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# Serum kallikrein-8 correlates with skin activity, but not psoriatic arthritis, in patients with psoriatic disease

## Abstract

**Background:** About 30% of cutaneous psoriasis (PsC) patients develop psoriatic arthritis (PsA) in the joint, which is under-recognized by dermatologists. Biomarkers for PsA are needed so that early referral to a rheumatologist is made. Kallikreins (KLKs) are secreted serine proteases implicated in skin desquamation and inflammation. This study examined KLK potential as serum biomarkers of PsA in cutaneous psoriasis patients.

**Methods:** KLKs were measured by ELISAs in synovial fluids of three PsA patients and three control early osteoarthritis (OA) patients, as well as in a cohort of 152 serum samples collected from age- and sex-matched PsC patients, with (n=76) or without PsA (n=76). KLK expression in psoriatic plaques was examined by immunohistochemistry. Univariate and multivariate logistic regression analyses were conducted to analyze the association between serum KLK levels and disease class (PsC, PsA). Serum KLKs that associated with PsA were correlated with clinical parameters of skin and joint activity.

**Results:** Among the seven KLKs tested, KLK6 and KLK8 were elevated in both PsA synovial fluids and psoriatic plaques, but only serum KLK8 levels were associated with psoriatic disease (odds ratio=2.56, p=0.03). Although significantly elevated in PsC and PsA sera compared to healthy controls, KLK8 did not discriminate PsA from PsC patients. KLK8 correlated positively with the psoriasis area and severity index (PASI) (r=0.43, p=0.001) independent of age, sex and psoriasis duration ( $\beta$ =1.153, p=0.0003) and exhibited no correlations with tender or swollen joint counts.

**Conclusions:** Increased KLK8 serum level in PsA patients reflects disease activity in the skin but not in the joints. Serum KLK levels are not useful for screening psoriasis patients for PsA.

**Keywords:** biomarker; ELISA; kallikreins; psoriasis; psoriasis area and severity index (PASI); psoriatic arthritis; screening.

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## Introduction

Psoriasis is a common inflammatory skin disease affecting about 3% of the North American population [1]. This

multifactorial chronic disease is characterized by epidermal hyper-proliferation and dermal inflammation that vary in severity from minor, localized patches to involvement of the entire skin surface [2]. About one-third of patients with psoriasis suffer from moderate-to-severe disease and report that the disease has a substantial negative impact on their quality of life. The concept of 'psoriatic disease' encompasses additional manifestations that may be associated with the occurrence of psoriatic skin lesions, including musculoskeletal and cardiovascular systems [3, 4]. Approximately 30% of psoriasis patients develop arthritis, which contributes additional morbidity to psoriasis patients [5]. Psoriatic arthritis (PsA) is an inflammatory joint disease associated with cutaneous psoriasis (PsC) and seronegativity for rheumatoid factor. There is a high prevalence of undiagnosed PsA among psoriasis patients seen in dermatology clinics [6]. Patients are usually diagnosed with PsA 8 to 10 years after skin psoriasis manifests. The diagnosis of PsA is usually made by a rheumatologist after a clinical evaluation, but no diagnostic test is available. Soluble biomarkers of PsA are of particular interest to dermatologists and rheumatologists, as they may aid in screening, early detection and treatment, leading to amelioration of progressive joint damage and disability, and improvement in patients' quality of life and function.

Kallikreins (KLKs) are a family of 15 trypsin- or chymotrypsin-like serine proteases encoded by a large cluster of genes on chromosome 19q13.4 [7, 8]. Prostate specific antigen (PSA or KLK3) is the most widely known KLK, currently used in the clinic as a biomarker of prostate cancer. Although many KLKs are primarily known for their potential as cancer biomarkers, they may also play a role as serum biomarkers for inflammatory diseases, such as PsC and PsA. To date, ten KLKs are known to be present in normal human epidermis. KLK5 and KLK7 were originally isolated from the outermost epidermal layer, the stratum corneum (SC), as the stratum corneum trypsin-like and chymotrypsin-like enzymes, respectively [9]. These proteases, in addition to KLK8 and KLK14, are physiologically active in the normal human skin-surface [10, 11] and regulate important barrier functions, such as desquamation and keratinocyte cell turnover, processing and activation of cathelicidin antimicrobial peptides (LL-37) during infection and activation of keratinocyte-expressed proteinase-activated receptor-2 (PAR2), leading to NF- $\kappa$ B-mediated skin inflammation and allergy [12–14].

