

To investigate this possibility, we performed additional predictive analysis of the GRSs from the SHARE consortium in the PIAMA birth cohort by using more liberal *P* value thresholds of 1×10^{-6} and 1×10^{-4} . However, this did not improve genetic risk prediction for asthma ever in the first 8 years of life with combined familial, perinatal, and environmental score and GRS AUC values of 0.65 and 0.65, respectively (see the Results section in this article's Online Repository at www.jacionline.org).

Identifying children at high risk for asthma development is important for prevention and installation of early treatment. However, the GRSs based on SNPs from the largest asthma GWAS did not improve asthma prediction over familial, perinatal, and environmental factors.

Asthma in childhood is a highly heterogeneous disease, with different genes being related to different subtypes of asthma. The fact that we used a more common asthma definition with the selection of all children with respiratory symptoms in the first 8 years of life and no selection on disease specificity could have influenced our results, although using a more strict definition (ie, doctor-diagnosed asthma) led to the same conclusion. For calculation of TAGC consortium GRSs, we added loci specifically related to pediatric asthma. In addition to this, an even more specific (subtype-related) selection of SNPs could be beneficial for generating GRSs to improve the prediction of asthma subtypes.

Asthma has a strong genetic contribution. However, based on the most recent insights in asthma genetics, genetic variants have no added value in predicting nonspecific asthma. This can be explained in several ways. The known heritability of asthma is due to common SNPs of modest effect, resulting in many children carrying risk alleles but not having asthma. Second, although the number of risk SNPs has increased considerably in the past years, these SNPs still explain only a small fraction of asthma heritability. We also acknowledge that a substantially larger study might have yielded a significant but small increase in AUC values after inclusion of the GRS. We show in our article that variation in *P* value thresholds for GRS SNP selection made no difference in asthma prediction. This is underlined by the findings of Zhang et al,⁹ who propose subsequently to focus more on effect size distributions than *P* values for selection of SNPs for disease prediction. These novel methodological approaches might benefit future genetic risk prediction in asthmatic patients. Better prediction might also depend on our ability to define different subtypes of asthma with shared causes. Moreover, better modeling of potential interactions between genes and environmental factors^{E1} might be needed to accurately predict asthma in future studies.

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REFERENCES

1. Sears MR. Predicting asthma outcomes. *J Allergy Clin Immunol* 2015;136:829-36.
2. Caudri D, Wijga A, Schipper CM, Hoekstra M, Postma DS, Koppelman GH, et al. Predicting the long-term prognosis of children with symptoms suggestive of asthma at preschool age. *J Allergy Clin Immunol* 2009;124:903-10.
3. Hafkamp-De Groen E, Lingsma HF, Caudri D, Levie D, Wijga A, Koppelman GH, et al. Predicting asthma in preschool children with asthma-like symptoms: validating and updating the PIAMA risk score. *J Allergy Clin Immunol* 2013;132:1303-10.
4. Colicino S, Munblit D, Minelli C, Custovic A, Cullinan P. Validation of childhood asthma predictive tools: a systematic review. *Clin Exp Allergy* 2019;49:410-8.
5. Demenais F, Margerite-Jeannin P, Barnes KC, Cookson WOC, Altmüller J, Ang W, et al. Multiancestry association study identifies new asthma risk loci that colocalize with immune-cell enhancer marks. *Nat Genet* 2018;50:42-50.
6. Ferreira MA, Vonk JM, Baurecht H, Marenholz I, Tian C, Hoffman JD, et al. Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. *Nat Genet* 2017;49:1752-7.
7. Wijga AH, Kerkhof M, Gehring U, de Jongste JC, Postma DS, Aalberse RC, et al. Cohort profile: the prevention and incidence of asthma and mite allergy (PIAMA) birth cohort. *Int J Epidemiol* 2014;43:527-35.
8. Kull I, Melen E, Alm J, Hallberg J, Svartengren M, van Hage M, et al. Breast-feeding in relation to asthma, lung function, and sensitization in young schoolchildren. *J Allergy Clin Immunol* 2010;125:1013-9.
9. Zhang Y, Qi G, Park JH, Chatterjee N. Estimation of complex effect-size distributions using summary-level statistics from genome-wide association studies across 32 complex traits. *Nat Genet* 2018;50:1318-26.

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Endolysin treatment against *Staphylococcus aureus* in adults with atopic dermatitis: A randomized controlled trial



To the Editor:

Staphylococcus aureus density is increased in many patients with atopic dermatitis (AD) and is thought to contribute to disease pathogenesis, interacting with an altered skin barrier and immunologic changes.¹ *S aureus* might induce or aggravate inflammation through different mechanisms, for example through excretion of virulence factors, even if the *S aureus* overgrowth is primarily caused by other factors.² Current guidelines only recommend antimicrobial therapy directed against *S aureus* in patients with clinically infected AD based on a Cochrane review in

which no clinical benefit of short-term antimicrobial treatment in patients with noninfected AD was found.³

Arguably, long-term antistaphylococcal treatment, such as antibiotics, might reduce symptoms in patients with AD.⁴ However, this is undesired because antibiotics can affect the commensal microbiota and could induce bacterial resistance.⁵ In contrast, long-term treatment of AD with an endolysin that targets only *S aureus* is feasible. It might improve AD symptoms and reduce the use of corticosteroids consecutively.^{2,6} Therefore we aimed to determine the topical corticosteroid (TCS)-sparing effect and safety of 12 weeks of endolysin treatment against *S aureus* in patients with AD.

