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A novel molecular disease classifier for psoriasis and eczema

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Abstract

Novel specific therapies for psoriasis and eczema have been developed and they mark a new era in the treatment of these complex inflammatory skin diseases. However, within their broad clinical spectrum, psoriasis and eczema phenotypes overlap making an accurate diagnosis impossible in special cases, not to speak about predicting the clinical outcome of an individual patient. Here, we present a novel robust molecular classifier (MC) consisting of *NOS2* and *CCL27* gene that diagnosed psoriasis and eczema with a sensitivity and specificity of >95 % in a cohort of 129 patients suffering from 1) classical forms, 2) subtypes and 3) clinically and histologically indistinct variants of psoriasis and eczema. *NOS2* and *CCL27* correlated with clinical and histological hallmarks of psoriasis and eczema in a mutually antagonistic way, thus highlighting their biological relevance. In line with this, the MC could be transferred to the level of immunofluorescence stainings for iNOS and CCL27 protein on paraffin-embedded sections, where patients were diagnosed with sensitivity and specificity >88 %. Our MC proved superiority over current gold standard methods to distinguish

psoriasis and eczema and may therefore build the basis for molecular diagnosis of chronic inflammatory skin diseases required to establish personalized medicine in the field.

Key Words

Diagnostic test; NOS2; CCL27; inflammatory skin diseases; precision medicine

Introduction

Genome-wide association studies and modern whole genome expression profiling methods have revolutionized our understanding of the pathogenesis of psoriasis and atopic eczema during the past decades [1-3]. This success has been paralleled by the development of diverse specific therapies targeting cytokines and other mediators of well-characterized signaling pathways that increasingly replace broadly acting immune-suppressive therapies. Secukinumab, an anti-IL-17A antibody for the treatment of psoriasis [4, 5] has been recently approved and other therapeutic options are tested in clinical trials such as dupilumab, an IL-4 receptor antagonist, for the treatment of atopic eczema [6]. Nevertheless, the development of biomarkers to predict therapeutic success - a prerequisite to establish personalized medicine in the field of inflammatory skin diseases – clearly lags behind these achievements. This discrepancy impeding the establishment of precision medicine is mainly due to the heterogeneity of both diseases, already causing difficulties at the level of diagnosis: While typical psoriasis plaques can easily be discriminated from classical atopic eczema, special phenotypes of both diseases overlap and may be diagnosed neither by clinical eye nor by histology. The appearance of erythematous plaques, for example, is shared by both entities and also histological features such as hyperparakeratosis and acanthosis in palmoplantar lesions occur in both diseases, thus complicating diagnostics [7]. In consequence, eczema

patients – when misdiagnosed as psoriasis - may experience impairment of disease if for example, a TNF alpha inhibitor is chosen [8] and psoriasis patients – when misdiagnosed as eczema - are unnecessarily detained from early induction of specific systemic therapy. Hence, there is an unmet need for novel diagnostic tests in the field of inflammatory skin diseases, in particular with regards to their high prevalence [2, 9].

We recently defined characteristic pathways and key players for psoriasis and eczema by analysing a unique patient group which - suffering concomitantly from psoriasis and eczema - represent an excellent model to study inflammatory responses independent of genetic background and environmental influences prior to tissue sampling [10, 11]. Based on these findings, several marker combinations to diagnose psoriasis or eczema were proposed, among them the combination of *NOS2*, the inducible nitric oxidase synthase which produces NO upon stimulation by proinflammatory cytokines [12] and *CCL27*, the cutaneous T-cell attracting chemokine (CTACK) eliciting a crucial role in T cell-mediated inflammation [13]. Here, we validated this molecular classifier for practical clinical use. We demonstrated that this classifier predicted the correct diagnosis on the level of RT-PCR with a test sensitivity and specificity of >95 % as compared to the gold standard diagnostics of histopathology. Moreover, it correctly identified several clinical subtypes of both diseases (n=31) and gave a clear hint on the diagnosis in clinically and histologically unclear patients (n=10) that was in line with the subsequent clinical course. Finally, we challenged our two markers on the protein level by fluorescent double stainings on paraffin-embedded sections evaluated by a multi-step image analysis program. Also here, patients were diagnosed correctly with a specificity and sensitivity higher than 88 %. Allowing classification of complex skin diseases on the basis of only two markers our classifier is on the cutting edge of novel objective diagnostics tools for inflammatory skin diseases.

