EXTENDED REPORT

Pretreatment macrophage infiltration of the synovium predicts the clinical effect of both radiation synovectomy and intra-articular glucocorticoids

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Objective: To explore whether pretreatment features of synovial tissue in patients with gonarthrosis could predict the clinical effect of radiation synovectomy with yttrium-90 (^{90}Y) and glucocorticoids or with intra-articular glucocorticoids alone.

Methods: A synovial biopsy was carried out blindly 2 weeks before treatment in 66 patients with persistent gonarthrosis, who were randomised to treatment either with ^{90}Y and triamcinolone or with placebo and triamcinolone. Immunohistochemistry was used to detect T cells, macrophages, B cells, plasma cells, fibroblast-like synoviocytes, adhesion molecules and pro-inflammatory cytokines. Stained sections were evaluated by digital image analysis. Individual patient improvement was expressed using a composite change index (CCI; range 0–12). Successful treatment was defined as CCI ≥ 6 after 6 months.

Results: Patients with rheumatoid arthritis, psoriatic arthritis, undifferentiated arthritis and other causes of gonarthrosis were included. The overall response rate was 47%. Clinical efficacy in both therapeutic groups was similar and not dependent on diagnosis. No significant differences were noted between baseline microscopic features of synovial tissue inflammation in patients with rheumatoid arthritis and in those with non-rheumatoid arthritis (ie, all diagnoses other than rheumatoid arthritis). The number of macrophages in the synovial sublining was significantly higher in responders than in non-responders ($p=0.002$), independent of treatment group and diagnosis. The clinical effect was positively correlated with pretreatment total macrophage numbers ($r=0.28$; $p=0.03$), sublining macrophage numbers ($r=0.34$; $p=0.005$) and vascular cell adhesion molecule 1 expression ($r=0.25$; $p=0.04$).

Conclusion: The observations support the view that intra-articular treatment either with ^{90}Y and glucocorticoids or with glucocorticoids alone is especially successful in patients with marked synovial inflammation.

Radiation synovectomy or radiosynoviorthesis (RSO), for recurrent synovitis of the knee, is carried out by intra-articular administration of yttrium-90 (^{90}Y), which has a mean soft-tissue penetration depth of 3.6 mm.¹ It has been suggested that in addition to the direct effects of local irradiation, phagocytosis of ^{90}Y by synoviocytes may be associated as well, leading to an even distribution over the synovial surface. This results in fibrosis and finally sclerosis of the synovial membrane.²

Treatment with ^{90}Y is often directly followed by treatment with glucocorticoid, using the same needle. The purpose is to prevent leakage with skin-and-needle tract burn after injection of ^{90}Y and to bridge the lag phase before the onset of the clinical effect of RSO. This lag phase is assumed to last 3–6 months.³ The relatively long half life of ^{90}Y (2.7 days) is an argument for post-injection immobilisation (usually 72 h) to minimise extra-articular leakage to the bloodstream by intermittent intra-articular pressure in the ambulant patient and as such improve retention in the knee.⁴

The clinical outcome of RSO has been suggested to depend on the primary disease and pre-existing radiological damage.⁵ Previous studies also indicated that the efficacy might depend on the diagnosis: patients with rheumatoid arthritis might respond better to RSO than those with other arthritides caused by a more pronounced synovial tissue proliferation in rheumatoid arthritis.^{5–7} Complete or almost complete remission of synovitis after RSO has been reported in 40–80% of treated joints.^{5–11} Other investigators have questioned the clinical effect of RSO,^{12–16} whereas superiority of RSO over glucocorticoids alone was debatable.¹⁷

To compare the efficacy between RSO combined with glucocorticoids and with glucocorticoids alone, we recently conducted a randomised, double-blind, placebo-controlled clinical trial (RCT) to evaluate clinical efficacy in patients with different forms of gonarthrosis.¹⁸ In the present study, we investigated whether clinical efficacy of either therapeutic regimen was related to features of synovial inflammation before treatment.