We performed a double-blind, vehicle-controlled superiority trial (MAAS trial, [ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02840955) NCT02840955) in 100 adult patients with TCS-treated, not clinically infected, moderate-to-severe AD, which was defined by an Eczema Area and Severity Index score of 7.1 to 50.0. After a 2-week run-in period to standardize the TCS treatment with triamcinolone acetonide 0.1% cream, patients were randomly assigned 1:1 to a 12-week intervention with either a topical endolysin against *S aureus* or a vehicle twice daily, followed by an 8-week follow-up period. The vehicle and recombinant chimeric endolysin, Staphefekt SA.100, were provided by Microcos Human Health (Bilthoven, The Netherlands) and topically applied in a cetomacrogol cream. Details on patient inclusion, randomization, and study procedures at 6 assessments are described in a previously published study protocol (see [Fig E1](#) in this article's Online Repository at www.jacionline.org).²

The primary outcome, the TCS-sparing effect of endolysin treatment against *S aureus*, was evaluated by the patients, who registered daily use of a TCS (yes/no) over 12 weeks. Secondary outcomes included differences in TCS use measured in grams, clinical efficacy, quality of life using the Skindex-29, *S aureus* load on the skin, and safety and tolerability of endolysin treatment. Clinical efficacy was measured by using the Eczema Area and Severity Index, Investigators Global Assessment, Patient-Oriented Eczema Measure, and pruritus Numeric Rating Scale and by registration of the number of flares. The *S aureus* load on the skin was assessed by using a semiquantitative culture and quantitative PCR (qPCR; see [Appendix E1](#) in this article's Online Repository at www.jacionline.org).

Generalized linear mixed-effects models for repeated measurements were used to analyze the primary and dichotomous secondary outcomes, and linear mixed-effects models were used for continuous secondary outcomes. Data were analyzed as intention-to-treat and per-protocol. Furthermore, a subgroup analysis was performed in patients with a positive *S aureus* skin culture at 2 time points before start of the intervention.

Eighty-eight (88.0%) patients completed the intervention, and 87 (87.0%) completed follow-up (13% dropout rate, see [Fig E2](#) in this article's Online Repository at www.jacionline.org). Patients' characteristics were comparable between the endolysin and vehicle groups ([Table I](#)). Over the 12-week intervention period (corresponding to 8400 days for 100 patients), patients in the endolysin group used a TCS for 1889 (45.0%) days compared with 1566 (37.3%) days in the vehicle group. There was no statistically significant difference in the probability of TCS use per day between the groups in the intention-to-treat and per-protocol analyses and in the subgroup of *S aureus*-positive patients ($P = .97$, $P = .40$, and $P = .08$, respectively; [Table II](#)). Sensitivity analyses showed no differences in the odds ratio of TCS use per assessment day. Except for the number of doctor-reported AD flares during the intervention period (per-protocol: $n = 2$ in the

endolysin group vs $n = 10$ in the vehicle group; $P = .03$), no statistically significant differences were found in the secondary outcomes after both intention-to-treat and per-protocol analyses (see [Tables E1 and E2](#) in this article's Online Repository at www.jacionline.org). At baseline, 62 (64.6%) patients had positive results for *S aureus* based on skin culture and 24 (24.7%) by qPCR. Both methods showed no significant difference in *S aureus* reduction (see [Tables E3 and E4](#) in this article's Online Repository at www.jacionline.org). During the study, 1 serious adverse event occurred in the endolysin group 8 weeks after the last application of endolysin cream (pleural effusion with hospitalization), which was considered unlikely to be related to the study intervention (see [Tables E5 and E6](#) in this article's Online Repository at www.jacionline.org).

Our results are in accordance with data from a Cochrane review showing no significant effect of short-term anti-*S aureus* therapy in patients with noninfected AD.³ We cannot confirm the positive results of other longer-term studies. However, these studies used broad-spectrum antimicrobials and mainly included patients with signs of bacterial infection.⁴ Patients with clinically infected AD were excluded from our study, and a possible effect of anti-*S aureus* endolysins in this patient group should be determined in future studies.

Several hypotheses could explain our results. First, use of triamcinolone in the run-in phase resulted in a decrease in AD severity ([Table I](#)), which might have masked a possible benefit of endolysin treatment.

Second, daily use of an emollient and good compliance with the treatment could have resulted in a reduction in triamcinolone use in both the endolysin and vehicle groups.⁷ Because AD is a heterogeneous disease, anti-*S aureus* treatment might not be suitable for all patients with AD, indicating the need for subphenotyping. Because only 56% of our study population had 2 consecutive positive *S aureus* skin cultures (indicating persistent colonization) before the start of the intervention, the target population that would probably benefit the most from endolysin treatment was small.

Our data suggest that endolysin treatment has no effect on *S aureus* *in vivo*. However, patients might have been recolonized with *S aureus* from the nose because 73% of them were nasal carriers (data not shown). Alternatively, cetomacrogol as the basis of the endolysin cream might have created a barrier on the skin that prevented the endolysin to reach and subsequently kill *S aureus*. However, some reduction in *S aureus* load would have been expected in both treatment groups because of the use of TCSs and emollients in this study, which both have been shown to reduce the *S aureus* load on the skin.^{8,9} Nonetheless, it is unclear whether complete eradication of *S aureus* is required for clinical improvement because a case series showed a clear clinical improvement without *S aureus* reduction using a qualitative culture in patients with *S aureus*-related dermatoses.⁶ In addition, the discrepant results between culture and qPCR indicate the complexity of the interpretation of *S aureus* testing. Despite the limitations and outcome, this study provides estimates of AD symptoms, use of TCSs, and percentages of persistent *S aureus* carriers that can be used for future clinical studies.

In conclusion, long-term targeted endolysin treatment against *S aureus* in this study was well tolerated but had no TCS-sparing effect in patients with AD. However, an effect cannot be excluded because good compliance with the treatment and concurrent application of TCSs, emollients, or both might have masked a clinical benefit.

