Decreased Systemic Levels of Endocan-1 and CXCL16 in Psoriasis Are Restored following Narrowband UVB Treatment.

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Decreased systemic levels of endocan-1 and CXCL16 in psoriasis are restored following narrow-band UVB treatment

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Key message: Decreased systemic levels of cardiovascular markers endocan-1 and CXCL16 are restored following UVB therapy

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Abbreviations and acronyms

NB, narrowband; UVB, ultraviolet B; CXCL, chemokine (C-X-C motif) ligand; CCL, chemokine (C-C motif) ligand; TNF, tumor necrosis factor; WHR, waist-hip ratio; BMI, body mass index; PTX3, pentraxin 3; FABP, fatty acid binding protein; LDL, low density lipoprotein; HDL, high density lipoprotein; HbA1c, hemoglobin A1c; PASI, psoriasis Area Severity Index; sIL-1R, soluble interleukin-1 receptor; sIL-2Ra, soluble interleukin-2 receptor alpha; sTNFR, soluble tumor necrosis factor receptor; sVEGFR, soluble vascular endothelial growth factor receptor; IFN, interferon; LFA, lymphocyte function-associated antigen; ICAM, intercellular adhesion molecule
Abstract

Background: In psoriasis, a common immune mediated disease affecting 2-3% of the population worldwide, there is an increased prevalence of extra-cutaneous diseases including obesity, metabolic syndrome and cardiovascular disease. This is believed to be linked to systemic inflammation. In previous studies, we have explored various markers in plasma and serum to characterize the on-going systemic inflammation in psoriasis patients compared to controls. We have identified several markers that were altered in psoriasis patients, but which all were unresponsive to NB-UVB treatment.

Objective: The objective of the study was to evaluate the effect of NB-UVB treatment on markers of cardiovascular risk and systemic inflammation in psoriasis.

Methods: The levels of 17 potential biomarkers with an association with cardiovascular risk were quantitated in plasma from 37 age- and gender-matched psoriasis patients and controls at baseline, and in 21 psoriasis patients after 12 weeks of NB-UVB treatment to identify a systemic treatment response.

Results: We identify mediators, endocan-1, CXCL16, and sVEGFR1 that are systemically decreased in psoriasis at baseline and FABP3, FABP4 and sIL-1R1 that show normal baseline levels. Upon 10-12 weeks of NB-UVB treatment endocan-1 and CXCL16 are restored to normal levels while sVEGF1, FABP3, FABP4 and sIL1R1 show a significant reduction.

Conclusion: The current study expands the number of potential biomarkers in psoriasis by including a greater number and greater variety of mediators, approaching the systemic inflammation from additional vantage points, including soluble immune receptors and adipocyte contribution, to provide a more complete picture of the systemic inflammatory state in psoriasis.
**Introduction**

In psoriasis, a common immune mediated disease affecting 2-3% of the population worldwide [1,2], there is an increased prevalence of extra-cutaneous diseases including obesity, the metabolic syndrome and cardiovascular disease. An increase in mortality, especially in severe psoriasis, has been identified [3-6], and several studies suggest that psoriasis is an independent risk factor for cardiovascular events [5,7-9]. In fact, psoriasis patients have been shown to be in a so called ‘prothrombotic state’, implying a disturbance in the homeostasis between coagulation and fibrinolysis along with an increased platelet activity, predisposing for cardiovascular thrombotic events [10,11].

In previous studies, we have explored various markers in plasma and serum to characterize the on-going systemic inflammation in psoriasis patients compared to controls. We have identified several circulating cytokines, chemokines and factors associated with cardiovascular disease that were elevated systemically in psoriasis, but were unresponsive to narrowband (NB)-UVB treatment [12,13].