Given that the majority of PsA patients initially present with PsC, we hypothesized that epidermal proteins implicated in skin barrier function and inflammation, such as KLKs, may act as serum biomarkers of

psoriasis severity and may aid in screening and early detection of PsA.

Currently, there is no validated single or panel of blood/serum biomarkers for PsA in the clinic. The diagnosis of PsA is considered when inflammatory musculoskeletal disease is recognised in the presence of psoriasis. PsA is classified using the CASPAR (CLASSification criteria for Psoriatic ARthritis) criteria [15] and PsA disease activity is primarily assessed by counting the number of tender or swollen joints. As early diagnosis of PsA is associated with less joint damage progression [16], it is imperative that psoriasis patients are screened regularly for the presence of PsA. Screening tools for PsA are being investigated, including questionnaires, imaging, genetic and cellular biomarkers [17]. Potential PsA biomarkers are likely to be overexpressed at sites of inflammation (skin and joint) and subsequently to enter systemic circulation. Preliminary studies have suggested soluble PsA markers, including acute phase reactants (such as high-sensitivity C-reactive protein, hsCRP), markers of extracellular matrix-destruction (matrix metalloproteinases, MMPs) and cytokines, such as tumor necrosis factor (TNF- $\alpha$ ) may discriminate patients with PsA from those with PsC alone [17]. These potential serum biomarkers were overexpressed in the lesional psoriatic skin and/or the inflamed synovial fluid of psoriasis and PsA patients, respectively [18, 19]. Herein, we examined KLK protein expression in inflamed PsA synovial fluids and psoriatic skin under the hypothesis that KLKs may mediate both skin and joint inflammation in PsA and hence may be useful as screening serum biomarkers of PsA in psoriasis patients. We then measured the protein levels of a panel of epidermal and synovial fluid-expressed KLKs in serum samples of well-phenotyped psoriasis patients, with or without PsA, to determine the utility of KLKs as soluble screening biomarkers for PsA.

## Materials and methods

### Collection of synovial fluids (SF) from PsA and control patients

Synovial fluids were aspirated from inflamed knee joints of three PsA patients and three non-inflammatory (early osteoarthritis, OA) controls. Non-inflammatory SF was defined according to a white blood cell count of  $<2000/\text{mm}^3$ , and a clear appearance [20]. Unfortunately there are no currently available biomarkers that allow the differentiation between PsA and OA, therefore such markers will also be clinically relevant. Each individual sample was subjected to a BCA total protein assay (Pierce Biotechnology Inc., Rockford, IL, USA) prior to loading 800  $\mu\text{g}$  of protein into antibody-coated plates to measure KLK levels by KLK-specific ELISAs.

## Immunohistochemistry of psoriatic skin

Rabbit anti human KLKs 6 and 8 antibodies were purchased from R&D Systems Inc. (Minneapolis, MN, USA). Punch biopsies (4mm) were obtained from four psoriasis patients with ethics approval. Formalin-fixed paraffin sections were deparaffinized with 2× xylene, and serial dilutions of ethanol (100%, 95%, 80%, 50%) and water, 5 min per step. Slides were heated at 80°C for 20 min in an autoclave, and cooled on ice for 20 min. Endogenous peroxidase activity was quenched with Dako peroxidase quenching buffer for 20 min at room temperature (RT). After washing with phosphate buffered saline (PBS), sections were blocked with 2% BSA in PBS for 1 h at RT. Sections were incubated first with antibodies diluted in 2% BSA (1:250, 1:400, 1:500, 1:1000 dilutions) overnight at 4°C in a humid chamber. After rinsing with PBS, slides were incubated for 1 h at RT in a humid chamber with rabbit horseradish peroxidase-conjugated secondary antibodies diluted 1:400 in blocking buffer. The immunoreactivity was detected with the Liquid DAB+ Substrate Chromogen System (Dako Cytomation). Nuclei were counterstained with Hematoxylin QS (Vector Laboratories, Burlingame, CA, USA) and mounted with Aquamount (BDH, Poole, UK). Slides were dehydrated via reverse order of deparaffinization steps and mounted with Permount mounting medium.

