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2022

Salava , A , Perälä , M , Pelkonen , A , Mäkelä , M & Remitz , A 2022 , ' Safety of tacrolimus 0.03% and 0.1% ointments in young children with atopic dermatitis: a 36-month follow-up study ' , Clinical and Experimental Dermatology , vol. 47 , no. 5 , pp. 889-902 . https://doi.org/10.1111/ced.15024

http://hdl.handle.net/10138/353423
https://doi.org/10.1111/ced.15024

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Article type : Original Article

Safety of tacrolimus 0.03% and 0.1% ointments in young children with atopic dermatitis - a 36-month follow-up study

Running head: Safety of tacrolimus 0.03% and 0.1% ointments in young children with AD

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/CED.15024

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Funding: The work was supported by: The Pediatric Research Foundation, Helsinki University Hospital Research Fund, Sigrid Juselius Foundation, Päivikki and Sakari Sohlberg Foundation, Finnish Dermatological Society, Allergy Research Foundation, Väinö and Laina Kivi Foundation, Orion Research Foundation, Ida Montin Foundation, Orion Pharma Finland and Astellas Pharma. The sponsors had no influence on the study.

Conflicts of interest: All authors: no conflicts of interest

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

What's already known about this topic?

There is a risk of undertreatment in pediatric AD based on safety issues concerning the use of topical calcineurin inhibitors or moderate potency topical corticosteroids. Topical tacrolimus is used off-label in young children, but data remains limited regarding children under 2 years of age and long-term treatment.

What does this study ad?

In our prospective clinical study with 152 young children from one to three years of age tacrolimus 0.03% and 0.1% ointments and mild and moderate potency topical corticosteroids were effective and showed similar safety profiles in the treatment of atopic dermatitis.

Summary

Background: Topical tacrolimus is used off-label in young children, but data remains limited regarding children under 2 years of age and long-term treatment.

Objectives: To compare safety differences between tacrolimus 0.03% and 0.1% ointments with mild and moderate potency topical corticosteroids in young children with atopic dermatitis.

Methods: We conducted a 36-month follow-up study with 152 young children from one to three years of age with moderate-to-severe atopic dermatitis. Children were followed prospectively and data concerning infections, disease severity, growth parameters, vaccination responses, and other relevant laboratory tests were gathered.

Results: There were no differences between the treatment groups in skin-related infections ($p = 0.198$), other infections ($p = 0.498$), growth parameters height ($p = 0.601$) and weight ($p = 0.812$), EASI scores ($p = 0.187$), vaccination responses ($p = 0.620$), serum cortisone levels ($p = 0.228$) and serum levels of IL-4, IL-10, IL-12, IL-31 and IFN gamma. EASI decreased significantly in both groups ($p < 0.0001$). In the tacrolimus group, nine patients (11.68%) had detectable tacrolimus blood concentrations at the 1-week visit. We observed no malignancies or severe infections during the study. Blood eosinophil counts were similar in both groups.

Conclusions: Topical tacrolimus (0.03% and 0.1%) and topical corticosteroids (mild and moderate potency) are safe to use in young children with moderate-to severe-atopic dermatitis and have comparable efficacy and safety profiles.

Introduction

Atopic dermatitis (AD) is one of the most frequent skin diseases of early childhood and causes a notable burden to young children, families and the health care system[1]. Intermittent courses of mild topical corticosteroids (TCS) are used as first line treatment in young children[2]. In non-responsive cases, moderate potency TCS or topical calcineurin

inhibitors are required. There is a risk of undertreatment in pediatric AD based on safety issues concerning the use of moderate potency TCS or topical calcineurin inhibitors[3].

Topical tacrolimus (TAC) 0.03 % ointment is approved from 2-years of age, but there have been concerns regarding its immunosuppressive potential[4]. The usage of 0.1% TAC ointment has been off-label in young children, still lacking data regarding long-term treatment in children under 2-years of age[5]. TAC 0.03% and 0.1% ointments were approved for use in 2002, and so far, no observations have directly linked their topical use to systemic immunosuppression or increased incidence of malignancies[6-7]. However, there have been reports associating severe and uncontrolled AD with increased cutaneous infections and a slightly elevated risk of developing malignancies, especially cutaneous lymphomas[8]. If topical treatments were to induce systemic immunosuppression and increase the infections rate, detectable blood levels or lowered vaccination responses should be observable[9-10].

We aimed to compare safety differences in the use of TAC 0.03% and 0.1% ointments with mild and moderate potency TCS in a cohort of young children with moderate-to-severe AD.

Patients and methods

We performed a single-center investigator initiated clinical study at the Helsinki University Hospital, Finland, comparing two topical therapies in the treatment of children with atopic dermatitis: TCS creams (hydrocortisone acetate 1% and hydrocortison-17-butyrate 0.1%) and TAC ointments (0.03% and 0.1%). The study was conducted as a randomized non-blinded follow-up study with a prior one-week washout period. Selection, inclusion and randomization of the study patients was carried out in the pediatric unit.

Patient characteristics and treatments

The investigated patient cohort comprised 152 young children, aged 1 to 3 years (mean age 1.43 years) with equal gender distribution and moderate (score 4.5-7.5) to severe (score 8-

9) AD based on the Rajka & Langeland severity score (Table 1)[11]. The study size was based on statistical power calculations. The same experienced dermatologists carried out the diagnosis of AD and subsequent follow-up visits. Patients were randomized 1:1 into two treatment groups: 1. TCS group (1% hydrocortisone acetate cream, and if needed, hydrocortisone-17-butyrate 0.1% cream) and 2. TAC group (topical 0.03% TAC ointment, and if needed, 0.1% TAC ointment). Parents and caregivers were thoroughly instructed regarding topical treatments. They were advised to apply sufficient amounts of TCS twice daily for courses of 3-7 days (TCS group) or topical TAC twice daily until clearance was achieved, and afterwards twice weekly, if needed (TAC group). Parents or caregivers could switch to the more potent topical treatment (in the TCS group hydrocortisone-17-butyrate 0.1% ointment and in the TAC group tacrolimus 0.1% ointment), if necessary. Study follow-up visits (including assessment of treatments and responses, infections, AD severity) were performed at baseline, after the first week and at months 1, 3, 6, 9 and 12, and thereafter every 6 months. The complete follow-up period comprised 36 months.

Safety concerns (infection rate, growth parameters) of TAC 0.03% and 0.1% ointment compared to mild and moderate potency TCS were determined as primary endpoints of the study analysis. Secondary endpoints were significant differences in treatment response (EASI), vaccination responses and other safety relevant laboratory values, between the treatment groups. Data about skin-related infections (SRI) and other infections was acquired retrospectively and was based on the parents and caregivers information. Infection type and severity could not be confirmed by health record data or other documented data. Growth parameters (height and weight) and EASI scores were measured at each follow-up visit[12].

