Subtyping of osteoarthritic synoviopathy

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Abstract

Objective

Osteoarthritis research is traditionally concentrating on events within the degenerated articular cartilage. Changes in the synovial membrane are largely neglected. In fact, they are generally interpreted as secondary to the cartilage changes and not pathogenetically involved in the disease process. In this study, we present a systematic analysis of the synovial reaction pattern in early and late stages of the osteoarthritic disease process.

Methods

A large series of synovial specimens derived from early and late stage osteoarthritic cartilage disease were investigated by histological and immunohistochemical means for tissue architecture and inflammatory cell infiltrates. For comparison, also samples with rheumatoid arthritis, seronegative arthritis, and septic arthritis were included as well as normal synovial membrane specimens.

Results

In all specimens derived from patients with diagnosed osteoarthritis alterations of the synovial tissue were observed. A large spectrum of alterations was found in different stages of osteoarthritic joint disease and four different basic pattern of synovial reactions could be identified: (i) hyperplastic, (ii) inflammatory, (iii) fibrotic, and (iv) detritus-rich synoviopathy.

Conclusion

We show that in all cases of clinically ouvert osteoarthritic joint disease significant synovial pathology is associated. Furthermore, our study clearly documents that in osteoarthritic synovium significant inflammation can occur. This is suggestive of a distinct pathogenetic role of the synovium also in osteoarthritic cartilage degeneration at least in a subset of cases.

Key words
Inflammation, synovia, osteoarthritis.

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Introduction

Osteoarthritic joint disease represents one of the most crippling diseases of an aging population. Though it originates most likely from matrix turnover disturbances of the articular cartilage, other joint structures such as the subchondral bone plate and the synovium play their own roles. The role of the synovium in terms of disease symptoms is beyond any doubt, but its role in the pathogenetic process of the cartilage destruction itself is largely neglected. The synovial reaction observed in osteoarthritic joint disease is considered to be secondary due to cartilage debris from the damaged cartilage (1-5), largely in contrast to the situation found in rheumatoid arthritis, which is considered to originate from a synovial inflammatory autoimmune reaction.

In this study, we investigated the spectrum of alterations found in different stages of osteoarthritic joint disease and were able to identify four different basic pattern of synovial reactions. The observed patterns suggest that osteoarthritic synoviopathy plays not only a role as far as the symptoms are concerned, but has also a pathogenetic potential within the disease process.

Materials and methods

Clinical cases

Samples of early and late stage osteoarthritis (derived from arthroscopy (n= 29; male:female = 16:13; age (mean) 20 – 81/42.8) and joint replacement [n=37; male:female = 10:27; age (mean) 38 – 91/63.5)] were studied. Duration of (symptomatic) disease was 9 months on the average in early stage and above 60 months in late stage, respectively. For comparison 22 samples of rheumatoid arthritis (male: female = 3:19; age 22 – 75/53.5), 12 samples of seronegative arthritis (male: female = 5:7; age 21 – 67/35,6), and 10 samples of bacterial arthritis (male: female = 6:4; age 21 – 68/33.9) were analyzed. For control, also 7 cases of posttraumatic arthropathy (male:female = 6:1; age 11 – 83/ 44.9) and 8 normal synovial specimens (from autopsy; male:female 4:4; age 32 – 57/47.4) were included in the study.

Histology and histochemistry

Routine histology (hematoxylin-eosin stainings) was performed in order to evaluate basic histomorphological features of the specimens.

The collagen content in the extracellular matrix and the amount of fibrinous exsudate overlaying the synovial lining or incorporated within the synovial membrane were evaluated by van Gieson staining.

Immunohistochemistry

Immunohistochemical studies were performed using a streptavidin-biotin-complex technique (Biogenex, san Ramon, CA, USA) detected with alkaline phosphatase as described previously (6). The antibodies and the pretreatments used are shown in Table I.

Control experiments: In control experiments, primary or secondary antibodies were replaced by PBS and the samples processed as described above. Additionally, in some test specimens similar dilutions of non-immune serum replaced primary antibodies. Control samples were consistently negative.

Histological evaluation

All areas of each biopsy section were examined and histological features were scored independently by two observers. Tissues were scored separately according to the degree of infiltration by lymphocytes, plasma cells, and polymorphonuclear cells (PMNs). A score of 0 represented minimal infiltration, while a score of 4 represented infiltration by numerous inflammatory cells. In addition, each tissue was assigned a score for hyperplasia of the synovial lining according to the cell layers present.