PATIENTS AND METHODS

Patients

All consecutive patients referred to four rheumatology clinics (University Medical Center Utrecht, Utrecht, The Netherlands; Medical Center Alkmaar, Alkmaar, The Netherlands; Westfries Gasthuis, Hoorn, The Netherlands; and St Antonius Hospital, Nieuwegein, The Netherlands), from July 1999 to July 2002 for persistent and recurrent arthritis of the knee joint were invited to participate in the RCT. The results of the clinical evaluation in the RCT are reported elsewhere.¹⁸ The ethics committee of each participating institution approved the study protocol. From all patients consenting to the RCT ($n=72$), only patients who voluntarily agreed to a synovial biopsy with written informed

Abbreviations: anti-ICAM1, anti-intercellular adhesion molecule 1; anti-VCAM1, anti-vascular cell adhesion molecule 1; CCI, composite change index; DMARD, disease-modifying antirheumatic drug; FLS, fibroblast-like synoviocytes; IOD, integrated optical density; mAb, monoclonal antibody; RCT, randomised clinical trial; RSO, radiation synovectomy; TNF, tumour necrosis factor; ^{90}Y , yttrium-90

consent were included in the present study ($n = 68$, 94%). Arthritis had to be persistent despite at least two intra-articular glucocorticoid injections in an outpatient setting, and ongoing for at least 4 weeks after the last glucocorticoid injection. Patient demographics, medical history and diagnosis, as well as previous and current treatment, were derived from the patient's chart.

Biopsy procedure

Two weeks before treatment, synovial biopsy specimens were taken blindly from the suprapatellar pouch and medial parapatellar gutter after intra-articular anaesthesia with lidocaine (University Medical Centre, Utrecht, The Netherlands). The biopsy was carried out with a straight arthroforce III biopsy forceps (Storz, Tuttlingen, Germany) with a diameter of 2.3 mm, through a biopsy canule (Storz) with a diameter of 3.2 mm and a length of 7 cm. A total of 20–30 synovial tissue specimens were taken per patient in a single procedure. In four patients both knees were affected—synovial biopsy specimens were retrieved from the right knee.

Synovial tissue

From each patient, an average of 10–15 synovial biopsy specimens were snap frozen en-block in Tissue Tek OCT (Miles Inc Diagnostic Division, Elkhart, Indianapolis, USA) by immersion in methylbutane (-70°C) immediately after retrieval. Frozen blocks were stored in liquid nitrogen until sectioned for staining. Sections, 5 μm thick, were cut in a cryostat and mounted on glass slides (Star Frost adhesive slides, Knittelgläser, Braunschweig, Germany). The slides were air dried overnight and sealed by wrapping in tin foil to store at -70°C until immunohistological analysis.

An average of additional five pieces of synovial tissue were fixed in formalin and embedded in paraffin wax for histological analysis; sections were cut and stained with haematoxylin and eosin.

Immunohistological analysis

Serial sections were stained with the following mouse monoclonal antibodies (mAbs): anti-CD3 (SK7; Becton-Dickinson, San Jose, California, USA; to detect T lymphocytes), anti-CD68 (EBM11; Dako, Glostrup, Denmark; to detect macrophages) and anti-CD55 (clone 67; Serotec, Oxford, UK; to detect CD55 or fibroblast-like synoviocytes (FLS)), also indicating the presence of the intimal lining layer. If the intimal lining layer was clearly present, further staining was carried out with anti-CD4 (SK3; Becton-Dickinson) and anti-CD8 (DK25; Dako; to detect this subset of T cells), anti-CD22 (CLB-B-Ly/1, 6B11; CLB, Amsterdam, The Netherlands; to detect B cells), anti-CD38 (HB-7; Becton-Dickinson; to detect plasma cells) and mAbs to detect adhesion molecules: anti-intercellular adhesion molecule 1 (anti-ICAM1; MEM-111; Sanbio, Uden, The Netherlands), anti-vascular cell adhesion molecule 1 (anti-VCAM1; 1G11B1; Sanbio) and anti-E selectin (BBIG-E4, R&D Systems, Abingdon, UK). Staining was also carried out with the following mAbs to detect pro-inflammatory cytokines: anti-tumour necrosis factor (TNF) α (52B83, Monosan, Uden, The Netherlands) and anti-interleukin (IL)1 β (2D8, Immunokontakt, Frankfurt, Germany). Staining was carried out according to a three-step immunoperoxidase method, as described previously.¹⁹ For control sections, the primary antibodies were omitted or irrelevant isotype-matched mouse mAbs were used.