Patients with recurrent infections (otitis, pneumonia, and recurrent skin infections), signs of immune deficiency, chronic diseases (asthma, autoimmune diseases, gastrointestinal diseases, malignancies, and kidney diseases), continuous medications, or need for continuous inhaled corticosteroids were excluded. There was a drop-out of 27 patients during the follow-up.

The local ethics committee of the Helsinki University Hospital and the Finnish Medicines agency approved the study protocol. Parents and caregivers gave a written consent for their children to participate in the study. General patient characteristics are presented in Table 1. In a former publication, we have characterized the baseline patient characteristics in more detail[5].

Laboratory tests

Serum cytokine levels (IL-4, IL-10, IL-13, IL-31 and INF gamma) were measured with sensitive ELISA assays in both TCS and TAC groups at baseline and 36 months (endpoint of the follow-up). Total serum cortisone levels were measured at baseline, 12, 24 and 36 months (immunochemiluminometric assay). In the TAC group, blood tacrolimus concentrations were measured at the 1-week and at the 12, 24 and 36 months follow-up visits (fluid chromatography-tandem mass spectrometry). In both groups, differential blood counts with total blood eosinophil counts (flow cytometry) were investigated at baseline, at the 12 months and 36 months follow-up visits. All laboratory tests were performed in the Laboratory of Helsinki University Hospital (HUSLAB®) and were based on accredited methods.

Statistical analyses

Descriptive statistics and group comparisons were calculated with IBM SPSS 27.0. The Mann-Whitney U-test was used to compare quantitative data and the Pearson's Chi-square-test for nominal data. In both tests, statistical significance was set at a P-value of < 0.05.

Results

Skin-related infections

There were 223 SRI observed (mean 1.50 SRI per patient) during the follow up; 105 bacterial SRI (impetigo, folliculitis, boils, erysipelas; mean 0.7 per patient), 88 viral SRI (warts, molluscum, herpes simplex; mean 0.59 per patient) and 31 other SRI (tinea, candida; 0.21 per patient) (Table 2, Figure 1a). In the TCS group there were 100 SRI (mean 1.35 per patient), with 51 bacterial SRI (mean 0.69 per patient), 38 viral SRI (mean 0.51 per patient) and 11 other SRI (mean 0.15 per patient) detected. In the TAC group, there were altogether 123 SRI (mean 1.64 per patient), with 54 bacterial SRI (mean 0.72 per patient), 50 viral SRI (mean 0.67 per patient) and 20 other SRI (mean 0.27 per patient).

Group comparisons between TCS and TAC groups did not show significant differences concerning total SRI ($p = 0.198$, 95% CI 0.152-0.283), bacterial SRI ($p = 0.890$, 95% CI 0.870-0.959), viral SRI ($p = 0.155$, 95% CI 0.094-0.208) or other SRI ($p = 0.342$, 95% CI 0.242-0.390) (Table 2, Figure 1b). There was a trend to higher amounts of viral SRI in the TAC group, but the difference was not statistically significant.

Other infections

Besides SRI, there were 1357 other infections observed (mean 9.11 per patient) (Table 2, Figure 1a). Most of these were respiratory infections (upper respiratory infections, pharyngitis, tonsillitis and laryngitis) with a total of 980 infections (mean 6.58 per patient). In addition, there were 74 viral rashes (mean 0.5 per patient), 163 otitis media (mean 1.09 per patient) and 139 other not SRI (mostly gastrointestinal and urogenital infections, mean 0.92 per patient). In the TCS group, there were 671 other infections (mean 9.07 per patient). These comprised 498 respiratory infections (mean 6.73 per patient), 33 viral rashes (mean 0.45 per patient), 78 otitis media (mean 1.05 per patient) and 61 other not SRI (mean 0.82 per patient). In the TAC group, there were 686 other infections (mean 9.15 per patient), with 482 respiratory infections (mean 6.43 per patient), 41 viral rashes (mean 0.55 per patient), 85 otitis media (mean 1.13 per patient) and 78 other not SRI (mean 1.04 per patient).

Group comparisons showed no significant differences concerning other infections (p = 0.498, 95% CI 0.440-0.599). There were no significant differences between respiratory infections (p = 0.267, 95% CI 0.236-0.382), viral rashes (p = 0.661, 95% CI 0.589-0.740), otitis media (p = 0.851, 95% CI 0.838-0.486) and other non-SRI (p = 0.495, 95% CI 0.330-0.486) (Table 2, Figure 1b). During follow up, there were no severe or life-threatening infections observed.

Growth parameters

At baseline, physical growth parameters were below standard deviation for young children in Finland in both treatment groups (TCS and TAC groups)[13]. The median height at baseline was 81.2 cm (IQR 78.2-89.5) and median SD was -0.20 (IQR -1.20-0.1) in the TCS group and 82.0 cm (IQR 78.2-88.1) and -0.63 (IQR -1.60-0.45) in the TAC group. After 36 months, there was a significant increase in height in both treatment groups, but both groups were still below Finnish standard deviations. The median height at 36 months was in the TCS group 108.5 cm (IQR 104.8-113.7) and median SD -0.17 (IQR -0.80-0.35) and in the TAC group 108.0 cm (IQR 104.9-113.0) and SD -0.50 (IQR -1.30-0.30). Weight measurements showed similar results (Table 3, Figure 2a). Comparisons between the TCS and TAC groups showed no significant differences regarding height (p = 0.601, 95% CI 0.541-0.696) and weight (p = 0.812, 95% CI 0.747-0.872) at baseline or at 36 months (Table 3, Figure 2b).

Cutaneous or internal malignancies

In both treatment groups, there were no cutaneous or internal malignancies identified during the 36 months follow-up.

EASI

In both treatment groups, EASI decreased significantly ($p < 0.0001$, 95% CI 0.000-0.02) during follow-up. At baseline the median EASI was 9.00 (IQR 6.50-17.10) in the TCS group and 10.1 (IQR 6.30-17.47) in the TAC group. At 36 months the median EASI was 1.20 (IQR 0.00-3.40) and 0.80 (IQR 0.00-2.27), respectively. Comparisons of both treatment groups showed no significant differences at baseline ($p = 0.451$, 95% CI 0.401-0.560) or at 36 months ($p = 0.187$, 95% CI 0.169-0.304) (Table 3).

