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Indocyanine Green–Enhanced Fluorescence Optical Imaging in Patients With Early and Very Early Arthritis

A Comparative Study With Magnetic Resonance Imaging

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Objective. **Indocyanine green–enhanced fluorescence optical imaging (FOI) is a novel diagnostic tool for the assessment of inflammation in arthritis. We undertook this study to compare FOI with magnetic resonance imaging (MRI) in 32 patients with early and very early untreated arthritis (mean disease duration 7.1 months).**

Methods. **FOI images were acquired with the commercially available Xiralite system. Image interpretation was done for an early phase (phase 1), an intermediate phase (phase 2), and a late phase (phase 3), and for an electronically generated composite image. The results were compared with those of clinical examination (960 joints) and contrast (gadolinium)–enhanced 1.5T MRI (382 joints) of the clinically more affected hand. Additionally, we evaluated FOI in a control group of 46 subjects without any signs of inflammatory joint disease (1,380 joints).**

Results. **With MRI as the reference method, the sensitivity of FOI was 86% and the specificity was 63%, while the composite image, phase 1, and phase 3 reached high specificities (87%, 90%, and 88%, respectively). The results differed considerably between the composite image and the phases. FOI did not detect inflammation in 11 joint regions that showed palmar tenosynovitis on MRI. Intrareader and interreader agreements were moderate to substantial (κ = 0.55–0.73). In the control group, FOI showed positive findings in 5% of normal joints in phase 2.**

Conclusion. **Further multicenter studies will address the question of whether FOI allows sensitive and reliable detection of inflammatory changes in early arthritis, as suggested by our initial findings. If this is confirmed, FOI has the potential to be a sensitive and valuable tool for monitoring disease activity on site in clinical settings and for serving as an outcome parameter in clinical trials.**

There is substantial evidence that early treatment of rheumatoid arthritis (RA) will decrease the rate of disease progression and will change the disease course (1). Despite recent advances in classification and management of early arthritis (2–4), valid diagnosis and assessment of involved joints still remain a major challenge. Careful history taking and clinical examination are crucial but may miss subclinical inflammation, particularly in subjects with insidious onset, few if any

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systemic signs of inflammation, and low disease activity at presentation. Conventional plain radiography is commonly used as an indicator of prognosis and represents the standard outcome measure of disease progression in clinical studies, but it is not diagnostic in early stages of disease (5,6). Gray-scale ultrasonography, power Doppler ultrasonography (PDUS), and magnetic resonance imaging (MRI) have enlarged the diagnostic armamentarium and are more useful than radiography in early arthritis, detecting tenosynovitis, synovitis, erosions, and, in the case of MRI, bone marrow edema in the absence of erosions on radiography (7–13). However, broader use in routine clinical settings may be restricted by workflow considerations, experience of the examiner, and, in the case of MRI, cost and limited availability.

Indocyanine green (ICG)–enhanced fluorescence optical imaging (FOI) has been applied for >30 years for various indications and has been evaluated for imaging of inflammation in a variety of animal models (14). The technology has been adapted to rheumatologic applications and enables visualization of active synovitis in experimental arthritis (15–17) and in humans (18,19). A first validation study showed good agreement and a correlation of FOI with ultrasonography and MRI results in patients with active arthritis (20). To our knowledge, the present study is the first to compare FOI with clinical examination and 1.5T MRI in patients with early arthritis (disease duration ≤24 months) and very early arthritis (disease duration ≤3 months).

PATIENTS AND METHODS

Patients. Thirty-two consecutive patients with untreated early arthritis (disease duration ≤24 months) at their first visit to an early arthritis clinic were selected for a comparative study of FOI with clinical examination and MRI. FOI and MRI examinations were performed within 7 days without any treatment changes. As a control, we evaluated FOI findings in 46 subjects (mean age 41 years, 18 female) without any evidence of inflammatory joint disease. The study was in compliance with the Declaration of Helsinki and was approved by the local ethics committee (Aerztekammer Nordrhein and ethics committee of the Charité University Clinic Berlin).

