

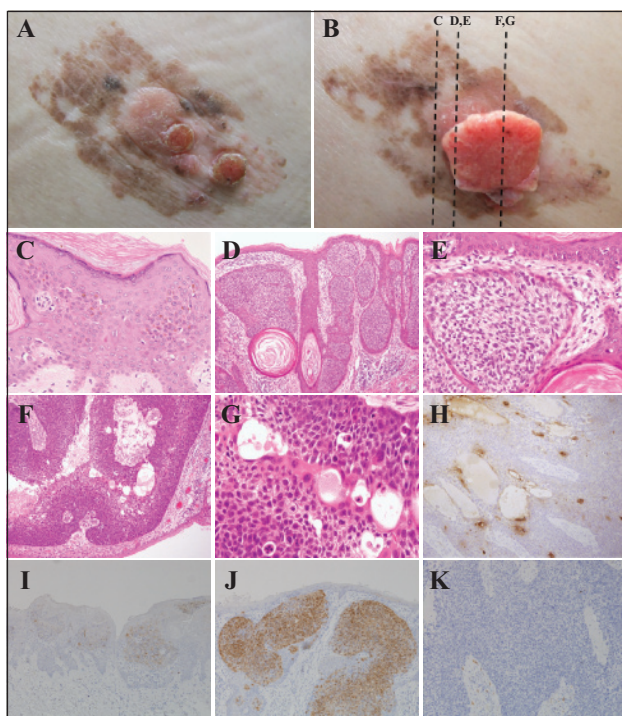


Figure 1. A) A 55×30-mm brown plaque with two nodules on the patient's back at her first visit. B) A 23×21-mm pedunculated tumour on the brown plaque at four months after the initial biopsy. Dotted lines labelled as C, D, E, F, and G indicate the histological areas shown in panels C, D, E, F, and G, respectively. C) SK portion: intraepidermal nests of basaloid cells were scattered in the epidermis and some cells in the nests were pale cells (H&E stain; original magnification: ×400). D) HL portion: intraepidermal tumour nests of atypical basaloid and/or pale cells proliferated downward into the papillary dermis (H&E stain; original magnification: ×100). E) HL portion: tumour cells in the nests had a vacuolated cytoplasm and atypical nuclei (H&E stain; original magnification, ×400). F) PC portion: anastomosing tumour nests of atypical basaloid and pale cells proliferated (H&E stain; original magnification: ×100). G) PC portion: in the anastomosing tumour nests, acrosyringal structures were observed (H&E stain; original magnification: ×400). H) Immunohistochemistry for CEA at the PC portion (original magnification: ×100). The expression of p16 was slightly positive at the SK portion (I), strongly positive at the HL portion (J), and negative at the PC portion (K) (H&E stain; original magnification: ×40).

and porocarcinoma (PC) was made, respectively. Immunohistochemical examinations showed that the expression of p16 was slightly positive at the SK portion, strongly positive at the HL portion, and negative at the PC portion (figures 1I-K).

Malignant tumours arising from seborrheic keratosis are relatively rare [1-3]. As mechanisms involved in tumourigenesis have been clarified, recent studies have revealed that some melanomas are derived from benign melanocytic nevi with a multistep nature of carcinogenesis [4, 5]. The Intraepidermal Epithelioma of Borst-Jadassohn/Borst-Jadassohn Phenomenon (IEBJ/BJP) has been speculated to be a precursor lesion or condition of malignant tumours in seborrheic keratosis. In our case, three different histopathological lesions of SK, HL and PC were observed in one

contiguous lesion. An association between SK and PC has been debated in terms of its tumourigenesis. The controversy whether PC arises from SK or occurs by chance remains to be elucidated [2]. Recent studies revealed a distinct expression pattern of cell cycle regulators, keratins, and epidermal growth factor receptors (EGF-R) in IEBJ [6-8]. We tentatively examined p16 expression and found that p16 was slightly expressed at the SK portion, but was dramatically up-regulated at the HL portion, and then switched off at the PC portion. This change in p16 expression in the SK portion to the PC portion is consistent with the change in expression of p16 in the transformation of benign melanocytic nevi to melanomas [5].

In conclusion, we present a case of a porocarcinoma with hidroacanthoma simplex-like features in association with seborrheic keratosis. The accumulation of such cases will reveal the neoplastic potential of SK lesions. ■

Disclosure. Financial support: none. Conflict of interest: none.

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doi:10.1684/ejd.2017.3013

Diagnostic properties of provocation tests for cold, heat, and delayed-pressure urticaria

Chronic inducible urticaria (CIndU), or physical urticaria, is a debilitating subtype of chronic urticaria, which occurs after direct physical stimuli such as cold, heat, or pressure

Table 1. Sensitivity and specificity.

