CsA) is the only registered oral immunosuppressive drug for the treatment of AD. The efficacy of CsA has been proven in patients with AD[79-82], but treatment with CsA may be limited by side effects such as nephrotoxicity and hypertension.[83]
Alternative oral immunosuppressive drugs are azathioprine (Imuran®), methotrexate (MTX) and mycophenolate mofetil (MMF, CellCept®). However, limited evidence for their effectivity in patients with severe AD is available.
Two randomized placebo-controlled trials[84;85] and several case series[86;87] showed that treatment with azathioprine may induce a clinically relevant improvement in AD patients. However, gastrointestinal side effects were found in up to 50%, which necessitated a dose reduction in some patients.[84;85;87] Also, genetic polymorphism in thiopurine methyltransferase (TPMT) has been linked to interindividual differences in myelotoxicity upon treatment with azathioprine. Therefore, its use is limited in patients with homozygous, but also heterozygous TPMT mutations.[86] Some case series [88-90] and in one prospective open-label study[91] show that MTX also seems to be effective in patients with AD. However, a randomized-controlled trial has not been performed sofar. Furthermore, side effects of MTX therapy in patients with AD are bone-marrow suppression, gastrointestinal toxicity and liver function abnormalities.[91]
Treatment with MMF has also been found effective in AD in several case series[92-94], but gastrointestinal side effects may limit its use.[95] Enteric-coated mycophenolate sodium (EC-MPS, Myfortic®) has been developed to improve the upper gastrointestinal tolerability. We performed an open label study to investigate the efficacy and safety of EC-MPS (chapter 8). Everolimus (Certican®), an immunosuppressive and antiproliferative macrolide derived from sirolimus has never been investigated in patients with AD. However, sirolimus and everolimus have been found to be effective in patients with psoriasis when treatment with sirolimus was combined with a low dose of CsA.[96;97] We investigated the efficacy and safety of everolimus in two patients with severe AD in chapter 9.

EC-MPS in the treatment of atopic dermatitis
In chapter 8 we found that treatment with the standard dosing regime of twice daily 720 mg of EC-MPS leads to an improvement of AD disease activity (measured by the

objective SCORAD) of 40% after 8 weeks of treatment. Only mild (gastrointestinal, tiredness, headache) side effects were seen and none of the patients had to discontinue treatment because of side effects.

Compared to CsA, patients treated with EC-MPS show a delayed clinical response.[83] In a recent randomized controlled trial the clinical efficacy of maintenance therapy with EC-MPS has been compared with CsA in adult patients with AD.[98] In the first ten weeks patients who received treatment with EC-MPS required more oral and topical corticosteroids compared to patients who received treatment with CsA. However, overall efficacy was found comparable between EC-MPS and CsA and only mild side effects were reported. Relapses were observed after CsA was stopped, but not after EC-MPS was stopped during a 12-week follow-up period.[98] In this group of AD patients EC-MPS is found to be an attractive treatment option for maintenance therapy.
In the study of Haeck et al.[98] patients were included if they had not been treated with CsA before, or when previous treatment with CsA was successful. In chapter 8 we included a different patient group (n=10): adult AD patients who had to discontinue previous treatment with CsA because of side effects (mainly hypertension or nephrotoxicity) Furthermore, one patient in this study did not show a clinical response during treatment with CsA, but a good response on EC-MPS was seen.
Other studies on the efficacy of MMF in adult patients with AD also selected AD patients who had not responded or experienced side effects to previous therapy with CsA.[92-94] These pilot data suggest that EC-MPS may be a good alternative for AD patients who were unresponsive to previous CsA therapy. A future study may be started in a larger group of patients to verify our results.

Effect of B-cell proliferation inhibition by EC-MPS

In contrast to CsA, where total serum IgE levels do not change during therapy[98], or even increase in a small subpopulation of patients[99], treatment with MMF results in a decrease of serum total IgE levels.[93] In chapter 8 we also observed a significant decrease in serum total IgE levels during treatment with EC-MPS. Furthermore, Haeck at al. reported a significant decrease in total serum IgE levels in AD patients treated with EC-MPS compared to CsA.[98] The decrease of serum total IgE levels may be due to an inhibitory effect of EC-MPS on B-cell proliferation. This suggests that different immunosuppressives may have different effects on serum IgE levels. However, a decrease in serum total IgE may also reflect a decrease in disease activity since it has been suggested that serum IgE levels are correlated with AD severity.[100;101]

Everolimus in the treatment of severe atopic dermatitis

The case report in chapter 9 shows that treatment with everolimus either in combination with prednisone or with CsA did not result in an improvement of AD disease activity.

Everolimus in atopic dermatitis; not effective?

