Markers of cartilage metabolism in arthrosis

A review

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The mechanisms involved in the disease process in arthrosis are largely unknown, with genetics, joint malalignment, overload or trauma, obesity, and aging as some of the known or suspected contributing factors. Even less well known is how these general factors are translated into disease mechanisms at the cell and tissue levels. However, it may be argued that degradation of cartilage matrix is a key event at some time in the development of arthrosis. During this process, fragments of matrix molecules and other chondrocyte products are released into the joint fluid and eventually into other body fluids. These molecules can be used as markers of cartilage metabolism to monitor joint disease. In addition, by identifying the proteases and the structure of the released matrix fragments, we may improve our understanding of the cellular mechanisms active in cartilage degradation. Such information offers improved diagnostic and prognostic tools for rational treatment aimed at retarding cartilage destruction in arthrosis.

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We diagnose arthrosis (osteoarthrosis, osteoarthritis) on the basis of combined clinical symptoms and radiographic findings (Ahlbäck 1968, Altman et al. 1986, 1987). Because the diagnosis is based on obligatory radiographic signs, notably a decrease in the joint space, it is dependent on the actual destruction of joint cartilage. By definition, then, the diagnosis will only be made late in the disease. However, the arthrosis is initiated long before the radiographic diagnosis can be effected, and our inability to diagnose the disease during the earlier stages reflects the dearth of available methods to monitor the health of the joint cartilage. Thus, current methods of diagnosis and staging of arthrosis are better suited for the preoperative planning by the orthopedic surgeon (e.g., for an osteotomy or a joint replacement) than for the diagnosis or monitoring of the early stages of the disease.

Treatment of the early, symptomatic stages of arthrosis centers on the use of physiotherapy, non-steroidal anti-inflammatory drugs, and sometimes intraarticular or intramuscular injections of steroids, hyaluronan, sulfated glycosaminoglycans, or other compounds. Although some of these treatments have evidenced promising effects in animal model arthrosis (Dean et al. 1991), none of them have been shown to influence the progress of the human disease or to delay the destruction of the joint cartilage in human arthrosis. On the other hand, tibial osteotomy when used in early stages of medial compartment arthrosis of the knee has been found to delay joint replacement for many years (Odenbring et al. 1990). Interestingly, postoperative regrowth of cartilage on the damaged joint surfaces has been observed in some patients' series (Odenbring et al. 1991b), although it is unclear whether the good outcome of the procedure is related to cartilage regeneration (Odenbring et al. 1991a, b). However, the fact that some regrowth of cartilage on damaged joint surfaces may take place at all is significant. If we are to use the apparently limited healing capacity of joint cartilage associated with surgical or pharmacologic treatment, it is obviously advantageous to diagnose the disease in its early phase.

Injury to the cruciate ligaments and menisci of the knee carries with it an increased risk of posttraumatic arthrosis (Graham et al. 1988, Sherman et al. 1988, Kannus et al. 1989). Much effort is now being invested in improving the techniques for surgical repair or replacement of injured ligaments and menisci, with the aim of decreasing joint instability and the hope of decreasing the risk of future posttraumatic arthrosis. However, the rationale for these efforts is often limited to the mechanical aspects of the procedure and blatantly ignores the biology of the cartilage and other tissues in the joint. Today, the situation as regards these surgical procedures is the same as that of the pharmacologic treatment of arthrosis: the treatment sometimes decreases the symptoms, but none of the surgical procedures have been found to unequivocally decrease the frequency or rate of development of posttraumatic arthrosis. The situation in

arthrosis therapy is thus similar to that in rheumatoid arthritis therapy, where current routine drug therapy with nonsteroidal anti-inflammatory drugs, steroids, or surgical synovectomy decreases the symptoms associated with the synovitis, but does not, with certainty, change the course of the joint destruction (Ianuzzi et al. 1983, McEwen 1988, Doets et al. 1989).