Analysis by microscopy

Sections stained by immunohistochemistry were coded and randomly analysed by blinded observers using digital image analysis, as described previously.²⁰ Measurements for CD

markers were expressed in cell counts/ mm^2 , and those for cytokines and adhesion molecules in integrated optical density (IOD)/ mm^2 .

Sections stained with haematoxylin and eosin were also coded and randomly analysed. All areas of each biopsy section were examined and histological features were scored independently by two blinded observers (ZNJ and MJGW). Tissues were separately scored for the degree of infiltration with lymphocytes, plasma cells and polymorphonuclear cells, using a 0–4 scale (0, minimal infiltration; 4, infiltration with numerous inflammatory cells) according to a previously described method.²¹ In addition, each tissue was scored for intimal lining hyperplasia (0–3 scale, where 0 indicates 1–2; 1 indicates 3–4; 2 indicates 5–6 and 3 indicates ≥ 7 cell layers).²¹ An inflammation score was determined by summing the scores for the four components: intimal lining hyperplasia, and infiltration with lymphocytes, plasma cells and polymorphonuclear cells. Finally, tissues were assigned a vessel score representing the number of blood vessels present in each tissue (0 indicates ≤ 3 ; 1 indicates 4–9; 2 indicates 10–15; 3 indicates 16–21 and 4 indicates ≥ 22 vessels).²¹ Capillaries, venules and arterioles were weighted equally for this score.

Randomisation of treatment

Patients were randomly allocated to intra-articular treatment with either 185 MBq (5 mCi) ^{90}Y citrate (CIS bio international, Paris, France) and 20 mg triamcinolone hexacetonide (Lederspan, Lederle BV, The Netherlands) (^{90}Y and glucocorticoids group), or placebo and 20 mg triamcinolone hexacetonide (placebo and glucocorticoid group). The injection was given after aspiration of synovial fluid as much as possible. In each group, 20% of the knee joints were treated with an equivalent dose of 40 mg triamcinolone acetate (Kenacort, Bristol-Meyers Squibb BV, The Netherlands) because triamcinolone hexacetonide has not been available in the Netherlands since 2002. Treatment was followed by immobilisation of the knee by a brace and 72 h clinical bed rest.

Clinical assessment

The clinical effect of treatment was assessed after 6 months as 3–6 months is generally assumed to be the lag phase before onset of clinical efficacy of RSO.³ To evaluate individual patient improvement, the composite change index (CCI) was calculated, based on the previously reported index.²² In this evaluation, acute-phase reactants (erythrocyte sedimentation rate and C reactive protein levels) were not included as patients with oligoarthritis were treated as well, and therefore these test results could be influenced by persistent arthritis of the non-treated joints.

In this CCI, a core set of variables of the knee is incorporated, including a functional disability score, visual analogue score of pain, clinical assessments of the knee (joint tenderness at palpation, joint swelling and effusion) and both patient's and doctor's global assessment of the effect of treatment. Calculation of the CCI was based on changes of the first five variables from baseline, whereas for the last two variables the score at the moment of evaluation was used. The total CCI ranged from 0 (no effect or deterioration) to 12 (maximal effect). We arbitrarily defined successful treatment as CCI ≥ 6 , whereas a score < 6 indicated failure. In the RCT, the cut-off point of CCI = 6 for the outcome of treatment was in accordance with clinical judgement.¹⁸

In the RCT, the individual patient's outcome (successful treatment or failure) was the primary outcome measure for efficacy; the CCI was the secondary outcome.