TABLE I. Baseline characteristics

	Total (n = 100)	Staphefekt (n = 50)	Vehicle (n = 50)
Age (y), median (IQR)	33.5 (25.5-47.5)	36.5 (25.0-51.0)	32.5 (24.0-44.0)
Male sex, no. (%)	55 (55.0)	24 (48.0)	31 (62.0)
Race, no. (%)			
American Indian or Alaska Native	5 (5.0)	2 (4.0)	3 (6.0)
Asian	10 (10.0)	2 (4.0)	8 (16.0)
Black or African American	8 (8.0)	5 (10.0)	3 (6.0)
White	77 (77.0)	41 (82.0)	36 (72.0)
Atopic disease, no. (%)			
Food allergy	43 (43.0)	18 (36.0)	25 (50.0)
Rhinoconjunctivitis	63 (63.0)	28 (56.0)	35 (70.0)
Asthma	47 (47.0)	25 (50.0)	22 (44.0)
EASI score, median (IQR)			
Screening (V1)	12.9 (9.2-19.0)	13.7 (8.9-19.1)	12.5 (9.2-19.0)
Baseline (V2)	8.0 (5.0-13.5)*	8.3 (5.0-14.7)†	8.0 (4.9-12.9)‡
IGA, median (IQR), baseline (V2)	2.0 (2.0-3.0)*	2.0 (2.0-3.0)†	2.0 (2.0-3.0)‡
POEM, mean (SD), baseline (V2)	12.9 (6.2)*	14.5 (8.3-17.0)†	13.0 (8.0-15.0)‡
Pruritus NRS, median (IQR), baseline (V2)	3.0 (2.0-4.0)*	3.0 (2.0-4.0)†	3.0 (2.0-4.0)‡
Skindex-29, mean (SD), baseline (V2)	35.1 (17.1)*	37.3 (15.3)†	32.9 (18.7)‡
Use of TCSs at screening, no. (%)			
Class 1	3 (3.0)	1 (2.0)	2 (4.0)
Class 2	13 (13.0)	6 (12.0)	7 (14.0)
Class 2-3	11 (11.0)	3 (6.0)	8 (16.0)
Class 3	44 (44.0)	22 (44.0)	22 (44.0)
Class 3-4	3 (3.0)	2 (4.0)	1 (2.0)
Class 4	18 (18.0)	11 (22.0)	7 (14.0)
Unknown	8 (8.0)	6 (12.0)	4 (8.0)
<i>Staphylococcus aureus</i> skin culture,§ no. (%)			
Positive	56 (56.0)	32 (64.0)	24 (48.0)
Intermediate	20 (20.0)	7 (14.0)	13 (26.0)
Negative	20 (20.0)	9 (18.0)	11 (22.0)
Missing	4 (4.0)	2 (4.0)	2 (4.0)

EASI, Eczema Area and Severity Index; IGA, Investigators Global Assessment; IQR, interquartile range; NRS, Numeric Rating Scale; POEM, Patient-Oriented Eczema Measure. Missing values: *n = 3 (3.0%), †n = 2 (4.0%), and ‡n = 1 (2.0%).

§Positive is defined as having a positive culture at visit 1 and visit 2a, intermediate is defined as having 1 positive culture and 1 negative culture at visit 1 and visit 2a, and negative is defined as having 2 negative cultures at visit 1 and visit 2a.

TABLE II. Generalized linear mixed-effects model results for the difference in TCS use per day during the 12-week intervention period

Analysis	Time period or time point in days from baseline	Patients included in analysis		TCS use "yes," no. (%)		OR (95% CI)	P value*
		Staphefekt, no.	Vehicle, no.	Staphefekt, no. (%)	Vehicle, no. (%)		
Intention-to-treat	Intervention						.97
	14	40	37	26 (65.00)	20 (54.05)	0.99 (0.95-1.03)	.49
	42	37	37	26 (70.27)	22 (59.46)	0.99 (0.78-1.24)	.91
	84	30	34	18 (60.00)	21 (61.76)	1.04 (0.87-1.23)	.68
Per-protocol	Intervention						.40
	14	22	21	14 (63.64)	10 (47.62)	1.09 (0.98-1.21)	.10
	42	17	18	12 (70.59)	11 (61.11)	1.36 (0.84-2.20)	.21
	84	13	13	7 (53.85)	5 (38.46)	1.69 (1.14-2.51)	.01
<i>Staphylococcus aureus</i> positive†	Intervention						.08
	14	26	16	17 (65.38)	7 (43.75)	0.87 (0.78-0.96)	.01
	42	22	17	16 (72.73)	8 (47.06)	0.85 (0.49-1.48)	.56
	84	18	15	11 (61.11)	11 (73.33)	1.29 (0.84-1.96)	.24

Sensitivity analyses, performed by adding/subtracting 0.25 times the SD to/from the odds, showed no differences in the odds ratio of TCS use per assessment day. Note: Given the low number of patients using escape medication, ie, TCS with higher potency (n = 5), we did not correct for its use.

OR, Odds ratio.

*Overall effect of endolysin treatment during the intervention period was calculated with a likelihood ratio test, and the effect per time point was calculated with a Wald test with a t-distribution.

†Defined as having a positive culture at both visit 1 and visit 2a (endolysin, n = 32; vehicle, n = 24).

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REFERENCES

1. Totté JE, van der Feltz WT, Hennekam M, van Belkum A, van Zuuren EJ, Pasmans SG. Prevalence and odds of *Staphylococcus aureus* carriage in atopic dermatitis: a systematic review and meta-analysis. *Br J Dermatol* 2016;175:687-95.
2. Totté J, de Wit J, Pardo L, Schuren F, van Doorn M, Pasmans S. Targeted anti-staphylococcal therapy with endolysins in atopic dermatitis and the effect on steroid use, disease severity and the microbiome: study protocol for a randomized controlled trial (MAAS trial). *Trials* 2017;18:404.
3. Bath-Hextall FJ, Birnie AJ, Ravenscroft JC, Williams HC. Interventions to reduce *Staphylococcus aureus* in the management of atopic eczema: an updated Cochrane review. *Br J Dermatol* 2010;163:12-26.
4. Wong SM, Ng TG, Baba R. Efficacy and safety of sodium hypochlorite (bleach) baths in patients with moderate to severe atopic dermatitis in Malaysia. *J Dermatol* 2013;40:874-80.
5. Niebuhr M, Mai U, Kapp A, Werfel T. Antibiotic treatment of cutaneous infections with *Staphylococcus aureus* in patients with atopic dermatitis: current antimicrobial resistances and susceptibilities. *Exp Dermatol* 2008;17:953-7.
6. Totté JEE, van Doorn MB, Pasmans S. Successful treatment of chronic *Staphylococcus aureus*-related dermatoses with the topical endolysin Staphfect SA.100: a report of 3 cases. *Case Rep Dermatol* 2017;9:19-25.
7. Ng JP, Liew HM, Ang SB. Use of emollients in atopic dermatitis. *J Eur Acad Dermatol Venereol* 2015;29:854-7.
8. Angelova-Fischer I, Neufang G, Jung K, Fischer TW, Zillikens D. A randomized, investigator-blinded efficacy assessment study of stand-alone emollient use in mild to moderately severe atopic dermatitis flares. *J Eur Acad Dermatol Venereol* 2014;28(suppl 3):9-15.
9. Gonzalez ME, Schaffer JV, Orlov SJ, Gao Z, Li H, Alekseyenko AV, et al. Cutaneous microbiome effects of fluticasone propionate cream and adjunctive bleach baths in childhood atopic dermatitis. *J Am Acad Dermatol* 2016;75:481-93.e8.

