Longitudinal IP-10 Serum Levels Are Associated with the Course of Disease Activity and Remission in Patients with Rheumatoid Arthritis

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ABSTRACT

Although rheumatoid arthritis (RA) is a chronic, persistent autoimmune disease, 10 to 15% of RA patients achieve sustained disease-modifying antirheumatic drug (DMARD)-free remission over time. The biological mechanisms underlying the resolution of persistent inflammation in RA are still unidentified, and there is a lack of prognostic markers. It is well established that increased serum levels of gamma interferon-induced protein 10 (IP-10) are associated with (acute) increased inflammatory responses (e.g., in leprosy). In order to assess the potential of IP-10 as a diagnostic tool for inflammatory episodes of RA, we performed a retrospective study and assessed IP-10 levels in longitudinally banked serum samples obtained from patients upon first diagnosis of RA. The selection consisted of 15 persistent RA patients and 19 patients who achieved DMARD-free sustained remission. IP-10 levels, measured by use of a user-friendly quantitative lateral flow assay (LFA), showed up to 170-fold variation interindividually, and baseline IP-10 levels could not be differentiated between the two patient groups. However, a difference in the change in IP-10 levels between the first and last visits (ΔIP-10) was observed (P = 0.003) between DMARD-free (median ΔIP-10, −662 pg/ml [decrease]) and persistent (median ΔIP-10, 468 pg/ml [increase]) RA patients. Moreover, intraindividual changes in IP-10 levels during the course of disease corresponded to the disease activity score (DAS) (P = 0.05). These data indicate that IP-10 is associated with disease activity and perseverance of RA. The association of IP-10 with DAS indicates that this tool may be a practical diagnostic aid to help in monitoring disease progression in RA patients and may also find applications in other chronic diseases with exacerbated inflammatory episodes.

KEYWORDS DAS, DMARD-free, immune monitoring, IP-10, longitudinal, point-of-care assay, rheumatoid arthritis, user-friendly

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Rheumatoid arthritis (RA), an autoimmune disease characterized by joint destruction and persistent inflammation, can be treated adequately using disease-modifying antirheumatic drugs (DMARDs). Clinically relevant joint destruction has therefore become scarce in Western countries, although persistent inflammation is still observed in the majority of patients. However, 10 to 15% of RA patients achieve DMARD-free remission, which is defined as the sustained absence of synovitis after termination of DMARD therapy for at least 1 year. This is an important long-term outcome, since it approximates a definitive cure of the disease (1). Several factors are known to be associated with DMARD-free remission, such as short symptom duration at treatment initiation and the absence of autoantibodies (2). The biological mechanisms underlying...
the persistence of RA are unknown, but persistent inflammation is induced by an imbalance between pro- and anti-inflammatory cytokines, which are numerously present in the synovial tissue (3).

The occurrence of RA is strongly associated with several disease susceptibility genes (4). In this respect, it is notable that RA shares genetic risk factors with completely different inflammatory diseases, such as leprosy (5, 6). In leprosy, a chronic infectious disease caused by Mycobacterium leprae, an increase of gamma interferon-induced protein 10 (IP-10) can be used to monitor the onset of leprosy reactions, acute episodes of increased inflammation which are the major cause of leprosy-associated tissue damage (7–9). The chemokine IP-10 is secreted by various cell types, and through its receptor (CXCR3), it regulates immune responses by activation and recruitment of leukocytes (T cells, monocytes, and NK cells) (10). Leprosy reactions have considerable overlap with acute immunological complications (flares) in other chronic diseases, such as RA (11), Buruli ulcer (12, 13), and Crohn’s disease (14, 15). Flares of these chronic diseases are characterized by a sudden increase in inflammation as well, leading to a deterioration of disease symptoms and often to irreversible tissue damage. Moreover, leprosy can even induce inflammatory arthritis and thereby mimic RA (16). In view of the shared susceptibility genes between RA and leprosy, we hypothesized that the tissue-destructive episodes of RA (flares) and leprosy (reactions) may share a pathway characterized by increased IP-10 production, and thus that IP-10 measurements may similarly be useful to monitor RA disease progression.