Immunohistochemical identification of inflammatory cell types

In order to identify the different cell populations within the specimens, a selection of immunohistochemical markers (Table I) were used.

CD3, CD4, CD8, CD20, CD68, and CD138 expression in the synovial sublining was scored on a five-point scale (0-4) by two independent observers. For the evaluation of CD4+ cells, only cells with lymphocytic morphology

Table I. Primary antibodies and enzymatic pretreatments used for immunohistochemical analysis (m: mouse monoclonal; r: rabbit polyclonal; h:heat pretreatment; hy: hyaluronidase (ovine testis in 2 mg/ml,phosphate buffered saline (PBS),pH 5,60 min at 37°C); pn:pronase (2 mg/ml, PBS, pH 7.3,60 min at 37°C; Boehringer Mannheim; FRG); pt:protease XXIV (0.02 mg/ml, PBS, pH 7.3,60 min at RT; Sigma, Berlin, FRG).

Antigen	Specificity	Clone	Species	Dilution	Pretreatment	Source
CD3	T-cells	CD3-12	rat	1:500	h	Biotrend
CD4	T-helper-cells, monocytes	1F6	m	1:200	h	Dako Denmark
CD8	cytotoxic/suppressor T-cells	C8/144B	m	1:2000	h	Dako Denmark
CD20	B-cells	L26	m	1:500	h	Dako Denmark
CD138	plasma cells	ID4	m	1:100	h	Dianova FRG
CD68	lysosomal protein: phagocytes	PGM-1	m	1:5000	pn	Dianova FRG
Neutrophil elastase	neutrophils	NP 57	m	1:200	—-	Dako Denmark

were included, since CD4 may also be expressed by macrophages. Individual readings of the two observers were identical or differed by only 1 point. Minor differences between the observers were resolved by mutual agreement.
The scoring system was calibrated for each marker and was adapted for the different cell populations. Thus, the scoring system should not be used to compare the expression of different markers within patient groups.

Statistical analysis
Statistical analysis was performed using a conventional statistical data bank (SPSS D 8.0®). For the evaluation of the variables non-parametrical tests (Mann-Whitney for disconnected and the Wilcoxon test for connected variables) were applied. Correlation analysis was done by the Kendalls tau' b test. Statistical significance was assumed if p was less than 0.05.

Results

General histological evaluation
In all specimens derived from patients with diagnosed osteoarthritis alterations of the synovial tissue were observed. In principle, four different basic pattern of osteoarthritic synoviopathies were found with characteristic histomorphological features: (i) hyperplastic, (ii) inflammatory, (iii) fibrotic, and (iv) detritus-rich synoviopathy. The histomorphological features are summarized in Table II.

Synovial hyperplasia - Hyperplastic osteoarthritic synoviopathy
Three forms of alterations of the synovial surface could be observed: first, the usually flat synovial lining cells (Fig. 1b) showed increased cytoplasmic volume and became cuboidal or even cylindrical suggesting strong activation of these cells (fig. 1d). Secondly, the normally single cell layer (Fig. 1 a,b) proliferated and could form more than fife cell layers. Finally, the whole synovial surface including the underlying stroma was hyperplastic and formed the classical synovial villi (Fig. 1c, 2a, 3a, 4d). Synovial hyperplasia could be found in all forms of osteoarthritic synoviopathy and in chronic synovites. Thus, villous hyperplasia was a non-specific feature of (chronic) synovial alteration. In fact, the synovial lining was in particular proliferated and activated in the detritus-rich synoviopathy and less pronounced in inflammatory or fibrotic synoviopathies. The on the average lowest activation and proliferation was found in hyperplastic synoviopathy.
The hyperplastic variant of osteoarthritic synoviopathy could be distinguished from the other variants by the absence of histomorphological parameters mentioned in Table II (inflammatory infiltrate, capsular fibrosis, macromolecular cartilage and bone detritus) (Fig. 1 c,d). A rather normal stroma was subjacent a hyperplastic and activated lining. This form of synoviopathy was found almost exclusively in early stage OA.