Clinical and laboratory assessment. A clinical assessment (tender joint count [TJC] and swollen joint count [SJC]), the Disease Activity Score in 28 joints using the erythrocyte sedimentation rate (DAS28-ESR) (21), the patient's global assessment of disease activity on a 0–100-mm visual analog scale, and laboratory tests (ESR and C-reactive protein [CRP] level) were performed/administered immediately prior to FOI by an independent investigator who was blinded with regard to the status of the subjects. Additionally, tenderness and swelling were graded separately for 30 individual joints of both hands (the wrist as a whole, metacarpophalangeal [MCP] joints 1–5, proximal interphalangeal [PIP] joints 2–5, distal interphalangeal [DIP] joints 2–5, and the interphalangeal [IP] joint) on a semiquantitative scale (0 = none, 1 = slight, 2 = definite) according to the Ritchie Articular Index (22), with the above modifications. Additionally, the sum score (local disease activity index [locDAI], range 0–60) for the hand joints was calculated.

Fluorescence optical imaging. For FOI we used the commercially available Xiralite X4 device (Mivenion) with fixed geometry and dorsal illumination of the hands. Both hands were placed on a preformed hand rest. Ten seconds after starting the examination, 0.1 mg/kg body weight of an ICG bolus (ICG-Pulsion; Pulsion Medical Systems) was injected intravenously (IV). Starting 10 seconds before injection of the ICG bolus, the system acquired 1 image every second for 6 minutes with a corresponding image stack of 360 frames. FOI findings were analyzed by 1 reader (SGW, with 24 months of FOI experience) separately, both for an electronically generated composite image (PrimaVistaMode; XiraView Software, version 3.6) and for an individual sequence of 360 images with 3 phases (phases 1–3) predefined by the physiologic increased signal intensities seen in the fingertips. Phase 1 includes all images until clearly increased signal intensities are visible in the fingertips. Phase 2 includes all images during increased signal intensities in the fingertips. Phase 3 includes all images from the end of phase 2 until the end of the image stack.

We used a semiquantitative scoring system with a 4-point scale (0 = no signal enhancement, 1 = low signal enhancement [≤25% of affected joint area], 2 = moderate signal enhancement [>25% and ≤50% of affected joint area], 3 = strong signal enhancement [>50% of affected joint area]) for assessment of the fluorescence optical activity according to our previously described method (20). For individual subjects, we calculated an FOI activity score (FOIAS; range 0–90) as a sum score of these semiquantitative findings over all evaluated joints (the wrist as a whole, MCP joints 1–5, PIP joints 2–5, DIP joints 2–5, and the IP joint of both hands) for each electronically generated composite image and for phases 1, 2, and 3. For estimation of intrareader and interreader agreement, 2 other readers (H-EL, with 18 months of FOI experience, and FS, with 5 months of FOI experience) scored the FOI findings, and a second reading by SGW was performed 5 months later in a blinded manner.

Magnetic resonance imaging. FOI and MRI were performed within a time frame of ≤7 days (within 24 hours in 18 subjects). Blinded readings of the MRIs were performed in random order by 2 experienced readers (PS and BK), with consensus. We evaluated fat-suppressed coronal proton-density turbo spin-echo sequences, nonenhanced and enhanced T1-weighted turbo spin-echo sequences with subtraction, and coronal and axial fat-suppressed post–IV gadoterate meglumine (0.2 ml/kg body weight) T1-weighted turbo spin-echo sequences of the clinically leading hand using a 1.5T MRI machine (Siemens Magnetom Symphony). Assessment of the MRI findings of synovitis and tenosynovitis (in MCP joints 1–5, PIP joints 2–5, DIP joint 5, and the IP joint) was performed according to the Outcome Measures in Rheumatology scoring method (23–25). For calculations of agreement rates, sensitivity, and specificity, the wrist was scored as a whole. Due to technical limitations DIP joints 2–4 were excluded from the calculations. The Rheumatoid Arthritis Magnetic Resonance