## Patient population and collection of serum samples

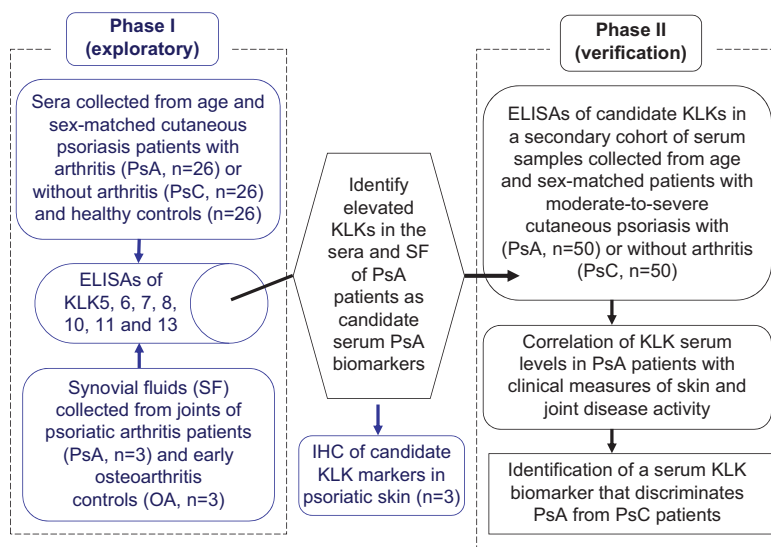
As outlined in the experimental flow chart in Figure 1, we recruited a total of 152 age- and sex-matched patients with active psoriasis, with or without arthritis, and 26 healthy volunteers from the University of Toronto PsA and psoriasis clinics. The study subjects were recruited between January 2006 and October 2010. The study protocol and informed consent forms were approved by the University Health Network Research Ethics Board and all patients signed a written consent form. Consenting patients are recruited into an observational cohort

and evaluated according to a standard protocol every 6–12 months. At each visit, symptoms, physical examination (including complete musculoskeletal examination and assessment of psoriasis severity), current use of medications and laboratory findings are recorded. The data are entered and stored in a computerized database. In phase I, 52 psoriasis patients and 26 healthy controls were recruited. Of the 52 psoriasis patients, 26 were diagnosed with PsA by a rheumatologist and satisfied the CASPAR criteria. PsA was excluded by a rheumatologist in the remaining 26 psoriasis patients. Psoriasis severity was evaluated by a dermatologist using the psoriasis area and severity index (PASI). Phase I was an ‘exploratory’ phase and thus we chose to test sera of mild-to-moderate cutaneous psoriasis patients ( $n=52$ ,  $PASI < 8$ ) to capture elevated serum KLKs, which would be the most promising markers.

Controls were healthy volunteers who did not have psoriasis or inflammatory arthritis. Patients with psoriasis and PsA were group-matched for age, sex and psoriasis duration, while controls were matched for age and sex. In phase II of the study, KLKs that showed promise were further investigated in a second independent cohort of 100 patients with moderate-to-severe psoriasis (PASI scores  $> 8$ ), 50 of whom had PsA. None of the 152 patients were treated with TNF inhibitors at the time of study participation. All recruited subjects patients reported European ethnicity. Blood samples were drawn at the time of clinical assessment, processed immediately, and serum aliquots were stored at  $-80^{\circ}\text{C}$  until KLK levels were determined by ELISAs.

## Enzyme-linked immunosorbent assays (ELISAs)

Serum levels of KLK5, KLK6, KLK7, KLK8, KLK10, KLK11 and KLK13 were determined using KLK-specific and sensitive immunofluorometric ELISAs developed in-house. The detailed procedure, specificity and sensitivity limits for each KLK ELISA assay are described elsewhere [21]. The assays were performed on stored serum samples



**Figure 1** Schematic flow chart of the experimental design.

linked to phenotypic information collected prospectively. The readers of the laboratory tests were blinded to the diagnosis and clinical information.

