

# Contrast-enhanced MRI compared to histological findings in the temporomandibular joint of antigen-induced arthritis in young rabbits

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## Abstract

### Objective

To study the correlation between histological findings and Magnetic Resonance Imaging (MRI) findings in experimentally induced arthritis in the temporomandibular joint (TMJ) of growing rabbits and to study the effect of intraarticular corticosteroid injections.

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### Methods

Arthritis was induced by ovalbumin in the left TMJ of 44 pre-sensitized rabbits. Nine animals died during this procedure. Eight of the remaining animals with induced arthritis were treated with intraarticular corticosteroid injections one week after induction of arthritis. Twelve rabbits served as controls. MRI enhanced with Gadolinium-DTPA was performed on all animals 1 to 2 weeks after induction of arthritis and again before sacrifice and the degree of enhancement was calculated. Histology of the condyle was performed and degree of villous hyperplasia, synovial thickness, infiltration of inflammatory cells and pannus was graded.

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### Results

TMJ arthritis was successfully induced in the rabbits and was verified by enhancement of the MRI and by histological changes one week after the induction. Joints treated with intraarticular corticosteroid injections revealed complete inhibition of the inflammation.

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### Conclusion

Enhancement of MRI in antigen-induced arthritis in the TMJ associated well with inflammatory changes shown histologically. An intraarticular corticosteroid injection prevents the initial inflammatory response in experimentally induced TMJ arthritis.

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### Key words

Experimental arthritis, temporomandibular joint, intraarticular injection, magnetic resonance imaging.

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## Introduction

Juvenile idiopathic arthritis (JIA) of the temporomandibular joint (TMJ) can cause severe growth disturbances in children probably caused by the intra-capsular position of the growth zone (1-3). The course of TMJ arthritis is not fully understood and the problem is to diagnose the initial stage of inflammatory changes in the temporomandibular joint, since involvement of this joint is often without symptoms and clinical findings (4). A treatment approach to control inflammation is intraarticular injection with corticosteroid in the arthritic TMJ in patients with rheumatoid arthritis (RA) (5,6). No studies have evaluated the treatment outcome of intraarticular corticosteroid injections in the TMJ of children with JIA.

Recent studies suggest that magnetic resonance imaging (MRI) with contrast agents such as gadolinium-DTPA may facilitate the diagnosis of early inflammatory changes in the TMJ (7). Information about the correlation between the MRI with contrast agent and the actual histological findings in the arthritic TMJ in growing individuals is, however, sparse. For ethical reasons an animal model of experimental arthritis is needed.

Our aim was to study the association between histological and MRI findings in antigen-induced arthritis in the TMJ of growing rabbits and to investigate the effect of intraarticular corticosteroid injection.

## Materials and methods

### Induction of arthritis

TMJ arthritis was induced in 44 ten-week-old male New Zealand white rabbits according to a method developed by Kapila *et al.* 1995 (8). The rabbits were sensitized with ovalbumin (Sigma Chemical) and Freund's complete adjuvant® (IFA, Sigma Chemicals). Two weeks after the procedure was repeated with Freund's incomplete adjuvant®. One week after sensitization the animals were injected with ovalbumin in the left TMJ under general anaesthesia. This intraarticular injection is referred to as "baseline". Nine animals died under this procedure due to respiratory insufficiency caused by the

anaesthesia.

Twelve age-matched rabbits served as controls.

### Experimental protocol

The animals were divided into five groups (A to E) (Fig.1). Group A (n = 21) with antigen-induced arthritis receiving no treatment had MRI performed 2 weeks after the intraarticular ovalbumin injection (baseline) and again 7-10 weeks later. Group B (n = 8) with induced arthritis were injected with 0.1 ml (2 mg) of triamcinolone hexacetonide (Lederspan®, Wyeth Lederle) in the left joint one week after baseline and scanned 2 and 7 weeks after baseline. Group C (n = 6) were scanned 1 to 2 weeks after baseline and again 9 weeks later. Group D (n = 6) with induced arthritis and group E (n = 6) serving as controls had MRI performed one week after baseline.

### MRI examination

Magnetic resonance imaging was performed two times at different intervals on 35 animals (Fig. 1) and one time on 12 animals. MR images were obtained on all animals under anaesthesia.