Although the rate of development of posttraumatic arthrosis is often rapid as compared with primary arthrosis, it still stretches over years, and sometimes decades; thus, the diagnosis is delayed because of its dependence on our current "gold standard," the weight-bearing radiographs (Ahlbäck 1968, Altman et al. 1987). As a consequence, patients in a clinical trial designed to decrease the rate of posttraumatic arthrosis or the rate of progress of early stage primary arthrosis must be followed for many years before the efficacy of the treatment can be evaluated as regards its influence on the progress of the disease. This makes such trials difficult and costly to perform. Thus, the development of improved methods to diagnose and monitor arthrosis is of interest not only to the patient, orthopedic surgeon, and rheumatologist, but also to the pharmaceutical industry. Such new methods need to monitor the present in vivo state of health of the joint cartilage, not merely provide a historical record of past destructive disease.

The precise mechanisms involved in the disease process in arthrosis are not known. Presumably, the pathogenesis is multifactorial, with genetics, joint malalignment, joint overload or trauma, obesity, and aging as some of the known or suspected contributing factors. Even less well known is how these general factors are translated into disease mechanisms on the cell and tissue levels. It should further be observed that the initiation and progression of arthrosis may be controlled by different factors. However, because changes in the properties of joint cartilage and loss of matrix components are an integral part of the disease process, it can be argued that degradation of cartilage matrix is a key event at some time in the development of arthrosis. During this process, matrix molecules or fragments thereof are released into the joint fluid and eventually into other body fluids. These molecules and fragments could be used as markers of cartilage turnover in both arthrosis (Lohmander 1988, 1990a, b) and arthritis (Saxne et al. 1986, 1987).

Mechanisms of cartilage matrix metabolism

The physiologic turnover of adult joint cartilage matrix is slow (Maroudas 1975), with average half-lives of months and years. The turnover of different matrix components is, however, not homogeneous, and several pools of matrix proteoglycans turn over at different rates (Lohmander et al. 1977). Little or nothing is known about the turnover rates of the more recently characterized components of the cartilage matrix, such as the minor collagens, the cartilage matrix proteins, or the small proteoglycans (Heinegård and Oldberg 1989). Experimental evidence suggests, though, that the mechanisms of matrix degradation are similar in both normal and stimulated cartilage turnover (Ratcliffe et al. 1986).

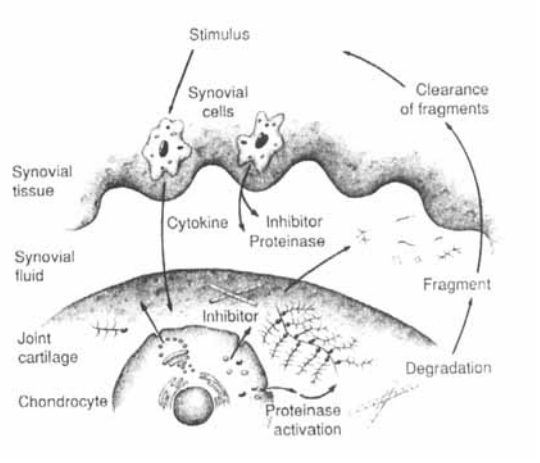

Figure 1. The chondrocyte has an important role in joint cartilage matrix metabolism.

The chondrocytes play a major role in both the physiologic metabolism of cartilage matrix in development and growth, and in the pathologic degradation that occurs in joint disease (Figure 1). Degradative enzymes derived from synovial cells may be important in certain conditions. The role of enzymes derived from leukocytes in matrix degradation in arthritis can be questioned, however, for joint destruction is not inhibited in neutropenic arthritic animals or in arthritic animals genetically deficient in neutrophil elastase or cathepsin G (Pettipher et al. 1988, Schalwijk et al. 1988). The degradative activity of the chondrocytes is greatly stimulated by cytokines, such as interleukin 1 or tumor necrosis factor released from cells of the synovium (Saklatvala et al. 1985), and this has been suggested to be an important disease mechanism in inflammatory joint disease. However, the significance of this signal pathway for the stimulation of cartilage destruction in arthrosis has not been demonstrated.