Combined treatment with sirolimus (3 mg/m^2), which has the same mechanism of action as everolimus, and CsA (1.25 mg/kg) resulted in a clinically relevant decrease in disease activity in patients with psoriasis. This effect was comparable to CsA monotherapy 5 mg/kg, but levels of serum creatinine were significantly higher with this regimen.[96] However, treatment with sirolimus as monotherapy resulted in a low success rate of <30% decrease in disease activity.[96]
The combination of everolimus (3 mg/day) and CsA (1 mg/kg) in a patient with psoriasis resulted in a 60% decrease in the Psoriasis Area and Severity Index (PASI).[97] However, in the fifth week of treatment leucocytopenia forced to stop the administration of everolimus and CsA.
We performed a pilot study on treatment with everolimus in two patients with AD. In the first patient we decided to investigate if monotherapy with everolimus could be effective. Our goal was to taper the dosage of prednisone during everolimus treatment.
The other patient was treated with a combination of everolimus and CsA because this was effective in psoriasis patients. Unfortunately, although the maximum dose of everolimus was given (3 mg/day) in combination with either prednisone 10-30 mg/day or CsA 3 mg/kg we had to stop treatment with everolimus because of lack of clinical response and even increase of disease activity in our patients. Everolimus in a dose higher than 3 mg/day is not recommended due to an increased risk of side effects such as leucocyopenia and thrombocytopenia.[102;103] Although denied by our patients, we cannot exclude lack of patients' adherence to therapy because patient 1 had blood everolimus levels below the therapeutic range, despite a dosage of 3.0 mg/day. Another explanation for the lack of clinical effect may be that the mechanism of action is not sufficient to inhibit T- and B-cell proliferation in AD. Furthermore, the therapeutic success of everolimus in psoriasis may be explained by the presence of two therapeutic targets for everolimus (T-cells and keratinocyte hyperproliferation).
Although we investigated the effect of everolimus in only two patients with AD, the results do not support a clinical trial with everolimus in patients with severe AD.

Final conclusions of this thesis

1. In adult patients with controlled AD, all patients treated with 0.05% clobetasol propionate ointment (10-30 g/day) had low serum cortisol levels, but only 40% of the patients treated with 0.1% betamethasone valerate ointment (10-30 g/day) and none of the patients treated with 0.005% fluticasone propionate ointment (10-30 g/day). (*chapter 2*)

2. The safe amounts of class III and IV topical corticosteroids as mentioned in the Dutch guideline on topical corticosteroids do not induce low serum cortisol levels in patients with moderate to severe AD. (*chapter 2*)

3. Liquid chromatography-tandem mass spectrometry (LC/MS/MS) offers a new method to measure clobetasol propionate in human serum. (*chapters 3 and 4*)

4. In a two-year follow-up study BMD did not differ between adult patients with moderate to severe AD who had used <75 g or ≥ 75 g of topical corticosteroids per month. (*chapter 5*)

5. In adult patients with moderate to severe AD, the change in BMD during a two-year follow-up period did not differ between patients with a T-score < -1 at baseline compared to patients with a T-score ≥ -1 at baseline. (*chapter 5*)

6. In contrast to adult patients, low BMD is not observed in children with moderate to severe AD. (*chapter 6*)

7. The correlation between the body surface area measurements of the SA-EASI and the objective SCORAD is higher than the correlation between the severity measurements of both disease activity scores in children with moderate to severe AD. (*chapter 7*)

8. The correlation between the body surface area measurement of the SA-EASI and serum TARC levels is higher than the correlation between the severity measurement of the SA-EASI and serum TARC levels in children with moderate to severe AD. (*chapter 7*)

9. EC-MPS may be an attractive treatment option for adult patients with moderate to severe AD who had to discontinue CsA due to side effects or unresponsiveness. (*chapter 8*)

10. Everolimus does not seem to be effective in the treatment of adult patients with severe AD. (*chapter 9*)

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Summary

Atopic dermatitis (AD) is a chronic inflammatory skin disease in which disease activity fluctuates in time. Topical corticosteroids are very effective in controlling disease activity and are the mainstay of treatment. The use of topical corticosteroids is associated with side effects. Local side effects include for example atrophy of the skin and teleangiectasia. Furthermore, systemic side effects may occur when topically applied corticosteroids are absorbed through the skin and lead to serum levels high enough to inhibit the hypothalamus-pituitary-adrenal axis. Indeed, it has been shown that a single application (30g) of both class III and IV topical corticosteroids in patients with an exacerbation of AD leads to inhibition of adrenal gland cortisol production. Prolonged systemic levels of exogenous corticosteroids, for example during oral corticosteroid therapy, may lead to Cushing's syndrome and osteoporosis.
In the first part of this thesis we investigated the systemic effects of topically applied corticosteroids on the adrenal gland (chapters 2, 3 and 4) and on bone mineral density (BMD) (chapters 5 and 6).

In chapter 2 we reported that a single application of 20-30 g of either 0.1% betamethasone valerate ointment or 0.05% clobetasol propionate ointment resulted in a decrease of serum cortisol levels within 24 hours in 89.5 % of adult patients with an exacerbation of AD. During hospital admission the amount of topical corticosteroids was tapered. Time to recovery of serum cortisol levels at the end of the hospital admission (mean days 16 ± 6) differed between equal amounts (10-30 g/day) of three topical corticosteroids wheras differences in disease activity were small. Recovery occurred in all patients when 0.005% fluticasone propionate ointment was used prior to discharge. Recovery of serum cortisol levels occurred in 60% when 0.1% betamethasone valerate ointment was used, but recovery was not observed when 0.05% clobetasol propionate ointment was used prior to discharge. After discharge, serum cortisol levels had normalized in all patients. The amounts of topical corticosteroids used at that moment were within the limits of the guideline on the safe use of topical corticosteroids (i.e. 100 g/week for class III and 50 g/week for class IV topical corticosteroids).

