titre ≤1:16.[4] Titres up to 1:512 were also observed.[4] Detection of the prozone effect thus requires to test both undiluted and diluted sera to prevent missed diagnoses.[6] Optimal dilutions to use are still debated, although dilutions of 1:8 and 1:16 have been reported as optimal in the literature.[4,7,8] Other factors may also contribute to the prozone phenomenon, such as low centrifuge temperatures.[9]

In conclusion, in case of a negative RPR test result and discordance with the other syphilis diagnostic tests, RPR should be repeated with diluted serum in order to reach the correct diagnosis. Further evidence is needed to provide specific recommendations for optimal serum titrations, although these may differ between automated and manual nontreponemal testing. Finally, our case highlights the importance of careful skin examination that must be routinely performed in individuals undergoing STI screening, especially in the current era of syphilis epidemic.[10]

Acknowledgement

The patient in this manuscript has given written informed consent to publication of the case details.

Conflict of interest

Nothing to disclose.

Funding source

None.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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DOI: 10.1111/jdv.18089

Reduction of cutaneous advanced glycation end products levels after effective psoriasis treatment

Dear Editor,

Psoriasis is a chronic immune-mediated inflammatory disease associated with various cardio-metabolic disorders.[1,2] A direct association between severity of chronic plaque psoriasis and skin and serum-advanced glycation end product (AGE) levels has been observed.[3–5] AGEs comprise different compounds formed non-enzymatically on proteins when reducing sugars react to amino acid residues. AGE accumulation occurs physiologically through ageing as a result of chronic low-grade inflammation, and it is markedly amplified in type 2 diabetes, obesity, metabolic syndrome, rheumatoid arthritis and fatty liver disease, consequently to hyperglycaemia, hyperlipidaemia and oxidative stress.[3] AGEs elicit biological function by activating a membrane receptor, which is expressed on the surface of inflammatory, endothelial and epithelial cells, and promotes innate immunity.[6] AGEs may be also involved in the pathogenesis of psoriasis.[3,7] Cutaneous AGEs reflect systemic AGE accumulation, and can be easily measured as skin autofluorescence (SA) by a non-invasive, standardized and reproducible technique.[8]

The aim of this observational study was to assess whether treatment of psoriasis with different biologics is associated with reduction in the levels of cutaneous AGEs. Variations in SA was measured in adult patients affected by moderate-to-severe chronic plaque psoriasis (Psoriasis Area and Severity Index, PASI ≥10) following 3 and 6 months of treatment with the anti-TNF-α drug, adalimumab, IL-17 or IL-23 inhibitors. Patients affected by psoriatic arthritis, smoking habit, diabetes, dyslipidaemia, hypercholesterolaemia, hypertension, any systemic inflammatory, metabolic or autoimmune diseases, were excluded from the study. Clinical and laboratory data collected included age, sex, body mass index, disease duration, special sites of involvement, serum C-reactive protein and glycated haemoglobin (HbA1c) levels, PASI and SA at baseline and at 3- and 6-months follow-

Table 1 Clinical and laboratory characteristics of the patients at the baseline and biological drugs used

	***N* = 79**
Sex, male, *n* (%)	55 (69.6)
Age, median (IQR)	54 (40–64)
Body mass index, mean ± SD	27.01 ± 5.01
Duration, years, mean ± SD	19.70 ± 10.10
C reactive protein (mg/dL), mean ± SD	2.58 ± 2.56
Hb1Ac (%), mean ± SD	5.91 ± 1.75
Anti-TNF-α (Adalimumab)	48
Anti-IL-17 (Ixekizumab, brodalumab)	10
Anti-IL-23 (Risankizumab, tildrakizumab, guselkumab)	21

Descriptive variables are presented as mean value ± standard deviation or median and interquartile range when appropriate. SD, standard deviation; IQR, interquartile range.