The primary cleavage of the cartilage matrix molecules is extracellular and is mediated by

proteinases, the majority of which are probably released by the chondrocytes themselves. A cardinal role in cartilage matrix degradation has been proposed for the metalloproteinase family, with stromelysin, collagenase, and gelatinase as its most prominent members (Dean et al. 1989, Docherty and Murphy 1990). These enzymes are closely related, exhibit extensive sequence similarity, and require Zn^{2+} and Ca^{2+}. The metalloproteinases are secreted in a latent proenzyme form, are activated extracellularly, and the active forms are inhibited by strong binding to tissue inhibitors of metalloproteinase (TIMPs) or to alpha-2-macroglobulin. Several levels of regulation of cartilage matrix degradation therefore exist: enzyme gene translation and expression, secretion of enzyme protein, extracellular activation of proenzyme, and inhibition of activated enzyme by TIMPs and other inhibitors. The TIMPs are also synthesized by the chondrocytes. The potential thus exists for a very close and local regulation of proteinase activity in the cartilage matrix, perhaps explaining some of the heterogeneity observed in the turnover of matrix components.

Collagenase primarily cleaves the native triple helix of Types II and X cartilage collagens, whereas gelatinase cleaves denatured collagen, as well as Types IV and V collagen. Stromelysin, on the other hand, has a broader specificity and cleaves not only the core protein of the large cartilage proteoglycan, but also Types II and IX collagen and other components of the cartilage matrix (Wu et al. 1991). The chondrocyte thus produces a range of proteinases that can degrade most or all the structural components of the cartilage matrix.

Release of matrix fragments into body fluid compartments

Fragments of cartilage matrix molecules resulting from extracellular enzyme action are either taken up by the chondrocytes and further degraded by the intracellular lysosomal enzymes or are lost to the joint fluid by diffusion (Figure 2). It is possible that in addition small amounts of matrix components are lost from the tissue by diffusion, independent of enzyme action (Bolis et al. 1989, Sah et al. 1991). The cartilage tissue does not, however, accumulate any appreciable amounts of diffusible matrix molecule fragments. In line with these observations, even vigorous physical exercise does not markedly increase the concentration of proteoglycan fragments in human knee joint fluid (Dahlberg et al. 1991).

Fragments of matrix molecules released into joint fluid may be taken up by and further degraded in the synovial cells, or may be removed by bulk flow with the circulating synovial fluid to the lymph (Fraser et al. 1988). In addition, tissue fragments and collagen fibrils can be phagocytosed by cells in the joint fluid (Moreland et al. 1989). A substantial proportion of the matrix molecule fragments removed by the lymphatic circulation are probably eliminated or, at least, further degraded in the regional lymph nodes (Fraser et al. 1988, Tzaikos et al. 1989). The majority of the remaining fragments that reach the blood stream are within minutes removed from the circulation, most likely by the liver cells (Fraser et al. 1984, Maldonado et al. 1989, Engström-Laurent and Hellström 1990, Smedsröd et al. 1990). The collagen crosslinks, however, survive the circulation and are found enriched in the urine (Eyre et al. 1988, Seibel et al. 1989).

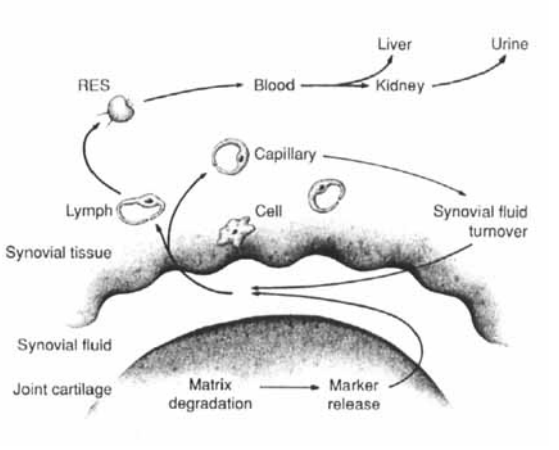

Figure 2. Turnover of joint cartilage matrix fragments released into the joint fluid and other compartments.