Statistics

Efficacy in both treatment groups was compared by applying intention-to-treat analyses and testing for differences

between both groups using Fisher's exact test (primary outcome measure) and t test or the Mann-Whitney U test where appropriate (secondary outcome measure).

Means of the histology scores of both investigators were used for all statistical analyses. Within non-responders and responders from both treatment groups, synovial tissue variables were compared by the t test and the Mann-Whitney U test where appropriate. The relationship between synovial tissue variables and CCI at 6 months was explored using Spearman's correlation coefficients.

For all analyses, $p < 0.05$ was considered to be significant. All tests were two sided. Data were analysed using the Number Cruncher Statistical System 2000 and Statistical Package for Social Sciences V.10.

RESULTS

Sixty eight patients were enrolled and underwent synovial biopsies. Synovial tissue sections of two patients (3%) did not contain representative tissue; these patients were excluded from analyses.

Baseline characteristics

Table 1 shows the clinical features of patients from the two treatment groups. ^{90}Y and glucocorticoids were administered in 37 knees (56%), whereas placebo and glucocorticoids in 29 knees (44%). Patients mainly had undifferentiated arthritis (monoarthritis or oligoarthritis; 41%) or rheumatoid arthritis (35%). Of the patients taking disease-modifying antirheumatic drugs (DMARDs) or oral glucocorticoids, most had rheumatoid arthritis (69% and 73%, respectively). Methotrexate was the DMARD most often used (58%). Synovectomy was carried out in the past in 20% of all treated knees: in 11% by RSO, in 6% by surgery and in 5% by chemical synovectomy. In one patient (2%), synovectomy had been carried out previously by several methods. Accidentally, three joints (5%) with severe radiological damage were treated, which was an exclusion criterion. Only usage of DMARDs or oral glucocorticoids was significantly different between both groups, higher in the placebo and glucocorticoids group ($p = 0.04$ for both variables). Before biopsy, synovial fluid was increased in 83% of patients from the ^{90}Y and glucocorticoids group versus 76% of those from the placebo and glucocorticoids group. Of these, this synovial fluid was opaque in 83% and 74%, respectively, indicating numerous inflammatory cells.

Immunohistochemistry

Table 2 shows the immunohistological features of synovial tissue of all treated knees ($n = 66$). The features of synovial tissue specimens of patients from both treatment groups did not differ significantly (table 2), despite the fact that more patients from the placebo and glucocorticoids group were taking DMARDs and oral glucocorticoids, and also if stratified to the diagnosis rheumatoid arthritis versus non-rheumatoid arthritis (ie, all diagnoses other than rheumatoid arthritis; data not shown). Macrophages were the predominant cells in the synovial infiltrate, whereas the T cell population consisted of slightly more CD4+ cells than CD8+ cells. High numbers of both FLS and plasma cells were seen, both reflecting chronic synovitis. There was marked expression of adhesion molecules and pro-inflammatory cytokines (table 2).

Histology

Table 3 shows the histology scores of 59 treated knees; synovial tissue sections of the other knee joints did not contain representative tissue. Again, if stratified to the assigned treatment (table 3) or the diagnosis rheumatoid arthritis versus

Table 1 Baseline characteristics ($n = 66$ patients or knees)

	$^{90}\text{Y}+\text{GC}$ ($n = 37$)	Placebo+GC ($n = 29$)
Women:men	20 (54): 17 (46)	15 (52): 14 (48)
Age, years	50 (15, 19–72)	48 (18, 19–82)
Diagnosis		
Rheumatoid arthritis	10 (27)	13 (45)
Undifferentiated arthritis	16 (43)	11 (38)
Psoriatic arthritis	6 (16)	4 (14)
Miscellaneous*	5 (14)	1 (3)
Rheumatoid arthritis (%)		
RF positive	80	69
Erosive disease	60	62
Disease duration, years	7 (7, 1–29)	9 (10, 1–46)
Concurrent treatment		
NSAIDs	23 (62)	20 (69)
DMARDs	14 (38)	19 (66)
Oral GC	3 (8)	8 (28)
Total duration of synovitis, months	39 (44, 6–240)	39 (33, 5–108)
Duration of arthritis after the last GC injection, months	10 (12, 1–48)	7 (10, 1–48)
Radiological status†		
I	10 (27)	9 (31)
II	26 (70)	16 (55)
III	0	3 (10)
History of synovectomy of knee	7 (19)	6 (21)
Aspirated SF before injection, ml	28 (29, 0–100)	24 (27, 0–86)
ESR, mm at the end of the first hour	25 (24, 2–119)	34 (30, 2–110)
CRP, mg/l	12 (12, 4–57)	32 (43, 4–140)