Lymphocytic infiltrates - Inflammatory osteoarthritic synoviopathy
By counting lymphocytes, the most prominent (up to moderate) lymphocytic infiltrate among the different types of osteoarthritic synoviopathies was found in the inflammatory sub-

Table II. Table listing the major histopathological features of the four pattern of osteoarthritis associated synoviopathy in comparison to each other and to normal synovial membrane. Bold letters indicate key diagnostic criteria.

	Normal	Hyperplastic	Inflammatory	Fibrotic	Detritus-rich
Villous hyperplasia	-	**++(+)**	++(+)	++(+)	++(+)
Synovial lining – proliferation	-	+	++	++	++(+)
Synovial lining – activation	-	+	++	+	+
Fibrinous exsudate	-	-	(+)	+	**++(+)**
Capsular fibrosis	-	-	(+)	**+++**	+++
(Macromolecular) cartilage and bone debris	-	-	(+)	-	**+++**
Granulocytic infiltrate	-	-	-	-	+
Lymphoplasmacellular infiltrate – diffuse	-	-	**++**	(+)	+(+)
Lymphoplasmacellular infiltrate – aggregates/follicles	-	-	**++**	(+)	(+)

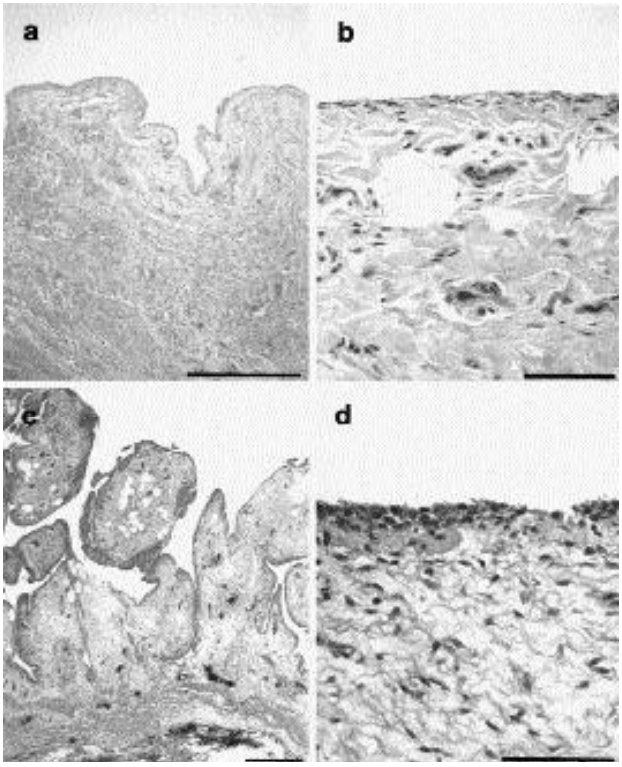

Fig. 1. Hematoxylin eosine stainings of normal synovia (a, b) and hyperplastic osteoarthritic synoviopathy (c, d). Whereas normal synovia shows a smooth surface with a single layer of flat synoviocytes (b), hyperplastic synovoviopathy shows villous hyperplasia (c) and proliferated and activated synoviocytes at the surface (d). (Magnification bars a, c: 500 µm; b, d: 100 µm).

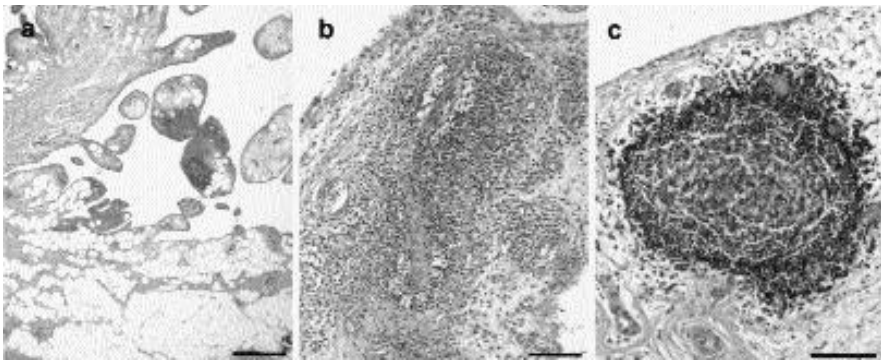

Fig. 2. Hematoxylin eosine stainings of inflammatory osteoarthritic synoviopathy: typically, synovial hyperplasia (a) and a slight to moderate lymphocytic infiltrate mostly around vascular structures (b) or lying in aggregates and even follicles (c) are found. (magnification bars a: 500 µm; b, c: 100 µm).