Material and Methods

Patients and material sampling

In total, 129 patients were enrolled for the study. Patients with plaque psoriasis or eczema (psoriasis, n=45; eczema, n=43) were included into the first cohort of the study. Four patients within this first cohort (n=1 for psoriasis, n=3 for eczema) contributed two biopsies of lesional skin, respectively (n=92 samples). Within this cohort of 88 patients and 92 samples, no autologous healthy skin could be obtained from 2 eczema patients and 1 psoriasis patient (in total 89 samples with corresponding autologous skin). The second cohort consisted of 31 patients with clinical variants and subtypes of eczema and psoriasis, respectively: nummular eczema, n=8, palmoplantar and scalp variants, n=6, erythroderma, n=2, guttate psoriasis, n=6, inverse psoriasis, n=3 and patients with co-existing psoriasis and eczema, n=6. 5 patients showing discrepancies between clinical and histological picture and 5 patients showing both clinical and histological unclear phenotypes build up the third cohort. Mean age of all patients was 47 ± 18.3 years, 57.6 % of patients were male, 36.3 % were smokers and mean body-mass index (BMI) was 27.6 ± 6.3. Twenty-three patients suffering from atopic eczema, 21 patients suffering from psoriasis, and 3 patients suffering from both psoriasis and atopic eczema had been previously published [11]. For all cohorts, patients treated with immune-efficient medication prior to material sampling were excluded from the study if not indicated otherwise (wash-out phase 6 weeks for systemic, 2 weeks for local treatment). Moreover, all patients were deeply analyzed for anamnestic, clinical, histological and laboratory criteria. Severity scores were obtained using the SCORAD and the PASI system, respectively.

Six-mm skin punch biopsies were obtained under local anesthesia from affected skin of all patients and in 119 out of 129 cases also from clinically non-involved autologous skin. Biopsies were divided into two parts: one part for routine histological evaluation and the

remaining part for isolation of total RNA and consecutive RT-PCR (see supplementary material).

Molecular classifier (MC)

RNA from lesional and nonlesional skin was isolated and transcripts of *NOS2* and *CCL27* from lesional and non-involved skin were measured by Real Time PCR. Data were expressed as mRNA fold change, relative to non-involved skin as calibrator. Relative quantification (RQ) was determined and the molecular classifier was then built using logistic regression on the RQ values transformed to the logarithm to the base of 10.

The MC predicts disease state (psoriasis or eczema) directly from transformed RQ values of *NOS2* and *CCL27*. The model was calculated using the generalized linear model function in R, with the family binomial and the logit link function. The model was trained on 88 clear patients and tested with patients of unclear disease state. To infer the robustness of the model a 10-fold cross validation (CV) was performed. The CV result for sensitivity was 98 ± 8.4 % and for specificity 100 ± 0 %.

Immunofluorescence of formalin-fixed paraffin-embedded skin sections

Paraffin mounted sections were dewaxed at 65 °C for 25 min and rehydrated by consecutive washes in Roticlear (Carl Roth GmbH, Germany), isopropanol, ethanol (96% vol/vol and 70% vol/vol, respectively) and distilled H_2O. Antigen retrieval was performed in the pressure cooker with boiling citrate buffer (pH=6) for seven minutes. Sections were blocked with 10 % normal goat serum and 10 % normal donkey serum diluted in antibody diluent (Leica, Germany) for one hour and incubated with primary antibody mix (anti-iNOS antibody,

Novus Biologicals, Colorado, 1: 250, anti-CCL27 antibody, R&D Systems, Minnesota, 1:20) for one hour at room temperature and then overnight. For negative controls, antibody diluent only was applied. After overnight incubation, secondary antibodies (488 goat-anti rabbit antibody, Life Technologies, California, 1:500, and 557 donkey anti-mouse antibody, R&D Systems, Minnesota, 1:500) were applied in the dark for 1 hour. To quench autofluorescence, sections were incubated in 0.1% Sudan Black B, diluted in 70% ethanol vol/vol followed by a washing step with 0.02% Tween 20 diluted in PBS and several changes of dH_2O. After DAPI staining sections were mounted in Vectashield Mounting Medium (Vector Laboratories, Califoria). Stainings were visualized in the blue (DAPI), red (CCL27) and green (iNOS) channel of an Olympus IX73 inverted fluorescence microscope. Image acquisition was corrected for unspecific background autofluorescence using sections stained with secondary antibody only.

Statistical analysis

To correlate expression of *NOS2* and *CCL27* with clinical and histological features, feature data type appropriate statistical tests were used. Statistical significance for categorical features with two levels was determined using a Welch two sample t-test, and for those with more than two levels the analysis of variance (ANOVA) was applied. For features on the interval scale significance was determined using Pearson's product moment correlation coefficient. The term association refers to categorical features; the term correlation refers to features on the interval scale. Only associations with a controlled false discovery rate of less than 10 % were selected. All listed p-values were adjusted using the Benjamini Hochberg procedure unless indicated otherwise. Significance levels were chosen as follows: * =

p<0.05; ** = p<0.01 *** = p<0.001. Results are given as mean ± SD unless indicated otherwise.

Supplementary Material

Additional materials and methods for this manuscript can be found in the supplements.