CRP, C reactive protein; DMARD, disease-modifying antirheumatic drug; ESR, erythrocyte sedimentation rate; GC, glucocorticoid; NSAID, non-steroidal anti-inflammatory drug; RF, rheumatoid factor; SF, synovial fluid; ^{90}Y , yttrium-90.

The number of patients taking DMARDs and those taking oral GC was different between both groups ($p = 0.04$ for both).

Values are n (%) or mean (SD, range) as appropriate.

*Miscellaneous diagnosis: $^{90}\text{Y}+\text{GC}$ group: pigmented villonodular synovitis ($n = 1$), synovial chondromatosis ($n = 1$), seronegative spondyloarthropathy ($n = 1$), osteoarthritis ($n = 2$). Placebo+GC group: osteoarthritis ($n = 1$).

†According to Steinbrocker radiological criteria.

non-rheumatoid arthritis (data not shown), we found no significant differences between both groups ($p > 0.05$).

Clinical effect

The overall response rate (ie, patients with $\text{CCI} \geq 6$) at 6 months was 47%, which was similar in both groups (46% in the ^{90}Y and glucocorticoids group v 48% in the placebo and glucocorticoids group; $p = 0.85$). If expressed by CCI, clinical efficacy of both therapeutic regimens was still similar (mean (SD, range) overall group CCI was 5 (3, 0–12); in the ^{90}Y and glucocorticoids group, CCI was 6 (4, 0–11); in the placebo and glucocorticoids group, CCI was 5 (3, 0–12); $p = 0.57$) and not dependent on diagnosis of rheumatoid arthritis versus non-rheumatoid arthritis (data not shown). During follow-up (18 months), efficacy remained similar in both groups.

Non-responders versus responders

Table 4 shows the synovial tissue features of non-responders and responders, regardless of the treatment strategy or diagnosis. Overall, only the number of macrophages in the synovial sublining was significantly higher in responders than in non-responders ($p = 0.002$). This was also found in patients from both treatment groups and in those with rheumatoid arthritis, but not in those with non-rheumatoid arthritis (fig 1). Additionally, in the ^{90}Y and glucocorticoids group, responders had more plasma cells than non-responders ($p = 0.03$), whereas responders in the

Table 2 Baseline immunohistological features of synovial tissue (n = 66 patients or knees)

	⁹⁰ Y+GC (n = 37)	Placebo+GC (n = 29)
Inflammatory cells		
T cells		
CD3	353 (338)	361 (301)
CD4	37 (72)	51 (9)
CD8	23 (54)	24 (46)
Macrophages		
CD68 lining	524 (301)	551 (317)
CD68 sublining	349 (204)	323 (174)
CD68 total	822 (419)	824 (379)
FLS		
CD55	591 (364)	582 (543)
B cells or plasma cells		
CD22	16 (32)	34 (98)
CD38	97 (142)	123 (184)
Adhesion molecules		
ICAM1	341 801 (178 814)	347 259 (228 862)
VCAM1	57 009 (48 825)	38 499 (38 733)
E-selectin	158 706 (96 068)	140 298 (82 230)
Pro-inflammatory cytokines		
TNF α	57 955 (52 426)	60 211 (35 177)
IL1 β	67 100 (50 934)	62 673 (44 601)

FLS, fibroblast-like synoviocytes; GC, glucocorticoids; ICAM1, intercellular adhesion molecule 1; IL, interleukin; TNF, tumour necrosis factor; VCAM1, vascular cell adhesion molecule 1; ⁹⁰Y, yttrium-90. Values are mean (SD).
CD markers are expressed in cell counts/mm², adhesion molecules and cytokines in integrated optical density/mm².
No significant differences were seen in immunohistochemistry data of patients from both treatment groups (p>0.05).

non-rheumatoid arthritis group had more B cells (p = 0.03). The histology scores were not significantly different between responders and non-responders (p>0.05).