Table 1. Study population and outcomes*

Patient	SJC	TJC	Local disease activity index, 0–60	ESR, mm/hour	CRP, mg/dl	Patient's global assessment of disease activity, 0–100-mm VAS	Patient's assessment of pain, 0–100-mm VAS	FOIAS, 0–90				DAS28-ESR	RAMRIS total score†	RAMRIS synovitis score
								Composite image	Phase 1	Phase 2	Phase 3			
1	3	5	21	5	0.3	4.0	4.0	3	0	12	1	2.5	0	0
2	7	0	15	2	0.6	6.0	6.0	0	0	15	1	2.6	1	1
3	0	1	3	12	0.2	9.0	9.0	6	5	23	7	3.0	2	1
4	16	12	56	26	0.3	9.0	9.0	22	5	51	13	6.7	28	18
5	1	1	6	10	0.8	4.0	5.0	3	4	16	1	3.8	1	1
6	0	2	6	14	0.1	8.0	7.0	3	0	7	2	3.8	0	0
7	0	0	0	11	0.3	2.0	5.0	3	0	13	1	2.2	0	0
8	8	7	30	5	0.1	8.0	7.0	28	0	34	14	5.0	3	2
9	0	1	2	2	0.1	2.0	2.0	0	13	19	4	1.7	0	0
10	5	3	16	55	0.3	7.0	6.0	3	16	16	2	6.3	5	5
11	9	9	37	28	0.3	3.0	8.0	38	42	55	21	5.3	15	7
12	5	1	12	56	4.1	2.0	2.0	3	0	5	2	4.3	2	0
13	0	0	4	14	0.1	7.5	7.5	0	0	4	0	4.1	0	0
14	2	2	8	30	0.1	0.0	0.0	5	0	11	4	3.6	1	1
15	0	1	3	50	1.3	1.0	1.0	0	0	10	0	3.4	0	0
16	5	2	26	10	0.1	7.0	7.0	16	18	48	21	4.4	15	11
17	1	0	8	50	3.0	3.5	4.0	0	0	7	1	3.5	12	7
18	6	0	16	4	0.1	5.0	2.0	20	11	37	7	2.4	10	5
19	2	2	14	18	0.4	5.0	5.0	33	1	42	19	3.9	16	9
20	4	4	17	50	0.3	10.0	8.0	3	15	24	7	6.3	12	8
21	0	4	13	12	0.5	6.0	5.0	5	0	10	4	3.5	1	1
22	0	13	31	49	0.6	6.0	5.5	8	0	17	2	5.9	1	1
23	2	0	9	4	0.1	5.0	5.0	4	2	19	4	2.6	7	3
24	0	0	2	20	1.8	7.0	7.0	0	0	4	2	3.1	0	0
25	0	8	20	26	0.1	4.0	4.0	5	4	10	1	4.5	1	1
26	0	0	4	90	9.8	4.5	8.0	3	6	8	2	4.9	6	1
27	0	2	10	38	0.3	9.0	9.0	4	4	5	4	5.2	6	6
28	1	1	6	31	0.1	5.0	6.0	6	3	5	2	3.7	3	3
29	0	14	30	5	0.1	4.0	4.0	8	24	11	3	3.3	0	0
30	0	0	2	8	0.1	6.0	5.0	1	1	3	1	3.4	6	0
31	2	0	6	1	0.1	3.0	4.0	0	0	1	0	0.8	0	0
32	3	1	14	10	0.4	10.0	8.0	11	14	21	1	4.0	2	2
Mean ± SD	2.6 ± 2.7	3 ± 3	14 ± 9.2	23 ± 17	0.8 ± 1	5.4 ± 2.2	5.5 ± 1.9	7.6 ± 7.2	5.9 ± 6.6	17.6 ± 11.2	4.8 ± 4.4	3.9 ± 10	4.9 ± 5.0	2.9 ± 3.1
Range	0–16	0–13	0–56	0–56	0.1–4.1	0–10	0–9	0–38	0–42	0–55	0–21	1.7–6.7	0–28	0–18
Median	1	1	11	14	0.3	5	5.3	3.5	1.5	12.5	2	3.75	2	1

* SJC = swollen joint count; TJC = tender joint count; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; VAS = visual analog scale; FOIAS = fluorescence optical imaging activity score; DAS28-ESR = Disease Activity Score in 28 joints using the ESR; RAMRIS = Rheumatoid Arthritis Magnetic Resonance Imaging Scoring system.
† Bone edema, synovitis, and erosions.