Tacrolimus blood concentration

The therapeutic blood concentration of tacrolimus is 5.0 to 15.0 ug/l when used systemically after solid organ transplantations. In the TAC group, nine patients (11.68 %) had detectable tacrolimus blood concentrations (range 1.5-5.6 ug/l) at the first week visit (i.e. after 1 week of topical therapy). Most of these patients had more severe disease with extensive areas of skin involvement. In four patients, the concentration was 1.5 ug/l (EASI 17.5, 7.8, 11.8, 5.1, respectively). In the other patients, the concentrations were 2.2 ug/l (EASI 11.3), 2.3 ug/l (EASI 32.0), 3.0 ug/l (EASI 6.0), 3.2 ug/l (EASI 45.0) and 5.6 ug/l (EASI 10.2), respectively. The patients had used topical tacrolimus on the same morning before blood sampling contrary to recommendations. None of these patients had detectable tacrolimus in the control sample (< 1.5 ug/l). Two of the patients had as an additional diagnosis ichthyosis vulgaris. At the 1-year visit, only one patient had a detectable tacrolimus blood concentration of 4.7 ug/l (EASI 1.70). This patient had used tacrolimus ointment on the same morning prior to blood sampling and was experiencing an acute flare of AD due to undertreatment and non-adherence. In the control blood sample, tacrolimus was not anymore detectable. At the 24 and 36 months visits, no patients had detectable tacrolimus blood concentrations (all patients had < 1.5 ug/l).

Vaccination responses

In the complete study cohort, 28 patients (18.4 %) had pathologic vaccination responses. 15 patients (20.0 %) in the TCS group and 13 patients (16.8 %) in the TAC group. There were no significant differences between the treatment groups (p = 0.620, 95% CI 0.387-0.679). The features of these patients regarding AD were similar in both groups and there were no significant differences in baseline EASI (p = 0.565, 95% CI 0.280-0.649). Patients with pathologic vaccination responses showed similar serum concentrations of IL-4, IL-10, IL-13, IL-31 and IFN gamma. There were more infections observed in patients with pathologic vaccination responses than in patients with normal vaccination responses. In the TCS group 2.33 SRI per patient and 10.2 other infections per patient (all patients TCS group: SRI 1.35 and other infections 9.07), and in the TAC group 1.69 SRI per patient and 12.31 other infections per patient (all patients TAC group: SRI 1.64 and other infections 9.15). Concerning patients with pathologic vaccination responses, there were no differences between the treatment groups regarding SRI (p = 0.240, 95% CI 0.062-0.071) and other infections (p = 0.120, 95% CI 0.023-0.029). Characteristics of the patients with pathologic vaccination responses are presented in Tables S5 and S6.

Serum cortisone concentration

Serum cortisone concentrations were similar in both treatment groups throughout the follow-up with no clinical signs of adrenal insufficiency. In the TCS group, the median serum cortisone concentration was 168.0 nmol/l (IQR 138.0-218.0) at baseline and 206.0 nmol/l (IQR 153.5-280.0) at 36 months. In the TAC group, the median serum cortisone level was 152.0 nmol/l (IQR 123.5-187.7) at baseline and 170.5 nmol/l (IQR 137.5-170.5) at 36 months (Table 3, Figure 3a). In the TCS group, there was one patient with a concentration below the reference area for children with 55 nmol/l (reference: 69-632 nmol/l) at 3 months but the level was normal at subsequent visits. There were no significant differences between the groups at baseline (p = 0.358, 95% CI 0.254-0.404) or at 36 months (p = 0.228, 95% CI 0.157-0.290) (Table 3, Figure 3b).

Blood eosinophil counts

In both groups, blood eosinophil counts were similar at baseline and at 36 months. In the TCS group, the median blood eosinophil count was 0.44 E9/l (IQR 0.25-0.68) at baseline and 0.37 E9/l (IQR 0.23-0.71) at 36 months. In the TAC group, the median eosinophil count at baseline was 0.41 E9/l (IQR 0.26-0.69) and 0.37 E9/l (IQR 0.23-0.76) at 36 months. There were no significant differences between the treatment groups (p = 0.563, 95% CI 0.494-0.651) (Table 1).

Discussion

TAC ointment has been used for nearly 20 years for the treatment of AD in adults and children[14]. The 0.03% TAC ointment has an official approval for children from 2 to 15 years, although off-label use of the more potent 0.1% ointment is common because of its higher efficacy[15-16]. However, we have still only limited clinical data on the use of topical TAC for children under 2 years of age and the use of TAC 0.1% ointment in children[17].

In our cohort of young children with moderate-to-severe atopic dermatitis both TAC 0.03% and 0.1% ointments and 1% hydrocortisone acetate and hydrocortisone-17-butyrate 0.1% creams were effective and safe topical treatments. Similar observations have been reported previously, mostly in older children and toddlers. Doss et al. conducted a non-inferiority study on 479 children aged 2-15 years with moderate-to-severe AD and compared TAC 0.03% and fluticasone 0.005% (moderate potency corticosteroid) ointments. Efficacy of TAC 0.03% ointment as a second-line treatment was not inferior to that of fluticasone 0.005% ointment, with similar effect on clinical improvement[18]. Reitamo et al. performed a 2-week pharmacokinetic study in 53 infants of 3 to 24 months of age with 0.03% TAC ointment. 97% of blood samples assayed contained tacrolimus concentrations < 1 ng/ml[19]. The blood levels were comparable to older children and adults. The same children were then allowed to participate in 2-year safety study[20]. There were significant clinical improvements with treatment tolerability and TAC blood levels comparable to older children or adults. There are

few studies where 0.1% TAC ointment has been used in children. Remitz et al. investigated children from 2 to 15 years and treated them with 0.03% TAC ointment, and if needed, 0.1% TAC and followed them for up to four years[21]. About 70% of the patients needed the more potent 0.1% TAC ointment. No safety concerns were observed in this study.

To our knowledge, our study presented here is the first comparative long-term study where TAC 0.03% and 0.1% ointments have been compared with TCS in children from 1 to 3 years of age. The follow-up period of 36 months was relatively long compared with former studies. We wanted to focus particularly on safety issues such as infection rate, growth parameters and vaccination responses. These are very relevant from the clinical perspective, because there have been issues of concern regarding the use of TAC ointment in young children, which may have limited its usage in pediatric AD.

In both treatment groups, there were only minor side effects noticed (mainly skin burning sensation after TAC application) and dropouts were mostly due to non-adherence or to moving away. We observed SRI and other, mainly respiratory, infections, but there were no significant differences between the TCS and TAC groups. Although growth parameters were below standard deviation both at baseline and at the end of follow-up, patients showed a consistent increase in height and weight in both treatment groups (closer to standard deviation of Finnish children). Our observations underline the results of former studies, which have shown that topical tacrolimus used in young children with AD does not impair physical growth and generally does not lead to a higher incidence of infections[22-23].