IP-10 is known to be overexpressed in synovial tissue of RA patients (17) and is elevated in sera of RA patients as well as prior to development of RA (18). These elevated levels implicate an immunopathogenic role for IP-10 in RA, which is supported by the observed decline in IP-10 levels in combination with improved disease activity scores (DAS) (19). DAS is used in the clinic to monitor disease activity and is based on the numbers of tender joints and swollen joints, the erythrocyte sedimentation rate (ESR), and general health. Although IP-10 seemed to be associated with DAS (19), longitudinal measurements of IP-10 in association with DMARD-free sustained remission had not yet been performed. In this study, we therefore monitored the levels of IP-10 for up to 7 years for both persistent and DMARD-free RA patients to examine the association of this inflammatory marker with disease activity and persistence.

IP-10 levels were assessed by use of quantitative up-converting phosphor lateral flow assays (UCP-LFAs), which were previously applied to examine IP-10 levels during reactional episodes in leprosy patients (7). These UCP-LFAs proved to be sensitive, user-friendly tools and have great potential for point-of-care (POC) monitoring of intraindividual differences in blood components over time.

RESULTS

Baseline characteristics. Persistent RA patients (n = 15) and patients who achieved DMARD-free sustained remission (n = 19) were selected from the Leiden Early Arthritis Clinic (EAC) (20), and their baseline characteristics were compared (Table 1). In line with previous observations (2), the symptom duration at inclusion was significantly longer for persistent RA patients. Additionally, several disease characteristics (swollen joint count [SJC], DAS44 [disease activity score based on the swollen joint count for 44 joints, the Ritchie articular index, the erythrocyte sedimentation rate, and a visual analogue scale for patient global assessment of disease activity], and ESR) were higher for these patients, and persistent RA patients were positive for anti-citrullinated protein antibodies (ACPA) more frequently than patients achieving DMARD-free remission.

IP-10 levels decrease in DMARD-free RA patients. In order to examine the association of IP-10 with RA persistence, IP-10 levels were measured longitudinally by use of UCP-LFAs. IP-10 levels at baseline varied from 316 to 53,685 pg/ml among RA patients but did not differ significantly (P = 0.19) between the two groups (median for persistent RA group, 1,991 pg/ml [range, 316 to 4,680 pg/ml]; median for DMARD-free sustained remission group, 3,292 pg/ml [range, 365 to 53,685 pg/ml]). However, after first diagnosis, the DMARD-free sustained remission patients gradually showed a de-
creased trend in IP-10 levels, which was not observed for persistent RA patients (Fig. 1). This was clearly demonstrated by the significant difference in ΔIP-10 (IP-10 serum level at last visit minus that at first visit) during the course of disease between persistent RA patients (median ΔIP-10, 468 pg/ml) and DMARD-free sustained remission patients (median ΔIP-10, 662 pg/ml) (\(P = 0.003\)). Furthermore, repeated-measurement analysis incorporating all serial IP-10 measurements showed a significant difference in IP-10 levels between the two groups (\(P = 0.001\)).

**IP-10 levels are associated with disease activity.** Since IP-10 levels decreased in patients achieving DMARD-free remission, representing patients with low disease activity, we examined the relationship between DAS and IP-10. For individual patients, IP-10 levels corresponded to the DAS, as evident from decreased DAS values accompanied by diminished IP-10 serum concentrations (Fig. 2; see Fig. S1 in the supplemental material). This association was observed for patients with persistent RA as well as for

### TABLE 1 Baseline characteristics of patients

| Variable | Persistent RA patients (n = 15) | DMARD-free remission patients (n = 19) | P value |
|----------|----------------------------------|---------------------------------------|---------|
| Age (yr) (mean [SD]) | 60 (13) | 50 (19) | 0.082 |
| No. (%) of females | 11 (73) | 12 (63) | 0.72 |
| Symptom duration (wk) (median [IQR]) | 19.9 (31) | 8.6 (15.5) | 0.019 |
| TJC (median [IQR]) | 5 (9) | 6 (11) | 0.63 |
| SJC (median [IQR]) | 12 (16) | 5 (16) | 0.01 |
| CRP concn (mg/liter) (median [IQR]) | 20 (32) | 17 (30) | 0.51 |
| ESR (mm/h) (median [IQR]) | 58 (68) | 28 (24) | 0.018 |
| DAS44 (median [IQR]) | 4.5 (2.6) | 3.2 (1.0) | 0.009 |
| ACPA positivity (no. [%]) | 11 (73) | 4 (21) | 0.005 |
| RF positivity (no. [%]) | 9 (60) | 8 (42) | 0.30 |