Imaging Scoring (RAMRIS) system (26) was used to calculate the total score for bone edema, erosions, and synovitis together as well as for synovitis separately.

Statistical analysis. Data evaluation and statistical analysis were performed using SAS/STAT software: release 9.2 (SAS Institute). Analyses were conducted at the patient and individual joint level. MRI was used as the standard reference method for calculation of sensitivity and specificity. Pairwise agreement rates between FOI, clinical examination, and MRI findings were calculated as the primary efficacy variable. The agreement rate was determined taking into consideration all joints with nonmissing data. Agreement was reached when a joint was assessed as affected (>0) or not affected (0) with both modalities. Ninety-five percent confidence intervals were calculated using a modified adjusted chi-square test to cover correlations of multiple measurements (joints) within the same patient (27). Further analyses of efficacy, summary statistics, frequency counts, and confidence intervals were computed, as appropriate. Correlations were calculated using Spearman's rank correlation coefficient (r_s). Correlations were valued as follows: $0 < r_s \leq 0.2$ = no correlation, $0.2 < r_s \leq 0.4$ = weak-to-moderate correlation, $0.4 < r_s \leq 0.8$ = distinct correlation, $0.8 < r_s \leq 1$ = high-to-perfect correlation. Inter-reader variability was evaluated by means of Cohen's kappa coefficient. Two-sided *P* values less than 0.05 were considered significant.

RESULTS

Study population. Thirty-two patients were selected for the study (23 with RA, 4 with psoriatic arthritis, 5 with peripheral spondyloarthritis) (median age 55 years, 24 female) (Table 1). Their mean disease duration was 7.1 months, and 13 patients had very early arthritis with disease duration of <3 months. No patient was being treated with disease-modifying antirheumatic drugs (DMARDs), and 21 of 32 patients (66%) had never been treated with any antirheumatic drug (DMARDs, glucocorticoids, nonsteroidal antiinflammatory drugs). Five of 32 patients (16%) were taking glucocorticoids at the time of assessment (mean ± SD dosage 15 ± 14 mg/day, range 5–50 mg/day). All patients' treatments were stable within the days between the 2 imaging procedures. At clinical examination, 25 of 32 patients (78%) had active disease defined by a DAS28-ESR of >2.6. Mean values were as follows: DAS28-ESR 3.9, SJC 2.6, TJC 3, ESR 23 mm/hour, CRP level 0.8 mg/dl, RAMRIS total score 4.9, RAMRIS synovitis score 2.9, RAMRIS erosion score 1.3, RAMRIS edema score 0.7. Clinical parameters are shown in detail in Table 1.

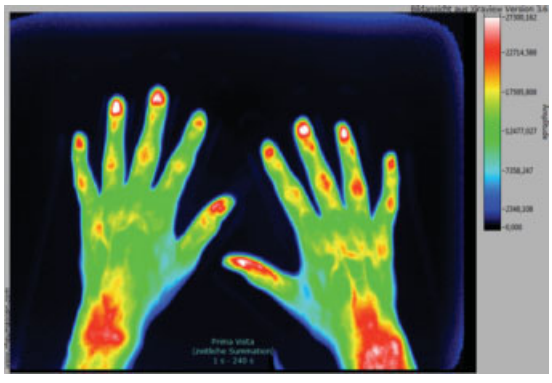

Figure 1. Early arthritis on fluorescence optical imaging. Shown are increased signal intensities in both wrists, metacarpophalangeal joint 5 in both hands, proximal interphalangeal (PIP) joints 3–5 in the left hand, PIP joints 2–5 in the right hand, and distal interphalangeal joint 3 in the right hand.