It should be observed that only a small proportion (< 10 percent) of the body cartilage mass is located in the joints; the rest is found in ribs, airways, intervertebral disks, and the like (Attencia et al. 1989). In addition, the concentration of a marker, e.g., in joint fluid or serum, cannot be interpreted quantitatively unless the clearance rate for that fragment is also known (Levick 1990). Because the clearance rate of synovial fluid components has been shown to vary in different joint diseases (Wallis et al. 1987), great caution should be exercised when attempting to quantitatively interpret data on cartilage markers in body fluids.

A choice of markers and models

Even if we, at this time, must exercise caution when translating cartilage marker data into terms of quantitative disease, this area of research is developing

rapidly, and the results of current work should help us to better identify disease mechanisms, and in time provide the means to monitor treatment.

Markers

In the broad sense, diverse markers of cartilage turnover in arthrosis can be identified: cytokines, proenzymes, active proteinases, proteinase inhibitors, fragments of matrix molecules produced by enzymes, serum antibodies to cartilage components, matrix molecules synthesized as an adaptive response to matrix degradation, and possibly growth factors. Sensitive and specific assays are now available for several molecular species within each of these groups:

A. Cytokines can be determined in synovial fluid (Saxne et al. 1988a, b, Feldmann et al. 1990).

B. Proteinases and their inhibitors can be assayed, by either enzyme activity or enzyme protein content (Martel-Pelletier 1988, Dean et al. 1989, Henderson et al. 1990, Cooksley et al. 1990, Walakovits et al. 1991).

C. Matrix components and their fragments can be assayed in the form of glycosaminoglycans (Carroll 1989, Silverman et al. 1990), hyaluronan (Engström-Laurent 1985, Fosang et al. 1990), keratan sulfate (Thonar et al. 1985, Sweet et al. 1988), different forms of chondroitin sulfate (Caterson et al. 1990), proteoglycans (Heinegård et al. 1985, Saxne et al. 1986, 1987, Witter et al. 1987, Ratcliffe et al. 1988, Lohmander et al. 1989), matrix proteins (Fife 1988), collagen crosslinks (Eyre et al. 1988, Seibel et al. 1989), and collagen propeptides (Madsen et al. 1990, Shinmei et al. 1991).

D. Serum antibodies to cartilage collagen and chondrocyte membrane proteins have been detected in joint disease (Niebauer et al. 1987, Mollenhauer et al. 1988, Paróczai and Nemeth-Csóka 1988, Choi et al. 1988).

E. The availability of monoclonal antibodies to specific structures on the chondroitin sulfate chain allows the assay of proteoglycan subpopulations synthesized in increased amounts as a response to joint disease (Caterson et al. 1990).

F. Growth factors may play a role in the regeneration of cartilage observed in animal and human arthrosis (Dean et al. 1991, Odenbring et al. 1991b). Our knowledge of the role of these factors in the development and growth of cartilage and bone is rapidly increasing (Wozney et al. 1990).

Compartments

The interpretation of data obtained with any of the above assays will depend on the compartment chosen for sampling: a joint fluid sample will reflect the condition of the cartilage contained within that joint, whereas both serum and urine samples will provide an integrated measure of cartilage turnover in all the joints, and potentially in all the body cartilage. Although it may certainly be argued that serum samples are easier to interpret on the basis of more straightforward clearance calculations (Levick 1990), marker concentrations are much lower in serum than in synovial fluid, complicating assay techniques. Additionally, many joint cartilage markers may be degraded en route to the blood circulation, and they are also diluted by markers from healthy joints and nonarticular cartilage. An exception to this case seems to be the collagen crosslinks, which survive endogenous metabolism and are concentrated in the urine. In fact, the levels of Type II collagen hydroxypyridinium crosslink in joint fluid are below the detection limit for current HPLC-based methods, and are less than 1 percent of the urinary levels.