*\(^{a}\)Differences in baseline characteristics for persistent rheumatoid arthritis (RA) patients (n = 15) and patients achieving disease-modifying antirheumatic drug (DMARD)-free remission (n = 19). Significant P values are indicated in bold. n, number of patients; SD, standard deviation; IQR, interquartile range; symptom duration, time between symptom onset and inclusion in cohort; TJC, tender joint count for 68 joints; SJC, swollen joint count for 66 joints; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; DAS44, disease activity score based on swollen joint count for 44 joints, the Ritchie articular index, the erythrocyte sedimentation rate, and a visual analogue scale for patient global assessment of disease activity; ACPA, anti-citrullinated protein antibodies; RF, rheumatoid factor.*

**FIG 1** Time course of IP-10 levels in persistent rheumatoid arthritis (RA) patients and patients achieving disease-modifying antirheumatic drug (DMARD)-free remission. The box-and-whisker plot shows IP-10 levels at different time points (0 to 84 months) for DMARD-free (open boxes) and persistent RA (striped boxes) patients. The whiskers show the minimum and maximum values per time point. For DMARD-free remission patients, the number of patients at each time point (months) was as follows: 0, n = 17; 3, n = 11; 12, n = 16; 24, n = 18; 36, n = 14; 48, n = 10; 60, n = 4; 72, n = 2; and 84, n = 1. For persistent RA patients, the number of patients at each time point (months) was as follows: 0, n = 8; 3, n = 0; 12, n = 12; 24, n = 2; 36, n = 14; 48, n = 10; 60, n = 0; 72, n = 0; and 84, n = 0.
patients achieving DMARD-free remission, as illustrated by the data for four patients representative of the persistent RA (Fig. 2A) and DMARD-free remission (Fig. 2B) groups. For these four patients, the commonly used ESR and C-reactive protein (CRP) markers to determine disease activity correlated less well with DAS than IP-10 did (Fig. S2). In contrast to IP-10 levels, median ESR and CRP levels decreased in both patient groups (Fig. S3A and B). Moreover, there was no significant correlation observed between IP-10 and CRP or between IP-10 and ESR, whereas ESR and CRP did correlate with each other \((P < 0.0001)\) (Fig. S3C to E), showing an added value for IP-10 in monitoring disease activity.

To further examine whether the differences in DAS (ΔDAS) and IP-10 (ΔIP-10) corresponded between two sequential visits, we calculated ΔIP-10 and ΔDAS ratios. These ratios are quantified differences in IP-10 level or DAS and reflect the change in IP-10 level or DAS between two visits. In Fig. 3, the data are divided into two groups based on the ΔDAS ratio; a negative ΔDAS ratio reflects a decrease in DAS over time, and a positive ΔDAS ratio reflects an increase in DAS. The corresponding ΔIP-10 ratios were plotted per group; positive ratios corresponded to an increase in either IP-10 level, whereas negative ratios corresponded to a decrease over time. In general, a negative ΔDAS ratio was accompanied by a negative ΔIP-10 ratio (median, ≈−0.34), and vice versa (median ΔIP-10 ratio, 0.27) (Fig. 3). Moreover, the ΔIP-10 ratio significantly differed between time points with negative and those with positive DAS ratios \((P = 0.05)\), showing the association of IP-10 and DAS.

**DISCUSSION**

Although RA is a persistent disease in the majority of cases, a small proportion of patients (10 to 15%) are able to achieve DMARD-free remission, thereby approximating a definitive cure (1). In this study, we examined the association of IP-10 with the persistence of RA by using longitudinal follow-up serum samples from patients who achieved DMARD-free remission or patients with persistent RA. The observed decline of IP-10 in patients achieving DMARD-free remission and the association of IP-10 with
disease activity implicate an immunopathogenic role for IP-10 in the persistent and more inflammatory state of RA, which may be exploited for diagnostic purposes.

The role of IP-10 as a disease activity marker is supported by a recent study of early untreated RA patients in which, among 15 chemokines measured, only IP-10 was shown to correlate with multiple disease activity measures (21). However, we showed that IP-10 not only is relevant in early RA but remains associated with disease activity during the course of disease.

A phase II trial using anti-IP-10 monoclonal antibodies to treat RA patients who did not respond to methotrexate therapy showed significant improvements in several clinical characteristics upon blocking of IP-10 (22), supporting a detrimental role for IP-10 in disease characteristics. Patients responding to the anti-IP-10 monoclonal antibody showed increased mRNA expression of FoxP3, a marker of regulatory T cells (Tregs) (22), implying that a decrease in IP-10 can restore the disrupted balance between pro- and anti-inflammatory cytokines. The role of IP-10 was quite clear in animal models, as IP-10-deficient mice demonstrated an enhancement of regulatory T-cell activity as well (23), whereas an increase in FoxP3 Tregs was accompanied by a regression in paw inflammation in induced arthritic rats (24). Whether the absence of synovitis in DMARD-free remission can be explained by the observed reduction of IP-10, and thereby an improved activity of Tregs, still requires further examination. Furthermore, the measurement of IP-10 in an entire cohort of RA patients instead of a selection of extremes would provide further insights into the association of IP-10 with disease activity.