In chapter 3, we describe a method to measure systemic levels of clobetasol propionate by liquid chromatography-tandem mass spectrometry (LC/MS/MS) during use of

0.05% clobetasol propionate ointment in patients with severe AD. Using this method, we reported in chapter 4 that a single application of 20-30 g 0.05% clobetasol propionate ointment in adult patients with an exacerbation of AD resulted in a rapid decrease of serum cortisol levels paralleled by serum levels of clobetasol propionate.

With respect to the potential systemic effects of the investigated topical corticosteroids we conclude from chapters 2, 3 and 4 that the chance on high systemic levels of the topically applied corticosteroid is minimal when 0.005% fluticasone propionate ointment is used. On the contrary, we hypothesize that systemic side effects may occur most likely during long-term application of >10 g/day of 0.05% clobetasol propionate ointment. These amounts of 0.05% clobetasol propionate ointment should only be used in AD patients who do not respond to treatment with either 0.005% fluticasone propionate ointment or 0.1% betamethasone valerate ointment.

In chapter 5 we performed a longitudinal study in adult patients with moderate to severe AD in which BMD was measured at baseline and after a follow-up period of two years. The amount of topical corticosteroids was calculated during follow-up. The observed percentage of osteopenia (37.3%) and osteoporosis (4.0%) in our study was significantly higher compared to the general population. We did not find a difference in BMD between baseline and follow-up. Also, the number of patients with a T-score <-1 at baseline did not differ between the number of patients with a T-score <-1 at follow-up. We found no significant difference in the change of BMD between patients who had used <75 g/month or ≥ 75 g/month of topical corticosteroids.
The influence of cutaneous inflammation on BMD is difficult to investigate because topical corticosteroids will parallel disease activity in AD. Long-term follow-up studies are necessary to investigate which patients are at risk for developing a clinically relevant decrease in BMD. Although we could not demonstrate a correlation between topical corticosteroid use and a decrease in BMD, adult patients with moderate to severe AD may be at risk for developing low BMD because we found a high incidence of both osteopenia and osteoporosis. Therefore, we advice to optimalize bone health by giving life-style advice: encourage attendance to sports, encourage dairy intake (4 portions/day) and encourage daily exposure to sunlight of 15 min/day.

In chapter 6 we investigated BMD in children with moderate to severe AD.
We found that the difference between the observed and expected number of children with a low BMD (Z-score ≤ -2) was not significant. A non-significant decrease in BMD was found when children had used systemic treatment, especially when both oral corticosteroids and cyclosporin A (CsA) were used next to topical/inhalation/nasal corticosteroids in the previous five years. Therefore, there is no reason to emphasize on life-style advice in children with moderate to severe AD.

When topical corticosteroids fail to alleviate the symptoms of AD, patients with severe AD can be treated with oral immunosuppressive drugs such as cyclosporin A (CsA). Upon treatment with CsA disease activity can improve within weeks, but its use can be limited by side effects such as hypertension or nephrotoxicity. Alternative oral immunosuppressive drugs may be enteric-coated mycophenolate sodium (EC-MPS) and everolimus and we investigated this in chapter 8 and 9.
To determine the effect of therapy, many objective scoring systems are used. Self-assessment of AD by the patient can facilitate scoring of AD and therefore we investigated the correlation between the Self-Administered Eczema Area and Severity Index (SA-EASI) and investigator based scoring systems (objective SCORAD and SASSAD) and with a laboratory marker (Thymus and Activation-Regulated Chemokine; TARC) in children with moderate to severe AD (chapter 7).

In chapter 7 we found a high correlation between the SA-EASI and the objective SCORAD. We found a moderate correlation between the SA-EASI and TARC. These results were mainly based on correlations of the BSA measurements and less on the severity measurements of each scoring system. Training patients and parents in assessing the severity of AD may improve the agreement between the SA-EASI and other AD scoring systems. Additional use of the SA-EASI in clinical practice or in clinical trials may then facilitate more frequent assessment of AD disease activity.

In chapter 8 we reported that AD disease activity (measured by the objective SCORAD) improves with 40% after 8 weeks of treatment with EC-MPS in patients with severe AD who had to discontinue previous therapy with CsA due to side-effects or unresponsiveness. Only mild side effects were seen. Although EC-MPS shows a delayed clinical response compared to CsA, it may be a good alternative for patients with AD who had to discontinue treatment with CsA due to side effects or unresponsiveness.

Treatment with everolimus in two adult patients with severe AD did not result in an improvement of disease activity. This is described in chapter 9. Although we cannot exclude lack of patients' adherence to therapy, the mechanism of action of everolimus may not be sufficient to inhibit T-cell proliferation in AD. These results do not support a clinical trial with everolimus in adult patients with severe AD.