On the basis of these arguments, it would seem that much speaks in favor of focusing on synovial fluid samples in the initial stages of marker investigations. When promising markers have been identified in joint fluid, a search can then be made in other and more easily accessible body fluid compartments. Above all, efforts should be made to correlate marker levels in several compartments using the same assays and the same sample donors. Moreover, much can be gained by coordinating assays of more than one marker in the same sample set. It is difficult to draw conclusions on the basis of data on different markers assayed in different patients' series when the patients' selection criteria vary and are often ill-defined.

Stratification

All the reports on body fluid markers of cartilage turnover demonstrate a considerable range of values among individual patients within each diagnostic group. Whereas a significant portion of this variability must be due to "biological" variation among individuals, it is also clear that many patients' groups are heterogeneous and with sometimes ill-defined criteria for inclusion or exclusion. Suggested minimum requirements in a study on arthrosis patients should be specified inclusion and exclusion criteria, age, sex, previous joint trauma and/or injury, duration of symptoms or time since joint trauma, previous joint surgery, medication for joint disease, arthroscopic or radiographic data or both, and staging of disease if possible.

Several studies have demonstrated that stratification of the patients within each diagnostic group will

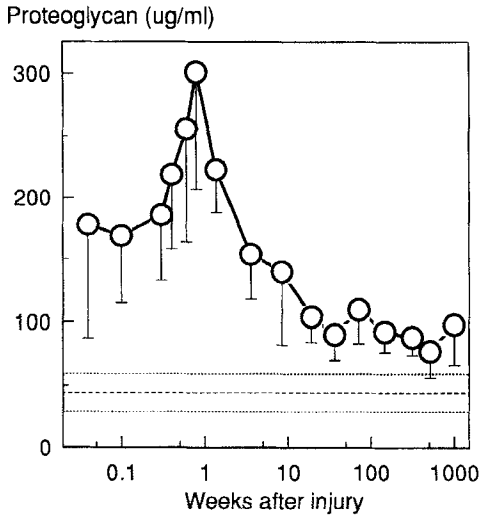

Figure 3. Proteoglycan fragment concentration in joint fluid after knee injury. Joint fluid samples (one sample/patient) were obtained at different times after trauma from 638 patients with injury to the cruciate ligament that was either isolated or combined with an additional meniscus and ligament injury or with only a meniscus injury. Proteoglycan fragments were quantified by immunoassay (Heinegård et al. 1985, Saxne et al. 1986). Bars indicate 95 percent confidence interval for the mean. The dashed and dotted lines indicate mean and 95 percent confidence interval for controls with normal joint cartilage and no trauma. Note logarithmic time scale.

