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Minimally-invasive Sampling of Interleukin-1 α and Interleukin-1 Receptor Antagonist from the Skin: A Systematic Review of *In vivo* Studies in Humans

Denise FALCONE¹, Pieter SPEE², Peter C. M. VAN DE KERKHOF¹ and Piet E. J. VAN ERP¹

¹Department of Dermatology, Radboud University Medical Center, Nijmegen, The Netherlands, and ²FibroTX LLC, Tallinn, Estonia

Interleukin-1 α (IL-1 α) and its receptor antagonist IL-1RA play a pivotal role in skin homeostasis and disease. Although the use of biopsies to sample these cytokines from human skin is widely employed in dermatological practice, knowledge about less invasive, *in vivo* sampling methods is scarce. The aim of this study was to provide an overview of such methods by systematically reviewing studies in Medline, EMBASE, Web of Science and Cochrane Library using combinations of the terms "IL-1 α ", "IL-1RA", "skin", "human", including all possible synonyms. Quality was assessed using the STrengthening the Reporting of OBServational studies in Epidemiology (STROBE) checklist. The search, performed on 14 October 2016, revealed 10 different sampling methods, with varying degrees of invasiveness and wide application spectrum, including assessment of both normal and diseased skin, from several body sites. The possibility to sample quantifiable amounts of cytokines from human skin with no or minimal discomfort holds promise for linking clinical outcomes to molecular profiles of skin inflammation.

Key words: IL-1 α ; IL-1RA; stratum corneum; *in vivo* evaluation.

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Corr: Denise Falcone, Department of Dermatology, Radboud University Medical Center, René Descartesdreef 1, PO Box 9101, NL-6500 HB Nijmegen, The Netherlands. E-mail: denise.falcone@radboudumc.nl

The primary function of the skin is to provide a physical barrier between the internal milieu and the external environment (1, 2). In addition, the skin was more recently recognized to have strong immunological role (3). Keratinocytes and resident skin cells, such as fibroblasts, mast cells, Langerhans cells and other dermal dendritic cells, have been shown to release a wide variety of mediators, both in the maintenance of tissue homeostasis (immune-surveillance) as well as in case of injury or pathogen invasion (4). Among the most characterized mediators are interleukins (IL) belonging to the IL-1 family, namely IL-1 α , IL-1 β and their receptor antagonist IL-1RA (5). These cytokines are among the first mediators to be released in acute or chronic skin inflammation and are involved in a wide spectrum of (skin) diseases (4, 5). In this respect, blocking IL-1 activity for therapeutic purposes has entered clinical practice (6). Of

note, unlike IL-1 β , IL-1 α and IL-1RA have also been shown to be detectable in normal skin (4).

Evidence for the presence of biologically active IL-1 in normal epidermis and stratum corneum (SC) emerged in the mid 1980s by means of bioassays, which measured the ability of IL-1 to augment proliferation of specific cell lines (e.g. murine thymocytes in the thymocyte co-stimulation assay) or to stimulate release of collagenase or prostaglandin from human dermal fibroblasts (7, 8). Bioassays were limited by the impossibility of discriminating between IL-1 α and IL-1 β as well as between IL-1 and other cytokines (9), prompting some authors to cautiously refer to their findings as IL-1 or IL-1 α "like" material (10). In the late 1980s these obstacles were overcome by the advent of monoclonal antibodies against IL-1 α and IL-1 β , allowing the 2 isoforms to be determined and distinguished with high sensitivity and specificity (9). Since then, enzyme immunoassays (EIA) and enzyme-linked immunosorbent assays (ELISA) have become widely used for the evaluation of soluble analytes in biological samples (11, 12). The major shortcomings of ELISA are that it allows the measurement of only one analyte at a time in a given sample and that it requires relatively large sample amounts (typically 100 μ l) (13). Building on the principles of ELISA, in the late 1990s multiplex arrays were introduced with the purpose of measuring multiple analytes in the same sample at the same time (13, 14). An example is constituted by multiplex bead-based assays, in which sets of microscopic colour-coded beads (microspheres) coated with capture antibodies for specific analytes are simultaneously used (13, 14). By flow cytometric analysis, the signal coming from the different bead sets can be distinguished and the binding events between the detection antibodies and the analyte-capture antibody complex on each bead set can be quantified (13, 14). Comparability analyses between ELISA and multiplex bead-based assays yielded positive results (15).