Another inflammatory mediator that we measured in this study was macrophage inflammatory protein 1β (MIP-1β), which was shown to be involved in synovitis (25); however, we did not observe an association for MIP-1β and DAS (data not shown), which was confirmed by a recent study (21). Serum levels of MIP-1β are therefore not useful for monitoring disease activity in RA.

Note that while intra-individual IP-10 differences corresponded nicely with fluctuations in disease activity, baseline levels of IP-10 differed inter-individually within patient groups. The level of IP-10 at a single time point is therefore not informative whatsoever with respect to disease activity, as it may fluctuate significantly between different individuals, regardless of their clinical status. Similar results were obtained for sera derived from leprosy patients with or without leprosy reactions (8, 26). Longitudinal monitoring is therefore vital for management of inflammatory diseases and can provide
immediate insight into the course of disease at a point-of-care level. Moreover, the user-friendly UCP-LFA can also be combined with finger-stick blood instead of serum derived from venous blood (27). This allows (self-)monitoring of IP-10 levels as well as other relevant markers in blood that are indicative of DMARD-free remission.

Inclusion of IP-10 as a marker to assess disease activity in RA can contribute to a biomarker profile for disease activity, as biomarker profiles with multiple parameters have proven to be more accurate than single markers for tuberculosis (28, 29) and leprosy (30, 31).

The increase in IP-10 serum levels during acute inflammatory episodes of leprosy (7–9), the exaggerated inflammatory responses in Behçet’s syndrome (32), and the association with augmented RA disease activity observed in this study suggest a longitudinal increase in IP-10 as a general marker for episodes of exacerbated inflammation. The association of IP-10 with DAS shows that rapid assessment of IP-10 levels by use of user-friendly UCP-LFAs can potentially be used as an easy and cheap alternative or additional tool to help monitor disease activity in RA. With the shared occurrence of acute episodes of exacerbated inflammation in diseases other than RA and leprosy, these rapid POC tests can possibly also be applied as tools for (self-)monitoring of diseases such as inflammatory bowel disease (IBD) and psoriasis (33, 34), allowing early treatment and subsequent reduction of tissue damage.

MATERIALS AND METHODS

Patients. A selection of 139 serum samples from 34 RA patients (according to the 1987 RA criteria) included in the Leiden Early Arthritis Clinic (EAC) (20) were studied, obtained at the time of diagnosis, at 3 months, and at yearly intervals thereafter. The EAC is an inception cohort that includes patients with clinically confirmed arthritis and a symptom duration of <2 years (35). Patients were included between 1993 and 2008, and all were DMARD naive at inclusion. The 15 patients with persistent RA were selected based on a high swollen joint count (SJC) within the first 3 years after disease onset and had never achieved DMARD-free remission. The other 19 patients were selected based on the achievement of DMARD-free remission. DMARD-free remission was defined as the sustained absence of synovitis (by physical examination) after discontinuation of DMARD therapy for at least 1 year after DMARD withdrawal. DMARD therapy includes the use of biologics and corticosteroids, but nonsteroidal anti-inflammatory drugs (NSAIDs) were not qualified as DMARDs (1). The 19 patients studied here achieved DMARD-free remission after a median follow-up of 2.3 years (interquartile range [IQR], 1.9 to 3.3 years).

Ethics. This study was performed according to the guidelines of the Helsinki Declaration as described previously (36). The study protocol was approved by the Medical Ethical Commission of the Leiden University Medical Center (LUMC) (protocol 05-2016; CME no. B15.015). Written informed consent was obtained before enrollment.