MRI was performed using a 1.5 Tesla Magnetom Vision (Siemens, Erlangen, Germany) with the rabbit placed in a prone position in a quadrature extremity coil. After a scout view dynamic sequences were obtained from both TMJs simultaneously with one slice through the centre of the condyle. The imaging protocol included a 2D flash with FA (TR/TE 43.0/4.1, FOV 160x160, 8 < 6 x 1280 matrix, 3.79s acquisition time, 90 images on both sides). An i.v. bolus injection 0.2 ml/kg of Gadolinium-DTPA (Magnevist, Schering, Berlin, Germany) was given in an ear vein 20 seconds after starting the dynamic sequence.

All animals were sacrificed with 1.0 ml Pentobarbital® i.v. after the last MRI was performed.

The ethical committee for experimental animal research approved all procedures in this study.

### Evaluation of MRI

The TMJs on both sides were traced freehand using the computer program

DimView (Atle Bjornerud, Nycomed) and the time courses of the average intensities of the region of interest (ROIs) were obtained. These were transferred into Excel where the relative enhancement (SI) was calculated by the following formula ((9):

$$SI = (SI_{post} - SI_{pre}) / SI_{pre}$$

where  $SI_{post}$  is the average intensity of the ROI in images 35 to 44 and  $SI_{pre}$  is the average ROI intensity of the five images acquired prior to bolus arrival (Fig. 2 a, b and c).

*Histology and evaluation of the synovium*

The TMJ was retrieved *en bloc*, dehydrated and infiltrated, and embedded in methylmethacrylate (MMA, Merck). The joints were placed in an orientation that allowed for the cutting of sections

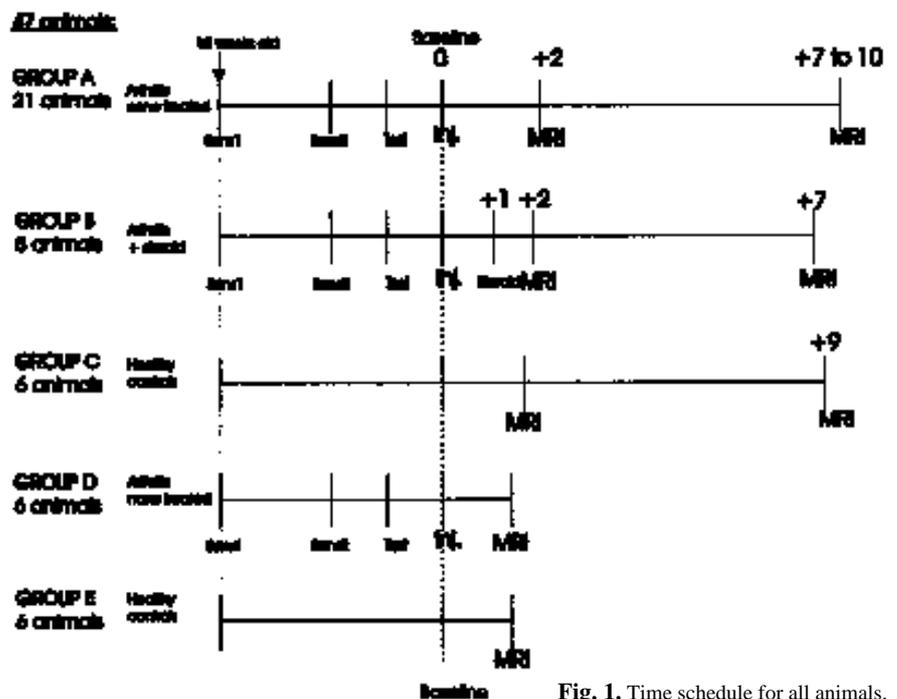
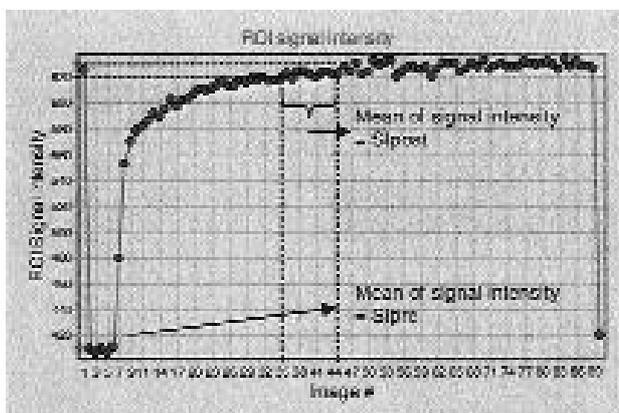


Fig. 1. Time schedule for all animals.