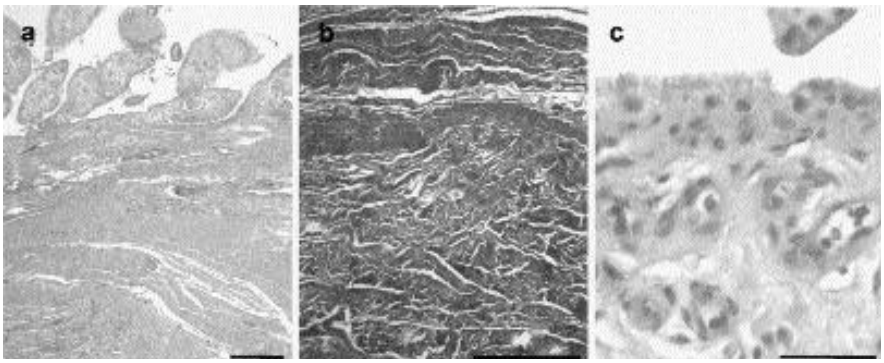

Fig. 3. Hematoxylin eosine stainings of fibrotic osteoarthritic synoviopathy: typically, a thickened (a) and fibrotic (b: van Giesons stain for collagen content) capsule is observed. Additionally, a variable proliferation and activation of synoviocytes is found (c). (magnification bars a,b: 500 µm; c: 50 µm).

form ($p < 0.01$ compared to other osteoarthritic synoviopathies) (Fig. 2). Immunotyping of the lymphocytic infiltrate (Fig. 6) showed highest scores for CD20 positive B-cells (Fig. 5h) and CD3 positive T-cells (Fig. 5e) in the inflammatory subform of osteoarthritic synoviopathy compared to the other subforms ($p < 0.05$). Also the CD 138 plasma cell count was highest in this subform (Fig. 5k), however, statistical significance was reached to the hyperplastic subform only ($p < 0.01$). Whereas the synovium of the inflammatory subform was infiltrated moderately by lymphocytes, it was still significantly less infiltrated than in RA synovium ($p < 0.001$) (Fig. 5 c,i,l). No lymphocytic infiltration was found in normal specimens (Fig. 5 d,g,j).

Staining for CD68 revealed an interesting feature. The lining cell layer of the inflammatory subform contained significantly more CD68 positive cells (Fig. 5b) than normal synovium (Fig. 5a) and all other synoviopathies including RA ($p < 0.05$).

In summary, the inflammatory subform of osteoarthritic synoviopathy was characterized by moderate lymphocytic infiltrates and synovial hyperplasia and activation whereas abundant macromolecular cartilage debris and prominent capsular fibrosis were absent. Synovial hyperplasia was found in specimens derived from patients with early as well as late stage osteoarthritis.

Capsular fibrosis – Fibrotic osteoarthritic synoviopathy

Capsular fibrosis was observed in the fibrotic (Fig. 3) and detritus-rich subforms of osteoarthritic synoviopathy ($p < 0.001$ (hyperplastic and inflammatory synoviopathy)) and, thus, only in late stage osteoarthritis. In contrast to the detritus-rich subform (Fig. 4) the fibrotic subform missed significant cartilage or bone debris, the rather extensive fibrinous exsudate and, largely, the inflammatory infiltrate. Of note, despite an overall long disease period of the cases of rheumatoid and seronegative arthritis (mean value 12.6 years), both disease entities showed much less capsular fibrosis than the two forms of