UCP-LFA for IP-10 detection. Quantitative lateral flow (LF)-based assays were applied to detect IP-10 as described previously (26, 37, 38). Briefly, a mixture of 100 ng of a cytokine-specific UCP reporter conjugate and a diluted (1:30) serum sample was incubated for 60 min on a thermal shaker at 37°C and 900 rpm. The mixture was applied to IP-10-specific LF strips (containing a test line with an anti-IP-10 antibody complementary to the antibody on the UCP particles), and immunochromatography was allowed to continue for at least 30 min. Dry LF strips were scanned in a Packard FluoroCount microtiter plate reader adapted for measurement of the UCP label (980-nm infrared [IR] excitation, 550-nm emission). Results are displayed as ratios of test and flow control values, based on the relative fluorescence units (RFUs) measured at the respective capture lines, as described earlier (39). Ratio values were translated to concentrations based on standard curves.

DAS. DAS is a continuous measure to objectify disease activity in RA patients. It includes assessment of the number of tender joints, number of swollen joints, erythrocyte sedimentation rate (ESR), and general health (measured on a visual analogue scale) (40).

Data analysis. (i) Baseline characteristics. Differences in baseline characteristics between DMARD-free and persistent RA patients were determined by the independent t test (age), Fisher’s exact test (sex and ACPA), the Mann-Whitney U test (symptom duration, tender joint count [TJC] for 68 joints, swollen joint count [SJC] for 66 joints, C-reactive protein [CRP] level, erythrocyte sedimentation rate [ESR], and DAS), and the chi-square test (rheumatoid factor [RF]).

(ii) DMARD-free versus persistent RA patients. IP-10 levels at baseline were compared for patients with persistent RA and patients achieving DMARD-free remission, using the Mann-Whitney U test. The difference between IP-10 levels at two different time points (∆IP-10) was calculated. Medians of the ∆IP-10 values for the last visit relative to the baseline visit were compared for patients with persistent RA and patients with DMARD-free RA by using the Mann-Whitney U test. To incorporate all serial measurements into one analysis, a linear mixed-model analysis was used with serial log10-transformed IP-10 levels as a response variable and time and group (persistent RA patients versus patients with DMARD-free sustained remission) as variables. To model the correlation over time, the compound symmetry matrix was used. An interaction term between time and group was added, thereby testing whether the courses of IP-10 levels differed between the two groups.
DAS over time were plotted for individual patients. To correct for interindividual differences, the delta values were divided by the first of the two sequential time points (e.g., 3 months versus baseline, 1 year versus 3 months, etc.).

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We declare that we have no financial/commercial conflicts of interest.