Given the relevance of IL-1 in skin homeostasis and disease, different sampling methods, followed by the above-mentioned immunoassay quantifications, have been reported to analyse it. While taking skin biopsies remains the most widely used approach in clinical practice, successful attempts using less invasive sampling methods have been reported. The aim of this study was to provide an overview of such minimally invasive methods by systematically reviewing studies in which

IL-1 α and/or IL-1RA were sampled from the skin of human volunteers *in vivo*. The choice was restricted to these 2 mediators because of their constitutive presence both in normal and diseased skin and their clinical relevance (4–6). For each method, applications as well as advantages and shortcomings were highlighted. We hope that this review will increase awareness of the additional insights offered by analysis of local skin inflammation at the molecular level and will foster research towards implementation of minimally-invasive biomarker analysis in dermatological practice.

METHODS

An extensive literature search was performed on 14 October 2016 in 4 computerized bibliographical databases: Medline, EMBASE, Web of Science and the Cochrane Library. Medical Subject Heading and free-text searches embracing the following terms were used: “interleukin 1 alpha”, “interleukin 1 receptor antagonist”, “skin”, “human”, including all possible abbreviations and synonyms. The complete search strategy is shown in Table S1[†]. The literature search was limited to publications in English. No date restrictions applied.

The article selection process followed 3 steps. In step one, a first screening was performed by one reviewer (DF) in which titles and abstracts, and materials and methods when the abstract was not available, were reviewed for relevance, taking into account a list of exclusion criteria. In case of uncertainty or if none of the exclusion criteria were met, in step 2 a second screening was performed in which the full text was retrieved and inclusion judged by 2 independent reviewers (DF and PvE) on the basis of a list of inclusion criteria. Consensus on inclusion was reached by discussion. In step 3, the reference lists of the eligible studies were additionally screened to ensure that all relevant studies were included. The schematic representation of the article selection process and the list of exclusion and inclusion criteria is shown in Fig. S1[†].

The methodological quality of the included studies was assessed by the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) criteria: each study received, independently by the 2 reviewers (DF and PvE), a score ranging from 0 to 22, which was then expressed as a percentage of the maximum score (16). Depending on the level of fulfilment of the criteria stated in STROBE, articles were categorized as A (>80%), B (60–80%) or C (<60%). When scores differed by more than 1.5, consensus was reached by discussion.

RESULTS

Article selection and quality

The search in the 4 bibliographical databases revealed a total of 6,598 hits. After exclusion of duplicates and of articles meeting any of the exclusion criteria in the first screening, 535 articles remained for full-text evaluation. For most articles, the exclusion criteria from the first screening applied following full-text evaluation. Four articles for which the inclusion criteria applied were excluded because of the lack of description of the human volunteers from which the skin material was taken or

of the method used to obtain the skin material. Manual screening of the reference lists resulted in the inclusion of one more article. In total, 63 articles were included in this review. The number of articles at each step of the selection process are shown in Fig. S2[†].

Article quality was, on average, good: 8 articles were classified as a category C (score <60%), 47 articles were classified as a category B (score 60–80%) and the remaining 8 articles were classified as a category A (score >80%). Shortcomings were mainly related to the lack of description of potential confounders and effect modifiers concerning the study participants (e.g. age, skin type) or the sampling procedure (e.g. season and experimental conditions in which the sampling was performed). In addition, only a few studies specified the number of participants included at each stage. The STROBE assessment for each article included in the review can be requested from the corresponding author.

Each sampling technique is presented in the following paragraphs. An overview of each technique, including collection time, applications, advantages and shortcomings is shown in **Table I**. A detailed summary of each study included in this review can be requested from the corresponding author.

Tape stripping

The majority of articles ($n=24$) sampled IL-1 α and/or IL-1RA using tape stripping. This technique was characterized by a high heterogeneity in the sampling procedure. Unfortunately, only a few articles thoroughly described the sampling procedure by specifying the type, area and number of adhesive tapes used, the application time of each tape on the skin, and whether constant pressure was applied, all variables known to influence the procedure (17). For the quantification of the protein levels of IL-1 α and/or IL-1RA, the majority used EIA/ELISA ($n=16$) (18–33) and the remaining used multiplex arrays (34–41). Concomitantly to the analysis of IL-1 α and/or IL-1RA, 9 articles used tape stripping to sample additional cytokines and chemokines (20–23, 34, 36–38, 40). The additional measurement of the total protein content on the tapes was used as a biomarker of the cohesiveness of the SC (20, 27, 36, 40, 41).