up. SA was measured as arbitrary unit (a.u.) with the AGE Reader mu® (Diagnoptics Technologies B.V., Groningen, the Netherlands) in normal appearing skin of the volar forearm. Mean SA at the baseline vs SA at 3- and 6-months control visits were compared with paired sample *t*-test, and normal distribution was assessed with Shapiro–Wilk test. Association between SA and PASI variation was tested using Pearson correlation coefficients and multivariate linear regression. *P*-values < 0.05 were considered statistically significant. Statistical analyses were performed using Stata version 13 (Stata Corp, College Station, TX, USA). A total of 79 patients were recruited, including 48 (61%), 10 (13%) and 21 (27%) receiving adalimumab, IL-17 or IL-23 inhibitors, respectively (Table 1). The mean PASI diminished from 15.5 ± 7.4 at the baseline, to 7.4 ± 4.0 at 3 months ($P < 0.001$), and to 2.5 ± 2.9 after 6 months ($P < 0.001$). The mean SA decreased from 2.8 ± 0.9 a.u. at the baseline, to a small but significant reduction at 3 months (2.6 ± 0.8, $P < 0.001$), and to 1.8 ± 0.8 after 6 months ($P < 0.001$; Fig. 1). The reduction in SA correlated with PASI variation, even adjusting for age, sex and disease duration (β = 0.54; 95% CI: 0.47–0.82, $P < 0.001$). Taken collectively, patients receiving IL-17 and IL-23 inhibitors showed a stronger PASI improvement (87.9% vs. 72.8%; $P = 0.003$) and a higher AGEs reduction compared to those treated with adalimumab (39.9% vs. 32.1%; $P = 0.046$). Only two patients showed no reduction in SA. HbA1c reduced at 6 months but not significantly.

Previous studies have shown a reduction in SA in patients with psoriasis or psoriatic arthritis treated with adalimumab,[9] or in patients under remission.[7] In this study, we show that a marked and sustained improvement of psoriasis with biologics, particularly anti-IL-17 and anti-IL-23, is associated with significant reduction in cutaneous AGEs. The study was open-label and non-randomized, but the patients were highly homogeneous. Although we did not measure blood AGE levels, AGEs quantified in the skin are believed to be a proxy of AGEs present in non-skin tissues.[3,6] Therefore, the reduction in cutaneous AGEs levels by effective treatments with biologics may have implications for the prevention of cardio-metabolic associated with psoriasis.[4,6]

Conflict of interest

The authors have no conflict of interest to declare.

Funding source

This article has no funding source.

Data availability statement

Data available on request from the authors.

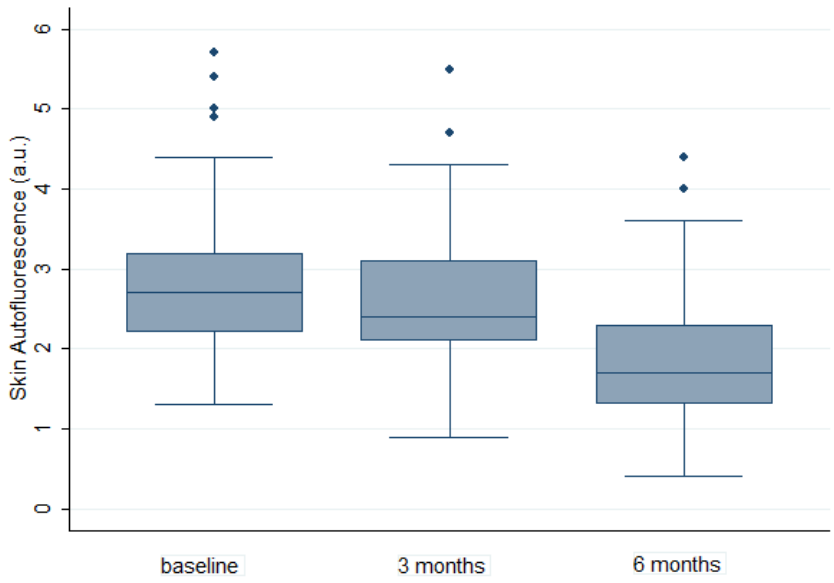

Figure 1 Box-plot showing reduction in skin autofluorescence (a.u.) after 3 months ($P < 0.001$), and 6 months ($P < 0.001$) of treatments with biological drugs.