Pathologic vaccination responses were observed in 28 patients (18.4%), but there were no significant differences between the treatment groups. There have been former reports on vaccination responses in children with AD with no signs that topical AD treatments decrease vaccination responses. Schneider et al. investigated immune responses to the varicella vaccine in children with AD compared to healthy controls and found similar responses in both groups[24]. In another open-label study with 23 children with AD, Stiehm et al. observed that 0.03% TAC ointment does not affect serologic responses to pneumococcal vaccine and does not affect T- and B-cell mediated immune responses[25]. In addition, Hofman et al.

showed in a controlled clinical trial that immune responses to meningococcal vaccine are comparable in children that were treated with either 0.03% TAC ointment or hydrocortisone 1% cream[26]. Thus, topical AD treatments do not seem to influence immediate responses to vaccination, immune memory or humoral and cell-mediated immunity.

Systemic absorption of tacrolimus after topical treatment has been shown to be very low in AD and occur mainly in patients with active and severe disease[27]. After response to therapy, systemic tacrolimus absorption discontinues[28]. In our study cohort, nine patients of the TAC group (11.6%) showed detectable tacrolimus blood concentrations. All of these were low (1.5-5.6 ug/l) and in control samples the tacrolimus levels were not anymore detected. Most of the patients had used tacrolimus ointment on the sampling day.

Mild and moderate potency TCS have been shown to be safe to use in young children if they are used short term, however data concerning long-term safety is limited. Axon et al. found in a recent meta-analysis no evidence of safety issues in children when TCS were used intermittently[29]. The risk for systemic absorption, immunosuppression and adrenal insufficiency seems to be very low[30]. Ghajar et al. conducted a meta-analysis regarding serum cortisone concentrations in small children with AD during therapy with mild and moderate potency TCS[31]. The amount of laboratory confirmed adrenal insufficiency with TCS use was 2.7% but no children had corresponding clinical symptoms. In our cohort, no clinical signs of adrenal insufficiency was observed and serum cortisone concentrations were similar in both TCS and TAC groups. Our observations support the current understanding that mild and moderate potency TCS are safe to use intermittently in young children[32].

Cytokines IL-4, IL-10, IL-13 and IL-31 are considered important markers of an enhanced Th2 inflammation seen in AD[33]. Recent studies have highlighted the importance of an immunologic Th2 shift and its relations with the skin immune system, epidermal barrier and the cutaneous microbiome in the pathogenesis of AD in infants and children[34]. IFN gamma has been recently linked to the NK-cell-ILC2 inhibitory axis, which may be a regulatory mechanism of innate immunity and constitute a part of the cutaneous immunological

barrier[35]. Many novel cytokine discoveries have now been translated clinically into more targeted therapies[36]. We did not observe significant changes in the serum concentrations of IL-4, IL-10, Il-13, IL-31 or IFN gamma in our study cohort. The concentrations of IL-4, IL-13 and IL-31 increased in both TCS and TAC groups when baseline levels were compared with levels at 36 months, but the changes were not statistically significant. IL-10 and IFN gamma levels did not show consistent patterns or significant changes. To our knowledge, serum concentrations of the investigated cytokines have not been formerly characterized to this extend in young children with AD.

The main limitation of the presented study is a relatively small patient cohort (n = 152). This is based on the fact, that the study was a single-center investigator-initiated clinical study with young children and relatively frequent follow-up visits. It is also important to notice that the study cohort represents selected patients of a tertiary-care center and that there were 27 dropouts registered during the follow-up (12 in the TCS group and 15 in the TAC group). In addition, we could not analyze long-term data regarding child development, growth parameters and disease course of AD since the follow-up time was limited to 36 months. Possible delayed effects after the end of follow-up such as difficulties in child development or incidence of malignancies could not be investigated. Another limitation was the fact that infection data was retrieved retrospectively on each follow-up visit and that this data was based mainly on the communication with parents or caregivers. Thus, the type, severity and treatments of infections could not be confirmed completely by health record data or from other documented sources.

Conclusions

Topical TAC (0.03% and 0.1% ointments) and TCS (mild and moderate potency) were safe to use in young children with moderate-to-severe atopic dermatitis and showed similar efficacy and generally a good safety profile. No safety issues were observed in the use of the more potent TAC 0.1% ointment in small children with AD[37]. In many cases of moderate-to-severe AD in young children, the use of moderate potency TCS or TAC 0.1% ointment is

necessary to achieve appropriate therapeutic responses[38-39]. As shown in our patient cohort, courses of TAC 0.1% ointment seems to be a good and safe alternative in small children that need recurrent courses of moderate potency TCS[40].

We think that it is reasonable to use TAC 0.03% ointment in younger children when AD is not sufficiently controlled with mild TCS. If needed, TAC 0.1% ointment can be used as a second or third line treatment in children, because it clearly shows higher efficacy than the TAC 0.03% ointment. This is often required especially on lichenified lesions of the extremities. There is a need for more studies on optimal course durations, effects of long-term and intermediate use of TAC 0.03% and 0.1% ointments or moderate potency TCS in young children with moderate-to-severe AD[41-42].

Acknowledgements

In memory of Professor Sakari Reitamo, our teacher, colleague and friend.

We would like to thank research nurse Anssi Koivuselkä for his assistance and medical statistician Paula Bergman, University of Helsinki, Finland, for her very valuable support.

Patient and parental advice, dermatologic treatments and follow up visits were carried out in the Skin and Allergy Hospital, Helsinki University Hospital, Finland. The study was approved by the Ethics Committee of Medicine of the Hospital District of Helsinki and Uusimaa (HUS), Finland. The patients and parents in this manuscript have given written informed consent to publication of their case details.