Patients	True Positive	False Positive	True Negative	False Negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Provocation								
Probable diagnosis								
Heat	0	1	183	14	0	99.5	0	92.3
Cold	9	7	205	8	52.9	96.7	56.3	96.2
Pressure	0	1	188	23	0	99.5	0	89.1
Possible diagnosis								
Heat	1	1*	183	55	1.8	99.5	50	76.9
Cold	4	7*	205	21	16	96.7	36.4	90.7
Pressure	1	1*	188	41	2.4	99.5	50	82.1

PPV: positive predictive value; NPV: negative predictive value; *same numbers as those used under “probable”.

[1]. The diagnosis is based on a combination of medical history, clinical evaluation, and response testing, exposing the patient to physical stimuli [2, 3]. The diagnosis and treatment of CIndU has been investigated and reviewed extensively [4-6]. However, provocation tests, which are widely used and referenced in the diagnostic process, have yet to be validated. In order to address this issue, we evaluated all standardized provocation test results from patients with CIndU during a 15-year period, from 2001-2016, in our department (Dermatology, Bispebjerg Hospital, Copenhagen, Denmark). Testing included exposure to cold (an ice cube), heat (bottled water at 37 °C), and pressure (weights strapped around the leg/arm). All patients were investigated by senior laboratory personnel using standard procedures. Symptom history was retrospectively obtained from the patients' files, including patients' subjective description of symptoms. Symptoms were classified as “probable” when consistent with a certain clinical diagnosis of urticaria (CIndU) in relation to specific physical stimuli, producing wheals, redness and itching. In less conclusive cases, the patients' symptoms were categorized as “possible”.

We included 254 consecutive patients with a mean age of 39.9 years (range: 8-89 years), with complete data for test results and symptoms. Of the patients included, 160 were female (63.0%) and 94 (37.0%) male. A total of 24 patients (9.5%) had a positive provocation test to at least one stimulus, *i.e.* 17 (10.6%) of the females and seven (7.4%) of the males. Cold was the most common stimulus to provoke a reaction in patients (20/24 positive reactions). One (female) patient reacted to both cold and heat.

Cold provocation exhibited the lowest specificity (96.7%) and highest sensitivity (52.9%) against a diagnosis of “probable” symptoms, whereas heat provocation (specificity = 99.5% and sensitivity = 0%) and pressure provocation (specificity = 99.5 and sensitivity = 0%) were more conclusive regarding specificity, but without any true positive cases to calculate sensitivity (*table 1*). Also, the sensitivity against a diagnosis of “possible” symptoms for the three types of CIndU were lower for heat and pressure compared to cold (cold: 16.0%; heat: 1.8%; pressure: 2.4%). Specificity estimates were similar to those for a “probable” diagnosis.

Our results showed high specificity, underlying that a lack of convincing symptoms is followed by a negative test result. The limited number of true positive cases highly interfered with both predictive values and sensitivity calculations (*table 1*), while sensitivity estimates approached

zero, except for cold urticaria (52.9% and 16%). This, however, was still far below the desired value for a diagnostic test. At best, patients with “probable” cold urticaria, judged from symptoms, present with a positive test in only 52.9% of the cases.

Komarov *et al.* [1] suggest patient testing whenever possible to verify the diagnosis. However, this approach implies the existence of validated tools with high sensitivity, which is so far unavailable. The variation in the threshold of symptom triggering and disease activity fluctuations [7] has set the challenge for the development of an efficient diagnostic tool. Such a tool should not only be used to diagnose the urticaria-inducing factor, but also provide estimates of threshold in order to help set goals for treatment. Magerl *et al.* [8] recently tested the fourth edition of the TempTest which is an instrument for temperature-induced urticaria. In addition to the diagnostic properties of the classic test, which was used in our patients, the TempTest offers measurements of the critical threshold temperature (CTT), which is improved with antihistamine treatment [9]. Test methods of similar validity are to be established for delayed pressure urticaria (DPU) in order to standardize the diagnostic process and its criteria, and hereby optimize the treatment approach according to the guidelines [8]. The low diagnostic accuracy observed for our test results supports the definition of CIndU as a complex multifactorial disease, of which the pathogenesis is not yet fully understood [6]. Provocation tests for cold, heat, and delayed-pressure urticaria have high diagnostic specificity, implying that a negative test with great certainty rules out CIndU in patients without convincing symptomatology. Conversely, symptoms consistent with CIndU are only rarely accompanied by a positive test, and therefore newer and more sensitive test methods for CIndU are warranted. ■

Disclosure. Financial support: none. Conflict of interest: none.