Among the advantages of tape stripping, the minimal invasiveness and the short duration of the procedure were mentioned (19, 20), suggesting that the cytokine amounts are hardly influenced by the sampling process (20). Among the drawbacks, the fact that in normal SC only IL-1 α and IL-1RA are present in sufficient amounts for routine cytokine determination (19). In agreement with this, some articles reported that other cytokines and chemokines were not or rarely detected (21–23, 34). Moreover, a study of de Jongh et al. evaluated the distribution of IL-1 α and IL-1RA in the SC, showing that, while in normal SC the distribution does not change with depth, after perturbation with chemical substances

[†]<https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-2709>

Table I. Overview of the minimally-invasive techniques to sample interleukin (IL)-1 α and/or IL-1RA from the skin of human volunteers *in vivo*

Method	<i>n</i>	Collection time	Body site(s) in which collection was performed	Applications	IL-1 α and/or IL-1RA detectable?	Other biomarkers detectable?	Advantages	Shortcomings
Tape stripping	24	Few minutes	Forearm, neck, scalp, cheek, forehead, hand, sole, upper arm, trunk, leg, vulvar area, buttock	Healthy skin, diseased skin (dandruff, SeD, AD, psoriasis, CID, acne, senile xerosis)	Yes	Variable	Short collection time Possibility to sample the distribution of biomarkers in the SC	Influence of several collection parameters on the outcome (e.g. pressure, type of tape)
Skin suction blistering	15	2–3 h	Forearm, hand, trunk, leg, buttock	Healthy skin, diseased skin (AD, psoriasis, SSC, PLE, BP, CRPS1)	Yes	Variable	Biomarkers in the blister fluid possibly more representative of the amount in the skin than biomarkers at the surface	Long collection time Cumbersome Possibility of pain/blood contamination Collected biomarkers possibly influenced by skin trauma
Adsorption by Sebutape	9	1–2 min	Forearm, scalp, cheek, forehead, upper arm, trunk, leg, buttock	Healthy skin, diseased skin (dandruff, SeD, rosacea, striae distensae, diaper dermatitis)	Yes	Variable	Short collection time	Influence of sebum Only biomarkers at the skin surface are collected
Skin chamber technique	4	3–24 h	Forearm	Healthy skin	Yes	Variable	<i>In vivo</i> model of cutaneous inflammation	Long collection time Cumbersome Possibility of pain
Microporation	3	3–4 h	Forearm	Healthy skin, diseased skin (AD)	Yes	Variable	Biomarkers in the transdermal fluid possibly more representative of the amount in the skin than biomarkers at the surface	Long collection time Collected biomarkers possibly influenced by skin trauma More data on the efficiency of transdermal fluid collection and on the depth of micropores needed
Swabbing	3	Few seconds	Scalp, arm, leg	Healthy skin, diseased skin (SeD, AD)	Yes	Variable	Short collection time	Only biomarkers at the skin surface are collected
Skin surface wash sampling	2	30 min	Arm	Healthy skin, diseased skin (AD, psoriasis)	Yes	Variable	Average collection time	Only biomarkers at the skin surface are collected
Microdialysis	1	1.5 h	Forearm	Healthy skin	Yes	Variable	Biomarkers in the dialysate possibly more representative of the amount in the skin than biomarkers at the surface	Long collection time Collected biomarkers possibly influenced by skin trauma Local anaesthesia needed
Scraping	1	Few minutes	Elbow	Healthy skin, diseased skin (psoriasis)	Yes	Variable	Short collection time	Poor control on the amount of SC removed
Transdermal analyses patch	1	20 min	Forearm, neck, cheek	Healthy skin	Yes	Variable	Average collection time	Only biomarkers at the skin surface are collected

AD: atopic dermatitis; BP: bullous pemphigoid; CID: chronic irritant contact dermatitis; CRPS1: complex regional pain syndrome type 1; PLE: polymorphic light eruption; SeD: seborrhoeic dermatitis; SC: stratum corneum; SSC: systemic sclerosis.

it changes significantly (20). The authors concluded that, in the study of irritated or diseased skin, it would be better to sample the whole SC. Observations about the variability of amounts across the SC were also reported in other studies (32, 40).