Correlation between synovial tissue variables and CCI

Overall, clinical effect was correlated with the total number of macrophages (r = 0.28; p = 0.03), the number of macrophages in the synovial sublining (r = 0.34; p = 0.005) and VCAM1 expression (r = 0.25; p = 0.04). Moreover, in the ⁹⁰Y and glucocorticoids group, clinical effect correlated with the number of synovial sublining macrophages (r = 0.34; p = 0.04) as well as the number of plasma cells (r = 0.39; p = 0.02). Similarly, in the placebo and glucocorticoids group, CCI correlated with the total number of macrophages (r = 0.43; p = 0.02) and the number of synovial sublining macrophages (r = 0.41; p = 0.03). We found no relationship between lining layer hyperplasia (intimal macrophages, FLS) and CCI. If stratified by diagnosis, we found no correlation in patients with rheumatoid arthritis, whereas clinical effect was inversely correlated with B cells (r = -0.37; p = 0.02) in patients with non-rheumatoid arthritis. The conventional histology scores were not correlated with the clinical effect.

DISCUSSION

This is the first sizeable controlled study in which prediction of the clinical effect of RSO with ⁹⁰Y and glucocorticoids or with glucocorticoids alone is investigated by synovial tissue biopsy before treatment.

We found that responders to either treatment regimen had markedly higher macrophage numbers in the synovial sublining than non-responders. Moreover, the number of sublining macrophages as well as the total number of macrophages were correlated with the clinical effect.

Synovial tissue samples were obtained by blind needle biopsy. It has previously been shown that measures of

Table 3 Histology scores (n = 59 patients or knees)

	⁹⁰ Y+GC (n = 32)	Placebo+GC (n = 27)
Lymphocytes (0–4)	1 (1, 0–3)	1 (1, 0–4)
Plasma cells (0–4)	1 (1, 0–3)	1 (1, 0–3)
PMNs (0–4)	0 (0, 0–2)	0 (0, 0–1)
Synovial lining (0–3)	1 (1, 0–2)	1 (1, 0–2)
Inflammation score (0–15)	3 (2, 0–7)	3 (2, 1–9)
Vessels (0–4)	3 (1, 0–4)	3 ± 1 (0–4)

GC, glucocorticoid; PMN, polymorphonuclear cell; ⁹⁰Y, yttrium-90. Values are mean (SD, range).
We found no statistically significant differences in histology scores of patients from both treatment groups (p>0.05).

inflammation in tissue samples taken from clinically inflamed joints with the blind needle technique are generally similar to samples obtained by arthroscopy under visual inspection.²³ Blind needle biopsy fails, especially in joints which are not swollen—for instance, after successful treatment.²⁴ As the patients were selected on the basis of clinically active arthritis and samples were obtained only before treatment, we chose to use blind needle biopsy with grasping forceps, which is a more inexpensive and technically easier method than arthroscopy. We can confirm that this approach results in adequate synovial tissue samples. To minimise the sampling error, we obtained and examined at least six samples from various areas of the knee, as described previously.^{25–27}

The results presented here show that intra-articular treatment as used in this study is especially effective in patients with marked synovial inflammation. Previous work in patients with rheumatoid arthritis treated with oral glucocorticoids as well as with other treatment for rheumatic diseases showed that the status of synovial tissue sublining macrophages as an optimal biomarker related with clinical response.^{28–29} Consistent with the notion that macrophage numbers are associated with clinical signs and symptoms,³⁰ our study shows, we believe, for the first time the relationship between macrophage numbers before treatment and clinical response after treatment with either ⁹⁰Y and glucocorticoids or with glucocorticoids alone. Both intra-articular treatments may directly interfere with macrophage accumulation and activation, and could result in a reduction in the numbers of these key effector cells. Thus, the association between pretreatment macrophage numbers and response to treatment interfering with these cells is expected. Although clinical effect was also correlated with other synovial tissue variables, we concentrated on correlations that we found in both therapeutic subgroups and were consistent with previous data in the literature. This, in light of the chance of correlations, is merely based on chance, owing to the large number of variables tested.