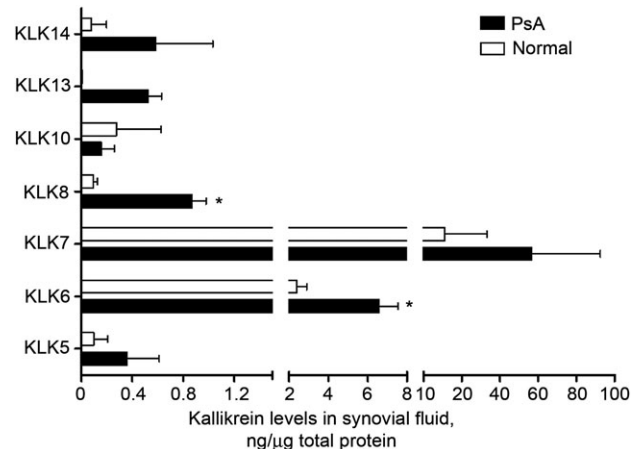
## Statistical analysis

In phase I, logistic regression models were fitted with disease classification as the outcome, using KLKs as explanatory variables while controlling for age and sex. Univariate, full multivariate and reduced multivariate logistic regression models were used to identify KLKs that are independently associated with disease class. Patients with PsC and PsA were grouped into one group to identify biomarkers for 'psoriatic disease'. Subsequently, patients with PsC, PsA, and controls were compared using polychotomous logistic regression. Finally, biomarkers that differentiate PsA from psoriasis were investigated by comparing patients with PsA to those with psoriasis alone. As phase I of the study was exploratory, results were considered to be statistically significant at  $p < 0.05$  and correction for multiple testing was not performed. In phase II, logistic regression models were fitted to examine the relationship between serum KLK6 and 8 levels and disease class. In order to determine the association between disease activity and KLK6 and 8 levels, correlation analyses and linear regression analyses were done with PASI score and joint counts as outcome and KLK6 and 8 levels, age, sex and disease duration as explanatory variables.

## Results

### KLK6 and KLK8 are elevated in PsA synovial fluids and lesional psoriatic skin

Synovial fluid samples were aspirated from the knee joints of three PsA patients and three patients with early OA. OA samples are commonly used as controls in rheumatology studies, because synovial fluid is not usually available from healthy individuals. Using ELISAs, we explored the presence of KLK proteases in the inflammatory synovial fluid from PsA patients, compared to non-inflammatory synovial fluid from patients with OA. As shown in Figure 2, many of the epidermal KLKs (such as KLK5, 6, 7, 8, 13 and 14) were detected in the synovial fluids of all three PsA patients, with significant elevation of KLK6 ( $p = 0.039$ ) and KLK8 ( $p = 0.013$ ) in PsA compared to OA. To our knowledge, this is the first report of KLK expression in human synovial fluids, although MMPs have been previously shown to be expressed in PsA synovial fluids and have been investigated as serum PsA biomarkers [22]. Given that among all the KLKs tested, KLK6 and KLK8 were significantly elevated in the synovial fluids of PsA patients, we next investigated their expression in psoriatic skin and confirmed the overexpression of these two KLKs in lesional psoriatic skin tissues by immunohistochemistry. Compared to



**Figure 2** KLK protein levels in PsA inflamed joint synovial fluids (n=3) and control (early OA) synovial fluids (n=3). KLK11 levels were below the detection level of the assay.

\*statistically significant differences between groups ( $p < 0.05$ ).

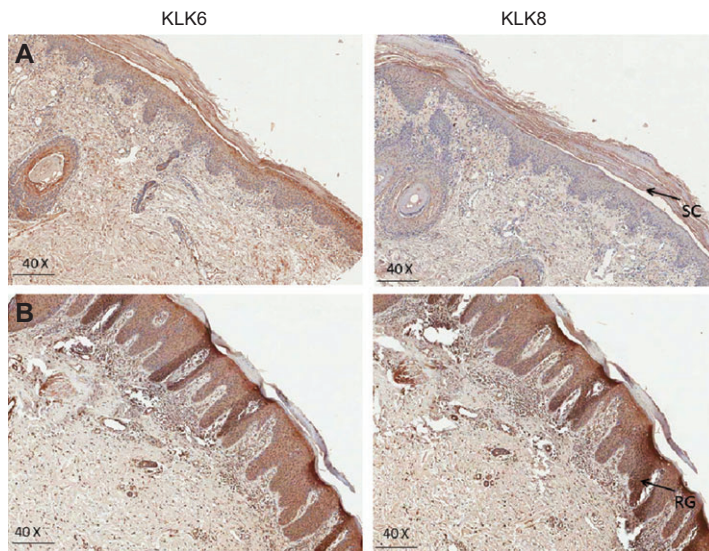
normal skin, where KLKs are normally expressed in the uppermost stratum corneum (SC) layer, KLK6 and KLK8 expression expanded below the SC into the spinous layer of the elongated rete ridges of psoriatic skin as shown in Figure 3.

### PsA and PsC patients

All PsC and PsA patients had well-established disease and were matched for age, sex and psoriasis duration. The demographics and disease characteristics of the study patients are summarized in Table 1.