Results

The molecular classifier (MC) consisting of *NOS2* and *CCL27* precisely separates classical cases of eczema and plaques psoriasis and identifies subtypes of psoriasis and eczema

In a first step, the molecular classifier (MC) consisting of *NOS2* and *CCL27* was validated in a cohort of 85 patients suffering from plaque psoriasis (44 patients, 45 samples) or eczema (41 patients, 44 samples) on the level of RT-PCR. The molecular classifier (MC) was trained using a logistic regression model (Supplementary Figure S1 a). For each sample, disease probability for both eczema and psoriasis was calculated. With a cut-off probability value of 55 % for clear prediction, our test diagnosed 87 out of 89 samples correctly. Compared to histopathology, which was considered as gold standard, test specificity for psoriasis (eczema) was 100 % (97.7%), sensitivity was 97.7 % (100 %) and the AUC (area under the ROC curve) was 0.9929. We tested the MC for robustness using a 10-fold cross validation yielding a specificity for psoriasis (eczema) of 100 % ± 0 % (96% ± 8.4 %), a sensitivity of 96 % ± 8.4 % (100 % ± 0 %) and an AUC of 99 % ± 3.2 %. One psoriasis patient was misclassified as eczema and another psoriasis patient showed a probability of 45 % for psoriasis and could thus not be classified definitely (Figure 1 a). Prediction probabilities of all samples are listed

in Table S1. To clarify if our test would also allow correct predictions if RNA of autologous healthy skin could not be obtained, we calculated a mean Ct value for *18S*, *NOS2* and *CCL27* from non-lesional skin samples of all patients and used these values as calibrators for relative quantification of *NOS2* and *CCL27* transcripts. The recalibrated MC performed with a comparable test sensitivity of 97.8 % and a specificity of 97.8 % for both the diagnosis of psoriasis and eczema (Supplementary Figure S1 b and c, Table S2). Beyond the initial 85 patients, 3 additional patients (in total n=88) without autologous healthy skin could be given an accurate prediction with this recalibrated MC (Supplementary Figure S1 c).

Being able to distinguish clear phenotypes of psoriasis and eczema, we challenged our MC further by applying it to subtypes of psoriasis and eczema (Figure 1 b-g). Therefore, samples of patients with nummular eczema (n= 8), psoriatic and eczematous hand, foot or scalp lesions (n=6), erythroderma (n=2), guttate psoriasis (n=6) and inverse psoriasis (n=3) were tested. Besides, patients who suffered from psoriatic and eczematous lesions concomitantly (n=6) were tested to confirm co-existence of both diseases. Out of the 31 patients in this second cohort, 29 patients were assigned to the correct diagnosis with the MC trained on autologous healthy skin (Figure 1 b-g, Table S3). Only one patient with hand eczema could not be given a clear diagnosis and two patients (1 patient with guttate psoriasis and 1 patient with co-existent psoriasis and eczema) were misclassified according to clinical and histological presentation.

The two markers iNOS and CCL27 separate psoriasis and eczema on protein level using immunofluorescence stainings

As classification of eczema and psoriasis on the level of RT-PCR was feasible without the prerequisite of comparing expression of *NOS2* and *CCL27* to the corresponding baseline

levels in autologous healthy skin, we next examined if the expression of the two markers also classified eczema and psoriasis on the protein level using immunofluorescence stainings on formalin-fixed paraffin-embedded (FFPE) sections. In total, 41 FFPE sections of lesional skin from the first cohort of clear patients were randomly picked and stained for iNOS and CCL27. Immunofluorescence was visualized using an inverted epifluorescence microscope (green channel: iNOS, red channel: CCL27, blue channel: DAPI). Normalized to unspecific background fluorescence, eczema samples were characterized by lower iNOS signal as compared to psoriasis samples (Figure 2 a-d, Supplementary Figure S2 a). Furthermore, CCL27 protein was detected in the nucleus in eczema samples, whereas in psoriasis CCL27 protein was characterized by cytoplasmic distribution (Figure 2 a and b, e-j). To quantify these morphological findings, an image analysis program was established based on the three criteria of mean intensity of green fluorescence (a), Fourier transformation analysis in the red and blue channel (b) as well as convolution analysis using the signals from the red and blue channel (c) (Supplementary figure S2). Samples were plotted in a 3D matrix to visualize the two separate diagnostic groups of eczema and psoriasis (Figure 2 k). 2 out of 18 eczema samples were plotted in the psoriasis cloud and one psoriasis sample out of 23 was plotted in the eczema cloud, resulting in a test specificity for psoriasis (eczema) of 88.9 % (95.7 %) and a test sensitivity for psoriasis (eczema) of 95.7 % (88.9 %).