Relatively little is known about the local effects of intra-articular treatment with glucocorticoids on the synovium, in contrary to systemic treatment with glucocorticoids. Systemic treatment reduces activation, proliferation, differentiation and survival of various inflammatory cells, including macrophages and T lymphocytes, and promotes apoptosis.³¹ Systemic glucocorticoids exert potent inhibitory effects on the transcription and action of most pro-inflammatory cytokines, including IL1 β , IL2, IL3, IL6, TNF α , interferon γ and granulocyte macrophage colony-stimulating factor.³² Intravenous treatment with glucocorticoids (methylprednisolone) reduces IL8 and TNF α levels in the synovial membrane within 24 h, which correlated with the clinical response to this treatment.³³ Conversely, the production of cytokines, such as IL4, IL10 and IL13, with anti-inflammatory

Figure 1 Pretreatment synovial tissue obtained from patients with rheumatoid arthritis (RA) and from those with non-rheumatoid arthritis (non-RA). Representative photographs of non-responders (left) and responders (right). The number of CD68+ macrophages (red) in the synovial sublining was higher in responders than in non-responders, independent of diagnosis (125×).

effects is either stimulated or not affected by glucocorticoids.³² Systemic treatment with glucocorticoids also reduces the expression of adhesion molecules,^{28 34 35} which may partly explain the decreased neutrophil trafficking into inflamed rheumatoid arthritis joints observed after intravenous treatment with glucocorticoids.³⁶ Similarly, intra-articular treatment with glucocorticoids is associated with a considerable

reduction of macrophage infiltration in the synovium,^{37 38} which clinically results in decreased arthritis activity and is reflected by a lower uptake of 99Tcm-labelled polyclonal human immunoglobulin G than pretreatment uptake.³⁸ Data on the local effects of treatment with ⁹⁰Y are limited. Autoradiography of synovial tissue sections has shown a homogeneous distribution of radioactivity in tissue samples

Table 4 Comparison of immunohistological scores between non-responders and responders

	Non-responders (n = 35 knees)*	Responders (n = 31 knees)*	p Value
Inflammatory cells			
T cells			
CD3	346 (332)	369 (311)	0.44
CD4	59 (98)	25 (63)	0.12
CD8	30 (58)	15 (38)	0.09
Macrophages			
CD68 lining	510 (307)	565 (306)	0.47
CD68 sublining†	272 (148)	411 (208)	0.002
CD68 total	740 (367)	917 (417)	0.07
FLS			
CD55	556 (384)	622 (515)	0.55
B cells or plasma cells			
CD22	34 (90)	13 (32)	0.18
CD38	97 (136)	122 (187)	0.24
Adhesion molecules			
ICAM1	317 212 (187 140)	374 669 (213 943)	0.25
VCAM1	39 861 (40 120)	59 054 (49 198)	0.11
E-selectin	141 075 (93 926)	161 391 (85 704)	0.36
Pro-inflammatory cytokines			
TNFα	59 768 (49 042)	71 375 (46 559)	0.20
IL1β	60 751 (40 795)	56 874 (50 549)	0.31

Non-responder has a composite change index score of <6. Responder has a composite change index score of ≥6. ICAM1, intercellular adhesion molecule 1; IL, interleukin; TNF, tumour necrosis factor; VCAM1, vascular cell adhesion molecule 1. CD markers are expressed in cell counts/mm², adhesion molecules and cytokines in integrated optical density/mm². *Values are the mean (SD). †p Value: only the number of CD68+ macrophages in the synovial sublining was significantly different (p=0.002) (higher in responders). The histology scores were not significantly different between responders and non-responders (p>0.05).