### KLK8 is independently elevated in serum samples of patients with psoriatic disease

We investigated KLKs as soluble biomarkers of psoriatic disease by measuring their concentration in the serum of psoriasis patients (PsC and PsA) and healthy controls in two subsequent phases (Phase I and Phase II). The KLK levels detected are reported in Supplementary Table1 (Supplemental data which accompanies the article at <http://www.degruyter.com/view/j/cclm.2013.51.issue-2/issue-files/cclm.2012.51.issue-2.xml>). In a phase I pilot study, epidermal and synovial fluid-expressed KLK5, 6, 7, 8, 10, 11, 13 were measured in 52 psoriatic disease serum samples compared to 26 healthy controls. Among all the KLKs tested, only KLK8 levels were significantly elevated in psoriatic disease patients compared to controls in univariate logistic regression analyses adjusted for age and sex. Increased serum levels of KLK8 associated with psoriatic



**Figure 3** Immunohistochemical expression of KLK6 and KLK8 in lesional psoriatic skin. (A) Normal skin; (B) Psoriatic skin. SC, stratum corneum; SG, stratum granulosum; RG, rete ridges. Note expression of both KLK6 and KLK8 below the SC into the rete ridges (RG) of psoriatic skin indicated with an arrow.

disease in a multivariate reduced model adjusted for age and sex [odds ratio per unit increase (OR) = 2.56, 95% CI (1.08, 6.12),  $p=0.03$ ]. Polychotomous logistic regression analysis showed that only KLK8 had significantly different effects when modelling PsC and PsA separately, controlling for age, sex and the other KLKs, as shown in Table 2. Binomial logistic regression analyses did not demonstrate a significant difference in serum KLK levels between PsC and PsA.

### KLK8 serum levels in PsA correlate with PASI score but not inflamed joint counts

As mentioned above, we detected significant elevation of KLK8 in the sera of a small set of PsC and PsA patients with mild-to-moderate plaque-type psoriasis. Furthermore, KLK6 and KLK8 were significantly elevated in the synovial fluids of PsA patients and lesional psoriatic skin. Thus, we subsequently aimed to re-examine and validate

#### Phase I

Characteristic	PsA (n=26)	PsC (n=26)	Controls (n=26)
Females/males	14/12	14/12	14/12
Age, years	46.9±10.4 <sup>a</sup>	45.0±12.1	42.5±14.1
Duration of psoriasis, years	16.9±13.8	16.7±13.8	–
Duration of PsA, years	13.4±10.7	–	–
Psoriasis area and severity index score	3.70±3.50	4.90±5.20	–
Number of tender or swollen joints	15.7±12.3	–	–
Number of swollen joints	4.90±4.20	–	–

#### Phase II

Characteristic	PsA (n=50)	PsC (n=50)	Controls (n=26) <sup>b</sup>
Females/males	18/32	17/33	14/12
Age, years	51.8±12.2	45.9±13.0	42.5±14.1
Duration of psoriasis, years	23.0±11.7	17.4±12.4	–
Duration of PsA, years	16.9±11.7	–	–
PASI score	18.1±10.0	16.5±9.40	–
Number of tender and/or swollen joints	11.4±12.0	–	–
Number of swollen joints	2.20±3.0	–	–

**Table 1** Demographics and clinical characteristics of study subjects.

PsA, psoriatic arthritis; PsC, cutaneous psoriasis without arthritis. <sup>a</sup>For all continuous variables mean ± standard deviation is reported.

<sup>b</sup>Controls are the same for Phase I (mild-to-moderate psoriasis, PASI<8) and Phase II (moderate-to-severe psoriasis, PASI>8) patients.

Covariate	Homogeneity	Psoriatic arthritis			Cutaneous psoriasis		
	p-Value <sup>a</sup>	OR	95% CI	p-Value	OR	95% CI	p-Value
KLK5	0.36	0.014	(<0.001, 5.335)	0.16	0.071	(<0.001, 16.067)	0.34
KLK6	0.47	1.363	(0.321, 5.781)	0.67	2.490	(0.566, 10.942)	0.23
KLK7	0.70	1.058	(0.928, 1.205)	0.40	1.054	(0.926, 1.201)	0.43
KLK8	0.03	4.274	(1.024, 17.846)	0.05	6.623	(1.62, 26.950)	0.01
KLK10	0.10	0.462	(0.099, 2.160)	0.33	0.143	(0.024, 0.858)	0.03
KLK11	0.49	1.877	(0.102, 34.674)	0.67	0.284	(0.006, 12.534)	0.51

**Table 2** Polychotomous logistic regression analysis to identify biomarkers associated with patients having cutaneous psoriasis alone and psoriatic arthritis.

<sup>a</sup>The homogeneity p-values indicate whether the markers have significantly different effects when modelling psoriasis and PsA separately, controlling for age, sex and the other KLKs listed. The p-values associated with PsA and psoriasis indicate the significance of difference between either patient group compared to controls. KLK13 levels were un-measurable and are not reported. Only KLK8 was statistically significant, for discussion see text. OR, odds ratio; CI, confidence interval.