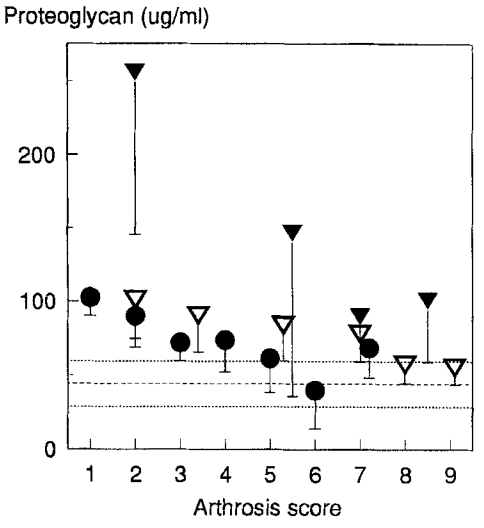

Figure 4. Proteoglycan fragment concentration in knee joint fluid in relation to arthrosis staging. Joint fluid samples were obtained (one sample/patient) from patients with primary arthrosis (▽), arthrosis combined with pyrophosphate arthritis (▼) or posttraumatic arthrosis after injury to the cruciate ligament isolated or combined with meniscus injury or meniscus injury only (●) (504 patients total). In the latter group, all the samples were taken more than 6 months after trauma. Joint fluid proteoglycan fragment concentration was determined as in Figure 3 and correlated with arthrosis staging as obtained by arthroscopy and radiography (Lohmander et al. 1990). A score of 1 is normal joint cartilage by arthroscopy, scores 2–5 indicate increasing arthroscopic cartilage changes but normal weight-bearing radiographs, and scores 6–10 indicate increasing radiographic changes (Ahlbäck 1968). Bars indicate a 95 percent confidence interval for the means. The horizontal dashed and dotted lines indicate mean and 95 percent confidence interval for controls with normal joint cartilage and no trauma.

decrease the scatter of the assay data (Sweet et al. 1988, Ratcliffe et al. 1988, Lohmander et al. 1989, Lohmander et al. 1990, Lohmander and Dahlberg 1990). For example, an assay of proteoglycan fragments in knee joint fluid after injury demonstrates a great decrease in the average marker concentration within the first 6 months after trauma and a plateau after 6 months: a recent joint trauma will greatly influence synovial fluid marker concentration (Figure 3). A stratification of arthrosis patients according to disease stage further demonstrates an inverse relation between arthrosis stage (as estimated by arthroscopy and radiography) and joint fluid proteoglycan fragment concentration: the advanced radiographic stages show lower concentrations than the early cases with only mild joint cartilage damage (Figure 4). Interestingly, the concentration of the proteoglycan fragment keratan sulfate in serum differs among patients with primary and posttraumatic arthrosis, even at the same stage of the disease (Figure 5). This again illustrates the importance of a careful stratification of patients that are included in marker studies.

Models

As remarked in the introduction of this review, human arthrosis has a heterogeneous pathogenesis, and the clinical diagnosis should probably not be regarded as a single disease entity, but perhaps as a final, common pathway of joint cartilage failure. The symptoms and radiographic presentation in this end stage are similar irrespective of the origin of the condition. Important information on the disease mechanisms of arthrosis has been gained by using a variety of animal models mimicking different aspects of the human disease (Lohmander 1990b). For example, naturally occurring arthrosis in monkeys and mice has been used as models of human primary arthrosis (Walton 1979, Chateauvert et al. 1989), whereas surgically induced

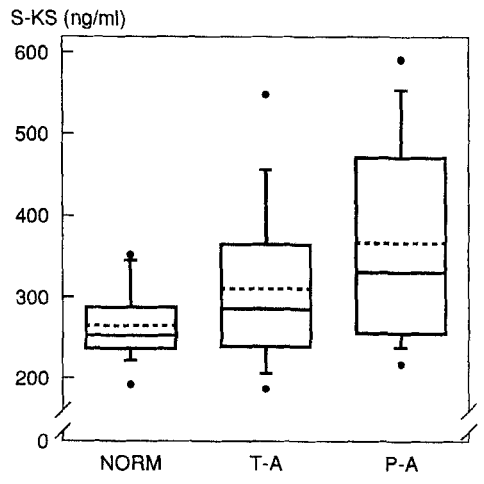

Figure 5. Concentration of keratan sulfate epitope in serum in patients with normal joint cartilage and no trauma (Norm, n 24), posttraumatic (T–A, n 39) or primary knee arthrosis (P–A, n 39). The average arthrosis score was 5 (see Figure 5 for explanation) in both arthrosis groups. Keratan sulfate epitope was assayed as described by Thonar et al (1985). Filled circles represent 5th and 95th percentiles, ends of whiskers 10th and 90th percentiles, ends of boxes 25th and 75th percentiles. The solid line in the box shows the median and the dashed line the average value for each group. The means of the three groups differ significantly.