### References

1. Ajeanov S, van Steenbergen HW, van Nies JA, Burgers LE, Huizenga TW, van der Helm-van Mil AH. 2016. Disease-modifying antirheumatic drug-free sustained remission in rheumatoid arthritis: an increasingly achievable outcome with subsidence of disease symptoms. Ann Rheum Dis 75:867–873. https://doi.org/10.1136/annrheumdis-2014-207080.
2. van der Woude D, Young A, Jayakumar K, Mertens BJ, Toes RE, van der Heijde D, Huizinga TW, van der Helm-van Mil AH. 2009. Prevalence of and predictive factors for sustained disease-modifying antirheumatic drug-free remission in rheumatoid arthritis: results from two large early arthritis cohorts. Arthritis Rheum 60:2262–2271. https://doi.org/10.1002/art.24661.
3. McInnes IB, Schett G. 2007. Cytokines in the pathogenesis of rheumatoid arthritis. Nat Rev Immunol 7:429–442. https://doi.org/10.1038/nri2094.
4. Koning F, Thomas R, Rossjohn J, Toes RE. 2015. Coeliac disease and rheumatoid arthritis: similar mechanisms, different antigens. Nat Rev Rheumatol 11:450–461. https://doi.org/10.1038/nrrheum.2015.59.
5. Zhang DF, Wang D, Li YY, Yao YG. 2016. Integrative analyses of leprosy susceptibility genes indicate a common autoimmune profile. J Dermatol Sci 82:18–27. https://doi.org/10.1016/j.jdermsci.2016.01.001.
6. Zhang Q, Fan HW, Zhang JZ, Wang YM, Xing HJ. 2015. NLRP3 rs35829419 polymorphism is associated with increased susceptibility to multiple diseases in humans. Genet Mol Res 14:13968–13980. https://doi.org/10.4238/2015.October.29.17.
7. Carstens PL, van Hooij A, Tjon Kon Fat EM, van den Eeden SJ, Wilson L, Geluk A. 2016. Field-friendly test for monitoring multiple immune response markers during onset and treatment of exacerbated immunity in leprosy. Clin Vaccine Immunol 23:515–519. https://doi.org/10.1128/CVI.00033-16.
8. Khadge S, Banu S, Bobosha K, van der Ploeg-van Schip JJ, Goulart LM, Thapa P, Kunwar CB, van Meijgaarden KE, van den Eeden SJ, Wilson L, Kabir S, Dey H, Goulart LR, Lobato J, Carvalho W, Bekele Y, Franken KL, Aseffa A, Spencer JS, Oskam L, Ottenhoff TH, Haggé DA, Geluk A. 2015. Longitudinal immune profiles in type 1 leprosy reactions in Bangladesh, Brazil, Ethiopia and Nepal. BMC Infect Dis 15:477. https://doi.org/10.1186/s12879-015-1128-0.
9. Scollard DM, Chauduvula MV, Martinez A, Fowlkes N, Nath I, Strjewskia BM, Kearney MT, Williams DL. 2011. Increased CXCL ligand 10 levels and gene expression in type 1 leprosy reactions. Clin Vaccine Immunol 18:947–953. https://doi.org/10.1128/CVI.00042-11.
10. Lee EE, Lee ZH, Song YW. 2013. The interaction between CXCL10 and cytokines in chronic inflammatory arthritis. Autoimmun Rev 12:554–557. https://doi.org/10.1016/j.autrev.2012.10.001.
11. Kuipper TM, Lammers-Karnebeek FB, Jacobs JW, Hazes JM, Luime JJ. 2015. Flare rate in patients with rheumatoid arthritis in low disease activity or remission when tapering or stopping synthetic or biologic DMARD: a systematic review. J Rheumatol 42:2012–2022. https://doi.org/10.3899/jrheum.141520.
12. Barogui YT, Kils SA, Johnson RC, Phillips RO, van der Veer E, van Diemen C, van der Werf TS, Stienstra Y. 2016. Genetic susceptibility and predictors of paradoxical reactions in Buruli ulcer. PLoS Negl Trop Dis 10:e0004594. https://doi.org/10.1371/journal.pntd.0004594.
13. Nienhuis WA, Stienstra Y, Abass KM, Tuah W, Thompson WA, Awuah PC, Awuah-Boateng NY, Adjei O, Bretzel G, Schouten JP, van der Werf TS. 2012. Paradoxical responses after start of antimicrobial treatment in Mycobacterium ulcerans infection. Clin Infect Dis 54:519–526. https://doi.org/10.1093/cid/cis586.
14. Grant AV, Alter A, Huong NT, Orlova M, Van TN, Ba NN, Thai VH, Abel L, Schurr E, Alcais A. 2012. Crohn’s disease susceptibility genes are associated with leprosy in the Vietnamese population. J Infect Dis 206:1763–1767. https://doi.org/10.1093/infdis/jis588.
15. Fava VM, Manry J, Cobat A, Orlova M, Van TN, Moraes MO, Sales-Marques C, Stefani MM, Latin AC, Belone AF, Thai VH, Abel L, Alcais A, Schurr E. 2017. A genome wide association study identifies a IncRNA as risk factor for pathological inflammatory responses in leprosy. PLoS Genet 13:e1006637. https://doi.org/10.1371/journal.pgen.1006637.
16. Gupta L, Zanwar A, Wakhlu A, Agarwal V. 2016. Leprosy in the rheumatology clinic: an update on this great mimic. Int J Rheum Dis 19:941–945. https://doi.org/10.1111/1756-185X.13023.
17. Yoshida S, Arakawa F, Higuchi F, Ishibashi Y, Goto M, Sugita Y, Nomura Y, Niino D, Shimizu K, Aoki R, Hashikawa K, Kimura Y, Yasuda K, Tashiro K, Kuhara S, Nagata K, Ohshima K. 2012. Gene expression analysis of rheumatoid arthritis synovial lining regions by cDNA microarray combined with laser microdissection: up-regulation of inflammation-associated STAT1, IRF1, CXCL9, CXCL10, and CCL5. Scand J Rheumatol 41:170–179. https://doi.org/10.3109/03009742.2011.623137.
18. Kokkonen H, Soderstrom I, Rocklov J, Hallmans G, Lejon K, Rantapaa DS. 2010. Up-regulation of cytokines and chemokines predates the onset of rheumatoid arthritis. Arthritis Rheum 62:383–391. https://doi.org/10.1002/art.27186.
19. Kuan WP, Tam LS, Wong CK, Ko FW, Li T, Zhu T, Li EK. 2010. CXCL 9 and...
CXCL 10 as sensitive markers of disease activity in patients with rheumatoid arthritis. J Rheumatol 37:257–264. https://doi.org/10.3899/jrheum.090769.

20. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG. 1988. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31:315–324. https://doi.org/10.1002/art.1780310302.

21. Pandya JM, Lundell AC, Andersson K, Nordstrom I, Theander E, Rudin A. 2017. Blood chemokine profile in untreated early rheumatoid arthritis: CXCL10 as a disease activity marker. Arthritis Res Ther 19:20. https://doi.org/10.1186/s13075-017-1224-1.

22. Yellan M, Palienko I, Balanescu A, Ter-Vartanian S, Tseluyko V, Xu LA, Tao X, Cardarelli PM, Leblanc H, Nichol G, Ancuta C, Chirieac L, Luo A. 2012. A phase II, randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of MDX-1100, a fully human anti-CXCL10 monoclonal antibody, in combination with methotrexate in patients with rheumatoid arthritis. Arthritis Rheum 64:1730–1739. https://doi.org/10.1002/art.34330.

23. Heller EA, Liu E, Tager AM, Yuan Q, Lin AY, Ahluwalia N, Jones K, Koehn SL, Lok VM, Aikawa E, Moore KJ, Luster AD, Gersten RE. 2006. Chemo-kine CXCL10 promotes arterogenesis by modulating the local balance of effector and regulatory T cells. Circulation 113:2301–2312. https://doi.org/10.1161/CIRCULATIONAHA.105.605121.

24. Eissa MM, Mostafa DK, Ghazy AA, El Azzouni MZ, Boulos LM, Younis LK. 2016. Anti-arthritis activity of Schistosoma mansoni and Trichinella spiralis derived-antigens in adjuvant arthritis in rats: role of FOXP3+ Treg cells. PLoS One 11:e0165916. https://doi.org/10.1371/journal.pone.0165916.

25. Andersen M, Boesen M, Ellegaard K, Christensen R, Soderstrom K, Soe N, Spee P, Morch UG, Torp-Pedersen S, Bartels EM, Danneskiold-Samsoe B, Vendel N, Karlsson L, Bliddal H. 2014. Synovial explant inflammatory mediator production corresponds to rheumatoid arthritis imaging hallmarks: a cross-sectional study. Arthritis Res Ther 16:R107. https://doi.org/10.1186/ar4557.

26. Bobosha K, Tjon Kon Fat EM, van den Eeden SJ, Bekele Y, van der Ploeg-van Schip JJ, de Dooij CJ, Dijkman K, Franken KL, Wilson L, Aseffa A, Spencer JS, Ottenhoff TH, Corstjens PL, Geluk A. 2014. Field-evaluation of a new lateral flow assay for detection of cellular and humoral immune responses. Clin Biochem 44:1241–1246. https://doi.org/10.1016/j.clinbiochem.2011.06.983.

27. van Dam GJ, de Dooij CJ, Lewis MD, Deelder AM, van Lieshout L, Tanke HJ, van Rooyen LH, Corstjens PL. 2013. A robust dry reagent lateral flow assay for diagnosis of active schistosomiasis by detection of Schistosoma circulating anodic antigen. Exp Parasitol 135:274–282. https://doi.org/10.1016/j.exppara.2013.06.017.

28. Chegou NN, Sutherland JS, Malherbe S, Crampin AC, Corstjens PL, Geluk A, Manjaay-Kizza H, Loxton AG, van der Spuy G, Stanley K, Kotze LA, van der Vyver M, Oelofse MC, Aikawa E, Moore KJ, Luster AD, Ottenhoff TH, Kaufmann SH, Walzl G. 2016. Diagnostic performance of a seven-marker serum protein biosignature for the diagnosis of active TB disease in African primary healthcare clinic attendees with signs and symptoms suggestive of TB. Thorax 71:785–794. https://doi.org/10.1136/thoraxjnl-2015-207999.

29. Jacobs R, Malherbe S, Loxton AG, Stanley K, van der Spuy G, Walzl G, Chegou NN. 2016. Identification of novel host biomarkers in plasma as candidates for the immunodiagnosis of tuberculosis disease and monitoring of tuberculosis treatment response. Oncotarget 7:57581–57592. https://doi.org/10.18632/oncotarget.11420.

30. van Hooij A, Tjon Kon Fat EM, Richardus R, van den Eeden SJ, Wilson L, de Dooij CJ, Faber R, Alam K, Richardus JH, Corstjens PL, Geluk A. 2016. Quantitative lateral flow strip assays as user-friendly tools to detect biomarker profiles for leprosy. Sci Rep 6:34260. https://doi.org/10.1038/srep34260.