NOS2 and *CCL27* correlate with well-established characteristics of psoriasis and eczema

As our MC offered clear separation between psoriasis and eczema not only in classical, but also in special phenotypes of psoriatic and eczematous lesions, we validated the discriminatory power of *NOS2* and *CCL27* in separating psoriasis from eczema by using anamnestic, clinical, histological and laboratory hallmarks of psoriasis and eczema. 42

parameters (11 clinical, 9 anamnestic, 15 histological and 7 laboratory characteristics) from all patients were examined for association or correlation with mRNA levels of *NOS2* and *CCL27* (Figure 3, Table S4). We found that *NOS2* levels were significantly associated with histological parameters assigned to psoriasis such as hypogranulosis ($p=1.65x10^{-7}$), microabscess ($p=4.02x10^{-6}$) and dilated dermal capillaries ($p=4.93x10^{-5}$). Moreover, there was a marked positive association of *NOS2* with clinical parameters such as BMI (p=0.012) and infect associated exacerbation (p=0.07). In contrast, *CCL27* was negatively associated with BMI (p=0.029), positively associated with allergic rhinoconjunctivitis (p=0.009) and on histological level negatively associated with dilated dermal capillaries (p=0.005). Apart from *NOS2* levels which showed a trend to be positively associated with PASI (p=n.s.), there was no further association of *NOS2* and *CCL27* with disease scores (Supplementary Figure S3).

MC proves to be a reliable tool for diagnostic purposes

Ultimately, we sought to apply our MC as new diagnostic tool in a third cohort of patients who initially presented for a diagnostic workup and remained unclear on the basis of clinical picture and dermatohistopathology. The first subcohort were five patients (Patients 120-124) who presented with clear clinical phenotype of psoriasis (Supplementary Figure S4*)*, however, in histology the diagnosis of eczema was made. All patients showed good therapeutic response to typical psoriasis treatments (Table S5). Our MC assigned all five cases to the diagnosis of psoriasis and confirmed the decision for psoriasis treatment (Table S6). The second subcohort of five patients remained unclear by both clinical and histological means. Patient 129 (Figure 4, Table S7) suffered from inflammatory skin lesions since early childhood. This 20 year old male suffered from allergic asthma and allergic rhinoconjunctivitis favoring eczema as underlying diagnosis of the skin disease. However,

the skin lesions which were rather on extensor than on flexural surfaces were only slightly itchy, not colonized by staphylococcus aureus and the family history was positive for psoriasis. Besides, histology was clearly consistent with psoriasis showing hyperparakeratosis, acanthosis, psoriasis-like papillomatosis, hypogranulosis, and dilated dermal capillaries. Eventually, under the diagnosis of psoriasis, the patient was prescribed fumaric acid, a well-established psoriasis but not eczema treatment[14]. However, under therapy of fumaric acid the lesions worsened but - under the new working diagnosis of eczema - cleared when therapy was switched to alitretinoin, a well-accepted therapy for palmoplantar eczema[15]. Both immunofluoresence and RT-PCR based MC clearly assigned the patient to the diagnosis of eczema (Table S6). Patient 127, a 78 year old male (Figure 4, Table S7) presented with itchy nummular plaques on feet and arms. Histology showed eczema based on the findings of hyperparakeratosis, hypo and - hypergranulosis, focal spongiosis and a mixed cellular infiltrate. Stable improvement could not be achieved by topical steroids. However, when starting a systemic therapy with fumaric acid, the patient's lesions improved quickly. In line with this, the patient reached 97.7 % probability for psoriasis in our MC (Table S6) and mapped in the field of psoriasis on the immunofluorescence based classifier. The remaining three patients are described in Supplementary Figure S5 and Table S6 and S7.

Discussion

Over the past years, the list of new specific therapeutic agents and candidate molecules for target-oriented therapies in the field of inflammatory skin diseases has broadly increased [16]. In contrast to melanoma research, for example, where the development of new therapeutic agents is paralleled by the establishment of diagnostic markers and risk prediction models [17-

[19] enabling to choose the most efficient therapy according to the patient's individual disease signature, approved diagnostic systems and biomarkers have not yet been established for psoriasis and eczema. Currently, this becomes a major issue, as now various specific therapies for patients with inflammatory skin diseases are available. With our classifier we present a novel diagnostic tool starting at the very beginning of any therapeutic concept, namely at the stage of diagnosis. In a cohort of 129 patients, our RT-PCR based MC correctly predicted psoriasis and eczema with test sensitivities and specificities of more than 95 %. Though most cases of psoriasis and eczema are clinically and histologically distinguishable, clinical phenotypes comprising the efflorescences of plaques, scales and erythema are shared by both entities and diagnoses of eczematized psoriasis or psoriasiform eczema are commonly found in histology [20-22]. Although a small percentage of patients fails to be correctly diagnosed from the very beginning, the total number of unclassified patients may be high due to the high prevalence rates of both diseases with approximately 2-4 % for psoriasis [23] and 2-10 % for eczema in adults [2]. In particular for psoriasis, a systemic inflammatory disease with increased risk rates for cardiovascular incidents [24, 25] rather than a pure dermatological disease with co-morbidities, prompt initiation of psoriasis therapy instead of delaying patients from the most efficient therapy is of high clinical relevance.