KLK6 and KLK8 as PsA screening biomarkers in a larger patient cohort consisting of 100 patients with moderate-to-severe psoriasis (PASI >8), with or without PsA (Phase II). Both KLK6 and KLK8 were elevated in the sera of PsA patients with moderate-to-severe psoriasis (Figure 4A). As listed in Supplementary Table 1, mean KLK6 levels in the sera of patients with mild-to-moderate PsC ( $1.95 \pm 0.49$   $\mu\text{g/L}$ ) was similar to age and sex-matched healthy controls ( $1.78 \pm 0.45$   $\mu\text{g/L}$ ), but was significantly higher in the sera of moderate-to-severe psoriasis patients ( $4.68 \pm 1.78$   $\mu\text{g/L}$ ). Conversely, mean KLK8 level was increased in the sera of both PsA patients with mild-to-moderate psoriasis ( $2.20 \pm 0.93$   $\mu\text{g/L}$ ) and patients with moderate-to-severe psoriasis ( $3.89 \pm 2.15$   $\mu\text{g/L}$ ), compared to healthy controls ( $1.67 \pm 0.5$   $\mu\text{g/L}$ ). A similar trend was observed for mean KLK6 and KLK8 serum levels in PsA patients. Logistic regression analysis demonstrated that neither KLK8 nor KLK6 levels distinguished PsA from PsC (Figure 4B).

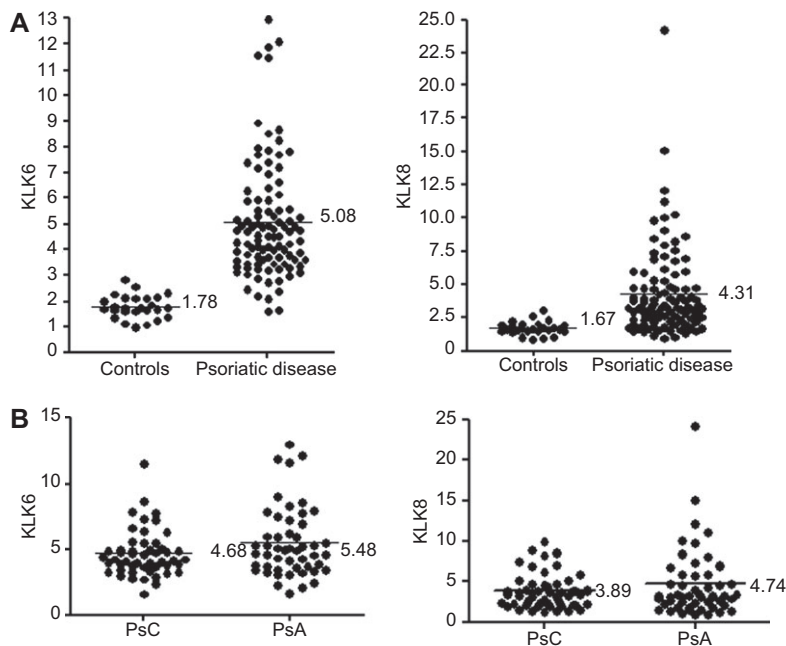
Furthermore, we investigated the association between serum levels of KLK6 and KLK8 with clinical parameters of PsA skin and joint activity, such as PASI scores and actively inflamed (tender and/or swollen) and swollen joint counts. As shown in Figure 5, both KLK6 ( $r=0.52$ ) and KLK8 ( $r=0.42$ ) serum levels correlated with the PASI scores of all patients ( $p<0.0001$ ). KLK8 correlated positively with PASI scores when patients with PsA ( $r=0.60$ ,  $p<0.0001$ ) and PsC ( $r=0.43$ ,  $p=0.001$ ) were considered separately. KLK6 correlated with PASI scores in PsA patients ( $r=0.63$ ,  $p<0.0001$ ) but not in PsC patients ( $r=0.036$ ,  $p=0.8$ ). When we adjusted for age, sex, psoriasis duration and disease group (PsA and PsC) in a linear regression model with PASI score as the outcome, only KLK8 was independently associated with the PASI score ( $\beta=1.153$ ,  $p=0.0003$ ); KLK6 was not ( $p=0.11$ ), Supplementary Table 2. In patients with PsA, although KLK8

serum levels correlated with PASI score independent of age, sex and psoriasis duration, there was no significant correlation with actively inflamed joint count ( $p=0.35$ ) or swollen joint count ( $p=0.12$ ). Thus, KLK8 serum level in PsA patients correlates with skin, but not joint arthritis, activity.