These general conclusions can be drawn from this thesis:

1. In adult patients with controlled AD, all patients treated with 0.05% clobetasol propionate ointment (10-30 g/day) had low serum cortisol levels, but only 40% of the patients treated with 0.1% betamethasone valerate ointment (10-30 g/day) and none of the patients treated with 0.005% fluticasone propionate ointment (10-30 g/day). (*chapter 2*)

2. The safe amounts of class III and IV topical corticosteroids as mentioned in the Dutch guideline on topical corticosteroids do not induce low serum cortisol levels in patients with moderate to severe AD. (*chapter 2*)

3. Liquid chromatography-tandem mass spectrometry (LC/MS/MS) offers a new method to measure clobetasol propionate in human serum. (*chapters 3 and 4*)

4. In a two-year follow-up study BMD did not differ between adult patients with moderate to severe AD who had used <75 g or ≥ 75 g of topical corticosteroids per month. (*chapter 5*)

5. In adult patients with moderate to severe AD, the change in BMD during a two-year follow-up period did not differ between patients with a T-score < -1 at baseline compared to patients with a T-score ≥ -1 at baseline. (*chapter 5*)

6. In contrast to adult patients, low BMD is not observed in children with moderate to severe AD. (*chapter 6*)

7. The correlation between the body surface area measurements of the SA-EASI and the objective SCORAD is higher than the correlation between the severity measurements of both disease activity scores in children with moderate to severe AD. (*chapter 7*)

8. The correlation between the body surface area measurement of the SA-EASI and serum TARC levels is higher than the correlation between the severity measurement of the SA-EASI and serum TARC levels in children with moderate to severe AD. (*chapter 7*)

9. EC-MPS may be an attractive treatment option for adult patients with moderate to severe AD who had to discontinue CsA due to side effects or unresponsiveness. (*chapter 8*)

10. Everolimus does not seem to be effective in the treatment of adult patients with severe AD. (*chapter 9*)

Samenvatting

In dit proefschrift onderzoeken we systemische effecten (*pitfalls*) van lokale corticosteroïden en onderzoeken we de effectiviteit van nieuwe therapieën (*opportunities*) bij patiënten met matig tot ernstig constitutioneel eczeem.

Constitutioneel eczeem (CE) is een chronische ontstekingsreactie in de huid, die gepaard gaat met roodheid, zwelling, schilfering, vochtafscheiding, kloofjes en jeuk. Lokale corticosteroïden (hormoonzalven) remmen de ontsteking in de huid en zijn een belangrijk onderdeel van de behandeling. Het gebruik van lokale corticosteroïden kan gepaard gaan met bijwerkingen. Lokale bijwerkingen zijn bijvoorbeeld atrofie van de huid en striae. Systemische bijwerkingen zouden kunnen optreden als lokale corticosteroïden door de huid geabsorbeerd worden in de bloedbaan (percutane absorptie). Hoge spiegels corticosteroïden in het bloed kunnen de hypothalamus-hypofyse-bijnierschors as onderdrukken waarbij het cortisol gehalte in het bloed daalt. Bij langdurige onderdrukking van deze as, zoals dat bijvoorbeeld gebeurt bij langdurig oraal corticosteroïd (prednison) gebruik, kunnen het syndroom van Cushing en botontkalking ontstaan. Omdat er weinig bekend is over de systemische bijwerkingen van lokale corticosteroïden onderzoeken we in het eerste deel van dit proefschrift de systemische effecten van lokale corticosteroïden op de bijnierschors (hoofdstukken 2, 3 en 4) en op botdichtheid (hoofdstuk 5 en 6).

In hoofdstuk 2 hebben we laten zien dat het cortisol gehalte in het bloed binnen 24 uur daalt nadat volwassenen met een opvlamming van CE eenmalig worden ingesmeerd met 0.1% betamethason valeraat zalf of 0.05% clobetasol propionaat zalf. De hoeveelheid van de lokale corticosteroïden werd afgebouwd tijdens de opname in het ziekenhuis. Aan het einde van de opname bleek het cortisol gehalte genormaliseerd te zijn bij alle patiënten die 0.005% fluticason propionaat zalf smeerden, bij 60% van de patiënten die 0.1% betamethason valeraat zalf smeerden, maar bij geen van de patiënten die 0.05% clobetasol propionaat zalf smeerden. Na ontslag bleek het cortisol gehalte bij alle patiënten genormaliseerd te zijn. Op dat moment smeerden deze patiënten met een hoeveelheid lokale corticosteroïden die veilig wordt geacht en ook in de richtlijn 'Dermatocorticosteroïden' wordt beschreven (100 g/week voor klasse III en 50 g/week voor klasse IV lokale corticosteroïden).

In hoofdstuk 3 wordt een nieuwe, gevoelige methode beschreven om spiegels van clobetasol propionaat in het bloed te kwantificeren tijdens gebruik van 0.05% clobetasol propionaat zalf. Er wordt gebruik gemaakt van vloeistofchromatografie met massaspectrometrie (LC/MS/MS). Met deze methoden tonen we in hoofdstuk 4 spiegels van clobetasol propionaat aan in het bloed na een eenmalige applicatie van 20-30 g 0.05% clobetasol propionaat zalf bij patiënten met ernstig CE. Tegelijkertijd wordt een daling gezien van het cortisol gehalte in bloed.

Uit hoofdstukken 2, 3 en 4 concluderen we dat de kans op hoge corticosteroïd spiegels in het bloed en dus mogelijke systemische bijwerkingen tijdens het gebruik van lokale corticosteroïden het kleinst is als 0.005% fluticason propionaat zalf gesmeerd wordt. Daarentegen is onze verwachting dat systemische bijwerkingen kunnen optreden als meer dan 10 g per dag 0.05% clobetasol propionaat zalf gesmeerd wordt door patiënten met CE. Daarom moet deze hoeveelheid van 0.05% clobetasol propionaat zalf alleen gebruikt worden als behandeling met 0.005% fluticason propionaat zalf of 0.1% betamethason valeraat zalf het eczeem niet voldoende onder controle brengt.