Table 2. Sensitivity and specificity of FOI and clinical examination, with MRI as the standard of reference*

FOI or clinical examination, MRI result	Sensitivity, % (95% CI)	Specificity, % (95% CI)
FOI		
Composite image		
Synovitis	59 (45–72)	87 (81–93)
Synovitis or tenosynovitis	51 (38–63)	88 (82–93)
Phase 1		
Synovitis	43 (22–64)	90 (83–97)
Synovitis or tenosynovitis	42 (23–61)	92 (86–98)
Phase 2		
Synovitis	79 (65–94)	69 (62–76)
Synovitis or tenosynovitis	70 (53–86)	70 (63–76)
Phase 3		
Synovitis	59 (44–73)	88 (82–93)
Synovitis or tenosynovitis	52 (36–67)	89 (84–94)
Any phase		
Synovitis	86 (74–97)	63 (55–71)
Synovitis or tenosynovitis	78 (66–91)	64 (56–72)
Clinical examination†		
Synovitis	62 (49–75)	86 (82–90)
Synovitis or tenosynovitis	55 (44–67)	88 (84–91)

* FOI = fluorescence optical imaging; MRI = magnetic resonance imaging; 95% CI = 95% confidence interval.
† Swollen joints.

Clinical examination, FOI, and MRI findings. FOI was compared with clinical examination findings in 960 individual joints and with MRI findings in 382 individual joints. Synovitis on MRI was seen in 63 of 382 joints (16%). FOI revealed focal, joint-related increased signal intensity as a sign of synovitis or tenosynovitis (Figure 1) in 54 of these 63 joints (86%). FOI did not detect inflammation in 11 joint regions (wrist and MCP) that showed palmar tenosynovitis on MRI.

Sensitivity and specificity. With MRI as the reference, clinical examination (swollen joints) had a sensitivity of 62% and a specificity of 86% for synovitis, and a sensitivity of 55% and a specificity of 88% for

Table 3. Agreement of FOI with MRI and clinical examination results*

MRI or clinical examination, FOI result	No. of agreements/ total joint count	Proportion of agreements, % (95% CI)
MRI		
Composite image		
Synovitis	314/382	82 (77–87)
Synovitis or tenosynovitis	304/382	80 (74–85)
Phase 1		
Synovitis	314/382	82 (75–89)
Synovitis or tenosynovitis	310/382	81 (75–88)
Phase 2		
Synovitis	270/382	71 (65–76)
Synovitis or tenosynovitis	266/382	70 (64–76)
Phase 3		
Synovitis	317/382	83 (78–88)
Synovitis or tenosynovitis	309/382	81 (76–86)
Any phase		
Synovitis	254/382	66 (60–73)
Synovitis or tenosynovitis	256/382	67 (60–74)
Clinical examination		
Composite image		
Swollen joints	772/960	80 (75–86)
Swollen and tender joints	805/960	84 (78–90)
Phase 1		
Swollen joints	770/960	80 (75–86)
Swollen and tender joints	823/960	86 (80–92)
Phase 2		
Swollen joints	675/960	70 (65–76)
Swollen and tender joints	648/960	68 (60–75)
Phase 3		
Swollen joints	799/960	83 (79–87)
Swollen and tender joints	842/960	88 (83–92)
Any phase		
Swollen joints	646/960	67 (61–74)
Swollen and tender joints	609/960	63 (55–71)

* See Table 2 for definitions.

synovitis or tenosynovitis. FOI had a sensitivity of 86% and a specificity of 63% for synovitis, and a sensitivity of 78% and a specificity of 64% for synovitis or tenosynovitis. Specificity of phases 1 and 3 was high (88–92%), but with corresponding lower sensitivity (42–59%) (Table 2).

Comparison of clinical examination findings with MRI and FOI. Agreement of clinical examination and MRI results was high (86% for swollen and tender joints, 82% for swollen joints). Agreement of clinical examination and FOI results was 63–88% for swollen and tender joints and 67–83% for swollen joints, but differed considerably between the composite image and the individual phases of FOI (phases 1–3). Lack of agreement between FOI and clinical examination results was primarily due to a higher proportion of positive findings on FOI compared to clinical examination, particularly in phase 2 of the FOI series. Detailed results are shown in Table 3, with the exception of those for tenosynovitis alone.