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Table legends

Table 1: Children with moderate to severe atopic dermatitis; characteristics of the treatment groups at baseline and at the 36 months follow-up visit, group comparisons; TCS = topical corticosteroids; TAC = tacrolimus; AD = atopic dermatitis; statistical significance was set at

P-value of < 0.05; CI = 95% confidence interval; IQR = interquartile range; [a]Pearson's Chi-Square test; [b]Mann-Whitney U-test; [c]Based on the Rajka & Langeland severity score

Table 2: Infections during the 36 months follow-up in children with moderate to severe atopic dermatitis, group comparisons; TCS = topical corticosteroids; TAC = tacrolimus; Statistical significance was set at P-value of < 0.05; CI = 95% confidence interval; IQR = interquartile range; SRI = skin-related infections; [a]Mann-Whitney U-test

Table 3: Growth parameters of children with moderate to severe atopic dermatitis, EASI scores and serum cortisone levels at baseline and at the 36 months follow-up, group comparisons; TCS = topical corticosteroids; TAC = tacrolimus; Statistical significance was set at P-value of < 0.05, CI = 95% confidence interval; IQR = interquartile range, SD = standard deviation; [a]Mann-Whitney U-test; [b]Saari et. al. Finnish growth references for children and adolescents aged 0 to 20 years. Ann Med 2011;43(3):235-248.; [c]Baseline serum cortisone concentration was measured at the 3 months visit

Table 4: Serum concentrations of the cytokines IL-4, IL-10, IL-13, IL-31 and IFN gamma in children with moderate to severe atopic dermatitis, group comparisons; BL = baseline; 36m = at the 36 months follow-up visit; TCS = topical corticosteroids; TAC = tacrolimus; IL = Interleukin, IFN = Interferon; statistical significance was set at P-value of < 0.05; CI = 95% confidence interval; [a]Mann-Whitney U-test

Table S5: Characteristics of patients with pathologic vaccination responses in the topical corticosteroid group (TCS), serum cytokine levels and infections; BL = baseline; 36m = at the 36 months follow-up visit; v/b/o = viral/bacterial/other; r/vr/om/o = respiratory/viral rash/otitis media/other; CodiAb = serum IgG antibodies against *N. diphteriae* (Diphteria); CIteAb = serum IgG antibodies against *C. tetani* (Tetanus), RubeAb = serum IgG antibodies against the Rubella-virus, MorbAb = serum IgG antibodies against the Morbilli-virus,

SpnAbVT = serum IgG antibodies against pneumococcal polysacharides, serotypes 4,6B,9V,14,18C,19F,23F (response to the 23-valent pneumococcal polysaccharide vaccine)

Table S6: Characteristics of patients with pathologic vaccination responses in the topical tacrolimus group (TAC), serum cytokine levels and infections; BL = baseline; 36m = at the 36 months follow up visit; v/b/o = viral/bacterial/other; r/vr/om/o = respiratory/viral rash/otitis media/other; CodiAb = serum IgG antibodies against *N. diphteriae* (Diphteria); ClteAb = serum IgG antibodies against *C. tetani* (Tetanus), RubeAb = serum IgG antibodies against Rubella-virus, MorbAb = serum IgG antibodies against Morbilli-virus, SpnAbVT = serum IgG antibodies against pneumococcal polysacharides, serotypes 4,6B,9V,14,18C,19F,23F (response to the 23-valent pneumococcal polysaccharide vaccine)

Figure legends

Figure 1 a.) Amount of infections during the 36 months follow-up in young children with moderate to severe atopic dermatitis; SRI = skin-related infections; TCS = topical corticosteroids; TAC = topical tacrolimus

Figure 1 b.) Amount of infections during the 36 months follow-up in young children with moderate to severe atopic dermatitis, group comparisons; N.S. = not significant; TCS = topical corticosteroids; TAC = topical tacrolimus

Figure 2 a.) Growth parameters and disease severity at baseline and at the 36 months follow-up in young children with moderate to severe atopic dermatitis; Height, weight and EASI scores; TCS = topical corticosteroids; TAC = topical tacrolimus

Figure 2 b.) Growth parameters and disease severity at baseline and at the 36 months follow-up in young children with moderate to severe atopic dermatitis; group comparisons; TCS = topical corticosteroids; TAC = topical tacrolimus, N.S. = not significant

Figure 3 a.) Serum cortisone levels (nmol/l) at baseline and at the 36 months follow-up in young children with moderate to severe atopic dermatitis; TCS = topical corticosteroids; TAC = topical tacrolimus

Figure 3 b.) Serum cortisone levels (nmol/l) at baseline and at the 36 months follow-up in young children with moderate to severe atopic dermatitis, group comparisons; TCS = topical corticosteroids; TAC = topical tacrolimus, N.S. = not significant

Table 1

	All patients	**TCS group**	**TAC group**	**Comparison TCS vs. TAC group, p-values (CI)**
Amount of patients, n (male/female)	152	75 (44/31)	77 (35/42)	
Mean age at baseline (years)	1.43	1.44	1.42	
Baseline AD severity[c], patients (%)				0.861 (CI 0.495-0.872)[a]
Mild	0	0	0	
Moderate	82 (53.9)	41 (54.6)	41 (53.2)	
Severe	70 (46.1)	34 (45.3)	36 (46.7)	
Amount of dropouts, n (%)	27 (17.7)	12 (16.0)	15 (19.4)	0.575 (CI 0.364-0.673)[a]
Patients with pathologic vaccination responses, n (%)	28 (18.4)	15 (20.0)	13 (16.8)	0.620 (CI 0.387-0.679)[a]
Blood eosinophil count (E9/l)				
Baseline, median, IQR (mean, range)	0.43, 0.25-0.68 (0.55, 0.01-3.09)	0.44, 0.25-0.68 (0.51, 0.05-1.83)	0.41, 0.26-0.69 0.59 (0.01-3.09)	0.954 (CI 0.939-0.995)[b]
36 months, median, IQR (mean, range)	0.37, 0.23-0.71 (0.51, 0.02-3.31)	0.37, 0.21-0.67 (0.51, 0.02-3.31)	0.37, 0.23-0.76 (0.51, 0.09-2.10)	0.563 (CI 0.494-0.651)[b]

Table 2

	All patients, n = 152	**TCS group, n = 75**	**TAC group, n = 77**	**Comparison TCS vs. TAC group, p-values (CI)[a]**
Skin-related infections, total, median, IQR (mean, range)	223, 1.00, 0.00-2.00 (1.50, 0-14)	100, 1.00, 0.00-2.00 (1.35, 0-12)	123, 1.00, 0.00-2.00 (1.64, 0-14)	0.198 (CI 0.152-0.283)
Bacterial SRI	105, 0.00, 0.00-1.00 (0.7, 0-12)	51, 0.00, 0.00-1.00 (0.69, 0-12)	54, 0.00, 0.00-1.00 (0.72, 0-12)	0.890 (CI 0.870-0.959)
Viral SRI	88, 0.00, 0.00-1.00 (0.59, 0-3)	38, 0.00, 0.00-1.00 (0.51, 0-3)	50, 0.00, 0.00-1.00 (0.67, 0-3)	0.155 (CI 0.094-0.208)
Other SRI	31, 0.00, 0.00-0.00 (0.21, 0-5)	11, 0.00, 0.00-0.00 (0.15, 0-3)	20, 0.00, 0.00-0.00 (0.27, 0-5)	0.342 (CI 0.242-0.390)
Other infections total, median, IQR (mean, range)	1357, 9.00, 6.00-11.50 (9.11, 0-30)	671, 9.00, 6.00-11.25 (9.07, 0-26)	686, 8.00, 5.00-12.00 (9.15, 0-30)	0.498 (CI 0.440-0.599)
Respiratory infections	980, 6.00, 3.00-9.00 (6.58, 0-26)	498, 7.00, 3.75-9.00 (6.73, 0-23)	482, 6.00, 3.00-8.00 (6.43, 0-26)	0.267 (CI 0.236-0.382)
Viral rash	74, 0.00, 0.00-1.00 (0.5, 0-5)	33, 0.00, 0.00-1.00 (0.45, 0-2)	41, 0.00, 0.00-1.00 (0.55, 0-5)	0.661 (CI 0.589-0.740)
Otitis media	163, 1.00, 0.00-2.00 (1.09, 0-8)	78, 1.00, 0.00-2.00 (1.05, 0-8)	85, 0.00, 0.00-2.00 (1.13, 0-6)	0.851 (CI 0.838-0.938)
Other infections	139, 1.00, 0.00-1.00 (0.93, 0-9)	61, 1.00, 0.00-1.00 (0.82, 0-4)	78, 1.00, 0.00-2.00 (1.04, 0-9)	0.495 (CI 0.330-0.486)