In the past, efforts have been made to develop classification systems for psoriasis and eczema using modern gene expression profiling techniques. Comparing mRNA expression signatures, Guttman-Yasky et al found a microarray based class prediction for both diseases using 13 significantly differently expressed genes for keratinocyte differentiation [26]. Another study brought gene expression microarrays of more than 300 samples from 16 different skin diseases together establishing a highly accurate multi-disease classifier [27]. However, reducing the size of these classifiers has remained a challenge mainly due to the complexity and heterogeneity of both diseases. A recent study found IL36γ as a predictor for psoriasis and

eczema thus offering a single-molecule based classifier. However, 6 out of 21 samples could not be classified clearly with this method leaving a substantial diagnostic gap [28]. With our approach of performing intra-individual genome expression analysis in patients affected by both psoriasis and eczema at the same time [10, 11], we were able to decipher disease specific signatures independent of environmental and genetic differences. Hence, the list of interesting candidate genes could be significantly minimized as compared to others and *NOS2* and *CCL27* was discovered as best classifying gene couple. We now showed that a molecular classifier based on these two markers successfully passed several validation levels: Beginning with its application in a large cohort of clear patients, going on to a cohort of subtypes and indistinct cases to its application on protein level, the classifier predicted with high sensitivity and specificity. For five unclear cases, our MC gave a clear prediction that was in line with therapeutic success. To prove the classifier's superiority over current gold standard methods, which are clinical eye and dermatohistopathology, thequestion of what defines a correct diagnosis was answered bychoosing the therapeutic response as main diagnostic criterion as this is eventually the most critical consequence resulting from a given diagnosis. For some unclear cases, response to alitretinoin and fumaric acid were the validation endpoints. Althoughboth are multimodal drugs, their application for either psoriasis or eczema, respectively, is clearly proven and no evidence exists for their beneficial application in the respective other disease. Fumaric acid esters have been found to act as allergic sensitizer triggering eczema [29, 30] and at least a transient increase of eosinophil granulocytes is frequently observed; such an increase would most likely worsen eczema [31]. Alitretinoin in contrast is broadly accepted and approved as therapy for chronic hand eczema [15], but there is no evidence for its successful application in psoriasis. A recent study for pustular psoriasis even found that alitretinoin had no superior effects over placebo [32]. As such our classifier is a

prime example of establishing reliable diagnostic tools by thorough validation of new parameters using new approaches and techniques.

The high sensitivity and specificity of our molecular test is based on its two components *NOS2* and *CCL27* which - working as mutually complementing couple - mirror the complex disease signature of psoriasis and eczema. *NOS2* is known to be a key player for metabolic and inflammatory processes in its function as NO producer [12]. We corroborated these findings as *NOS2* expression in lesional skin significantly correlated positively with patients' body-mass index. Moreover, in line with the findings that *NOS2* is significantly upregulated in psoriatic lesional skin compared to healthy skin or eczema [33-35] we showed that *NOS2* expression was highly associated with hallmarks of psoriasis such as hypogranulosis and neutrophils, but negatively associated with eosinophils and spongiosis which are characteristics of eczema. In contrast, levels of *CCL27* were shown to be positively associated with asthma and allergic rhinoconjunctivitis. This finding strengthens previous results showing correlation of *CCL27* serum levels with clinical severity score of eczema [36] , enhanced contact hypersensitivity to Th2, but not Th1 stimuli in CCL27-transgenic mice [37] and lower mRNA levels of CCL27 in psoriasis than in eczema [38]. More importantly, both markers reflect disease specific parameters beyond established clinical criteria and histological parameters. Thus, the presented diagnostic tool is robust and at the same time superior than current gold standard diagnostic methods in giving the correct diagnosis for overlapping phenotypes. Though our classifier clearly assigned each sample to either the diagnosis of eczema and psoriasis, this does not exclude that due to the heterogeneity and complexity of both diseases, there might be overlapping conditions, where e.g. a psoriasis can show hallmarks of eczema when it is manipulated by scratching. In summary, our proposed classifier is the first of its kind characterized by both small size and high test sensitivity and

specificity and could build the basis for establishing personalized medicine in the field of inflammatory skin diseases.

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Author contribution

NG, FL, AA, JT and SE performed experiments. LK, NM and FT established the RT-PCR based molecular classifier and the correlation of *NOS2* and *CCL27* with clinical parameter. SS established the molecular classifier on the level of immunofluorescence. RF performed histopathological examination. SW and AB provided samples. JR, CSW and TB critically revised the paper. SE, KE and NG designed the study. NG and KE wrote the paper.

Ethics approval

All patients gave their written consent to participate in the study, and the study was approved by the local ethical committee (project number 2773/10).

Conflict of Interests

The authors have declared no conflicting interests.