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Janus kinase 1/2 inhibition for the treatment of autoinflammation associated with heterozygous *TNFAIP3* mutation



To the Editor:

Heterozygous loss-of-function pathogenic variants in the TNF- α -induced protein 3 gene (*TNFAIP3*) cause autoinflammation due to haploinsufficiency of A20 protein (HA20). HA20 commonly manifests as severe orogenital ulceration and uveitis.¹

Central nervous system (CNS) inflammation is rare but is reported in patients with HA20, and animal studies have shown that mice with mutant *TNFAIP3* are prone to severe neuroinflammation.^{2,3} Loss of A20 function causes spontaneous cerebral inflammation, as demonstrated by robust microglial activation, reactive astrogliosis, endothelial activation, increased oxidative/nitrosative stress, and expression of nuclear factor κ (NF- κ B)-regulated proinflammatory soluble mediators, such as IL-1 β , TNF, IL-6, and monocyte chemoattractant protein 1 (MCP-1) in the brain.³ CNS involvement as the sole clinical manifestation of heterozygous *TNFAIP3* variants in human subjects has never been described.

We report a case of *TNFAIP3*-mediated autoinflammation manifesting as progressive neuroinflammation. We show that mutated A20 protein did not control interferon-dependent transcription, highlighting an entirely novel mechanism of autoinflammation in patients with HA20. Targeted treatment with the Janus kinase (JAK) inhibitor baricitinib led to marked clinical, radiologic, and immunologic improvement. Methods are provided in this [Methods](#) section in this article's Online Repository at www.jacionline.org.

The proband (III.1) was an 8-year-old girl of nonconsanguineous Pakistani-Indian descent ([Fig 1, A](#)) who presented with left-sided focal seizures and hemiparesis, uveitis, and cognitive decline. She had choreoretinitis from the age of 6 months considered secondary to congenital infection; however, no pathogen had been identified. There was no history of systemic symptoms, and she had occasional mouth ulcers but no genital ulcers. Magnetic resonance imaging of her brain revealed contrast-enhancing T2-hypointense intracranial mass lesions affecting predominantly the grey matter of the paracentral lobule and the thalamus on the left, with surrounding edema ([Fig 1, B-E](#)). Laboratory tests included negative autoantibody screen results; and negative normal complement function study results; targeted *NOD2* genetic testing; a nitroblue tetrazolium test; and immunoglobulin levels, lymphocyte subsets, and cerebrospinal fluid analysis (see [Table E1](#) in this article's Online Repository at www.jacionline.org). She had a modestly increased erythrocyte sedimentation rate of 25 mm/h (normal range, <5 mm/h) and normal C-reactive protein levels (5 mg/L; normal range, <5 mg/L). Brain histology revealed granulomatous inflammation, focal necrosis, and calcification ([Fig 1, F](#)). Tuberculosis was still considered a possible differential diagnosis, and empiric anti-tuberculosis treatment was started (isoniazid/rifampicin/pyrazinamide/moxifloxacin). However, results of brain tissue culture, PCR tests for mycobacteria, and QuantiFERON tests were all negative. There was progression of the mass lesion, worsening of left-sided hemiplegia, and new-onset ataxia.

At that point, the patient was considered to have an unclassified granulomatous neuroinflammatory disorder reminiscent of neurosarcoid. Based on this diagnosis, she received treatment with 2 mg/kg/d prednisolone, weaning to 0.5 mg/kg/d over 6 months and mycophenolate mofetil (1200 mg/m²/d). There was no response to this treatment, with a further increase in size of the brain lesion. Intravenous cyclophosphamide (6 doses, 500-750 mg/m²) was then given, and prednisolone continued at a higher dose (2 mg/kg/d). There was poor response to these treatments, and significant steroid-related side effects were noted (weight gain, Cushingoid appearance, and arterial hypertension).

A second brain biopsy again revealed necrotizing granulomatous inflammation and no evidence of infection. Whole-body