Table 3

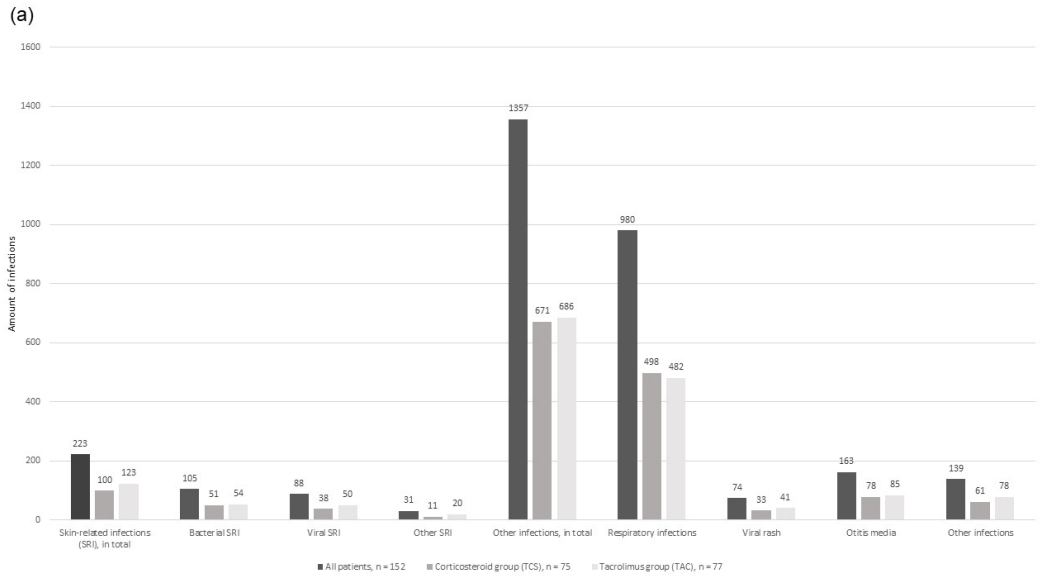
	TCS baseline	TCS at 36 months	TAC baseline	TAC at 36 months	Comparison TCS vs. TAC baseline, p-values (CI)[a]	Comparison TCS vs. TAC at 36 months (p-values)[a]
Height [cm] median, IQR (mean, range)	81.2, 78.2 - 89.5 (83.3, 71.0 - 104.1)	108.5, 104.8 - 113.7 (109.5, 98.4 - 122.0)	82.0, 78.2 - 88.1 (83.0, 66.5 - 100.9)	108.0, 104.9 - 113.0 (108.8, 96.3 - 129.9)	0.972 (CI 0.948 - 0.999)	0.601 (CI 0.541 - 0.696)
Height SD[b] median, IQR (mean, range)	-0.20, -1.20 - 0.1 (-0.40, -3.40 - 3.00)	-0.20, -0.80 - 0.35 (-0.17, -2.30 - 2.20)	-0.75, -1.60 - 0.45 (-0.63, -4.10 - 2.00)	-0.50, -1.30 - 0.30 -0.37, -2.70 - 2.80)	0.258 (CI 0.205 - 0.347)	0.285 (CI 0.248 - 0.397)
Weight [kg] median, IQR (mean, range)	11.00, 10.0 - 12.55 (11.61, 7.9 - 19.5)	18.50, 17.00 - 20.45 (19.93, 15.0-29.4)	11.50, 10.27 - 12.72 (11.73, 6.80 - 17.40)	19.30, 17.00 - 20.40 (18.79, 13.8-24.7)	0.364 (CI 0.349 - 0.506)	0.812 (CI 0.747 - 0.872)
EASI median, IQR (median, range)	9.00, 6.50 - 17.10 (11.35, 0.00 - 31.50)	1.20, 0.00 - 3.40 (2.53, 0.00 - 22.80)	10.1, 6.30 - 17.47 (13.32, 1.50 - 46.00)	0.80, 0.00 - 2.27 2.40 (2.28, 0.00-30.50)	0.451 (CI 0.401 - 0.560)	0.187 (CI 0.169 - 0.304)

Serum cortisone-concentration [nmol/l] median, IQR (mean, range)	168.0, 138.0 - 218.0 (176.4, 55.0 - 319.0)	206.0, 153.5 - 280.0 (224.6, 76.0 - 542.0)	152.0, 123.5 - 187.7 (165.1, 82.0 - 289.0)	170.5, 137.5 - 170.5 (204.9, 65.0 - 579.0)	0.358 (CI 0.254 - 0.404)[c]	0.228 (CI 0.157 - 0.290)

Table 4

	All patients, BL	**All patients, 36 m**	**TCS BL**	**TCS 36m**	**Comparison TCS BL vs. TCS 36m[a]**	**TAC BL**	**TAC 36m**	**Comparison TAC BL vs. TAC 36m[a]**	**Comparison TCS BL vs. TAC BL[a]**	**Comparison TCS 36m vs. TAC 36m[a]**