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Figure legends

Figure 1: **Molecular classifier (MC) for psoriasis and eczema on the level of RT-PCR tested on histologically and clinically clear cases of plaque psoriasis and eczema as well as on subtypes of both diseases.** First, classical cases of plaque psoriasis (indicated in red circles) and eczema (indicated in blue circles) were validated on MC (a), n= 89 samples. Then, MC was applied to subtypes, n=31, including nummular eczema (b), hand/feet and scalp lesions (c), erythroderma (d), guttate psoriasis (e), inverse psoriasis (f) and patients suffering from co-existing psoriasis and eczema (g). In (b-g), samples are represented as framed colored circles, grey dots in the background of (b-g) represent the clear cases of eczema and psoriasis from (a). Prediction probabilities for both diseases are indicated on upper and lower sides of each graph. Samples with missing corresponding autologous skin are depicted as triangles.

Figure 2: **Analysis of the two markers iNOS and CCL27 on protein level including multi-step image analysis of immunofluorescent stainings.** Immunofluorescent stainings of iNOS (green) and CCL27 (red) on FFPE sections of psoriasis (a) and eczema (b). Higher magnification view of boxes in (a) and (b) are shown in (c-j). iNOS expression is more prominent in psoriasis, n=23, (c) as compared to eczema, n=18 (d). CCL27 shows nuclear localization in eczema (h-j) as compared to psoriasis where CCL27 is localized in the cytoplasm (e-g). Insets in (e) and (h) highlight distribution differences of CCL27 and are at a magnification of 2x. Qualitative and quantitative differences of staining patterns in psoriasis and eczema (c-j) are quantified by image analysis based on the three criteria Fourier transformation, convolution and intensity of green fluorescent signal ($\sum m_{green}$). 3D plot based on these three criteria shows psoriasis (red) and eczema (blue) samples (k). Scale bars in (a) and (b): 100µm. Scale bars in (c-j): 50 µm.

Figure 3: **Correlation of *NOS2* and *CCL27* with hallmarks of psoriasis and eczema**. Validation of *NOS2* and *CCL27* as markers using anamnestic, clinical and laboratory parameters (all three indicated in dark blue) as well as histological parameters (indicated in light blue) (a). Positive associations are indicated as green lines, negative associations as red lines. Levels of significance are represented by the size of lines with thick lines indicating high significance levels. n1 = feature present, n2= feature not present. The most significant associations for both *CCL27* and *NOS2* are shown separately in (b).

Figure 4: **Application of MC on clinically and histologically unclear patients.** Patient 129 was diagnosed as psoriasis based on clinical picture (a-c) and histology (d, e) but both RT-PCR (f) and immunofluorescence based MC (g, h) diagnosed eczema in this patient which was consistent with the patient's therapeutic course. Patient 127 was diagnosed as eczema as histology showed eczema (d, e) and clinical picture was unclear (a-c). Both RT-PCR (f) and immunofluorescence based MC (g, h) classified the patient as psoriasis which was in line with his therapeutic response to fumaric acid. Circles indicate the patients' probability when MC trained on autologous healthy skin was applied, Triangles in F indicate the patients' probability when *NOS2* and *CCL27* are normalized to *NOS2*, *CCL27* and *18S* of pooled healthy skin. Scale bars: 100 µm in d, 50 µm in e and g.