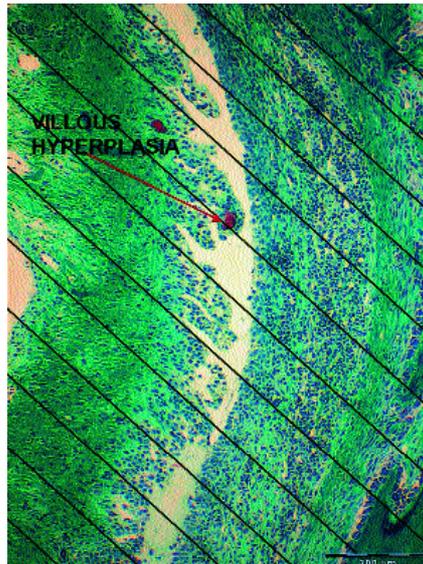
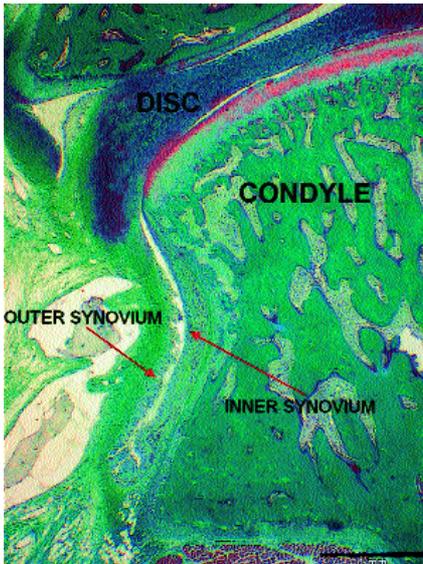
(a)

(b)

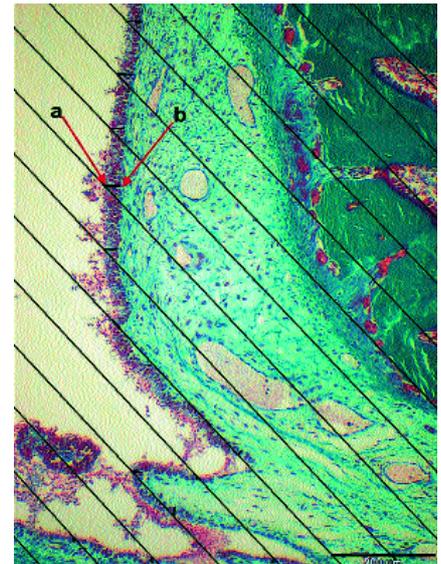


(c)

Fig. 2. (a) Contrast-enhanced MRI of a rabbit; (b) Contrast-enhanced MRI with traced TMJ. (c) Typical enhancement curve with the pre-contrast signal intensity value ( $SI_{pre}$ ) and the post-contrast signal intensity value ( $SI_{post}$ ) indicated.



**Fig. 3.** (a) Histological section of the rabbit TMJ (stained with Goldener-trichrome, original magnified x 4). (b) Larger magnification of (a) (x 26) showing villous hyperplasia of the outer synovial membrane of the antigen-induced left joint in an animal from group D with arthritis of one-week's duration.



**Fig. 4.** The distances from the point of intersection with a randomly placed grid and the synovial membrane (a), and a line perpendicular to the outer surface through the synovial membrane (b) were digitized. The mean of these distances was calculated, and the thickness of the synovial membrane was expressed as the mean x /4 (original x magnification x 21).

in the parasagittal plane starting from the most lateral part. Serial sections 7  $\mu$ m were cut with an interval of 100 sections in between. Sections were stained with Goldener-trichrome.

One representative section per joint was chosen. Sections were evaluated blindly in an Olympus BH2 microscope (26x) and an Olympus standard grid (100/25) was used. The number of intersections with normal, hyperplastic and villous hyperplasia of the inner and outer synovial membrane was counted (Fig. 3 a,b). The percentage of intersections of these parameters in relation to total amount of intersections was calculated.

The thickness of the synovial membrane was calculated using the computer program Sigma-Scan with an attached digitiser and again the Olympus standard grid (100/25) randomly placed on the section in a 21x magnification. The point of intersection between the grid and the synovial membrane and a perpendicular line through the synovial membrane was digitised (Fig. 4). For each section 50-60 distances were digitised. A mean value per section (joint) was calculated and the synovial thickness was calculated by multiplying this mean by /4 (10).

A semi-quantitative assessment was made by an experienced histologist, who blindly scored the degree of in-

flammation based on the presence of plasma cells, which are the most prominent cells seen in inflamed joints in rheumatoid arthritis. They were scored from 0 to 4 (Table I). The degree of pannus was also scored 0-4 (Table I).