IL-4 [pg/ml], median, IQR (mean, range)	98.72, 0.00 - 388.05 (1088.00, 0.00 - 16850.00)	71.21, 0.00 - 459.75 (2553.28, 0.00 - 84480.00)	57.59, 0.00 - 333.77 (1052.44, 0.00 - 16850.00)	67.670, 0.00 - 326.67 (1993.10, 0.00 - 84480.00)	**0.908** (CI 0.841 - 0.973)	148.40, 0.00 - 464.70 (1122.92, 0.00 - 14970.00)	72.480, 0.00 - 593.20 (3103.28, 0.00- 77280.00)	**0.889** (CI 0.828 - 0.964)	**0.282** (CI 0.193 - 0.333)	**0.554** (CI 0.507 - 0.664)
IL-10 [pg/ml], median, IQR (mean, range)	227.85, 4.92 - 1539.00 (2863.04, 0.00 - 47410.00)	160.70, 11.84 - 1036.55 (4362.22, 0.00 - 120800.00)	208.70, 0.00 - 1485.00 (3421.24, 0.00 - 47410.00)	169.55, 0.00 - 831.75 (3035.24, 0.00 - 98240.00)	**0.704** (CI 0.546 - 0.761)	239.00, 27.79 - 1543.00 (2325.14, 0.00 - 35640.00)	143.30, 35.27 - 1150.00 (5665.08, 0.00 - 120800.00)	**0.897** (CI 0.795 - 0.945)	**0.662** (CI 0.610 - 0.758)	**0.673** (CI 0.667 - 0.807)
IL-13 [pg/ml], median, IQR (mean, range)	2075.00, 568.55 - 12755.00 (40900.04, 0.00 - 1582000.00)	1657.00, 610.15 - 10234.00 (61175.18, 0.00 - 1792000.00)	1715.50, 378.80 – 5643.75 (46961.67, 0.00 - 1582000.00)	1480.00, 578.07 – 6341.50 (51158.84, 0.00 - 1792000.00)	**0.763** (CI 0.679 - 0.868)	3534.00, 765.90 – 16530.00 (34948.62, 0.00 - 562000.00)	1983.00, 633.90 - 16600.00 (71009.43, (0.00 - 1532000.00)	**0.863** (CI 0.811 - 0.955)	**0.170** (CI 0.106 - 0.223)	**0.283** (CI 0.242 - 0.390)
IL-31 [ng/ml], median, IQR (mean, range)	52.750, 7.52 – 471.20 (723.57, 0.00 - 9380.00)	65.50, 11.45 - 404.55 (1044.29, 0.00- 30680.00)	46.615, 7.41 - 519.80 (888.24, 0.00- 9380.00)	69.735, 3.86 - 309.77 (1352.25, 0.26- 30680.00)	**0.773** (CI 0.589 - 0.798)	77.970, 8.93 - 389.10 (561.887, 0.00 - 8925.00)	65.20, 12.79 - 653.50 (741.92, 0.00 -13865.00)	**0.654** (CI 0.599 - 0.804)	**0.760** (CI 0.667 - 0.807)	**0.721** (CI 0.681 - 0.819)
IFNg [pg/ml], median, IQR (mean, range)	0.00, 0.00 - 39.95 (205.051, 0.00 - 9968.00)	0.00, 0.00 - 15.99 (179.420, 0.00 - 8735.00)	0.00, 0.00 - 56.54 (341.45, 0.00 - 9968.00)	0.00, 0.00 - 17.19 (159.60, 0.00 - 7173.00)	**0.119** (CI 0.98 - 0.275)	0.00, 0.00 - 36.41 (71.120, 0.00 - 763.30)	0.00, 0.00 - 16.98 (198.880, 0.00 - 8735.00)	**0.351** (CI 0.244 - 0.457)	**0.745** (CI 0.739 - 0.866)	**0.900** (CI 0.846 - 0.944)



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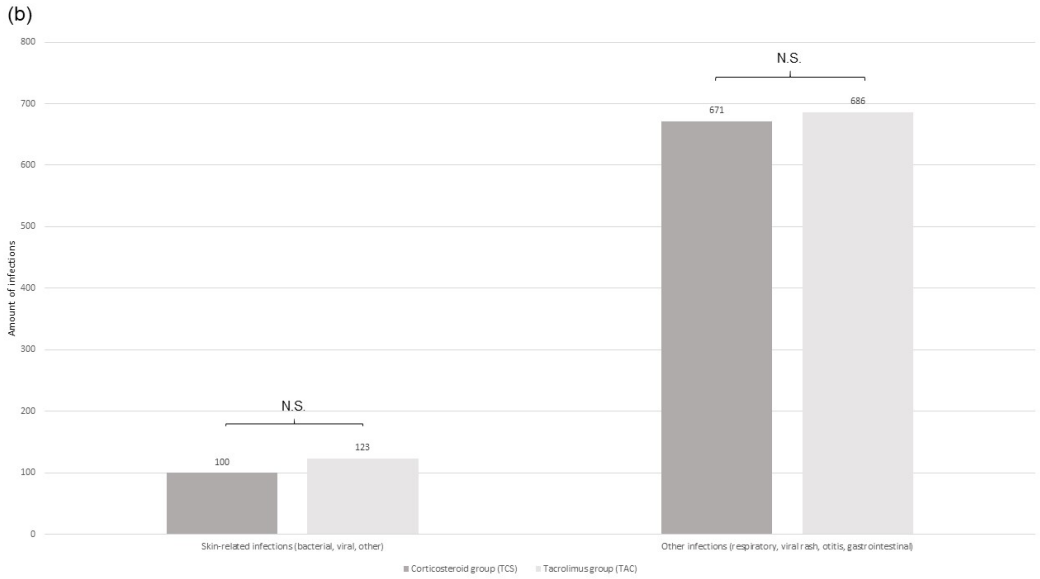

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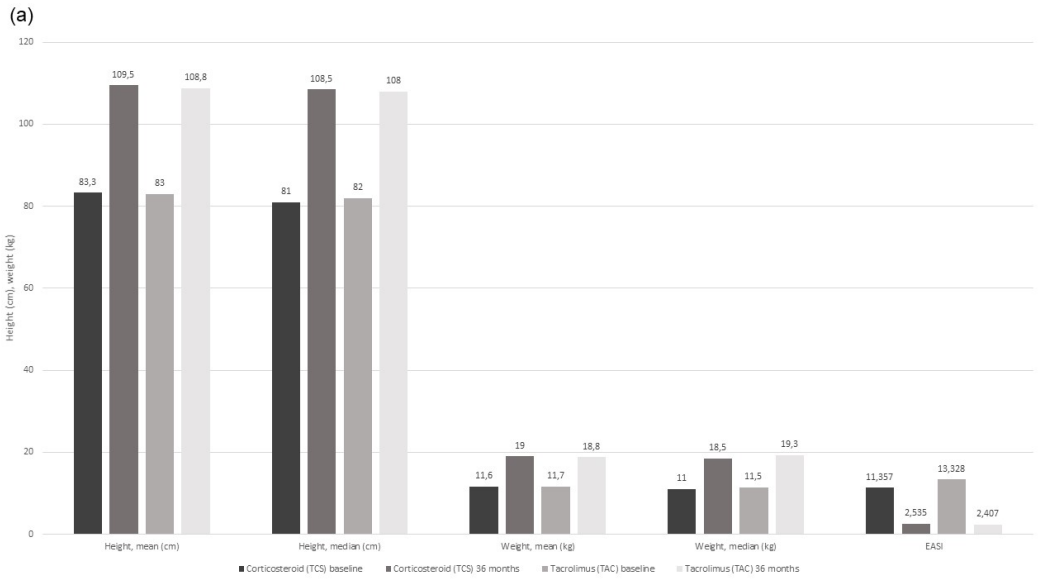