We here identify additional biomarkers which demonstrate a clear response to 10 -12 weeks of NB-UVB treatment. The baseline levels of endocan-1, CXCL16 and sVEGFR1 were significantly reduced in psoriasis patients compared to controls. Upon NB-UVB treatment, endocan-1 and CXCL16 were normalized to control levels, while sVEGFR1 showed a further decrease. We also observed that FABP3, FABP4 and sIL-1R1, which showed no significant differences between patients and controls at baseline were significantly reduced upon NB-UVB treatment.
Materials and methods

Study design

The patients and control subjects were recruited from the Departments of Dermatology at the University Hospital in Linköping and Sahlgrenska University Hospital in Gothenburg, Sweden. The participants were examined by a dermatologist who verified the diagnosis of psoriasis. The patients had not received phototherapy or systemic treatment for at least four weeks prior to inclusion in the study. The levels of biomarkers were quantitated in 37 age- and gender- or age-, gender-, waist hip ratio (WHR) and body mass index (BMI) matched pairs of patients and controls. 21 patients underwent NB-UVB therapy for 10-12 weeks. Plasma samples were collected before and after the NB-UVB treatment for the analysis of the selected biomarkers. Disease severity was assessed using the Psoriasis Area and Severity Index (PASI).

Measurements of biomarkers for endothelial dysfunction, inflammatory markers in fat tissue, lipid binding proteins together with markers of innate immune reactions

Blood samples were collected from patients and controls in sodium heparin coated CPT tubes. Plasma was collected and stored at -80°C until analysis. The levels of markers for endothelial dysfunction and prothrombotic state including thrombomodulin, pcam-1, pentraxin (PTX)3 and endocan-1, inflammatory markers produced in fat tissue including the adipokine oncostatin M, and the fatty-acid binding proteins (FABP)3 and FABP4 together with the chemokines CXCL16 and CXCL6 was determined. Measurements of the soluble interleukin receptors (sIL)-1R1, sIL-1R2, sIL-2Ra, soluble TNF receptors sTNFR1, sTNFR2 and soluble vascular endothelial growth factor receptors (VEGFR) 1, sVEGFR2 and sVEGFR3 were performed.

The levels of the selected biomarkers (table 1) were determined using multianalyte profiling from Milliplex®MAP (Millipore Corporation, Billerica, MA, USA), according to the manufacturer’s instructions. Sample data were collected on a Luminex 200 instrument.
(Biosource, Nivelles, Belgium) using the xPONENT software (Luminexcorp, Austin, TX) and analyzed in MasterPlex QT (MiraiBio, Alameda, CA).

**Measurement of clinical parameters of metabolic, heart- and vascular disease**

The levels of glucose and hemoglobin (Hb)A1c were measured and the lipid profile (total cholesterol, low density lipoprotein (LDL)- and high density lipoprotein (HDL)-cholesterols, triglycerides and apolipoproteins A1 and B) was characterized according to standard clinical procedures of the Department of Clinical Chemistry at the University Hospital in Linköping, Sweden.

**Statistical analysis**

Data analysis was performed in GraphPad Prism® version 6.01 (GraphPad Software Inc., San Diego, CA, USA). Data were compared using Mann-Whitney or Wilcoxon matched-pair signed rank test. Correlations were determined by Spearman’s test. A p-value of less than or equal to 0.05 was considered significant.
Results

Clinical parameters of metabolic and vascular disease

The psoriasis patients displayed a significantly higher BMI (median BMI 28.8, range 20.0-37.5) compared to the controls (median BMI 25.0, range 20.8-30.1), p=0.01. The WHR was also significantly higher in the patients (median 0.98, range 0.74-1.26) compared to the controls (median 0.90, range 0.68-1.06), p=0.02. Measurements of total cholesterol, LDL and HDL cholesterol, triglycerides and apolipoproteins A1 and B together with blood glucose and HbA1c did not differ between the psoriasis patients and the controls.

Levels of potential biomarkers in psoriasis

Measurement of a wide array of biomarkers of cardiovascular risk and systemic inflammation in 35-37 age-and gender matched patients and control was performed. The median baseline PASI score for the psoriasis patients was 7.5 (range 1.2-25.3). The level of endocan-1 was found to be markedly reduced in psoriasis patients compared to controls (p=0.009; Fig. 1a). Interestingly, endocan-1 also showed a negative correlation to PASI (r=-0.3, p=0.045), with an increasing severity correlating with lower endocan-1 levels. Furthermore, the level of the chemokine CXCL16 was decreased in psoriasis patients compared to the controls (p=0.02; Fig. 1b). When matched for WHR and BMI, these differences receded.

We found no statistically significant difference in the baseline levels of thrombomodulin, pecam-1, PTX3, oncostatin M, FABP3, FABP4 or CXCL6 between psoriasis patients and control subjects.