SUPPORTING INFORMATION

Figure E1. Study timeline

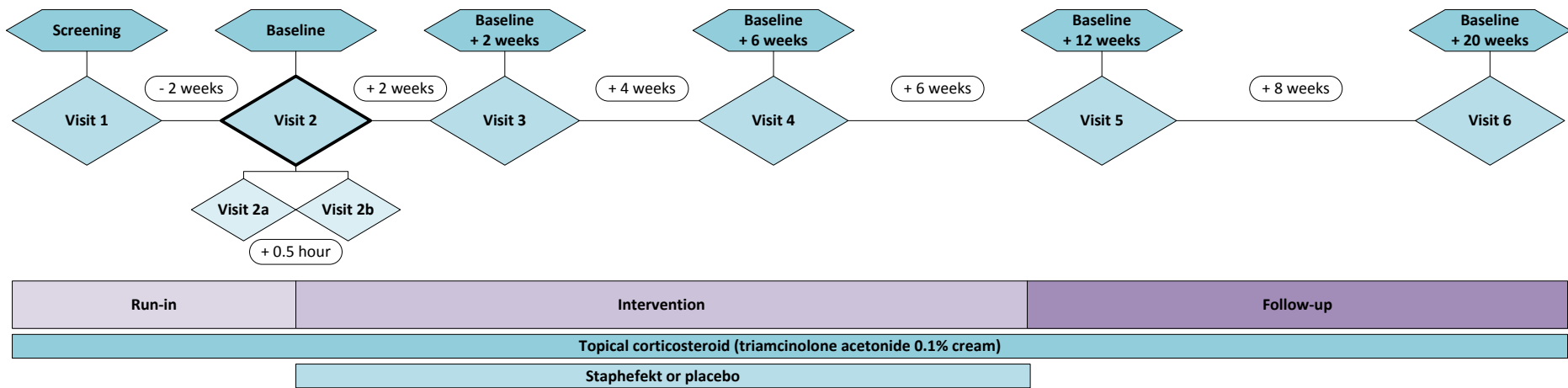
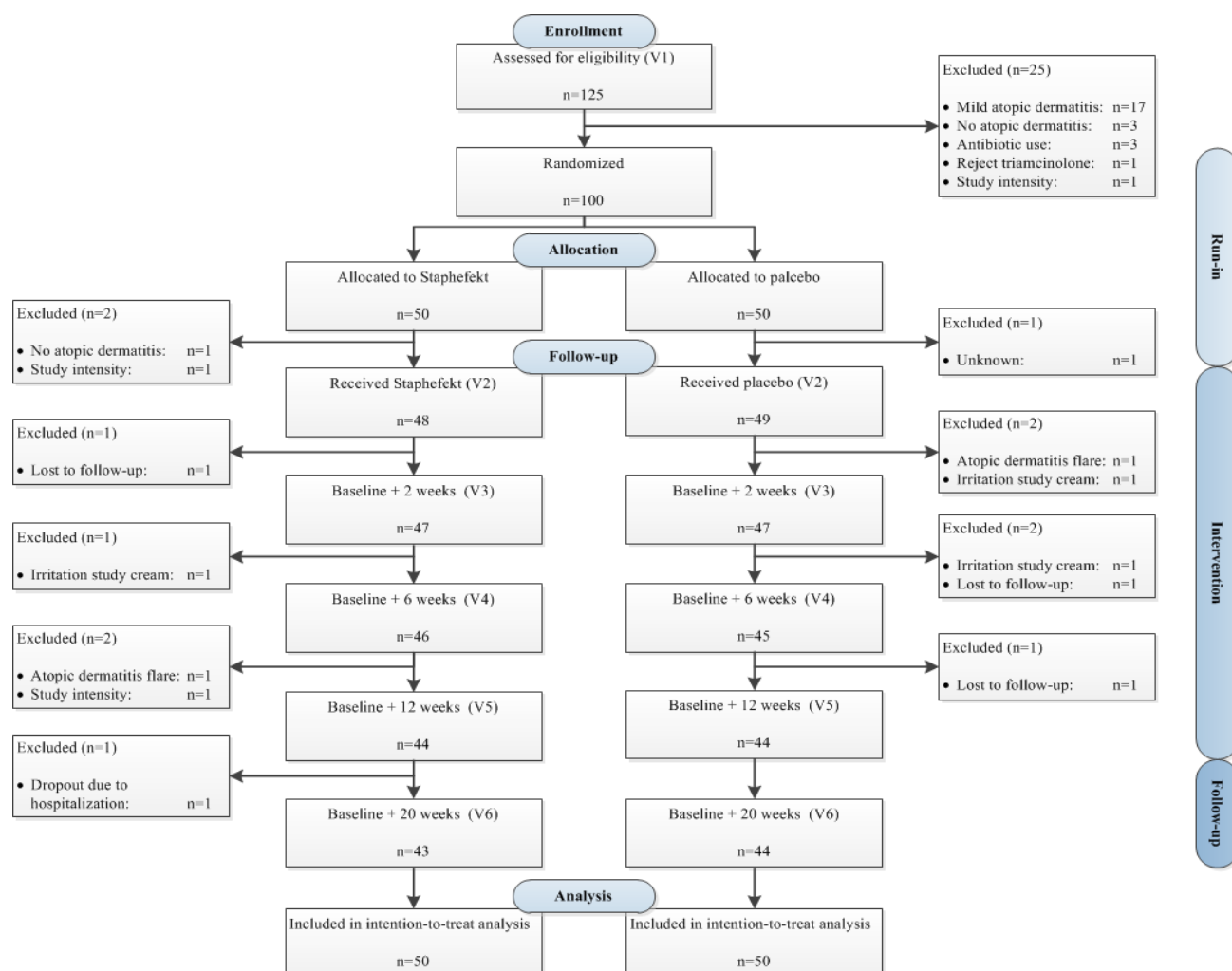


Figure E2. Flowchart of the study design



NOTE 1: One patient in the endolysin group was excluded between visit 1 and visit 2 (reason “No atopic dermatitis”) because of a dermatomycosis that was initially diagnosed as atopic dermatitis.

NOTE 2: Dropout patients had significantly higher atopic dermatitis severity scores (EASI, IGA and POEM), a poorer quality of life and less often rhinoconjunctivitis (data not shown). Dropout rates and lost to follow-up of patients were similar in both treatment groups.