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DOI: 10.1111/jdv.18090

Phenotypic alleviation in *LAMB3*-mutated severe junctional epidermolysis bullosa

Dear Editors,

Autosomal recessively inherited null mutations in the *LAMA3*, *LAMB3* or *LAMC2* genes, each coding for a subunit of the extracellular adhesion protein laminin-332 (LM-332), cause severe junctional epidermolysis bullosa (JEB). Total absence of functional protein leads to extensive blistering of skin and mucous membranes and usually causes early demise within the first two years of life.[1] Genotype–phenotype correlations in JEB suggest that even small amounts of residual protein expression can significantly improve the phenotype.[1,2]

We report here a case of a female patient, who first presented at 2 years of age with severe skin involvement since birth, enamel hypoplasia, corneal erosions, laryngeal synechiae with hoarseness and chronic inspiratory stridor, vesicoureteral reflux disease and chronic anaemia. Family history for skin diseases was negative for her non-consanguineous parents and her four siblings. After obtaining informed consent from her parents, immunofluorescence (IF) antigen mapping of lesional skin, using antibodies specific for the α3, β3 and γ2 chains of LM-332, demonstrated subepidermal blistering and total absence of LAMB3 expression along the dermoepidermal junction (Fig. 1a), while staining patterns of the α- and γ-chains were normal. DNA sequencing of peripheral blood genomic DNA (gDNA) additionally revealed two heterozygous null mutations consistent with severe JEB (sJEB; Fig. 2a). However, our patient was in a surprisingly good general condition and nutritional status (body-mass-index 13 at 2 years of age) compared to prototypic cases of sJEB. Moreover, the disease exhibited a stable and unexpectedly moderate course, with large skin areas remaining permanently free of blisters from birth until the current age of 8 years (Fig. 1b–d).

To reassess the molecular basis of this intriguing phenotype, a biopsy was taken from hitherto uninvolved, non-blistering skin of the left thigh. Although IF staining confirmed the previously observed LM-332-negative expression profile, residual LAMB3 protein could be detected by western blot analysis of protein lysates from cultured keratinocytes isolated from the biopsy (Fig. 1e). Subsequent laser microdissection (LMD) of keratinocytes from tissue sections, followed by next generation sequencing (NGS) of gDNA from the LMD-isolated samples, confirmed the presence of the paternal mutation c.430C>T (p.R144*), with 42% in heterozygous state. In contrast, the maternal duplication c.1969_1970dupTC, as well as a maternal SNP (Single Nucleotide Polymorphism, c.384C>T; p.P128P), could not be detected in DNA from the LMD-captured cells as compared to peripheral blood gDNA (Fig. 2b,c). The distribution of the paternal mutation and SNP on different alleles was confirmed by cloning of the amplified exon 6 PCR fragments into the StrataClone vector (Agilent) and Sanger sequencing. Sanger sequencing also excluded the presence of SNPs at the primer binding sites of the NGS panel, which could result in allelic dropout by this analysis method. Furthermore, subsequent analysis of total RNA isolated from uninvolved skin by RT-PCR, using primers complementary to exons 5 and 7, as well as exons 13 and 16, did not result in additional amplification products of unexpected sizes, arguing against aberrant splicing as an underlying cause for the alleviated phenotype.

Common molecular mechanisms of phenotypic alleviation in genetic diseases include loss of heterozygosity (LOH), gene deletion, nondisjunction mutations, as well as nonsense codon readthrough and RNA *trans*-splicing. In the JEB variant with pyloric atresia, amelioration has also been reported in the context of splice mutations and mRNA rescue alongside ageing and concomitant modulation of cellular factors and intercellular