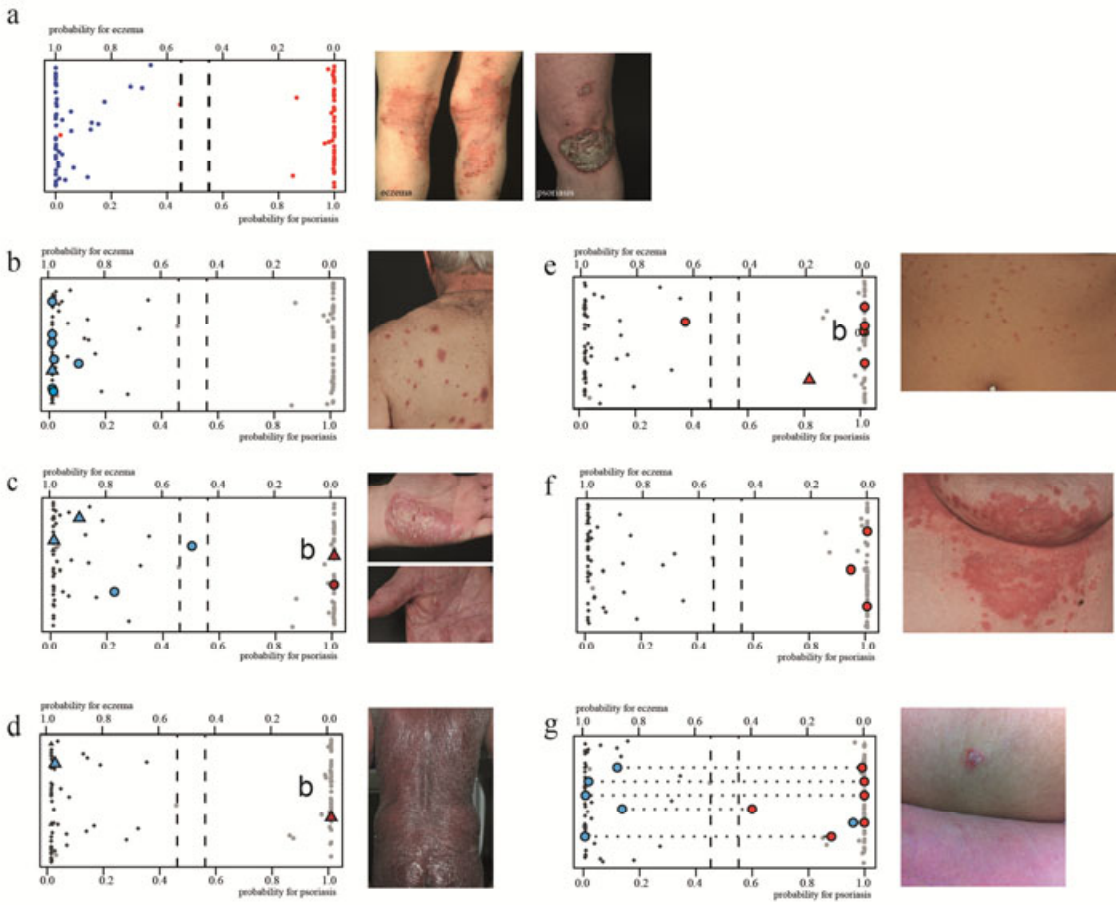

Figure 1

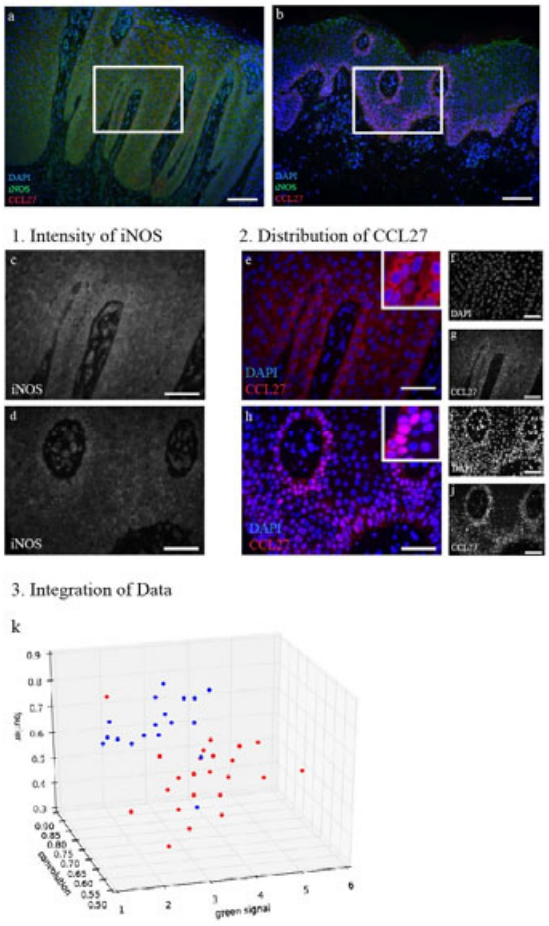

Figure 2