Comparison of FOI with MRI. Agreement of FOI and MRI results was good (71–83% for synovitis, 66–83% for tenosynovitis, and 70–81% for synovitis or tenosynovitis depending on the subset of images assessed) (Figure 2). In accordance with the comparison between FOI and clinical examination, agreement was lower in phase 2 (66–71%). Like the lack of agreement between FOI and clinical examination, lack of agreement between FOI and MRI was due to the higher proportion of positive findings on FOI compared to MRI.

Quantitative assessments of disease activity. The FOIAS was compared with the DAS28-ESR, the RAMRIS total and synovitis scores, the locDAI, the SJC, the TJC, and laboratory parameters of systemic inflammation (ESR, CRP level). Correlations of the scores were found to be moderate to distinct (r_s = 0.3–0.7). There was no correlation of these scores with

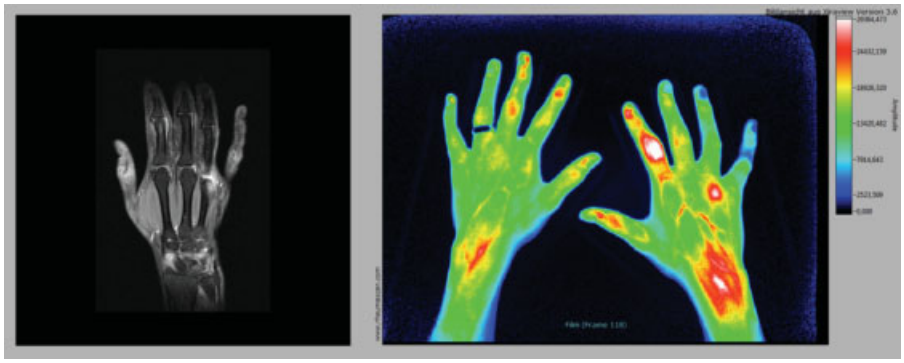

Figure 2. Comparison of magnetic resonance imaging (left) and fluorescence optical imaging (right) in a patient with early arthritis. Shown is synovitis in the interphalangeal joint, proximal interphalangeal joint 2, metacarpophalangeal joint 4, and wrist in the right hand (left), with corresponding increased signal intensities on fluorescence optical imaging (right).

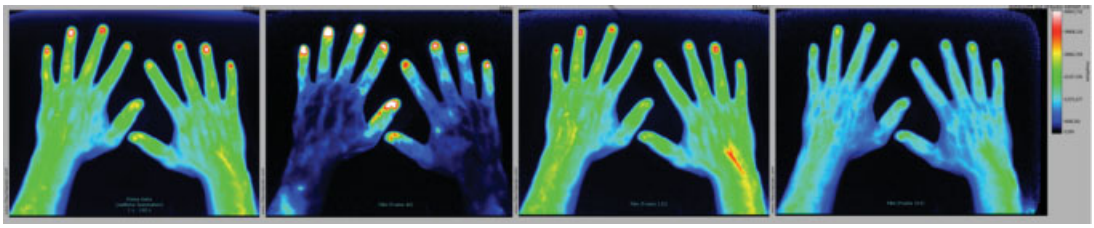

Figure 3. Fluorescence optical imaging in a control subject, showing normal findings without signs of inflammation. From left to right, Composite image, phase 1, phase 2, and phase 3.

parameters of systemic inflammation (further information is available at http://radiologie.charite.de/static/xiralite/Werner_et_al_Suppl_Table_S1.pdf).

Results excluding patients taking prednisolone. Five patients in the cohort were receiving therapy with prednisolone. Four patients received low-dose prednisolone (<7 mg/day), and 1 patient received prednisolone at 50 mg/day. Calculations of agreement rates, sensitivity, specificity, and correlations without these 5 patients did not show relevant differences compared to calculations with the whole cohort (data not shown).