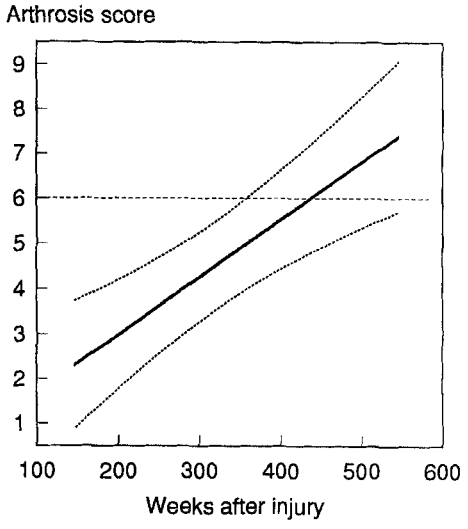

Figure 6. Relation between arthrosis score and time after knee trauma and injury to the cruciate ligament and/or meniscus (372 patients). The arthrosis score was plotted against the time since trauma for each patient. A least squares linear regression was performed, the dashed lines indicate the 95 percent confidence intervals of the mean. The horizontal dotted line indicates the lowest arthrosis score with radiographic changes.

ligament or meniscus lesions or blunt trauma of the knee in the rabbit or dog has been used to model the human posttraumatic arthrosis (Hulth et al. 1970, Pond and Nuki 1973, Donohue et al. 1983, Moskowitz 1984, Carney et al. 1985).

Human arthrosis and cartilage markers

In human studies, we need to select subgroups of patients to decrease scatter and to simplify the interpretation of marker data. The subpopulation of patients with posttraumatic arthrosis of the knee offers an attractive model for the general disease. It has the distinct advantage in that it is common; we can identify the beginning of the disease process by the time of trauma; we can provide an early and precise diagnosis of the injury by arthroscopy; we can follow the disease from its early stages in prospective studies in a high risk group; and, finally, the disease progress is comparatively rapid. It should be noted that these patients will, of course, also carry the same population risk of primary arthrosis, which may influence the course of the disease in the individual patient (Doherty and Dieppe 1983). Another model of considerable interest is the familial arthrosis due to a single base genetic defect in the gene for cartilage collagen Type II (Ala-Kokko et al. 1990). This defect results in the synthesis of abnormal Type II collagen chains (Eyre et al. 1991), and is associated with early-onset arthrosis (Katzenstein et al. 1990). It remains, however, to be shown whether such abnormalities are responsible for any significant proportion of human arthrosis.

In our sample of patients with injury to the cruciate ligament or meniscus and symptoms, the average length of time from injury to the appearance of radiographic changes consistent with arthrosis (Ahlbäck 1968) was about 10 years (Figure 6). Interestingly, the average rate of progression was about the same in patients with a cruciate ligament injury and in those with only a meniscus injury. However, the rate varied widely between patients; some acquired radiographic arthrosis within a year or 2 after the injury, whereas the joint cartilage of others apparently survived for decades (Figure 7). The reasons for this wide difference in susceptibility are unclear, but continued joint abuse and inborn factors may be involved (Doherty and Dieppe 1983, Noyes et al. 1983, Graham et al. 1988, Sherman et al. 1988, Kannus and Järvinen 1989). It is evident, though, that patients with posttraumatic arthrosis reach a radiographic stage of the disease 15–20 years earlier in life than patients