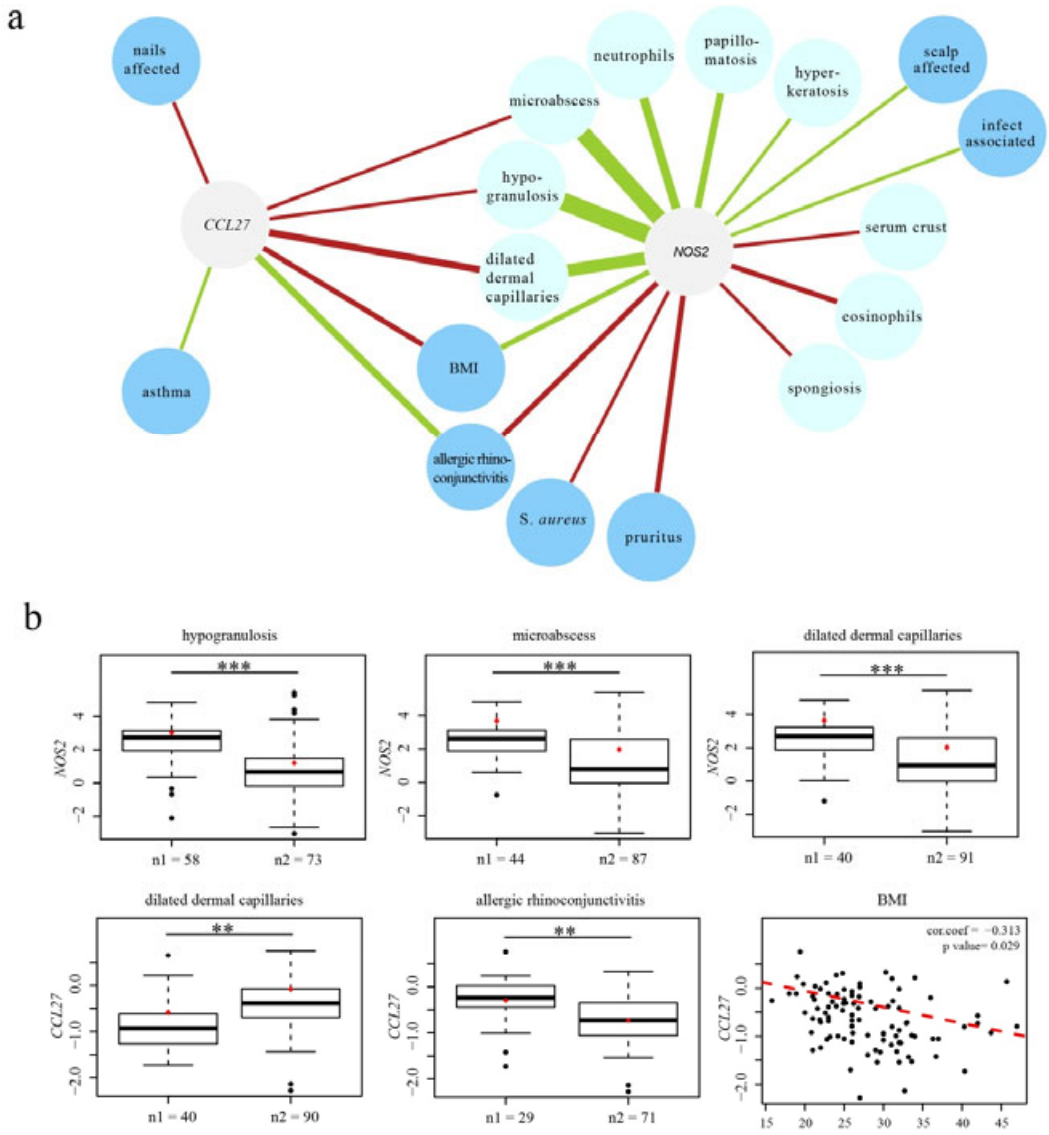

Figure 3

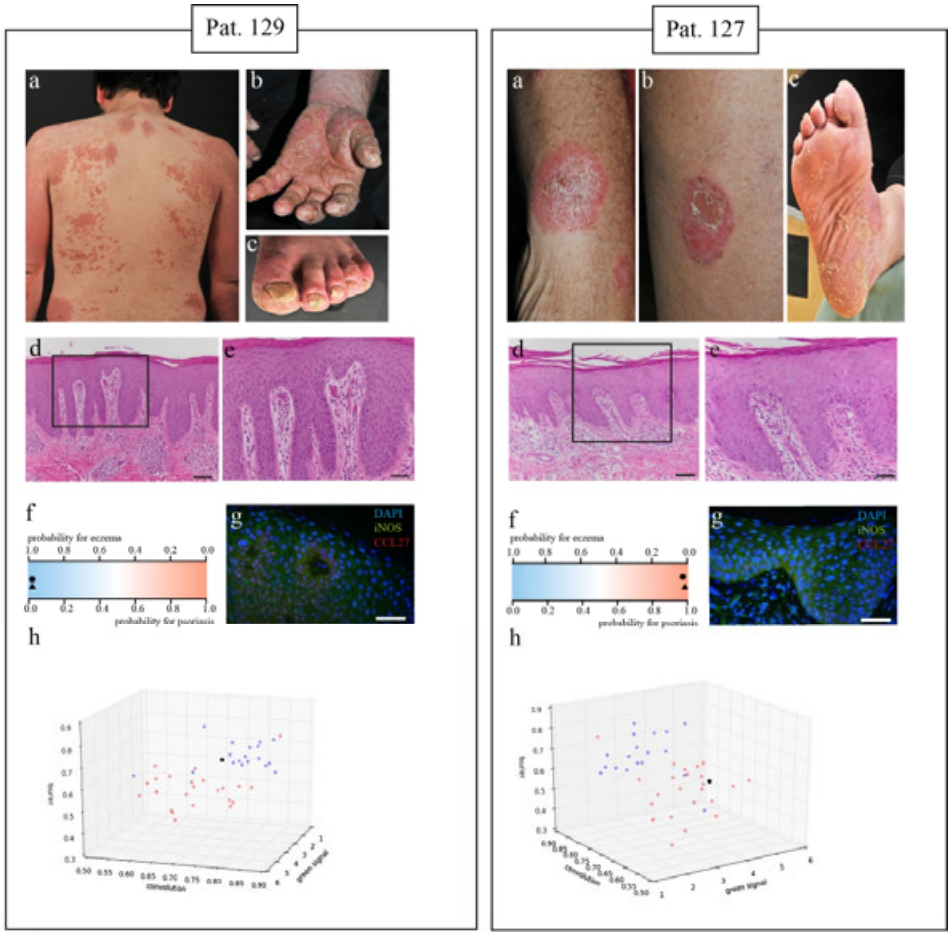

Figure 4