In hoofdstuk 5 beschrijven we de resultaten van de botdichtheidsstudie bij volwassen patiënten met matig tot ernstig CE. Er werd een botdichtheidsmeting gedaan bij de start van de studie en deze werd na twee jaar herhaald. De hoeveelheid voorgeschreven lokale corticosteroïden werd berekend voor deze periode. Bij de start van de studie hadden 37.3% van een groep van 75 patiënten osteopenie (verminderde botdichtheid) en 4.0% osteoporose (botontkalking). Deze getallen zijn hoger dan dat in de algemene bevolking verwacht zou worden (15.3% osteopenie en 0.62% osteoporose). Tijdens de studie bleef het aantal mensen met osteopenie of osteoporose gelijk. Verder was er geen significante verandering tussen patiënten die weinig (<75 g/maand) lokale corticosteroïden hadden gesmeerd en patiënten die meer (≥75 g/maand) lokale corticosteroïden hadden gesmeerd. Een andere factor die een negatieve invloed zou kunnen hebben op botdichtheid is de mate van ontsteking (inflammatie) in de huid. Echter, dit is lastig te onderzoeken omdat patiënten meer lokale corticosteroïden gaan smeren als de inflammatie van de huid toeneemt. Er zijn vervolgstudies nodig om te onderzoeken welke patiënten een risico

op een te lage botdichtheid hebben. Omdat we in deze studie vinden dat veel volwassenen met matig tot ernstig CE een te lage botdichtheid hebben adviseren we leefstijladviezen voor gezonde botten: sporten moet worden aanbevolen, de zuivel inname moet tenminste 4 porties per dag bedragen en iedereen moet tenminste 15 minuten per dag in de zon komen met tenminste het gezicht en de handen.

In hoofdstuk 6 hebben we de botdichtheid onderzocht bij kinderen met matig tot ernstig eczeem. In een groep van 60 kinderen was het aantal kinderen met een te lage botdichtheid niet anders dan in de algemene bevolking voorkomt. Verder bleek het gebruik van lokale corticosteroïden, inhalatie/intranasale corticosteroïden, systemische corticosteroïden en cyclosporine A in de voorgaande vijf jaar geen significante invloed te hebben op de botdichtheid. Bij kinderen met matig tot ernstig CE is er daarom geen reden om leefstijladviezen voor gezonde botten te benadrukken.

Als lokale corticosteroïden het eczeem niet goed onder controle kunnen brengen, worden patiënten met ernstig CE soms behandeld met orale immunosuppressiva zoals bijvoorbeeld cyclosporine A (CsA). Tijdens het gebruik van CsA daalt de ziekte activiteit binnen enkele weken, maar bijwerkingen zoals nierfunctiestoornissen en hypertensie (hoge bloeddruk) zorgen er soms voor dat het gebruik afgebouwd of gestaakt moet worden. Alternatieve orale immunosuppressiva zijn bijvoorbeeld mycofenolaat natrium met een maagsap beschermende coating (enteric-coated mycophenolate sodium; EC-MPS) en everolimus. In het tweede deel van dit proefschrift (hoofdstuk 8 en 9) onderzochten we de effectiviteit en veiligheid van deze middelen. Het effect van een therapie op de mate van ontsteking van de huid kan onderzocht worden met bepaalde huidscore instrumenten. De self-administered eczema area and severity score (SA-EASI) is een huidscore instrument dat patiënten zelf in kunnen vullen. Daardoor zou het effect van een therapie makkelijker gemeten kunnen worden. In hoofdstuk 7 onderzochten we of de SA-EASI goed correleert met veel gebruikte huidscore instrumenten die door een arts ingevuld worden (de objectieve SCORAD en de SASSAD) en of de SA-EASI goed correleert met een biomarker in het bloed (thymus and activation-regulated cytokine; TARC).

In hoofdstuk 7 vonden we dat de SA-EASI goed correleert met de objectieve SCORAD. Er werd een matige correlatie gevonden tussen de SA-EASI en TARC. Het bleek dat de gedeeltes van de instrumenten die de oppervlakte van het eczeem aangaven het beste correleerden. De gedeeltes van de instrumenten die de ernst van het eczeem aangaven correleerden minder. Als patiënten meer uitleg krijgen over de metingen van de ernst van het eczeem, dan zouden de correlaties tussen de SA-EASI en de objectieve SCORAD/TARC toe kunnen nemen. Dan zou de SA-EASI vaker gebruikt kunnen worden in de dermatologische praktijk of tijdens studies naar de effectiviteit van nieuwe therapieën.

Tijdens behandeling met EC-MPS neemt de ziekte activiteit van het eczeem af met 40% (objectieve SCORAD) na een behandelperiode van 8 weken bij volwassenen met ernstig CE. Er werden alleen milde bijwerkingen gevonden. In vergelijking met CsA duurt het bij de behandeling met EC-MPS langer voordat er een goed effect op het eczeem zichtbaar is. Toch zou EC-MPS een goed alternatief voor CsA zijn, zeker voor patiënten die eerdere behandeling met CsA moesten stoppen vanwege bijwerkingen of omdat CsA geen effect had op het eczeem.