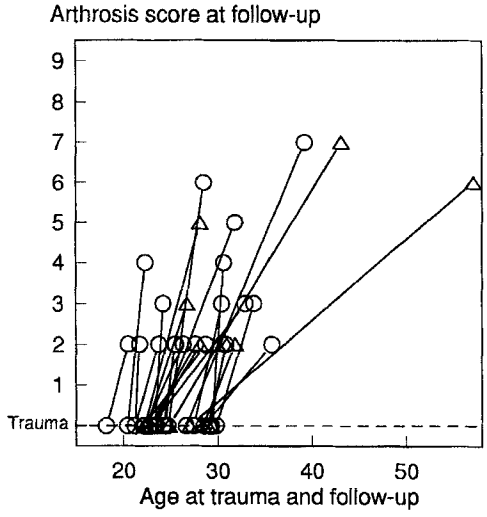

Figure 7. Individual rates of progress of arthrotic changes after knee injury. A total of 79 patients who sustained an injury of the cruciate ligament or the meniscus more than 6 months (average 4 years) before arthroscopy and between the ages of 18 and 30 years (average 23 years) were selected from a pool of some 600 patients with a joint injury. It was assumed that these patients had a normal joint cartilage (arthrosis score = 1) at the time of trauma. The age at trauma is marked (bottom dashed line) with a symbol and is connected with a line to a second symbol representing age and arthrosis score at the time of follow-up arthroscopy and radiography. Each patient is thus represented by two symbols connected by a line. The steeper the rise of the line, the more rapid is the rate of arthrosis progress for that patient. A total of 27 out of 79 patients had an arthrosis score higher than 1 at the follow-up examination. Injury to anterior cruciate ligament isolated or combined with meniscus injury (○), injury to meniscus (△).

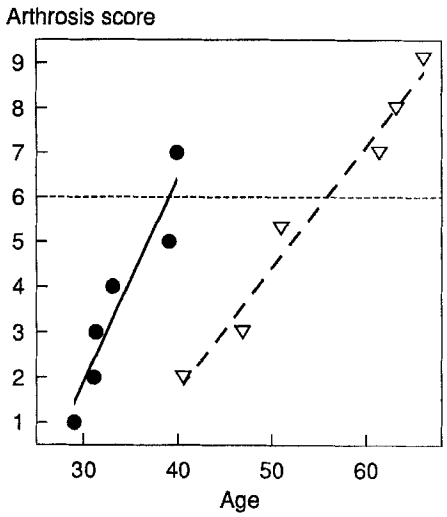

Figure 8. Relation between arthrosis score and age at examination in patients with posttraumatic (●, n 320) or primary knee arthrosis (▽ , n 156). Data shown represent average results for patients with knee symptoms and posttraumatic or primary joint cartilage changes as assessed by arthroscopy and radiography, taken from a total of some 3,000 records in our database.

with primary arthrosis (Figure 8). Patients with a recent joint trauma thus provide an important starting point for prospective studies on arthrosis and markers of cartilage turnover.

Investigations on animal posttraumatic arthrosis have demonstrated early and dramatic changes in joint cartilage composition and metabolism—showing the advantages of being able to monitor the earliest stages of the disease—before secondary cascade phenomena complicate the picture (Hoch et al. 1983, Carney et al. 1985). Indeed, if the concentration of proteoglycan fragments in joint fluid reflects disease activity in arthrosis, then, disease activity is highest before the condition can be diagnosed by routine radiography (Figure 4). Clearly, this should influence the strategies of both surgical and pharmacologic interventions that aim at inhibiting joint cartilage destruction.

Conclusions

Cartilage research in arthrosis is still in the early stage of identifying useful markers, suitable disease models, and patterns of correlation. The results of retrospective case-control studies have generated hypotheses that can be prospectively tested. Such longitudinal, prospective studies will be necessary to answer questions on the relevance of cartilage markers for the prediction of disease outcome.

Meanwhile, ongoing work is already providing us with a better understanding of the disease mechanisms involved in arthrosis on the cell and tissue levels: we can correlate quantitative data on molecular fragments, proteinases, and inhibitors with each other. We are also able to obtain structural information on matrix molecule fragments that are released into the joint fluid, as well as those remaining in the tissue, allowing us to draw conclusions on the identity of the enzymes that are active in the destruction of cartilage—a central issue in arthrosis research. Together, these areas of investigation should offer the means for improved diagnostic and prognostic tools in arthrosis, as well as provide a basis for the monitoring and rational development of treatment aimed at inhibiting cartilage destruction in arthrosis.

Acknowledgements

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