obtained 24 h after treatment with ^{90}Y of the knee joint in animal models.³⁹ After 24 h, very little radioactivity was detectable in synovial fluid, although it was still present in the synovium.^{39–40} It has been suggested that ^{90}Y is taken up from the articular cavity by phagocytosis of the colloidal particles by the intimal lining layer cells and polymorphonuclear cells.⁴¹ In agreement with this notion, the radioactive particles were densely placed over the cytoplasm of the synovial cells, whereas the nuclei stood out as areas free of activity.³⁹ The uptake of ^{90}Y from the articular cavity by synovial macrophages probably takes place before synovial macrophage infiltration has been reduced by cotreatment with glucocorticoids. The size of the colloidal particles is important for their retention in the synovial membrane; ^{90}Y citrate used in our study has small colloids and seems to be associated with more leakage from the joint, with subsequently more extra-articular spread than larger colloids such as ^{90}Y silicate.⁴²

RSO may result in coagulation necrosis of the superficial layer of the synovium within 2 weeks after treatment with ^{90}Y , as has been shown both in animal models^{41–43–45} and in patients.⁴⁶ Severe damage of the cytoplasmic ultrastructure of the synovial lining cells has been reported 3 days after RSO.⁴⁶ Initial radiation damage was followed by removal of the necrotic tissue by an intense inflammatory demarcation reaction, with infiltration of the synovial sublining with histiocytes, plasma cells and fibroblasts, as well as fibrin deposition.^{41–43–45} A striking feature was the thrombotic occlusion of numerous capillaries in the synovial membrane, which could explain the favourable therapeutic effects of RSO in patients with arthritis with effusion.⁴³

Besides coagulation necrosis and removal of the superficial layers of the synovium, enzymatic effects have also been described.⁴¹ In the synovial sublining, accumulation of ^{90}Y occurs in lysosomes (cytosomes) after phagocytosis by the synovial lining cells, and their uptake takes place via endocytosis.⁴¹ Severe disintegration has also been observed in FLS, in which distribution of the radioactive colloids takes place via the extracellular space, as they are not capable of phagocytosis.⁴¹ Animal studies have shown that most of the cellular infiltration of the synovial sublining is markedly decreased 4–8 weeks after RSO, followed by a reparative process with formation of collagen and interstitial fibrous tissue in the synovial membrane.^{41–43–45} Over time, this may result in a thick, fibrotic synovium with sclerosis of the synovial sublining and few inflammatory infiltrates.^{41–43–45}

Unexpectedly, we found no major difference in the clinical effect of both treatments: there was no additive effect of RSO compared with intra-articular treatment with glucocorticoids.¹⁸ Perhaps a stringent approach using treatment with glucocorticoids after aspiration of the synovial fluid and followed by immobilisation of the knee joint by a brace combined with 72 h of clinical bed rest is so effective that it is difficult to prove additional efficacy of RSO. Alternatively, we cannot exclude the possibility that RSO does not induce clinically relevant effects. Whether we believe that treatment of persistent gonarthrosis by RSO with ^{90}Y is justified or not is evaluated in the RCT on the overall study group, and is reported and discussed elsewhere.¹⁸

The effects shown here were not dependent on diagnosis of rheumatoid arthritis versus non-rheumatoid arthritis, which is in contrast with what has been suggested in the literature.^{3–7} This could at least partially be explained by the fact that all patients, regardless of the diagnosis, had a comparable degree of synovial inflammation, as the features of inflammation of the synovial tissue in patients with rheumatoid arthritis did not considerably differ from those in patients with non-rheumatoid arthritis.

CONCLUSIONS

The clinical effect of intra-articular treatment either with ^{90}Y and glucocorticoids or with glucocorticoids alone is related to macrophage infiltration of the synovium, regardless of the diagnosis. The underlying rheumatic disease did not affect the clinical effect, probably because the patients had a comparable degree of synovial inflammation. This observation supports the view that both therapeutic regimens are especially successful in patients with marked synovial inflammation.

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