Table E1. Results for the difference in secondary clinical outcomes – intention-to-treat analysis

Outcome	Time period or visit	Staphefekt Endolysin	Vehicle	Difference in change (95% CI) ¹	OR (95% CI) ²	P-value ³
Mean grams/week topical corticosteroid use, median (IQR)	Intervention ⁴	4.5 (2.2-8.7)	3.6 (1.9-7.4)			0.66
	Intervention + follow-up ⁵	5.1 (1.9-8.9)	4.1 (2.3-10.4)			0.91
Proportion of patients who indicate to have used less corticosteroids compared with baseline, n (%)	Intervention + follow-up					0.19
	3	36 (76.60)	34 (72.34)		0.77 (0.45 – 1.29)	0.32
	4	30 (65.22)	27 (60.00)		0.79 (0.51 – 1.22)	0.29
	5	22 (50.00)	20 (45.45)		0.91 (0.61 – 1.38)	0.67
	6	23 (53.49)	13 (29.55)		0.32 (0.21 – 0.51)	<0.001 [†]
Change in EASI from baseline, mean (SD)	Intervention + follow-up					0.57
	3	-0.61 (4.88)	-1.63 (5.09)	1.03 (-1.30 – 3.36)		0.38
	4	0.11 (6.40)	-0.88 (6.22)	1.08 (-1.41 – 3.57)		0.39
	5	0.29 (7.40)	-1.08 (6.16)	1.28 (-1.48 – 4.04)		0.36
	6	0.43 (7.83)	-1.71 (7.26)	2.61 (-0.53 – 5.75)		0.10
IGA corrected for baseline	Intervention + follow-up					0.78
Proportion of patients with a reduction of ≥2 points in IGA from baseline, n (%)	3	0 (0.00)	2 (4.26)			0.46 ⁶
	4	1 (2.17)	2 (4.44)			0.62 ⁶
	5	1 (2.27)	3 (6.82)			0.62 ⁶
	6	1 (2.33)	5 (11.36)			0.20 ⁶
Change in POEM from baseline, mean (SD)	Intervention + follow-up					0.98
	3	-3.15 (4.64)	-2.94 (5.52)	-0.23 (-2.55 – 2.09)		0.84
	4	-2.93 (5.80)	-2.09 (5.43)	-0.54 (-2.87 – 1.78)		0.64
	5	-2.43 (6.13)	-2.02 (6.76)	-0.57 (-3.01 – 1.88)		0.65
	6	-1.79 (6.32)	-1.09 (6.68)	-0.79 (-3.57 – 1.99)		0.57
Change in pruritus NRS from baseline, mean (SD)	Intervention + follow-up					0.56
	3	0.26 (1.57)	0.21 (1.64)	0.03 (-0.63 – 0.69)		0.92
	4	0.20 (1.77)	-0.09 (1.43)	0.37 (-0.31 – 1.05)		0.28
	5	0.23 (1.85)	-0.05 (1.70)	0.32 (-0.43 – 1.07)		0.39
	6	-0.05 (2.23)	0.14 (1.98)	-0.09 (-0.97 – 0.79)		0.84
Proportion of patients with a reduction of ≥2	Intervention +					0.65

⁵Endolysin group n=32, vehicle group n=32.

⁶Since the number of patients per cell are ≤ 5 a Fisher's Exact test per visit was used instead of a Generalized Linear Mixed-Effect model. A *P*-value of 0.0125 will be considered significant after Bonferroni correction.

⁷For every visit from baseline through week 12, 137 visits in endolysin group and 136 visits in vehicle group, it was registered if a flare occurred yes/no.

⁸Endolysin group n=14, vehicle group n=17.

⁹Endolysin group n=8, vehicle group n=5.

¹⁰Rate ratio with 95% CI.

[†]Significant result.

Table E2. Results for the difference in secondary clinical outcomes – per-protocol analysis

Outcome	Time period or visit	Endolysin	Vehicle	Difference in change (95% CI) ¹	OR (95% CI) ²	P-value ³
Mean grams/week topical corticosteroid use, median (IQR)	Intervention ⁴	6.0 (3.3-10.9)	4.1 (2.6-7.9)			0.44
	Intervention + follow-up ⁵	6.1 (4.2-7.3)	5.0 (2.6-14.7)			1.00
Proportion of patients who indicate to have used less corticosteroids compared with baseline, n (%)	Intervention + follow-up					0.40
	3	19 (76.00)	21 (72.41)		0.91 (0.41 – 2.00)	0.81
	4	8 (42.11)	11 (52.38)		1.52 (0.70 – 3.34)	0.30
	5	7 (43.75)	11 (73.33)		3.17 (1.06 – 9.48)	0.04
	6	8 (50.00)	5 (33.33)		0.50 (0.18 – 1.39)	0.18
Change in EASI from baseline, mean (SD)	Intervention + follow-up					0.81
	3	-1.17 (5.70)	-0.64 (4.99)	-0.45 (-3.64 – 2.73)		0.78
	4	-0.58 (7.19)	1.73 (6.14)	-1.95 (-5.55 – 1.65)		0.28
	5	-0.59 (7.79)	-0.71 (4.16)	-0.77 (-4.83 – 3.30)		0.71
	6	-0.94 (6.44)	-0.20 (5.61)	-1.45 (-5.92 – 3.03)		0.52
Change in IGA from baseline	Intervention + follow-up					0.86
Proportion of patients with a reduction of ≥2 points in IGA from baseline, n (%)	3	0 (0.00)	1 (3.45)			1.00 ⁶
	4	0 (0.00)	1 (4.76)			1.00 ⁶
	5	1 (6.25)	1 (6.67)			1.00 ⁶
	6	1 (6.25)	2 (13.33)			0.60 ⁶
Change in POEM from baseline, mean (SD)	Intervention + follow-up					0.77
	3	-4.04 (4.72)	-3.14 (5.78)	-0.91 (-4.11 – 2.30)		0.57
	4	-3.63 (5.56)	-2.76 (6.31)	-0.78 (-4.25 – 2.70)		0.66
	5	-3.06 (5.95)	-2.93 (7.44)	-0.14 (-3.83 – 3.54)		0.94
	6	-3.00 (3.93)	-0.87 (6.55)	-2.13 (-5.82 – 1.55)		0.25
Change in pruritus NRS from baseline, mean (SD)	Intervention + follow-up					0.50
	3	0.08 (1.53)	0.34 (1.88)	-0.24 (-1.13 – 0.65)		0.59
	4	-0.16 (1.34)	-0.10 (1.38)	-0.29 (-1.29 – 0.71)		0.56
	5	0.19 (1.56)	0.00 (1.69)	0.11 (-1.09 – 1.31)		0.85
	6	-0.06 (2.41)	0.80 (1.86)	-0.89 (-2.38 – 0.60)		0.24
Proportion of patients with a reduction of ≥2	Intervention +					0.58

considered significant after Bonferroni correction.