Skin suction blistering

The second most widespread method to sample IL-1 α and/or IL-1RA was based on skin suction blistering ($n=15$). In this technique, introduced by Kiistala in the 1960s (42), the viable epidermis is separated from the dermis by application of negative pressure, resulting in the generation of artificial blisters. In the articles included in this review, the negative pressure was between 200 and 400 mmHg, the suction cups ranged in size from 5 to 15 mm and the time needed for the formation of the blisters was mostly 2–3 h. Warming was sometimes added to aid blister formation. For the quantification of the protein

levels of IL-1 α and/or IL-1RA, the majority used EIA/ELISA ($n=10$) (43–52) and the remaining used multiplex arrays (53–57). All studies but 2 analysed additional cytokines and chemokines, albeit many reported that not all could be detected (43–45, 51, 52, 54, 55).

The advantage offered by the assessment of the local inflammatory profile in the suction blister fluid (54, 55) was counterbalanced by the suggestion that the local trauma caused by the suction process might induce cytokines release and formation, thereby influencing the measured amounts (46–48, 50). Other reported issues included the cumbersomeness of the technique (56), the possibility of pain during the procedure (57) and the possibility of blood contamination of the blister (52).

Adsorption by Sebutape

The third most common method consisted in the adsorption of IL-1 α and/or IL-1RA from the skin surface by

means of a lipophilic tape, Sebutape (CuDerm, Dallas, TX, USA) ($n=9$). Sebutape, originally developed for extraction of sebum from the skin, was demonstrated by Perkins et al. to be able to recover quantifiable levels of inflammatory proteins from normal skin (58). All articles used ELISA to quantify the protein levels of IL-1 α and/or IL-1RA (58–65), except one which used a multiplex array (66). In addition to the measurement of IL-1 α and/or IL-1RA, 4 articles sampled additional cytokines and chemokines (58, 63, 64, 66). However, authors reported that some of these additional biomarkers were frequently extremely low or not detected.

The minimal-invasiveness of the method was demonstrated by Perkins et al. by analysing the impact of 30 sequential applications of Sebutape on the skin and finding no upregulation of IL-1 α nor IL-8, another inflammatory cytokine, up to 24 h after stimulation (58). However, the same author warned about the problem of sebum deposition on the tape, which could compete with IL-1 α for adsorption. As a consequence, all articles employing this method used short (1–2 min) collection times.

Skin chamber technique

Four articles sampled IL-1 α and/or IL-1RA using a skin chamber technique. This technique, developed from the skin suction blistering method, was used as *in vivo* model to study the cutaneous inflammatory response in humans and, specifically, the dynamics of leukocyte migration (67). In this procedure, after removal of the blister roof obtained by application of negative pressure, the denuded dermis (“skin window”) is covered by a chamber containing a medium capable to induce a chemotactic response (mostly autologous serum) (67). All articles included in this review collected the skin chamber fluid between 3 and 24 h after removal of the blister roofs and, besides IL-1 α and/or IL-1RA, used ELISA to analyse additional cytokines and chemokines (68–71).

Microporation

Three articles used microporation to sample IL-1 α and/or IL-1RA. In this technique, an infrared laser is used to generate micropores (approximately 100 μm wide) up to the first tens of μm in the epidermis. Transdermal fluid (TDF) is then collected by application of negative pressure similarly to the skin suction blistering technique (around 300 mmHg for 3–4 h). One article analysed IL-1 α with ELISA and IL-1RA with a multiplex array (72), whereas the others used only multiplex arrays (73, 74). All studies also assessed additional cytokines and chemokines, but reported that some were extremely low or not detected.

While being less invasive than the skin blistering technique, as impact on the integrity of the tissue is relatively minor (74), concerns arise about the collection efficiency of TDF, since in one out of 9 volunteers (72),

7 out of 28 volunteers (73) and 6 out of 16 volunteers (74) collection of TDF failed or was too low. The possible impact of the procedure on the measured amounts of cytokines should be considered, and additional data on the reproducibility of the depth of the laser-generated micropores would be needed.

Swabbing

Three articles sampled IL-1 α and/or IL-1RA by swabbing the skin with cotton-tipped swabs or cotton buds impregnated with a solution of saline and Triton to lyse cells and extract proteins. All articles used ELISA to analyse IL-1 α and/or IL-1RA together with other cytokines and chemokines (75–77).

Skin surface wash sampling

Two articles sampled IL-1 α and IL-1RA by means of skin surface wash. In this technique, proposed by Portugal-Cohen et al. (78), a small well is attached to the skin by an adhesive pad and is filled with 0.5–1 ml extraction buffer consisting of phosphate-buffered saline (PBS), pH approximately 7. After sealing, the well is left on the skin for a 30-min incubation time. Collected samples were analysed with ELISA in the study of Portugal-Cohen et al. (78) and with multiplex arrays with varying detectability levels in a more recent study (79).