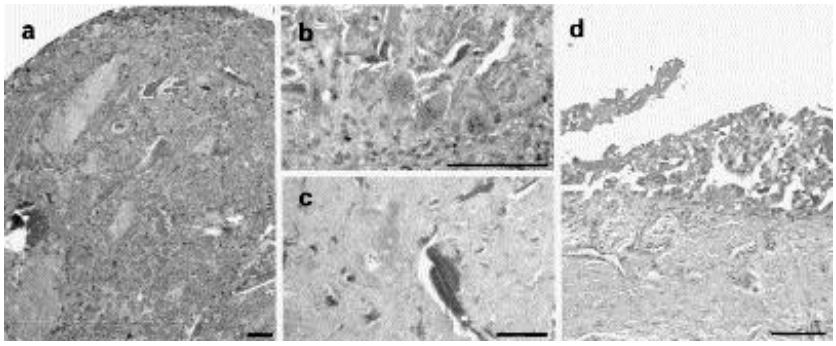

Fig. 4. Hematoxylin eosine stainings of detritus-rich osteoarthritic synoviopathy: typically, a lot of cartilage and bone fragments intermixed with multinucleated giant cells (b) in an abundant fibrinous exsudate are found (a, c; c: van Giesons stain for collagens shows red bone fragments and pink cartilage debris). In less affected portions of the synovial membrane a fibrinous exsudate is found (d) overlaying an often hyperplastic synovial membrane (magnification bars a-d: 100 µm).