Controls. In the control group, which included 46 subjects without any signs of inflammatory joint disease, FOI yielded unremarkable findings in 95–99.5% of the 1,380 evaluated individual joints (98% for the electronically generated composite image, 99.5% for phase 1, 95% for phase 2, 99.3% for phase 3) (Figure 3).

Intrareader and interreader agreement. Intrareader agreement ($\kappa = 0.73$) and interreader agreement ($\kappa = 0.71$) were found to be substantial for the experienced readers (28), and interreader agreement was moderate ($\kappa = 0.55$) for the less experienced reader.

DISCUSSION

Fluorescence optical imaging is a novel imaging modality for the detection of inflammation in arthritis and allied conditions. The ICG-enhanced FOI system that we used enables visualization of the altered microcirculation and dysregulated angiogenesis in inflammatory disorders (29,30). These changes are early events in the pathogenesis of RA (31,32) and have been proposed to be the link to bone destruction (33). In clinical studies, joint vascularity correlated with radiographic progression (34) and was inversely correlated with the therapeutic response in RA patients receiving biologic therapy (35).

The aim of this analysis was to compare FOI findings with findings on clinical examination and contrast-enhanced MRI in a cohort of patients with early arthritis and very early arthritis. In this population, we were able to reproduce our earlier findings of high sensitivity, specificity, agreement rates, and correlation of FOI with MRI and clinical examination results in patients with active arthritis (20).

With MRI as the reference, FOI was more sensitive than clinical examination. We found a high agreement of FOI with clinical findings (up to 88%), depending on the phase evaluated. Lack of agreement was primarily due to a higher rate of positive findings on FOI, particularly in phase 2 of an individual FOI sequence. These findings were also concordant with earlier results (20).

FOI had a high sensitivity (up to 86%) and a high specificity (up to 90%) for synovitis, depending on the phase evaluated. FOI findings were in close agreement with MRI findings (up to 83%), also depending on the phase evaluated. As with the comparison of FOI and clinical examination, lack of agreement between FOI and MRI was primarily due to a higher rate of positive findings on FOI, also mainly in phase 2.

In comparative studies of clinical examination and ultrasonography, PDUS, and MRI, inflammatory changes were documented in clinically asymptomatic joints and were associated with radiographic progression (36–38). Our data raise the hypothesis that FOI may also reveal subclinical inflammatory activity in clinically asymptomatic or MRI-negative joints in patients with arthritis.

To exclude an influence of prednisolone therapy on the results, we performed calculations without the 5 patients receiving prednisolone therapy. No relevant differences in sensitivity, specificity, agreement rates, or correlations were found. Thus, in this cohort of patients with early and very early arthritis, prednisolone seems to have no relevant influence on FOI assessment of inflammation in the hands (data not shown).

The low rate of false-positive findings (0.5–5%) in controls in this study is consistent with our previous

findings (20). Our standard of reference (MRI) is an established procedure, but FOI showed a higher rate of positive findings in patients with arthritis, with the abovementioned low rate of false-positive findings in controls. These methods are based on different mechanisms for enabling visualization of inflammation; thus, they could show different results. Further studies, potentially also including histology, will investigate whether the positive FOI findings may be due to a higher sensitivity for synovitis as compared to the gold standard of MRI.

FOI findings may vary in relation to the electronically generated composite image and the described phases. The clinical use of the phases is still under investigation. Of special interest is phase 1, with high specificity (90%) and high agreement with MRI (82%) for detection of synovitis. In a previous study (20), phase 1 also had high specificity (90%) and high agreement with power Doppler activity (82%); thus, phase 1 may reveal high activity, with increased vascularity corresponding to active synovitis on MRI and ultrasonography. Phase 2 seems to be the most sensitive. Phase 3 could show increased capillary permeability in which ICG is more persistent than normal. These hypotheses should be investigated in larger cohorts and, if possible, with histologic confirmation. For clinical use, phase 2 could be the phase for detecting subclinical inflammation in patients with early arthritis or patients with clinical remission. Patients with positive findings in phase 1 may have very active disease, and further diagnostic evaluation and perhaps therapy escalation could be necessary. These suggestions are currently only working hypotheses; the diagnostic performance of the individual phases will need further validation. However, differences in sensitivity, specificity, and agreement rates suggest that an adequate FOI interpretation requires a separate reading of the phases.