Tenslotte beschrijven we in hoofdstuk 9 dat behandeling met everolimus geen effect had op de ernst van het eczeem bij twee volwassenen met ernstig CE.
We weten niet zeker dat deze patiënten therapietrouw waren. Het lijkt alsof het werkingsmechanisme van everolimus niet geschikt is om de inflammatie in de huid bij patiënten met CE te remmen.

Publications

First experience with enteric-coated mycophenolate sodium (Myfortic®) in severe recalcitrant adult atopic dermatitis: an open label study.
S.G.A. van Velsen, I.M. Haeck, C.A.F.M. Bruijnzeel-Koomen, M.S. de Bruin-Weller.
British Journal of Dermatology 2009;160(3): 687-91.

Severe atopic dermatitis treated with everolimus.
S.G.A. van Velsen, I.M. Haeck, C.A.F.M. Bruijnzeel-Koomen.
Journal of Dermatological Treatment 2009;20(6):365-7.

Liquid chromatography-tandem mass spectrometric assay for clobetasol propionate in human serum from patients with atopic dermatitis.
R.W. Sparidans, S.G.A. van Velsen, M.P. de Roos, J.H. Schellens, C.A.F.M. Bruijnzeel-Koomen, J.H. Beijnen.
Journal Chromatography B 2010;878(23):2150-4.

The Self-administered Eczema Area and Severity Index in children with moderate to severe atopic dermatitis: better estimation of AD body surface than severity.
S.G.A. van Velsen, M.J. Knol, I.M. Haeck, C.A.F.M. Bruijnzeel-Koomen, S.G.M.A. Pasmans.
Pediatric Dermatology 2010;27(5):470-5.

Bone mineral density in children with moderate to severe atopic dermatitis.
S.G.A. van Velsen, M.J. Knol, R.L. van Eijk, M.A. de Vroede, T.C. de Wit, M.G. Lam, I.M. Haeck, M.S. de Bruin-Weller, C.A.F.M. Bruijnzeel-Koomen, S.G.M.A. Pasmans.
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Journal of the American Academy of Dermatology 2010;63(5):911-3.

Forthcoming publications

The potency of clobetasol propionate: serum levels of clobetasol propionate and adrenal function during therapy with 0.05% clobetasol propionate in patients with severe atopic dermatitis.
S.G.A. van Velsen, M.P. de Roos, I.M. Haeck, R.W. Sparidans, C.A.F.M. Bruijnzeel-Koomen.
Journal of Dermatological Treatment, in press, 2011.

Enteric-coated mycophenolate sodium versus cyclosporin A as long-term treatment in adult patients with severe atopic dermatitis: a randomized controlled trial.
I.M. Haeck, M.J. Knol, O. ten Berge, S.G.A. van Velsen, M.S. de Bruin-Weller, C.A.F.M. Bruijnzeel-Koomen.
Journal of the American Academy of Dermatology, in press 2010.

Moderate correlation between quality of life and disease activity in adult patients with atopic dermatitis.
I.M. Haeck, O. Ten Berge, S.G.A. van Velsen, M.S. de Bruin-Weller, C.A.F.M. Bruijnzeel-Koomen, M.J. Knol.
Journal of the European Academy of Dermatology & Venereology, in press, 2011.

Curriculum Vitae

Sara Gertrudes Anna van Velsen werd op 15 mei 1982 geboren te Hilversum.
In 2000 deed zij eindexamen Gymnasium aan het Alberdingk Thijm College te Hilversum en behaalde dit cum laude. Datzelfde jaar begon zij aan de studie Geneeskunde aan de Universiteit Utrecht. In het vijfde jaar van haar opleiding begon zij zich steeds meer te interesseren voor het specialisme Dermatologie.
Onder leiding van dr. M.S. de Bruin-Weller en prof. dr. C.A.F.M. Bruijnzeel-Koomen deed zij haar wetenschappelijke stage aan de afdeling Dermatologie van het UMC Utrecht. Het hoofdonderwerp van deze stage was het kwantificeren van DNA-schade in de huid na UVB expositie bij patiënten met constitutioneel eczeem voor en tijdens gebruik van lokale calcineurineremmers en lokale corticosteroïden.
In 2006 behaalde zij cum laude het artsexamen en kort daarna startte zij als basisarts interne geneeskunde in het Haga Ziekenhuis te Den Haag onder leiding van dr. R.M. Valentijn.
In 2007 werd zij gevraagd om te gaan werken als arts-onderzoeker aan de afdeling Dermatologie van het UMC Utrecht. Het onderzoek richtte zich nu op de behandeling van patiënten met constitutioneel eczeem en heeft geleid tot dit proefschrift.
In januari 2010 startte zij met veel plezier de opleiding tot dermatoloog aan het VUmc te Amsterdam onder leiding van prof. dr. Th. M. Starink.