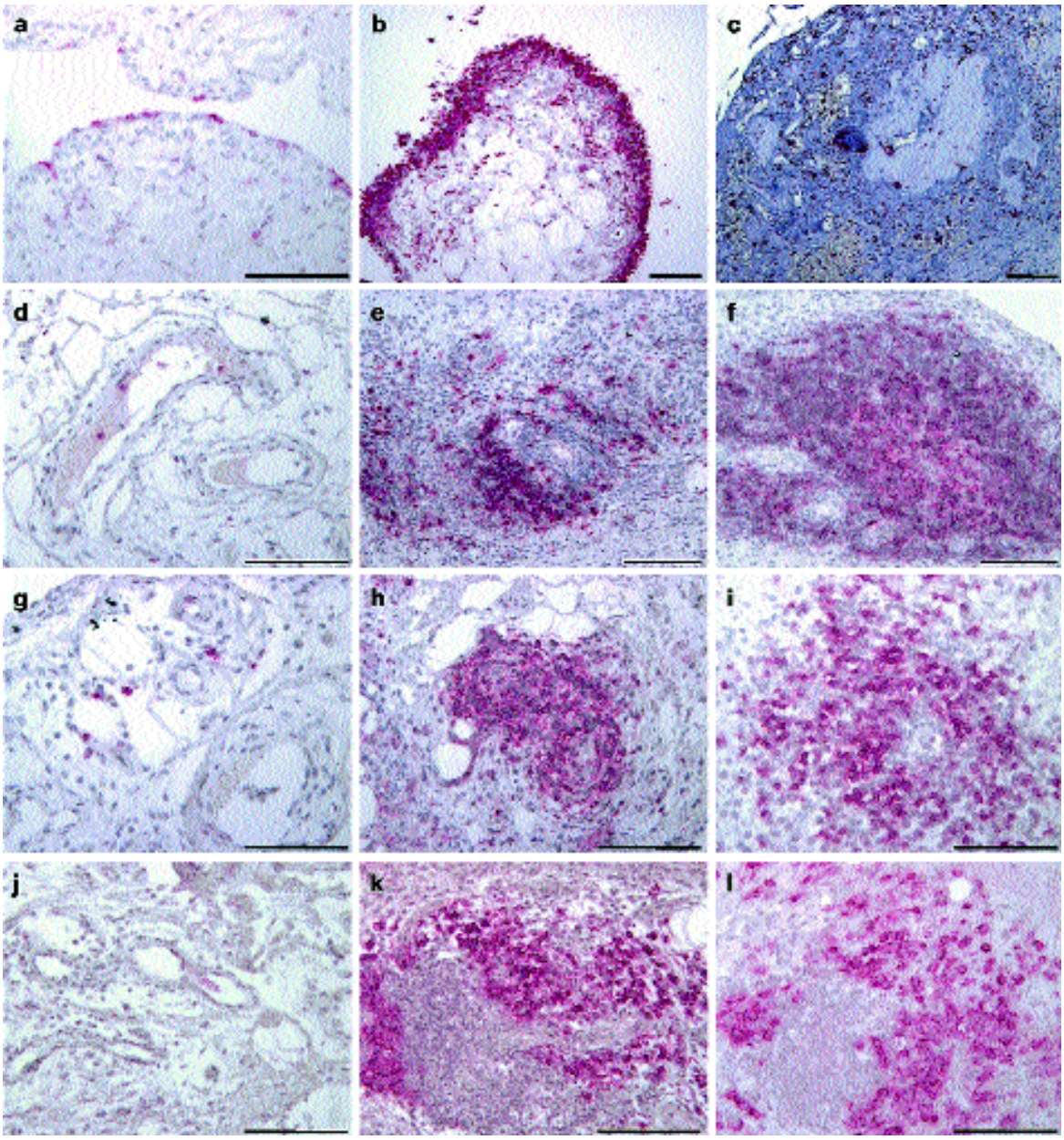

Fig. 5. a-c:Immunostainings for CD68 in normal synovial membrane (a) and inflammatory (b) and detritus-rich (c) osteoarthritic synoviopathy. d-f:Immunostainings for CD3 in normal synovial membrane (d),inflammatory osteoarthritic synoviopathy (e),and rheumatoid synovitis (f). g-i: Immunostainings for CD20 in normal synovial membrane (g), inflammatory osteoarthritic synoviopathy (h), and rheumatoid synovitis (i). j-l: Immunostainings for CD138 in normal synovial membrane (j), inflammatory osteoarthritic synoviopathy (k), and rheumatoid synovitis (l). (magnification bars: a-l: 100 µm).

osteoarthritic synoviopathy ($p < 0.01$ and $p < 0.05$). The only two patients of the rheumatoid group, who showed rather significant capsular fibrosis, have developed additionally a secondary osteoarthritic joint destruction requiring joint replacement.

Detritus-rich osteoarthritic synoviopathy

Cartilage and bone debris was mainly found in the detritus-rich synoviopathy (Fig. 4). Significant debris was also detectable in the RA cases. All other forms of osteoarthritic synoviopathy as well as seronegative arthritis showed no or only minor amounts of cartilage and bone fragments ($p < 0.01$). Besides the debris also a rather significant fibrinous exsudate was either found at the surface alone or combined with incorporated fibrin, with the latter reflecting longer ongoing fibrinous exudation. Detritus-rich synoviopathy was the only osteoarthritic synoviopathy in which (low numbers of) neutrophil granulocytes and multinucleated giant cells (Fig. 4b) were found.

Though the number of CD68 positive cells in the sublining and stroma was in all osteoarthritic synoviopathies increased compared to the normal synovial specimens ($p < 0.05$), the clearly highest presence was seen in the detritus-rich subform compared to the other subforms ($p < 0.01$) including multinucleate giant cells (Fig. 5c).

Early and late stage osteoarthritic synoviopathy

In this study, overall 29 cases of early osteoarthritic lesions as well as 37 cases of late stage osteoarthritic lesion were investigated. Most of the early cases showed the hyperplastic ($n = 21$) and some the inflammatory subform ($n = 8$). Late stage lesions showed the fibrotic ($n = 12$), inflammatory ($n = 14$) and detritus-rich ($n = 9$) subforms. In two cases, only synovial hyperplasia was observed ($n = 2$). Thus, the inflammatory subform was found in both stages of the disease process whereas the others were rather selectively observed either in early stage disease (hyperplastic) or late stage disease (fibrotic and detritus-rich subforms). On the histological level, no significant differences could be detected for the following parameters in early and late stage osteoarthritic synoviopathy: villous hyperplasia, number and activation of lining cells, overall number of inflammatory infiltrates, percentage of CD68-positive synoviocytes, CD68-positive histiocytes within the subsynovial stroma, incorporated fibrin. In contrast, significant differences were found for surface fibrin, detritus, capsular fibrosis, and plasma cells (each $p < 0.01$) (Fig. 7).

Discussion

Osteoarthritis research is traditionally concentrating on the understanding of events within the degenerated articular cartilage. Changes happening in the synovial membrane are largely neglected. They are generally interpreted as largely secondary to the cartilage changes and not pathogenetically involved in the disease process.

In this study, we present a systematic analysis of the synovial reaction pattern in early and late stages of the osteoarthritic disease process. We show that in all cases of clinically significant osteoarthritic joint disease some significant synovial pathology is associated, which fits to the assumption of the direct relation in between clinical symptoms and the synovial reaction in osteoarthritis. Furthermore, our study clearly documents that in osteoarthritic synovium significant inflammation can occur. This is suggestive of a pathogenetic role of the synovium also in this disease at least in a subset of cases.