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doi:10.1684/ejd.2017.3014

Accelerated features of senescence in cultured type 2 diabetic skin fibroblasts

Type 2 (non-insulin-dependent) diabetes mellitus (T2D) is a metabolic disease which causes chronic hyperglycaemia, biochemical and cellular disorders, and organ complications [1]. It predisposes to lower extremity ulcerations and modifies the healing process, leading to chronic wounds [1]. High-glucose conditions cause oxidative stress in diabetes, which, in turn, induces premature senescence [2]. Senescent cells display several changes, such as growth arrest, alterations in gene expression, and a senescence-associated secretory phenotype (SASP) which modifies their environment by releasing a wide variety of inflammatory proteins [2]. Here, we analysed replicative senescence of fibroblasts isolated from unwounded skin of T2D patients, and evaluated oxidative stress and senescence markers, specifically reactive oxygen species (ROS), ROS-inducers eicosanoids, SA- β -galactosidase (β -gal) staining, miR-200c expression, and inflammatory cytokine secretion.

Following written informed consent, we collected skin biopsies from clinically unaffected skin of four T2D patients and four age-matched control donors (under institutional approval and in adherence with the principles of the Declaration of Helsinki). Cultures of diabetic fibroblast (DF) and healthy fibroblast (HF) cultures were established and then serially amplified until their natural senescence (*i.e.* until they stopped proliferating) in low glucose condi-

tions, as previously reported [3]. We found that, at the end of their *in vitro* life span, HF and DF underwent an average population doubling of 31.91 ± 5.16 and 17.46 ± 2.53 , respectively (*figure 1A*). In addition, evaluation of senescence markers, p16^{INK4a} and p21^{WAF1}, by western blot showed a higher expression level during passages in DF when compared to HF (*figure 1B*). These results confirmed a markedly reduced proliferative potential of DF.

ROS are produced by several cellular oxidative processes, including arachidonic acid (AA) metabolism [4]. Thus, we studied the ROS basal levels in fibroblast cultures, as well as the production of some AA-derived metabolites, *i.e.* the hydroxyeicosatetraenoic acids 5-HETE, 8-HETE, 15-HETE, and 20-HETE, and the leukotriene LTB₄ in cell-conditioned medium [5]. These experiments revealed a significantly greater production of ROS (*figure 1C*) and of almost all the analysed eicosanoids (*figure 1D*) by DF in the last *in vitro* passages, with respect to HF.

5-HETE is a product of the 5-lipoxygenase (5-LOX) pathway [5]. To verify if eicosanoid production impacts on DF senescence, we treated DF and HF with the 5-LOX inhibitor BWA4C [6] and then evaluated 5-HETE production and β -gal staining. Results (*figure 1E*) confirmed that 5-HETE production was higher in DF than in HF and showed that BWA4C induced a significantly greater decrease of 5-HETE in DF. In addition, the β -gal senescence marker (*figure 1F*), which was significantly more expressed in DF than in HF, significantly decreased after 5-LOX inhibition in DF at the beginning of their *in vitro* life span, underlying the role of 5-HETE in DF senescence. Irreversible gene expression changes due to replicative senescence might explain the absence of β -gal staining modifications in DF at the end of their life span.

MicroRNAs (miRs) are non-coding RNA molecules that control the stability and/or the translational efficiency of target messenger RNAs, and are modulated in most biological processes [7]. It has been demonstrated that miR-200c, a miR-200 family member, is upregulated by oxidative stress [7]. Therefore, we analysed miR-200c expression and showed that it was significantly higher in DF than in HF (*figure 1G*). Interestingly, miR-200c overexpression itself induces apoptosis and senescence [7] and upregulates transforming growth factor- β 1 (TGF- β 1) [8]. TGF- β 1, in turn, enhances the expression of inflammatory genes, such as interleukin-6 (IL-6) and monocyte chemoattractant peptide-1 (MCP-1) [9, 10]. IL6 and MCP-1 are the predominant cytokines of fibroblast SASP and, together with transforming growth factor- β 1 (TGF- β 1), are considered SASP-generated factors that may aggravate the senescence cell burden in diabetic patients [2]. Thus, we also analysed these cytokines in the conditioned supernatants from HFs and DFs, and found that their levels in DF cultures were higher than in HF, with a very relevant increase of IL-6 in the last *in vitro* passages (*figure 1H*).

Our results indicate that accelerated features of senescence in diabetic fibroblasts may depend on oxidative stress and altered cytokine secretion, and that miR-200c might have a role in these senescence-associated alterations. Moreover, we show that dysfunction of diabetic fibroblasts persists upon prolonged cell culture, even when cells are derived from clinically unaffected skin and are no longer exposed to a high glucose environment. ■