Abbreviations

ACTH: adrenocorticotropic hormone

AD: atopic dermatitis

APCI: Atmospheric Pressure Chemical Ionization

APPI: Atmospheric Pressure Photoionization

β-CTX: C-terminal peptide of type I collagen

BMAD: Bone Mineral Apparent Density

BMD: Bone Mineral Density

BMI: Body Mass Index

BSA: Body Surface Area

BSAP: bone-specific alkaline phosphatase

CID: Collision Induced Dissociation

CRH: corticotropin-releasing hormone

CsA: Cyclosporin A

CTACK: Cutaneous T-cell-Attracting Chemokine

CV: Coefficient of Variation

DXA: Dual-energy X-ray Absorptiometry

EASI: Eczema Area and Severity Index

EC-MPS: Enteric-Coated Mycophenolate Sodium

ESI: Electrospray Ionization

HPA-axis: Hypothalamus-Pituitary-Adrenal-axis

IDEC: Inflammatory Dendritic Epidermal Cells

IgE: Immunoglobulin E

IL: Interleukin

IM-PDH: Inosine Monophosphate Dehydrogenase

INF-γ: Interferon-gamma

IQR: Inter Quartile Range

IS: Internal Standard

ISCD: International Society of Clinical Densitometry

LC/MS/MS: Liquid Chromatography-tandem Mass Spectrometry

LLOQ: Lower Limit of Quantification

LSC: Least Significant Change

LSS: Leicester Sign Score

MCP-1: Monocyte Chemotactic Protein-1

MMF: Mycophenolate Mofetil

MPA: mycophenolic-acid

mTOR: mammalian Target of Rapamycin

MTX: metothrexate

NFAT: Nuclear Factor of Activated T-cells

25-OH-vitamin D: 25-hydroxyvitamin D

PAR-2: Protease Activated Receptor-2

PTH: parathyroid hormone

QC: Quality Control

RANTES: Regulated upon Activation Normal T-cell Expressed and Secreted

SA-EASI: Self-Administered Eczema Area and Severity Index

SASSAD severity score: Six Area Six Sign Atopic Dermatitis severity score

SCORAD index: SCORing Atopic Dermatitis index

SD: Standard Deviation

SE: Standard Error

SEM: Standard Error of the Mean

SRM: Selected Reaction Monitoring

TARC: Thymus and Activation-Regulated Cytokine

TSLP: Thymic Stromal Lymphopoietin

T½ el: elimination half life time

VAS: Visual Analogue Scale

WHO: World Health Organization

Dankwoord

Omdat ik zo van koken hou…
De totstandkoming van een proefschrift is als een ingewikkeld recept waarvoor vele ingrediënten nodig zijn en een heel speciale bereidingswijze nodig is:

Pitfalls and opportunities in the treatment of atopic dermatitis

Voor 1 proefschrift

Ingrediënten:

- Een flinke dosis Carla Bruijnzeel-Koomen
- Ongeveer dezelfde hoeveelheid Suzanne Pasmans
- Een paar honderd patiënten met constitutioneel eczeem
- Een uitstekende polikliniek en kliniek Dermatologie
- Een significante hoeveelheid Mirjam Knol
- 1 onderzoeksverpleegkundige
- 150 m² laboratoria (dermatologie, endocrinologie en immunologie)
- 1 DEXA-scanner
- Een surfbordlengte Onno ten Berge
- Een gedegen onderzoeksbasis van Inge Haeck
- Pittige gesprekken met Peter Lee
- Een handje kritische noten van Yoony Gent
- 1078 koppen thee van Thuy-My Le
- Een enthousiaste onderzoeksgroep Dermatologie
- Een vleugje design van de hand van Ton van Velsen
- Een solide, liefdevolle, maar ook kritische thuisbasis
- Twee paranimfen: Linda Willemstein en Anke Lecluse

Bereidingswijze:

Als basis van dit proefschrift gebruiken we de onderzoeksvoorstellen die voortvloeiden uit het eerdere onderzoek van Inge Haeck. Deze onderzoeksvoorstellen werden voorzichtig bewerkt zodat er een nieuwe onderzoekslijn ontstond.
Hierbij heb ik de begeleiding van mijn promotor, Carla Bruijnzeel-Koomen als onmisbaar ervaren. Carla, je gaf mij veel vrijheid en je nam mij altijd serieus tijdens

onze besprekingen. Ook mijn co-promotor, Suzanne Pasmans heeft de basis voor een aantal onderzoeksvoorstellen gelegd en haar altijd enthousiaste houding en kritische opmerkingen zijn zeer belangrijke ingrediënten gebleken om onze kinderstudies tot een succes te maken. Marjolein de Bruin-Weller dank ik voor haar waardevolle bijdrage, onder andere aan het Myfortic stuk. Tijdens het MES heb ik veel van haar geleerd over de behandeling van patiënten met ernstig constitutioneel eczeem.

Op het moment dat de basis is gelegd kunnen we beginnen met het verzamelen van alle onderzoeksgegevens. Dit was niet mogelijk geweest zonder de gedrevenheid van vele patiënten met constitutioneel eczeem van de kliniek en polikliniek Dermatologie. Zonder deze patiënten had dit onderzoek niet kunnen plaatsvinden en ik ben hen dan ook enorm dankbaar. Al deze patiënten werden vaak voor de eerste keer benaderd via hun behandelend arts, waarbij ook de eczeem-verpleegkundigen en de verpleegkundigen van afdeling D2, onder leiding van Jan van der Woude, veel hebben bijgedragen. Mijn dank gaat daarom ook uit naar hen. Daarnaast heb ik twee student-onderzoekers mogen begeleiden en zij hebben ongelooflijk hun best gedaan de kinderstudie tot een succes te maken.
Lonneke Franken en Rachel van Eijk, bedankt daarvoor!