#### Statistical analysis

A paired t-test was used where variables were continuous and normally distributed in order to test intra-individual differences between the left and right joint. The non-paired t-test was used to test inter-individual differences between the left or the right joint, respectively, among the groups. For histological scores with discontinuous variables a Wilcoxon paired rank-sum test was employed.

Intra-observer variance was tested by repeating six times the measurements on six randomly chosen joints.

#### Results

Histological changes showed statistically more villous hyperplasia, increased synovial thickness, increased amount of inflammation and pannus in the antigen-induced left joint and MRI showed increased enhancement when compared to the non-treated right joint in animals sacrificed one week after the induction of arthritis in the left joint (group D) (Table I). This difference was not seen in animals treated with intra-

articular corticosteroid (group B) or in healthy animals (group C).

In group A the animals were sacrificed at 7-10 weeks after baseline. We found no significant differences in the histological changes and MRI enhancement between the left joint in group A and the left joints in the healthy animals of the same age in group C (Table II).

Intra-observer variance was 3.1% for the histological evaluation and 4-2% for the MRI assessment.

#### Discussion

In this animal model of experimental TMJ arthritis we found villous hyperplasia to be a characteristic of arthritis. We calculated the actual percentages of villous hyperplasia in each section, since villous hyperplasia is a feature that is less independent of the oblique cutting phenomenon than synovial lining hyperplasia and counting the actual number of inflammatory cells. Likewise, the assessment of the thickness of the synovium may be influenced by the oblique cutting phenomenon and we therefore used a well-known formula for the correction for oblique cutting for plate-like structures (10). The evaluation of the presence of inflammatory

**Table I.** Intra-group differences. Left joints were tested against right joints.

	Histology				MRI							
	Synovial hyperplasia % villous [mean, (SE)]	Synovial thickness µm [mean, (SE)]	Inflammation <sup>(a)</sup> [mean, (SE)]	Pannus <sup>(b)</sup> [mean, (SE)]	1stMRI enhancement at 1-2 weeks [mean, (SE)]	2nd MRI enhancement at 7-10 weeks [mean, (SE)]						
Group A (n=21) arthritis, 7-10 weeks	R	9.20 (2.66)	R	14.73 (1.02)	R	0.52 (0.13)	R	0.95 (0.20)	R	0.77 (0.16)	R	0.37 (0.02)
	L	5.50 (4.35)	L	14.36 (0.81)	L	0.57 (0.16)	L	0.86 (0.19)	L	0.92 (0.16)	L	0.43 (0.05)
Group B (n=8) arthritis + steroid, 7 weeks	R	14.27 (4.23)	R	13.46 (0.91)	R	0.25 (0.16)	R	0.50 (0.19)	R	0.29 (0.04)	R	0.35 (0.02)
	L	21.13 (5.52)	L	14.01 (1.44)	L	0.13 (0.13)	L	0.38 (0.18)	L	0.37 (0.03)	L	0.45 (0.04)
Group C (n=6) healthy, 9 weeks	R	21.93 (7.34)	R	16.56 (1.52)	R	0.67 (0.21)	R	1.33 (0.33)	R	0.32 (0.02)	R	0.32 (0.03)
	L	22.05 (7.89)	L	16.76 (2.11)	L	0.17 (0.17)	L	1.00 (0.37)	L	0.35 (0.03)	L	0.36 (0.01)
Group D (n=6) arthritis, 1 week	R	13.54 (4.45)	R	18.37 (2.34)	R	0.17 (0.17)	R	0.50 (0.22)	R	0.33 (0.04)		
	L	66.97 (10.55)	L	31.75 (3.08)	L	1.67 (0.33)	L	2.00 (0.26)	L	0.44 (0.03)		
	**		*	**	**	**	**	**	**			
Group E (n=6) healthy, 1 week	R	10.03 (4.96)	R	15.23 (1.68)	R	0.50 (0.22)	R	0.83 (0.31)	R	0.33 (0.01)		
	L	6.60 (3.68)	L	15.09 (1.61)	L	0.17 (0.17)	L	0.50 (0.34)	L	0.34 (0.02)		

\* p < 0.05 \*\* p < 0.01 \*\*\* p < 0.001, (SE) standard error.

<sup>(a)</sup> Inflammation was scored: 0 = no inflammatory cells; 1 = few, scattered; 2 = clearly present; 3 = multiple inflammatory cells organized in bands; and 4 = massive presence of inflammatory cells.

<sup>(b)</sup> Pannus was scored: 0 = no pannus; 1 = small areas with synovial proliferation; 2 = larger areas with synovial proliferation; 3 = invasion into the joint cavity by synovial proliferation; and 4 = joint cavity totally occupied by synovial proliferation.