Among the soluble receptors, we detected reduced levels of sVEGFR1 in the patients (p=0.05; Fig. 1c). No other significant differences could be detected between the patients and the controls in soluble interleukin, TNF or vascular endothelial growth factor receptors.
The influence of NB-UVB therapy on biomarker levels

We compared the levels of the biomarkers before and after 10-12 weeks of NB-UVB therapy in psoriasis patients. The levels of endocan-1 and CXCL16, which were both reduced at baseline in psoriasis patients, were normalized to control levels after the UVB therapy (p=0.008 and p=0.002, respectively; Fig. 2a and b). In the same patients, the NB-UVB therapy further reduced the already decreased levels of sVEGFR1 (p=0.04; Fig. 2c).

Furthermore, FABP3, FABP4 and sIL-1R1, which had similar levels in patients and controls at baseline demonstrated a significant decrease in the patients upon NB-UVB treatment (p=0.03, p=0.04 and p=0.005, respectively; Fig. 2d, e and f).

There was a significant reduction in PASI scores after the UV treatment, median PASI score before treatment being 8.3 (range 2.2-17.2) and after treatment 1.8 (range 0.0-5.6), p<0.0001.
Discussion

It has become increasingly evident that many immune-mediated diseases share an increased risk for cardiovascular disease. Inflammatory mechanisms affect the homeostasis of the vascular endothelium, leading to an endothelial dysfunction locally in the targeted tissue and often also systemically. Finding biomarkers that not only reflect the psoriasis disease state but are also indicative of associated comorbidities would be highly valuable.

In this study, we analyzed the levels of a multitude of potential biomarkers that are also markers of cardiovascular risk, to investigate their suitability as biomarker and whether they can be used as a quantitation of the treatment response to UVB. In doing so, we identified three mediators whose expression was reduced in psoriasis: endocan-1, CXCL16 and sVEGFR1.

Interestingly, while the biomarkers we have previously explored in psoriasis tended to remain unaffected by NB-UVB therapy, we here observe that NB-UVB therapy normalizes the levels of endocan-1 and CXCL16 to the levels of the control subjects, and further reduces the levels of sVEGFR1. This altered response indicates that these mediators might be susceptible enough to treatment to properly identify and characterize the response.

Endocan-1 has previously been linked to endothelial dysfunction, a hallmark of atherosclerosis, and is being explored as a prognostic factor/biomarker in sepsis, cancer and cardiovascular disease. Under normal physiological conditions, endocan-1 is vasoprotective, but the levels of endocan-1 are increased in many inflammatory and hypervascular states, perhaps as a protective response. Low levels may therefore pinpoint to diminished vasoprotective effects [14-17]. Furthermore, decreased endocan-1 levels are described in overweight and non-alcoholic fatty liver disease (NAFLD), conditions overexpressed in psoriasis [17-20].

The reduction of endocan-1 that we observe is also interesting in the context of leukocyte extravasation, where endocan-1 inhibits the extravasation process by preventing the binding of leukocyte function-associated antigen (LFA)-1 to intracellular adhesion molecule (ICAM)-1.
Interestingly, we have previously found elevated levels of soluble ICAM-1 in this psoriasis population [13]. It may be speculated that the reduced endocan-1 levels may be the result of a feedback loop stemming from the increased levels of ICAM-1, and that the low levels of endocan-1 may result in a lack of inhibition of the leukocyte transmigratory responses. Such a mechanism has also been proposed in atopic dermatitis [22].

Previous studies on endocan-1 in psoriasis are contradictory. Balta et al have identified elevated levels of endocan-1 in psoriasis, which also correlated positively to disease severity [23]. However, this elevation was not replicated by Toprak et al who found no differences in endocan-1 levels between psoriasis patients and controls [24]. This emphasizes that the role of endocan-1 in psoriasis is uncertain and not be fully explored.

We also found notably lower levels of CXCL16 in psoriasis patients than in controls. CXCL16 is produced constitutively by keratinocytes and expressed by macrophages in atherosclerosis lesions. The relationship between circulating level of soluble CXCL16 and atherosclerotic disorders remains controversial in clinical practice. Both decreased and increased CXCL16 levels have been reported in patients with atherosclerotic disorders [25-27]. CXCL16 is increased in the lesional psoriatic skin, where it is most prominent in the lower epidermis [28]. Compared to healthy and atopic dermatitis skin, monocytes, keratinocytes and dendritic cells in the psoriatic skin express a higher level of CXCL16 [29]. In contrast, previous studies on the systemic levels of CXCL16 found no increase in the serum of psoriasis patients, and no correlation between CXCL16 levels and disease severity [30].