De volgende stap in dit proces is het verwerken van de onderzoeksdata. Kennis van statistiek en de programma's SPSS en ook Access is essentieel. Carla, ik wil jou hiervoor ook bedanken omdat ik de benodigde cursussen mocht volgen en omdat onze afdeling een vast aanspreekpunt kreeg voor al onze statistische vragen: Mirjam Knol. Mirjam, heel erg bedankt voor al je hulp en je duidelijke uitleg.
Dataverwerking is een ongelooflijk karwei en als ik dat alleen had moeten doen zat ik, denk ik, nog achter de computer… Ik heb veel hulp gehad van Jos Beutler, onze onderzoeksverpleegkundige, en ook mijn moeder, Truus van Velsen, heeft een aantal weken flink gewerkt om alle datasets compleet te maken! Jullie zijn super!

Sommige data komen via een omweg bij de onderzoeker terecht.
Zo was de bepaling van verschillende botmarkers en TARC in serum een belangrijk ingrediënt voor enkele studies. Ik wil hiervoor Inge Maitimu van het Endocrinologisch laboratorium en Henny Otten van het Immunologisch laboratorium bedanken voor hun hulp bij de afwijkende aanlevering van de sera en het ontwikkelen van de TARC assay. De afdeling Nucleaire geneeskunde heeft alle data aangeleverd van de botdichtheidsmetingen. Samen met Marnix Lam en Tim de Wit is het mij duidelijk geworden hoe een DEXA-scan gemaakt wordt en wat er bij de interpretatie komt kijken. Heel erg bedankt voor de prettige samenwerking.

Ja, en als je dan alle data netjes op een rij hebt staan en deze hebt verwerkt tot resultaten is het erg prettig als je hierover kunt discussiëren om zo een basis te leggen voor het daadwerkelijke manuscript. Carla en Suzanne, hierbij spelen jullie een speciale rol en ik ben trots op het werk dat we samen geleverd hebben.
Ook dank ik hierbij Monique de Vroede, kinderendocrinoloog, voor haar hulp bij het beoordelen van de onderzoeksresultaten van de botdichtheidsstudie bij kinderen.

Tussen de dataverwerking en het schrijven van de manuscripten door is ook ontspanning belangrijk. Zo waren daar de dagelijkse theepauze en de lunchpauze, maar ook moet er soms tussendoor even bijgepraat worden over het een of ander. Met mijn mede-onderzoekers, Inge Haeck, Onno ten Berge, Peter Lee, Thuy-My Le en Yoony Gent heb ik heel wat gediscussieerd over onze onderzoeken. Ik heb veel steun aan jullie gehad! Fijn dat we elkaar nog regelmatig zien! Iedereen van de onderzoeksafdeling en in het bijzonder Stans, Marloes, Annemieke, Ans, Els, Ilse, Laura, Laury, Maarten, Kees, Huib, Ina, Feiko, Miranda en Jantine; bedankt voor jullie positiviteit en gezelligheid!

Wanneer we aan de laatste stap van het proefschrift kunnen beginnen, namelijk het schrijven van de introductie en algemene discussie, begint ook de opleiding tot dermatoloog aan het VUmc te Amsterdam. Het is niet makkelijk om de opleiding te combineren met de laatste, tijdrovende bereidingen van dit proefschrift. Daarom wil ik mijn opleider, prof. Th. M. Starink, ontzettend bedanken voor zijn steun, interesse en de tijd die ik heb gekregen om te schrijven. Dank ook aan de overige stafleden voor hun belangstelling en zelfs hulp bij de sponsoraanvragen!
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Nu lijkt het alsof we alle ingrediënten voor het proefschrift bij elkaar hebben, immers we hebben het manuscript geschreven. Toch missen we nog een aantal belangrijke ingrediënten die maken dat dit proefschrift er gekomen is: liefde en steun.
Mijn ouders, Truus en Ton van Velsen, hebben dit promotietraject met mij doorgemaakt en mijn verhalen altijd aangehoord om me daarna met raad en daad bij te staan. Samen met hen heb ik een aantal belangrijke beslissingen kunnen nemen.
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En lieve Koen en Bob, mijn broers, jullie zorgden voor ontspanning en vrolijkheid thuis bij onze ouders in de weekeinden. 'Kolonisten' vind ik stiekem toch nog steeds wel leuk hoor!

En als het proefschrift helemaal 'afgebakken' is en de dag aanbreekt dat we dit gaan vieren zijn daar nog twee mensen heel belangrijk: de paranimfen. Ik ben ontzettend blij dat jullie, Linda Willemstein en Anke Lecluse, mijn paranimfen willen zijn.
Linda, we kennen elkaar al heel lang en het is fijn zo'n goede vriendin te hebben!
Anke, dank voor al je steun en gezelligheid! Ik vind het erg leuk dat we uiteindelijk toch samen in opleiding zijn gegaan!

Welnu, mijn proefschrift is een feit! Hoera, het is vandaag! ©

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