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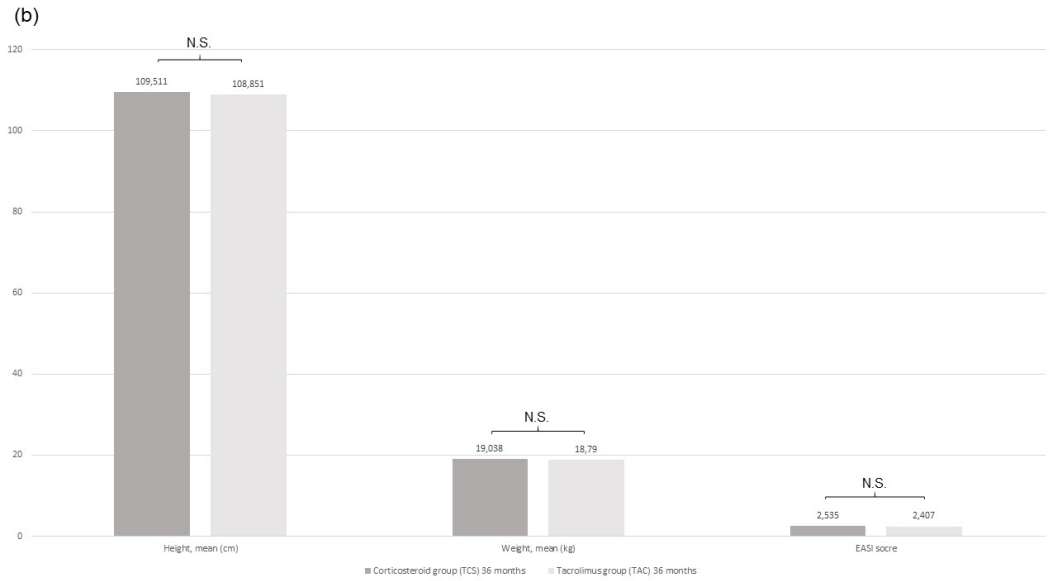

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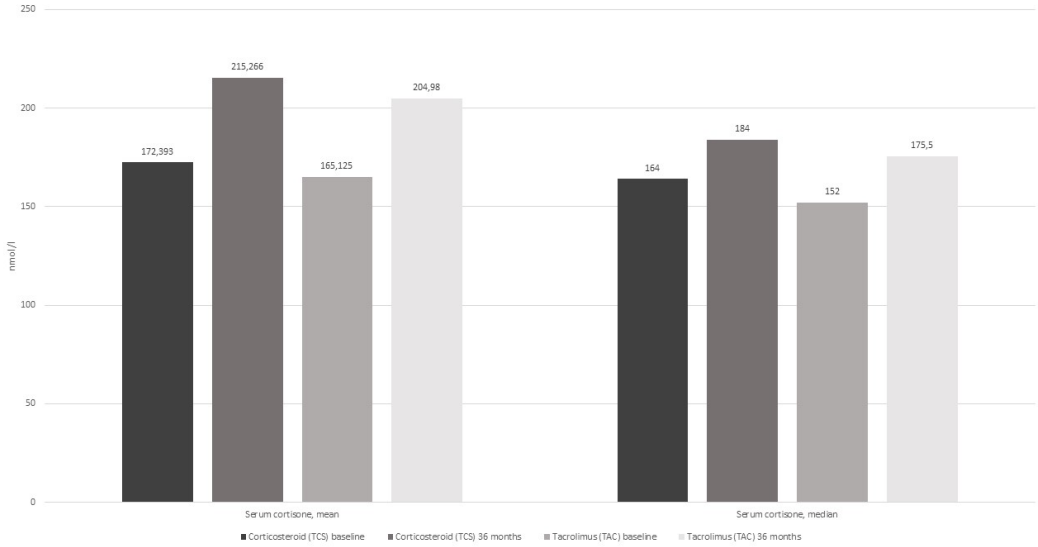

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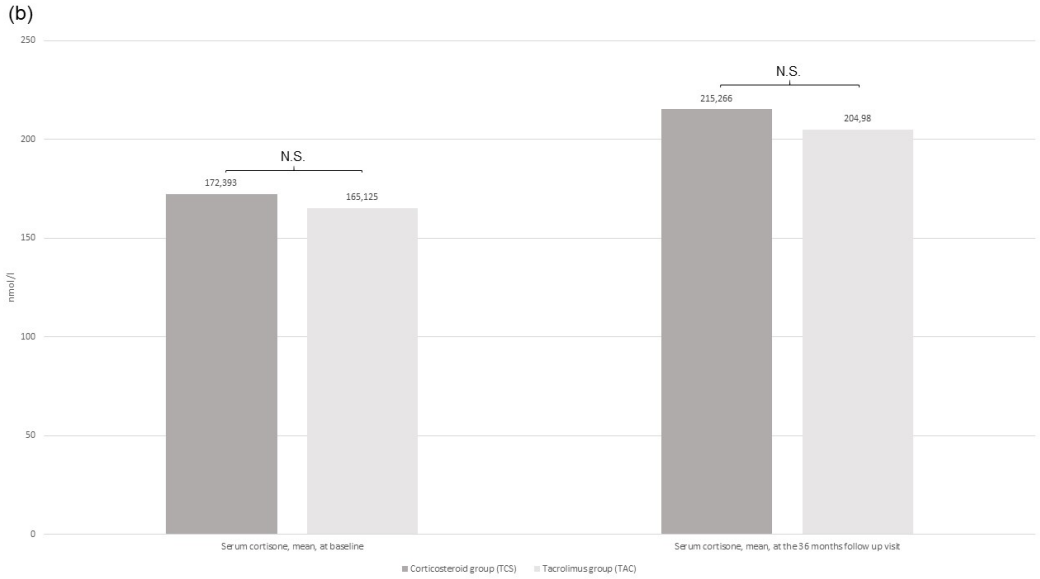

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