In the literature, largely two subforms of synovial reaction pattern in osteoarthritis are distinguished: synovial hyperplasia as well as the detritus-synovitis of late stages (1, 2, 7). The latter form represents a consequence of total joint destruction leading to abundant macromolecular cartilage and bone detritus (8). In our study, we characterize four pattern of osteoarthritic synoviopathies. Among them, the most interesting in terms of pathogenesis might be the inflammatory osteoarthritic synoviopathy showing significant lymphocytic infiltrates. In fact, also previously

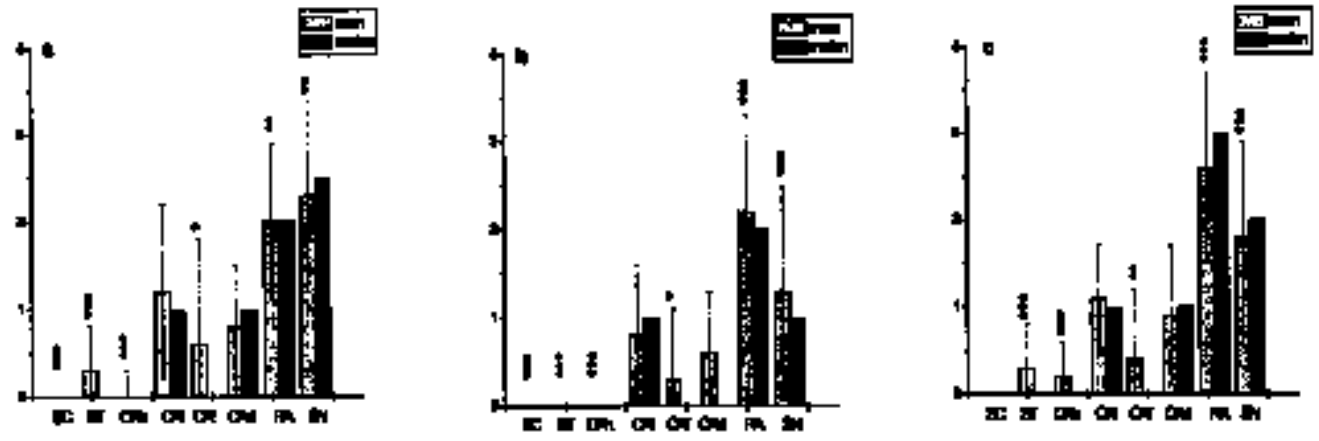

Fig. 6. Diagrammatic representation of CD20 (a),CD 138 (b),and CD3 (c) positive cellular infiltrates rated in control synovia (c),posttraumatic synovia (t) as well as hyperplastic (OAh),inflammatory (OAi), fibrotic (OAf), and detritus-rich (OAd) osteoarthritic synoviopathy. For comparison, results for rheumatoid arthritis (RA) and seronegative arthritis (SN) are shown. (stars indicate significance levels in between indicated entities and the inflammatory synoviopathy: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

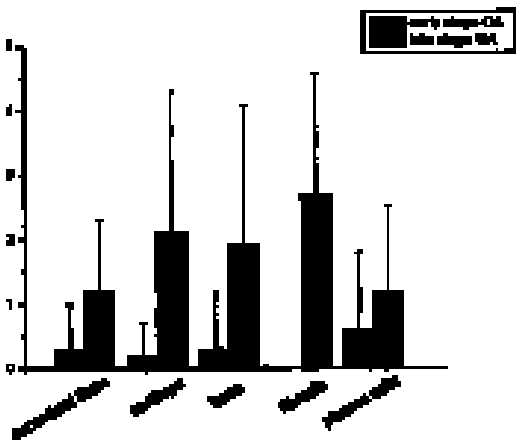

Fig. 7. Diagrammatic representation of basic features of osteoarthritic synoviopathy found significantly different in early and late stage osteoarthritic specimens ($p < 0.01$).

some authors noticed that osteoarthritic synovia might resemble rheumatoid synovitis (9). It is intriguing to speculate whether this subform reflects some kind of autoimmune aspect of a subset of osteoarthritic patients. Inflammatory osteoarthritic synoviopathy resembled rheumatoid synovitis in some respects, but there were also clear differences beyond the higher quantity of lymphocytic infiltrates in the latter condition: in particular the diffuse lymphocytic infiltrates in the sublining layer were not seen in osteoarthritic inflammatory synoviopathy ($p < 0.001$). More importantly, however, there was a difference in the composition of the lymphocytic cellular infiltrate. Whereas in rheumatoid synovitis in accordance with the literature a clear predominance of T-lymphocytes was found ($p < 0.01$) (10-14), in inflammatory osteoarthritic synoviopathy no such difference was noted (Fig. 3).