Our measurements of soluble receptors in psoriasis revealed decreased levels of the sVEGFR1. sVEGFR1 binds and sequesters VEGF, acting as an endogenous inhibitor of the VEGF response [31]. This is particularly interesting in the context of psoriasis, where VEGF is over-expressed both in the lesional skin and in serum [32]. sVEGFR1 is labelled a cardiovascular risk marker because of its association with endothelial dysfunction [33].
reduced levels of sVEGFR1 in plasma samples from psoriasis patients at baseline, along with the further reduction after NB-UVB, may illustrate the dysfunctional VEGF pathway in psoriasis and an insufficient inhibition of VEGF that despite the reduction of sVEGFR1 is rendered less noticeable after UVB due to the attenuating effect of UVB also on VEGF levels [34]. In serum from psoriasis patients with mild disease, Flisiak et al reported higher baseline level of sVEGF1 , which further increased upon topical treatment [35].

FABPs are key players in lipid homeostasis and comprise several isoforms. The heart and adipocyte isoforms FABP3-4, have been well studied in other systemic disorders but not fully investigated in psoriasis. FABP3 is highly expressed in the cardiac and skeletal muscles and its presence in blood is indicative of acute myocardial infarction [36]. FABP4, an adipokine and the most commonly occurring FABP isoform, is linked to obesity, diabetes, NALFD and atherosclerosis [37,38]

We did not observe increased baseline levels of either FABP3 or FABP4 in patients compared to controls. However, NB-UVB treatment led to a significant reduction in both FABP3 and FABP4 levels suggesting an influence of UVB on lipid metabolism through the above isoforms. Our results are partially in agreement with a recently published paper by Baran et al [39], reporting no differences in baseline levels of FABP3 in patients and controls. In contrast to our results, they report increased baseline FABP4 levels in the patients which does not alter upon topical treatment.

In this study, we have screened a large number of putative biomarkers for the systemic inflammation in psoriasis. They were selected based on previous association to the risk of cardiovascular disease. Numerous studies have been performed with the aim of establishing soluble biomarkers for the systemic inflammation in psoriasis. However, most of the biomarkers that have been studied have not succeeded in meeting the criteria for a clinically useful biomarker. This work contributes by the evaluation of a larger number of candidate
biomarkers in psoriasis. It is apparent that further studies are needed to elucidate the role of the investigated biomarkers in the pathogenesis of psoriasis and its comorbidities.
Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest

Ethical approval

Written informed consent was obtained from the patients and control subjects, and the ethical principles of the Declaration of Helsinki were followed. The study was approved by the local ethics committee.

Funding sources

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Acknowledgements

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### Table 1. The evaluated markers

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<th>Mediator, full name</th>
<th>Abbreviated name</th>
</tr>
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<tbody>
<tr>
<td>Chemokine (CXC-motif) ligand 6</td>
<td>CXCL6</td>
</tr>
<tr>
<td>Chemokine (CXC-motif) ligand 16</td>
<td>CXCL16</td>
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<td>PTX3</td>
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<td>Trombomodulin</td>
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**Figure legends**

**Fig. 1.** Baseline levels of (a) endocan-1 (n=35), (b) CXCL16 (n=35) and (c) sVEGFR1 (n=37) measured systemically in age-and gender matched psoriasis patients (psoriasis) and control subjects (controls). The line shows the median level. *p≤0.05, **p < 0.01.

**Fig. 2.** The levels of (a) endocan-1, (b) CXCL16, (c) sVEGFR1, (d) FABP3, (e) FABP4 (n=21) and (f) IL-1R1 (n=15) in the plasma of psoriasis patients before and after NB-UVB therapy. *p≤0.05, **p<0.01.
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18 Jensen P, Skov L: Psoriasis and Obesity. Dermatology 2017
37 psoriasis patients & 37 matched controls

Plasma

21 psoriasis patients

NB-UVB therapy for 10-12 weeks

Measurements of biomarkers

Measurements of clinical parameters of metabolic-, heart- and vascular disease