Together, our analyses show that multiple KLKs are co-expressed in PsA synovial fluids. KLK6 and KLK8 are significantly elevated in PsA synovial fluid compared to OA synovial fluid. Both KLK6 and KLK8 are involved in cutaneous psoriasis activity, with KLK8 being an independent serum biomarker of psoriasis skin activity among the KLKs tested. However, none of the skin and synovial-expressed KLKs identified in this study can function as markers for PsA in patients with psoriasis.

## Discussion

Psoriasis (PsC) and psoriatic arthritis (PsA) are relatively common inflammatory diseases of the skin and joints, respectively. Psoriatic arthritis (PsA) is an inflammatory arthritis that develops in up to one-third of psoriasis patients [5]. The varied manifestations of PsA make it difficult to recognize. Dermatologists managing a patient's psoriasis often do not inquire about symptoms of arthritis. Thus, the presence of PsA in psoriasis patients is often overlooked in dermatology clinics. Early diagnosis of PsA is essential to prevent joint damage progression and disability. The key to early PsA diagnosis is better recognition of PsA presence in psoriasis patients [23], which is difficult due to the lack of specific diagnostic tests. Soluble biomarkers have the potential to screen for arthritis in psoriasis patients so that an appropriate referral to a



**Figure 4** KLK6 and KLK8 cannot function as screening biomarkers for arthritis in psoriasis patients.

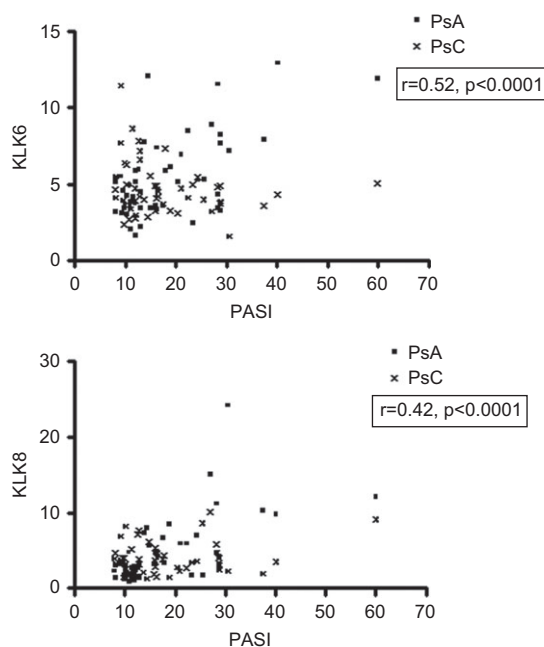
(A) KLK6 and KLK8 levels are elevated in moderate-to-severe psoriatic disease patients (combined PsC and PsA) compared to healthy controls. (B) KLK6 and KLK8 serum levels do not distinguish PsA from PsC patients.

rheumatologist is made for early PsA diagnosis. Candidate serum PsA biomarkers are likely to originate from target tissues, such as inflamed skin and joints. To date, few potential PsA screening biomarkers have been identified, none validated and additional biomarkers await discovery.

The main aim of this study was to evaluate various KLKs as potential PsA serum biomarkers in a cohort of psoriasis patients with or without PsA, with special emphasis on the relationships between KLKs and clinical parameters of PsA skin and joint activity.

Given that the majority of PsA patients initially present with cutaneous psoriasis, we hypothesized that KLKs may be overexpressed in the inflamed skin, joints and sera of PsA patients and may thus be potential serum markers of PsA. KLKs are secreted trypsin- and chymotrypsin-like serine proteases that are expressed in a wide range of tissues, including skin epidermis. They regulate skin barrier integrity via their ability to degrade adhesion molecules linking cells in the uppermost skin epidermis to result in skin desquamation and keratinocyte cell turnover every 2–4 weeks, and process antimicrobial peptides and cell surface proteinase-activated receptors (PARs) to activate the immune system when required [12–14, 24, 25]. KLK upregulation has been implicated in a number of inflammatory skin diseases, including psoriasis [26, 27], but their involvement in inflammatory joint arthritis, including PsA, has not been studied. The potential role of serum KLKs as soluble biomarkers of psoriasis severity and PsA has not been examined.