Hyperplastic osteoarthritic synoviopathy showed only synovial hyperplasia without significant capsular fibrosis, inflammatory infiltrates or macromolecular detritus. It was only found in the early stages disease stages in our investigation. Villous hyperplasia itself appears to be a rather unspecific synovial reaction in response to chronic activation as it was seen in all stages of the disease process in all subforms of osteoarthritic synoviopathy (15) and also in many other forms of synovitis irrespective of the etiology of the joint lesion: this process leads to an overall increase in synovial surface and might be, therefore, a reaction to a chronically increased need of synovial activity.

In contrast, synoviocyte activation can be considered as a short term reaction and synoviocyte proliferation as intermediate of both. In this respect, it is not of major importance whether synovial hyperplasia represents a result of increased proliferation (16, 17), reduced synovial apoptosis (18), both (19), or in the case of A-synoviocytes increased immigration from the hematopoietic system.

Our data suggest that in most cases of osteoarthritis (20) capsular fibrosis follows synovial hyperplasia both being a consequence of micro- and macromolecular detritus. A similar sequence of events has also been shown in a rabbit model after cartilage fragment injection (20). Thus, fibrotic osteoarthritic synoviopathy is found selectively in the late stage specimens. In fact, capsular fibrosis might have very detrimental effects due to shortening of the capsule and be, thus, responsible for part of the symptoms such as joint stiffness and joint pain. Additionally, it might easily further increase the biomechanical stress on the already damaged articular cartilage by increasing tension stress all over the joint.

Most likely, the initial event in osteoarthritic synoviopathy is molecular cartilage detritus, which exceeds the "physiological" molecular detritus derived from articular cartilage even in situations of normal matrix turnover. Synoviocyte activation, proliferation, and synovial hyperplasia presumably all represent reactive changes to increased demands of clearance of molecular debris in the synovial fluid of the joint (1, 3, 5). This is also suggested by the increase in the proportion of CD68-positive type A synoviocytes in the synovial lining layer, which have phagocytic capacity (21-24). Thus the percentage of type A synoviocytes increases from about 10% in normal joints to about 40% in osteoarthritic synoviopathy as shown by this and other studies (21, 25).

In line with this, the highest percentage of up to 60% type A synoviocytes was found in the inflammatory subform suggesting that this subform is associated with a very significant matrix catabolic activity. It is intriguing to speculate that the cartilage matrix catabolism might be at least in part induced or promoted by catabolic mediators such as Il-1ß and TNF-α secreted by the activated synoviocytes. This is not unexpected, because previous work could show that similar cell types expressing similar cytokines are present in osteoarthritic synoviopathy as in rheumatoid synovitis (26). Basically, inflammatory cytokines such as Il-1ß and TNF-α are top candidates because both are not only able to down-regulate matrix anabolism in articular chondrocytes (27), but also to induce expression and secretion of matrix degrading proteases (28, 29) and most likely also in an auto/paracrine manner in synoviocytes.

Alternatively, antagonistic molecules such as the Il-1-receptor-antagonist might be down-regulated (30). In fact, Smith and colleagues were able to demonstrate that the expression and synthesis of Il-1 and TNF-α increases with ongoing matrix destruction (15). Besides the production of cytokines and their inhibitors by the synoviocytes, also the secretion of cartilage matrix degrading proteases and their inhibitors is very likely to affect directly cartilage matrix integrity. In fact, clearly increased levels of matrix degrading enzymes were previously detected in synovial fluids of osteoarthritic patients (31).

Altogether, it appears that the production of mediators by the synoviocytes, in particular if they are activated, may play a very important role for cartilage matrix turnover and destruction. Interestingly, the lymphocytic infiltrate in

the subsynovial stroma appears to correlate directly with Il-1ß in the synovial fluid as well as MMP-1 expression by synoviocytes (32, 33) suggesting a direct stimulatory role of the inflammatory infiltrates on the activity of the synovial lining cells.

In conclusion, our work describes four different pattern of osteoarthritic synoviopathy with significant differences in overall tissue architecture and inflammatory cell infiltrates. They support a pathogenetic role of osteoarthritic synoviopathy at least in a subset of osteoarthritic cartilage degeneration.

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