⁷For every visit from baseline through week 12, 60 in endolysin group and 65 in vehicle group, it was registered if a flare occurred yes/no.

⁸Endolysin group n=2, vehicle group n=10.

⁹Endolysin group n=5, vehicle group n=6.

¹⁰Endolysin group n=1, vehicle group n=2.

¹¹No IQR because $n \leq 2$.

¹²AEs that occurred until 8 weeks (follow-up period in intention-to-treat analysis) after the first protocol deviation, i.e. no use of endolysin or vehicle on total skin surface twice daily, were analyzed.

¹³Rate ratio with 95% CI.

[†]Significant result.

Table E3. Results for the difference in reduction of *Staphylococcus aureus* 0.5 hour after first application determined by semi-quantitative culture

Analysis	Decrease in semi-quantitative culture of <i>S. aureus</i> ¹	Endolysin, n (%) ²	Vehicle, n (%) ³	P-value ⁴
Intention-to-treat	Yes	6 (12.5)	4 (8.3)	0.74
	No	42 (87.5)	44 (91.7)	
Abbreviations: <i>S. aureus</i> , <i>Staphylococcus aureus</i> ¹ Decrease is defined as a decrease of at least 1 point on semi-quantitative scale (scale ranges from 0-4). ² Patients included in in analysis in endolysin group: n=49. ³ Patients included in in analysis in vehicle group: n=48. ³ P-values were calculated using a Fisher’s Exact test for categorical data.				

Table E4. Results for the difference in reduction of *Staphylococcus aureus* from baseline determined by qPCR

Analysis	Time period	Log10 reduction in qPCR for <i>S. aureus</i>		P-value ¹
		Endolysin, n (%)	Vehicle, n (%)	
Intention-to-treat ²	Visit 2a to visit 3	3 (6.5) ⁶	4 (8.5) ⁶	1.00
	Visit 2a to visit 5	3 (6.8) ⁷	8 (18.2) ⁷	0.20
Per-protocol ³	Visit 2a to visit 3	2 (7.1)	3 (9.4)	1.00
	Visit 2a to visit 5	1 (6.3)	5 (35.6) ⁸	0.07
<i>Staphylococcus aureus</i> positive ⁴⁻⁶	Visit 2a to visit 3	3 (33.3)	4 (30.8)	1.00
	Visit 2a to visit 5	3 (33.3)	9 (66.7)	0.20
Abbreviations: <i>S. aureus</i> , <i>Staphylococcus aureus</i> ; qPCR, quantitative polymerase chain reaction ¹ P-values were calculated using a Fisher's Exact test for categorical data. ² Patients included in endolysin and vehicle group: visit 2a to visit 3, n=47 and n=47; visit 2a to visit 5, n=44 and n=44. ³ Patients included in endolysin and vehicle group: visit 2a to visit 3, n=25 and n=29; visit 2a to visit 5, n=16 and n=15. ⁴ Patients included in endolysin and vehicle group: visit 2a to visit 3, n=9 and n=13; visit 2a to visit 5, n=9 and n=12. ⁵ Analysis additionally to analyses described in the study protocol. ⁶ <i>Staphylococcus aureus</i> positive is defined as having a positive qPCR at visit 2a (endolysin group n=10, vehicle group n=14). Missing: ⁶ n=1 (2.1%), ⁷ n=1 (2.3%), ⁸ n=1 (6.7%).				

Table E5. Incidence of (non-) Treatment Emergent Adverse Events – Overall and per study phase

	Total (n=100)		Endolysin (n=50)		Vehicle (n=50)	
	Patients, n (%)	Events, n	Patients, n (%)	Events, n	Patients, n (%)	Events, n
Overall (V1-V6)	83 (83.0)	183	43 (86.0)	96	40 (80.0)	87
Run-in (V1-V2)						
At least 1 non-TEAE	21 (21.0)	27	11 (22.0)	14	10 (20.0)	13
At least 1 serious non-TEAE	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
At least 1 non-TEAE leading to study discontinuation	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
At least 1 non-TEAE leading to death	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Intervention (V2-V5)						
At least 1 TEAE	73 (73.0)	125	36 (72.0)	67	37 (74.0)	58
At least 1 serious TEAE	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
At least 1 TEAE leading to study discontinuation	3 (3.0)	3	1 (2.0)	1	2 (4.0)	2
At least 1 TEAE leading to death	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Follow-up (V5-V6)						
At least 1 TEAE	25 (25.0)	31	15 (30.0)	15	10 (20.0)	16
At least 1 serious TEAE	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
At least 1 TEAE leading to study discontinuation	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
At least 1 TEAE leading to death	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Abbreviations: TEAE, Treatment Emergent Adverse Event (adverse events after the first endolysin or vehicle administration); V, visit						