We detected multiple tissue KLKs in synovial fluids, with increased expression of KLK6 and KLK8 in PsA compared to non-inflammatory early OA patients. Non-inflammatory synovial fluid is defined as having <2000



**Figure 5** KLK6 and KLK8 correlate positively with the PASI scores in psoriatic disease (both PsA and PsC) patients.

leukocytes/mm<sup>3</sup> and a clear appearance [20]. Although the expression of tissue and plasma KLK with kinninogenase activity in the Kallikrein-kinin system has been detected in synovial fluids of arthritis patients [28], the expression of trypsin and chymotrypsin-like tissue KLKs in PsA synovial fluids has not been previously reported. In PsA, the synovial membrane becomes inflamed in response to proinflammatory cytokines, such as TNF- $\alpha$ , leading to the secretion of cartilage-digesting enzymes, such as MMPs by synovial fibroblasts [17, 29]. Similarly, KLK6 and KLK8 may be induced by cytokines to degrade collagenous and non-collagenous structural molecules in the joint. This hypothesis requires further scrutiny. These two KLKs were also elevated in lesional psoriatic skin in which they expanded lower into the spinous layer of the elongated rete ridges. Our immunohistochemistry results are consistent with previous reports of elevation of KLK6 and KLK8 transcripts within two separate gene clusters in a large-scale psoriasis gene expression analysis [30].

After examining KLK expression in the synovial fluids and lesional psoriatic skin, we measured the concentrations of KLK5, 6, 7, 8, 10, 11 and 13 in the sera of a cohort of PsA and PsC patients with mild-to-moderate psoriasis (PASI <8). Among all the KLKs tested, patients with PsC (n=26) and PsA (n=26) displayed significantly higher KLK8 serum levels compared to healthy controls (n=26). The remaining tested KLKs did not significantly vary in these patients. Thus, our initial exploratory analysis of KLK expression in the synovial fluids, lesional skin and serum of patients with psoriatic disease suggested KLK6 and KLK8 as candidate psoriatic disease biomarkers. Thus, we next measured the levels of these two KLKs in a larger independent cohort of serum samples of moderate-to-severe psoriasis patients, with or without arthritis (n=100, PASI >8). Although both KLK6 and KLK8 were elevated in the sera of these patients compared to healthy controls, the increase in KLK6 serum levels was not significant when we controlled for sex, age and disease duration, unlike KLK8.

Given that KLK8 was independently and significantly elevated in the sera of PsC and PsA patients, we next examined its correlation with clinical parameters of skin and joint activity. KLK8 correlated positively with the PASI score, but there was no significant correlation between serum KLK8 levels and actively inflamed joint or swollen joint counts, indicating that KLK8 is a soluble marker of skin activity, but not arthritis, in PsA patients.

We recently showed that KLK8 is a physiologically-active trypsin-like serine protease in normal human stratum corneum extracts [10]. KLK8 protein levels are significantly increased in lesional psoriatic skin-surface

compared to non-lesional and normal skin, whereby only the lesional psoriatic skin-surface exhibits higher trypsin-like activity [26]. Hence, the significant correlation between KLK8 serum levels and the PASI scores of both PsC and PsA patients reported herein indicates that KLK8 trypsin-like activity is related to the progressive skin barrier dysfunction in both PsC and PsA.

However, none of the epidermal and synovial-expressed KLKs identified in this study, including KLK6 and KLK8, which were elevated in PsA synovial fluids and psoriatic skin, can function as PsA screening serum biomarkers. Identifying PsA-specific soluble biomarkers remains a challenge to date. For instance, biomarkers from genetic and genomic studies, such as *HLA-Cw06*, *IL12B* and *IL23R* are associated with PsA, but their primary association is with psoriasis susceptibility [17, 22, 31].

TNF- $\alpha$  is present in the sera and skin of psoriasis patients and in the synovial fluids of PsA patients [32, 33]; however, TNF- $\alpha$  serum levels are not useful for screening PsA in psoriasis patients. Nonetheless, anti-TNF- $\alpha$  agents are effective in controlling skin and joint manifestations in PsA patients and preventing progression of joint damage [34]. Although our data show that KLKs cannot function as PsA screening biomarkers, we illuminate the importance of KLK8 as a marker of cutaneous psoriasis severity. As we demonstrate here that KLK8 can act as a surrogate serum biomarker of PASI, KLK8 may hold promise as a biomarker for monitoring response to PsC and PsA therapy and relapse. Furthermore, the detection of high levels of KLK8 in the sera of both PsC and PsA patients, as well as in the skin and synovial fluid of PsA patients, suggests that blockade of this protease may be beneficial in both skin and musculoskeletal manifestations of psoriasis.

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## Conflict of interest statement

**Authors' conflict of interest disclosure:** The authors state that there are no conflicts of interest regarding the publication of this article. Study support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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