The advantage of the method relies on its minimal invasiveness, whereas its drawback is the relatively long incubation time (80). Moreover, the extraction buffer is only suitable to extract hydrophilic compounds; lipophilic compounds could be sampled using a more suitable extraction buffer containing surfactants (80).

Of note, a similar version of this method (though defined as a “skin chamber technique”) was used by Reilly et al. to sample biomarkers from tape-stripped skin (48). Yields of IL-1 α increased by increasing the incubation time and the number of tape strips.

Microdialysis

One study used microdialysis to sample IL-1 α and other biomarkers and quantified them by multiplex array (81). Two linear semi-permeable membranes were inserted into the volar forearm of healthy volunteers at a depth of 0.7 mm for a length of 20 mm. Upon onset of probe perfusion with buffer, extracellular molecules would diffuse through the membranes into the probe lumen to be collected in the exiting fluid (dialysate). Dialysate collection lasted for 30 min and was performed at baseline and at several time-points following intradermal injection of allergen.

Although almost all biomarkers could be detected following allergen injection, the use of microdialysis for sampling large proteins, such as cytokines, is challenging (82). In order to increase relative recovery, an in-house

membrane with molecular mass cut-off of 3000 kDa was used, much higher than the molecular mass cut-off of 100 kDa usually employed in commercially available membranes for cytokine sampling (82, 83). This can expose the membranes to ultrafiltration, possibly causing significant loss of dialysate (82, 83). Other effects potentially hampering the recovery rate of cytokines in microdialysis include low analyte concentration in the extracellular milieu and the adsorption onto the polymeric materials used to construct the membranes and the outlet probe (82, 83). Finally, commonly to skin suction blistering and microporation, the trauma to the skin due to probe insertion and the possible effect on the measured amounts of biomarkers should be considered.

Scraping

In one article, delicate scraping with a surgical blade was used to obtain extracts of SC from healthy volunteers and scales from plaques of patients with psoriasis (84). Samples of 10 mg were homogenized in PBS and levels of IL-1RA and other biomarkers were subsequently analysed with a multiplex array.

Transdermal analyses patch

A novel method to sample IL-1 α and IL-1RA from the skin surface was introduced by Orro et al. (85). In this method, a transdermal analyses patch (TAP) is attached to the skin by means of a dermal adhesive plaster. On the side in contact with the skin, the patch contains a circular nitrocellulose micro-array (5 mm in diameter) coated with antibodies for 4 different protein biomarkers. On the upper side, covering the micro-array, a reservoir allows addition of a standardized amount of buffer (PBS at pH 7.3) for biomarker extraction and ensures close contact between the micro-array and the skin. In the study of Orro et al., following optimization of the protein capture and detection protocols, 20 min resulted a suitable incubation time for biomarkers extraction. After removal of TAP, biomarkers were analysed using ELISA.

In the same study, authors compared the biomarkers yield of TAP and of the skin surface wash sampling method, showing higher yield of proteins for the former. They suggested that the higher sensitivity of TAP might be due to higher concentration of proteins in the buffer, or to possible degradation of analytes in skin surface wash. Also Reilly et al., using a similar version of skin surface wash, suggested that the reason why some mediators could not be detected could be dilution of the small amount of inflammatory exudates in a large volume of PBS (48).

DISCUSSION

The primary objective of this study was to systematically review minimally-invasive methods whereby the pro-inflammatory cytokine IL-1 α and its receptor antagonist

IL-1RA were sampled from the skin of human volunteers *in vivo*, and subsequently analysed at the protein level by means of immunoassays.

Ten different methods were found. Common to all methods was the possibility to assess, in addition to IL-1 α and/or IL-1RA, other biomarkers including pro-inflammatory cytokines, anti-inflammatory cytokines, chemokines and various growth factors. Since not every article explicitly mentioned if IL-1 α , IL-1RA or the other biomarkers were measurable in all samples, a comparison of the different methods based on the detectability level of the collected biomarkers was not possible. As a general trend, IL-1 α and IL-1RA were often or consistently measurable, whereas the other biomarkers were sometimes rarely or not detectable. Possible reasons could be absence in the epidermis, low production or production in different time courses, rapid uptake by target cells, and insufficient sensitivity of the assay (21, 48, 55, 58, 74, 81). Another frequently reported aspect was the large inter-subject variability in the biomarkers amounts (21, 32, 36, 43, 45–47, 56, 58, 63, 64, 72, 73, 81), for which authors resorted to non-parametric statistical analyses or to log-transformation of the data.