31. Geluk A, Bobosha K, van der Ploeg-van Schip JJ, Spencer JS, Banu S, Martins MV, Cho SN, Franken KL, Kim HJ, Bekele Y, Uddin MK, Hadi SA, Aseffa A, Pessolani MC, Pereira GM, Dockrell HM, Ottenhoff TH. 2012. New biomarkers with relevance to leprosy diagnosis applicable in areas hyperendemic for leprosy. J Immunol 188:4782–4791. https://doi.org/10.4049/jimmunol.1103452.

32. Ambrose N, Khan E, Ravindran R, Lightstone L, Abraham S, Botto M, Johns M, Haskard DO. 2015. The exaggerated inflammatory response in Behcet’s syndrome: identification of dysfunctional post-transcriptional regulation of the IFN-gamma/CXCL10 IP-10 pathway. Clin Exp Immunol 181:427–433. https://doi.org/10.1111/cei.12655.

33. Ge X, Hu D, Cao Y, Liu Z, Ding C, Tian H, Gong J, Zhu W, Li N, Li J. 2016. Procalcitonin in Crohn’s disease with fever episodes, a variable to differentiate intra-abdominal abscess from disease flares. Int J Surg 36:34–39. https://doi.org/10.1016/j.ijsu.2016.10.011.

34. Osborne JE, Hutchinson PE. 2008. Demographic and clinical correlates of extent of psoriasis during stable disease and during flares in chronic plaque psoriasis. Br J Dermatol 158:721–726. https://doi.org/10.1111/j.1365-2133.2008.08447.x.

35. de Rooy DP, van der Linden MP, Knevel R, Huizinga TW, van der Helm-van Mil AH. 2011. Predicting arthritis outcomes—what can be learned from the Leiden Early Arthritis Clinic? Rheumatology (Oxford) 50:93–100. https://doi.org/10.1093/rheumatology/keq230.

36. Richardus RA, Alam K, Pahan D, Feenstra SG, Geluk A, Richardus JH. 2013. The combined effect of chemophrophylaxis with single dose rifampicin and immunophrophylaxis with BCG to prevent leprosy in contacts of newly diagnosed leprosy cases: a cluster randomized controlled trial (MALTALEP study). BMC Infect Dis 13:456. https://doi.org/10.1186/1471-2334-13-456.

37. Corstjens PL, de Dooij CJ, van der Ploeg-van Schip JJ, Wiesmeijer KC, Ruijtamaeki T, van Meijgaarden KE, Spencer JS, Tanke HJ, Ottenhoff TH, Geluk A. 2011. Lateral flow assay for simultaneous detection of cellular- and humoral immune responses. Clin Biochem 44:1241–1246. https://doi.org/10.1016/j.clinbiochem.2011.06.083.

38. Corstjens PL, Tjon Kon Fat EM, de Dooij CJ, van der Ploeg-van Schip JJ, Wiesmeijer KC, Ruijtmakere T, van Meijgaarden KE, Spencer JS, Tanke HJ, Ottenhoff TH, Geluk A. 2011. Lateral flow assay for simultaneous detection of cellular- and humoral immune responses. Clin Biochem 44:1241–1246. https://doi.org/10.1016/j.clinbiochem.2011.06.083.

39. Corstjens PL, Tjon Kon Fat EM, de Dooij CJ, van der Ploeg-van Schip JJ, Franken KL, Chegou NN, Sutherland JS, Howe R, Mihret A, Kassa D, van der Vyver M, Sheehama VJ, Simukonda F, Manjaay-Kiza F, Ottenhoff TH, Walzl G, Geluk A. 2016. Multi-center evaluation of a user-friendly lateral flow test to detect IP-10 and CCL4 levels in blood of TB and non-TB cases in Africa. Clin Biochem 49:22–31. https://doi.org/10.1016/j.clincbiochem.2015.08.013.

40. Corstjens PL, Zuidervijk M, Brink A, Li S, Feindt H, Niederbichler RS, Tanke H. 2001. Use of up-converting phosphor reporters in lateral-flow assays to detect specific nucleic acid sequences: a rapid, sensitive DNA test to identify human papillomavirus type 16 infection. Clin Chem 47:1885–1893.

41. van der Heijde DM, van’t Hof M, van Riel PL, van de Putte LB. 1993. Development of a disease activity score based on judgment in clinical practice by rheumatologists. J Rheumatol 20:579–581.