Semiquantitative analysis of FOI signal intensities revealed a moderate-to-distinct correlation with the RAMRIS score, notably with the RAMRIS synovitis score, but also with the corresponding clinical assessment of local disease activity measured in our study with the SJC, the TJC, and the locDAI. The low correlation with the assessments of global disease activity and laboratory parameters of systemic disease activity could be explained by a concept of disparity between systemic and localized disease activity in rheumatic diseases. Especially in patients with short disease duration (as in our cohort) or being treated with disease-modifying drugs or glucocorticoids, systemic inflammation may differ from local inflammation in the articular joints. This hypothesis is supported by the low systemic inflammation as measured by the ESR and CRP level, but moderate disease activity as measured by the SJC, TJC, RAMRIS score, and DAS28. The correlation with the assessments of local inflammation (RAMRIS score, SJC, TJC, locDAI) shows that FOI reliably detects localized disease activity.

The semiquantitative assessment depends on a subjective interpretation of the FOI findings. A substantial (28) intrareader agreement ($\kappa = 0.73$) and moderate-to-substantial interreader agreement ($\kappa = 0.55–0.71$) were achieved using this method. Thus, the scoring method with the described systematic image adjustment is valid and reliable.

We are aware of some specific limitations of the commercial device that was used. Generally, the technology of optical imaging is based on the transmission of light through a particular tissue. Depth of penetration depends on local factors and the tissue concentration of the fluorophor, and may reach 4 cm (39). In our series, we found that the detection of palmar inflammation was influenced in FOI by overlying anatomic structures, notably in the wrist and MCP region. However, this limitation could be overcome by technical modifications of the device, for example, using additional light-emitting diodes and a second camera on the palmar side of the imaging field.

Second, ICG-enhanced FOI of the hands is a novel imaging modality in humans. While the examination procedure itself has been standardized in detail, consistent standards are not yet fully established for the evaluation of a particular series of images. We have observed that diagnostic pitfalls may arise from optical interference, for example, reflections caused by external light sources, leading to incorrect adjustment of the automatic brightness gain. If pitfalls are not corrected by manual adjustment, positive findings on FOI may be missed and incorrectly interpreted as false-negative results. For the present study we used a standardized procedure for the evaluation of the correct automatic adjustment of gain and manual correction, if deemed necessary. In this manner we reached substantial interreader and intrareader reliabilities, as mentioned above, which underscore the quality of our evaluation algorithm and the data that we have gathered.

Particular attention should be directed to the fact that FOI detects any inflammation (e.g., psoriatic plaques, scratches, and wounds as well as synovitis or tenosynovitis). Differentiation of the inflamed structure may be possible by localization and temporal distribution of increased signal intensities, but this requires an

experienced observer. Thus, we believe that the interpretation of the FOI findings has to be evaluated in the clinical context of these settings.

All of our present findings were consistent not only with the findings in our previous study comparing FOI with clinical examination, MRI, and ultrasonography (20), but also with findings in the proof-of-concept study by Fischer et al, which compared FOI with low-field MRI (19). There was some discrepancy with the findings in the study by Meier et al, which compared FOI findings with 3T MRI (40). The interreader reliability ($\kappa = 0.47$) and intrareader reliability ($\kappa = 0.50$) in that study were more or less moderate, and the study population was heterogeneous and with limited clinical data. Since Meier et al's study population, design, and image interpretation differ from ours, the findings in the 2 studies are not fully comparable.

The strengths of our study are that all subjects had early or very early arthritis, none had ever been treated with DMARDs, and only a minority had been previously treated with glucocorticoids. Thus, our findings reflect the "natural" state of disease unaltered by therapeutic interventions. Multicenter studies are ongoing to establish the potential role of FOI as a sensitive and valuable tool for monitoring disease activity on site in clinical settings and for serving as an outcome parameter in clinical trials.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Burmester had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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