Table E6. Overall incidence of Treatment Emergent Adverse Events specified

	Total (n=100)		Endolysin (n=50)		Placebo (n=50)	
	Patients, n (%)	Events, n	Patients, n (%)	Events, n	Patients, n (%)	Events, n
Any Treatment Emergent Adverse Event	83 (83.0)	183	43 (86.0)	96	40 (80.0)	87
Infectious Diseases (including bacterial, viral and fungal infections)	47 (47.0)	65	26 (52.0)	40	21 (42.0)	25
Common cold	26 (26.0)	29	18 (36.0)	20	8 (16.0)	9
Dental inflammation	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
Ear infection	1 (1.0)	1	0 (0.0)	0	1 (2.0)	1
Influenza	15 (15.0)	15	7 (14.0)	7	8 (16.0)	8
Herpes labialis	5 (5.0)	6	3 (6.0)	4	2 (4.0)	2
Impetiginized dermatitis	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
Joint and muscle inflammation	3 (3.0)	3	2 (4.0)	2	1 (2.0)	1
Ophthalmic infection	7 (7.0)	7	4 (8.0)	4	3 (6.0)	3
Pneumonia	2 (2.0)	2	1 (2.0)	1	1 (2.0)	1
Allergies	6 (6.0)	6	0 (0.0)	0	6 (12.0)	6
Food allergy	1 (1.0)	1	0 (0.0)	0	1 (2.0)	13
Allergic rhinoconjunctivitis	3 (3.0)	3	0 (0.0)	0	3 (6.0)	2
Allergic rash	2 (2.0)	2	0 (0.0)	0	2 (4.0)	2
Metabolic/Endocrine/Nutritional	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Musculoskeletal	2 (2.0)	2	1 (2.0)	1	1 (2.0)	1
Joint and muscle pain	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
Lower back pain	1 (1.0)	1	0 (0.0)	0	1 (2.0)	1
Dermatologic	24 (24.0)	26	13 (26.0)	14	11 (22.0)	12
Culicosis	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
Dermatomycosis	5 (5.0)	5	4 (8.0)	4	1 (2.0)	1
Hyperkeratotic eczema of the hands	2 (2.0)	2	2 (4.0)	2	0 (0.0)	0
Intertrigo	1 (1.0)	1	0 (0.0)	0	1 (2.0)	1
Local dry skin	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
Papules	2 (2.0)	2	2 (4.0)	2	0 (0.0)	0
Paronychia	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
Perioral dermatitis	2 (2.0)	2	0 (0.0)	0	2 (4.0)	2
Pustulosis	6 (6.0)	6	3 (6.0)	3	3 (6.0)	3
Rosacea	2 (2.0)	2	0 (0.0)	0	2 (4.0)	2
Skin lesions of unknown origin	2 (2.0)	2	0 (0.0)	0	2 (4.0)	2
Tinea versicolor	1 (1.0)	1	0 (0.0)	0	1 (2.0)	1

HEENT¹	5 (5.0)	5	2 (4.0)	2	3 (6.0)	3
Increased eye pressure	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
Tinnitus	1 (1.0)	1	0 (0.0)	0	1 (2.0)	1
Tooth extraction	3 (3.0)	3	1 (2.0)	1	2 (4.0)	2
Respiratory	5 (5.0)	5	3 (6.0)	3	2 (4.0)	2
Breathing problems	1 (1.0)	1	0 (0.0)	0	1 (2.0)	1
Dyspnea	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
Exacerbation asthma	2 (2.0)	2	1 (2.0)	1	1 (2.0)	1
Pleural effusion	1 (1.0)	1	1 (2.0)	1	0 (0.0)	0
Cardiovascular	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Gastrointestinal/Hepatic	6 (6.0)	6	3 (6.0)	3	3 (6.0)	3
Gastroenteritis	6 (6.0)	6	3 (6.0)	3	3 (6.0)	3
Genitourinary/Renal	0 (0.0)	0	0 (0.0)	0	0 (0.0)	0
Neurologic	2 (2.0)	2	1 (2.0)	1	1 (2.0)	1
Headache	2 (2.0)	2	1 (2.0)	1	1 (2.0)	1
Psychiatric/Psychosocial	2 (2.0)	2	1 (2.0)	1	1 (2.0)	1
Depression	2 (2.0)	2	1 (2.0)	1	1 (2.0)	1
Gynaecologic/Obstetric	1 (1.0)	1	0 (0.0)	0	1 (2.0)	1
Non-specific menstrual complaints	1 (1.0)	1	0 (0.0)	0	1 (2.0)	1
Skin symptoms after application of study medication	45 (45.0)	48	21 (42.0)	23	24 (48.0)	25
Local reaction after application of study medication (e.g. wounds, itch, irritated skin)	45 (45.0)	48	21 (42.0)	23	24 (48.0)	25
Other	14 (14.0)	15	8 (16.0)	8	6 (12.0)	7
Bruises	1 (1.0)	1	0 (0.0)	0	1 (2.0)	1
Fatigue	2 (2.0)	2	1 (2.0)	1	1 (2.0)	1
Malaise	11 (11.0)	12	7 (14.0)	7	4 (8.0)	5

¹HEENT: Head, Eye, Ear, Nose and Throat

Appendix 1. Microbial methods

a. Microbial sampling methods

Samples of the skin were collected using sterile Copan 490CE.A swabs for culture analysis and skin scrubs for qPCR analysis. Skin swabs were collected from a skin lesion at the first visit, preferably located at the antecubital fold or the popliteal fold. For all consecutive visits the swab was collected from the location chosen at the first visit. The scrubs were collected from the lower arm, adjacent to the antecubital fold according to methods described previously.²³ Scrub samples were stored at -80 °C.

b. Semi-quantitative culture and qPCR for *Staphylococcus aureus*

Bacterial cultures were performed using routine diagnostic culture procedures, using blood agar plates and specific *S. aureus* culture plates (ChromID *S. aureus* Elite agar (SAIDE), Biomérieux, France) for overnight incubation and subsequent species determination by MALDI-TOF (Bruker Daltonics, Bremen, Germany). For DNA isolation 150 uL from the sample was added to 350 uL lysis buffer, 500 uL Phenol (Tris pH 8) and 500 uL 0.1 mm zirconium beads. This mixture was mechanically disrupted by bead beating twice for 2 minutes, followed by centrifuging for 10 minutes at 1690 RCF to separate the aqueous and phenolic phases. The aqueous phase was purified using AGOWA mag Mini DNA isolation kit. After elution, we used qPCR to determine the total load of *S. aureus* with the following primers and probes: 16S-*S.aur*-F1 (5'-GCG AAG AAC CTT ACC AAA TCT TG-3'), 16S-*S.aur*-R1 (5'-TGC ACC ACC TGT CAC TTT GTC-3'), 16S-*S.aur* MGB Taqman® probe (5'-CAT CCT TTG ACA ACT CT-3') with NED™ label.