**Table II.** Intergroup differences. Left joints were compared.

	Histology				MRI							
	Synovial hyperplasia % villous [mean, (SE)]	Synovial thickness µm [mean, (SE)]	Inflammation [mean, (SE)]	Pannus [mean, (SE)]	1stMRI enhancement at 1-2 weeks [mean, (SE)]	2nd MRI enhancement at 7-10 weeks [mean, (SE)]						
Group D vs. Group E arthritis, 1 week vs. healthy, 1 week	D	66.97 (10.55)	D	31.75 (3.08)	D	1.67 (0.33)	D	2.00 (0.26)	D	0.44 (0.03)		
	E	6.60 (3.68)	E	15.09 (1.61)	E	0.17 (0.17)	E	0.50 (0.34)	E	0.34 (0.02)		
	***		***	**	**	**	**	**	**			
Group D vs. Group B arthritis, 1 week vs. arthritis + steroid, 7 weeks	D	66.97 (10.55)	D	31.75 (3.08)	D	1.67 (0.33)	D	2.00 (0.26)	D	0.44 (0.03)		
	B	21.13 (5.52)	B	14.01 (1.44)	B	0.13 (0.13)	B	0.38 (0.18)	B	0.37 (0.03)		
	**		***	***	***	***	*	*	*			
Group B vs. Group E arthritis+steroid,7 weeks vs.healthy1week	B	21.13 (5.52)	B	14.01 (1.44)	B	0.13 (0.13)	B	0.38 (0.18)	B	0.37 (0.03)		
	E	6.60 (3.68)	E	15.09 (1.61)	E	0.17 (0.17)	E	0.50 (0.34)	E	0.34 (0.02)		
Group B vs. Group A arthritis + steroid, 7 weeks vs. arthritis 7-10 weeks	B	21.13 (5.52)	B	14.01 (1.44)	B	0.13 (0.13)	B	0.38 (0.18)	B	0.37 (0.03)	B	0.45 (0.04)
	A	15.50 (4.35)	A	14.36 (0.81)	A	0.57 (0.16)	A	0.86 (0.19)	A	0.92 (0.16)	A	0.43 (0.05)
									*			
Group A vs. Group C arthritis 7-10 weeks vs. healthy 9 weeks	A	15.50 (4.35)	A	14.36 (0.81)	A	0.57 (0.16)	A	0.86 (0.19)	A	0.92 (0.16)	A	0.43 (0.05)
	C	22.05 (7.89)	C	16.76 (2.11)	C	0.17 (0.17)	C	1.00 (0.37)	C	0.35 (0.03)	C	0.36 (0.01)
									**			

\* p < 0.05; \*\* p < 0.01; \*\*\* p < 0.001; (SE) standard error.

cells and pannus was done by an experienced histologist and therefore represents a semi-quantitative assessment

and we obtained similar results when the two different methods of assessing the histological sections were com-

pared. No generally accepted histological scoring system of synovial inflammation exists (9, 11-13).

The histological scores, as well as MRI findings were statistically significantly increased in the antigen-induced left joint as compared to the non-treated right joint within the same animal one week after induction of arthritis. However, when the animals were sacrificed 7 to 10 weeks after the induction of arthritis changes were not observed on either histology or MRI. The antigen-induced arthritis model proved to be useful in studies of initial inflammation of TMJ arthritis but did not result in permanent damage to the joint. Kapila (8) stated that the inflammatory changes of the TMJ can be seen from 5 to 55 days after intraarticular injection with 0.1 ml of 5 mg/ml ovalbumin in adolescent rabbits. However, in 9 animals from group A that were sacrificed 7 weeks (49 days) and 3 animals that were sacrificed 8 weeks (56 days) after the induction of arthritis we could not detect any statistically significant difference between the left and the right joints. This difference in results could have been caused by the different ages of the rabbits at the time of arthritis induction, since younger animals may have a larger capacity for recovery. We found a marked effect of intraarticular corticosteroid injection on TMJ arthritis. Symptomatic temporomandibular arthritis in humans with rheumatoid arthritis has been studied and a marked long-term effect of the intraarticular corticosteroid injections has been reported (5,6,15). However, the patients were adults with severe symptoms and signs that were already verifi-

able on radiographic examination. So far no studies have been performed in children with JIA on the effect of intraarticular corticosteroid injection in the TMJ.

In conclusion, we found an association between the histological and MRI findings after induction of arthritis. Intraarticular injection with corticosteroid seems to prevent the initial inflammatory response in TMJ arthritis.

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