The balance between the expression levels of cytokines of the IL-1 family is decisive in the generation of pro-inflammatory and homeostatic functions (5). In particular, the ratio between IL-1RA and IL-1 α can be used to assess skin inflammation (4). Compared with healthy volunteers, an increase in the IL-1RA/IL-1 α ratio was observed in patients with inflammatory skin diseases including psoriasis, atopic dermatitis (AD), dandruff, seborrhoeic dermatitis and rosacea (32, 36, 63, 64, 75). This indicates that the increased ratio could be considered a non-specific hallmark of various kinds of inflammation (32, 36, 63), and might reflect an attempt to downregulate the inflammatory response (64). This is supported by a decrease in the levels of IL-1 α and in the IL-1RA/IL-1 α ratio observed after topical treatments (18, 25, 27, 32, 35, 36, 39, 75, 77) and by a decreased IL-1 α expression found in full-thickness biopsy specimens of psoriatic skin (86). The ratio might also highlight differential expression of inflammatory markers following different modalities of skin irritation, since an increased ratio compared with normal skin was observed after repeated exposure to sodium dodecyl sulphate (SLS) (21, 72) and a lower ratio was reported after single exposure to SLS (58) or after tandem exposure to SLS and another irritant (34). Interestingly, an increased ratio could distinguish also non-lesional skin in patients with AD, psoriasis and rosacea from skin of healthy volunteers (32, 64), as well as sun-exposed skin on the face compared with unexposed skin (23, 32, 58, 85). This suggests that inflammatory changes might be present even in normal-appearing skin and supports what Professor Kligman called invisible dermatoses (87).

Among the extrinsic factors possibly affecting the recovered amounts of IL-1 α and IL-1RA figure season,

since a lower IL-1RA/IL-1 α ratio was found in summer compared with winter (41), and UV exposure, since release of IL-1 α and/or IL-1RA was observed following UV irradiation (23, 43, 56, 73). These factors indicate that data collection and skin phototype (e.g. Fitzpatrick skin type) should be specified, as they might constitute potential confounders. In addition, the presence of IL-1 α in sweat (22) and the increased expression of IL-1 α at low humidity (88) suggest that the temperature and relative humidity of the experimental room should be standardized, as for measurements with bioengineering techniques (89–91). Among the intrinsic factors, conflicting results emerged for sex, since differences were found in one study (19) and not in others (28, 35), as well as for age, since an effect was observed in some studies (23, 79) and not in others (31, 34). In the particular case of infant skin, higher IL-1 α levels without concomitant signs of inflammation were found in neonates compared with adults (38) and at one-month post-partum compared with birth (62), suggesting that, for this specific age group, IL-1 α might be used as a biomarker of skin barrier maturation (62).

The limitation of this study is that the restriction of the search strategy to IL-1 α and IL-1RA could have excluded minimally-invasive methods used to sample other biomarkers. The addition of relevant cytokines and chemokines, such as IL-1 β , IL-6 and IL-8 (4) would have strengthened and, possibly, broadened the overview of minimally-invasive sampling methods; on the other hand, it would have also complicated the search strategy and prolonged the article selection process. Despite this obvious limitation, we believe that the relevance of the chosen biomarkers and the systematic article selection process maintain the validity of the overview of minimally-invasive methods presented in this review.

In conclusion, this review showed that several methods are available to collect quantifiable amounts of IL-1 α , IL-1RA and other biomarkers from the skin, causing no or minimal discomfort. This is relevant on both practical and ethical grounds. Whereas it might be possible to argue that the levels of biomarkers measured in the fluid extracted by skin suction blistering, microporation and microdialysis are more representative of the overall levels present in the skin, the long collection time and the relative invasiveness make them less practical for use in routine testing. Tape stripping, adsorption with Sebutape, swabbing, skin surface wash sampling, scraping and TAP, characterized by shorter collection time and lower invasiveness, would be more fit for that purpose. However, it needs to be realized that these methods sample biomarkers at the skin surface, and that surface levels do not necessarily correspond to the ones in deeper skin layers. Independently of the method used, assessing the individual cytokine profile locally in the skin would bring additional insights than assessing it only systemically in blood (54, 55, 69, 81). We strongly encourage performing

clinical investigations to gain further insights into the relationship between clinical responses and biomarkers at the molecular level. This will hopefully lead to implementation of these minimally-invasive methods in clinical practice to target and monitor therapies, predict disease progression and response to treatment.

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