REVIEW

Synovial biopsy in arthritis research: five years of concerted European collaboration

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The term rheumatoid arthritis (RA) was first proposed by Garrod in 1859.[1] By 1959, the histopathological features of synovitis, the proliferating pannus, and cartilage degradation in longstanding RA had been well described.[2] Early histopathological studies were based on tissue samples obtained at surgery or at postmortem examinations. Occasionally, biopsy samples were obtained for analysis from patients with arthritis undergoing open arthrotomy.

Needle biopsy of synovium

The initial interest in developing synovial biopsy techniques was to aid the differential diagnosis of joint diseases. In 1932 Forestier described a technique for obtaining synovial tissue with a dental nerve extractor that was introduced into the joint through a large calibre needle.[3] He never published his results. Early experience with needle biopsy of the synovium was described in the 1950s.[4 5] It was concluded that if strict aseptic techniques were employed, the procedure was safe and practical for use in both hospital wards and outpatient clinics. However, the biopsy needles tended to cause considerable trauma to the penetrated tissues owing to their wide bore and the requirement for an incision. In 1963 Parker and Pearson developed a simplified 14-gauge biopsy needle that did not require a skin incision.[6] They described their experience of 125 procedures, almost all from the suprapatellar pouch of the knee joint, of which only five failed to yield adequate tissue for analysis. No serious complications were encountered. The potential of needle biopsy as a research tool in arthritis was highlighted in 1970 by Kinsella *et al* in their study of synovial lining layer cells in RA,[7] and in 1972 by Schumacher and Kitridou in their clinicopathological study of the early features of synovitis.[8]

Arthroscopic biopsy of the synovium

Arthroscopy was also initially developed as a diagnostic instrument. It was used primarily by orthopaedic surgeons.[9] In the 1970s and 1980s a number of academic rheumatology groups, notably in London, Stockholm, Paris and Ann Arbor, introduced arthroscopy as a research tool. With the development of small-bore (for example, 2.7 mm) arthroscopes, which can be used in a day-care environment under local anaesthesia, regional nerve block, or general anaesthesia, it became possible to select tissue samples from various large and small joints and from most regions within the joint, including the cartilage-pannus junction.[10–12] In addition, methods for quantifying intra-articular disease were validated.[13] These developments were of great interest to rheumatologists as they opened new and exciting opportunities in the field of synovial tissue research. Now, training courses in arthroscopy are regularly organised by EULAR and the ACR. A recently completed international survey of arthroscopy in rheumatology identified 24 academic centres in 10 European countries that regularly use arthroscopic techniques (Kane D, personal communication). Twenty three of these centres have started using arthroscopy since 1990. Arthroscopic biopsy is technically more complicated and more expensive than closed needle biopsy, but it provides larger tissue samples that can be selected under direct vision.

Advances in the analysis of synovial biopsy tissue

Many technological developments in fields that included electron microscopy, cytochemistry, immunohistochemistry, cell culture, and molecular biology have been successfully applied to synovial tissue research. As a result, detailed descriptions of the synovial membrane and pannus architecture have been published.[14–17] In addition, many of the pathophysiological mechanisms associated with chronic synovial inflammation and progressive matrix degradation have been identified.[18–28] It is known that the normal synovium at the cartilage-pannus junction contains mainly inactive fibroblasts and macrophages.[29] In RA and some other forms of arthritis these cell populations increase in number and become highly activated and transformed. They produce many proinflammatory and destructive mediators, which enable them to invade cartilage and bone.[30 31] In addition, these cells and their products may modulate other cell populations participating in tissue degradation.[32–34] At the same time the inflamed synovium demonstrates prominent new blood vessel formation,[35] and the accumulation of antigen-presenting cells, T and B lymphocytes, plasma cells, and neutrophils.[36–38] Another critical element in the inflamed synovium is the dysregulation of normal

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apoptosis.[39 40] More understanding of the relative contributions of these many factors to the pathogenesis and resolution of arthritis will be elucidated by relating them to the clinical course and outcome, and by evaluating their susceptibility to therapeutic modulation.

Recent application of synovial biopsy to arthritis research

Some of the early literature emphasised the heterogeneity of histological change in synovial tissue.[2 41–46] This caused concern about interpretation of the histopathological features in small samples obtained blindly from only one location. It was suggested that quantitative analysis of synovial tissue might be unreliable owing to unavoidable sampling error. In addition, some of the earlier studies produced conflicting results when correlations between the synovial membrane appearance and the clinical manifestations of RA, including joint damage, were evaluated. These issues were examined extensively in a series of studies which, when taken together, showed that despite the degree of histological variation, representative measures of synovial tissue inflammation may be obtained by examining a limited area of tissue.[47–49] Some measures of synovial tissue inflammation have been consistently correlated with variables of local or systemic disease activity, severity, and outcome.[47 50–53] In addition, the microscopic characteristics of rheumatoid synovitis are present even in joints that have not yet become overtly inflamed.[54–57] Finally, in RA the immunohistological features of synovial inflammation change as the clinical manifestations change in response to conventional disease modifying antirheumatic drugs, pulse methylprednisolone, or intra-articular glucocorticoids.[50 58–64] These observations from many clinicopathological research protocols have provided compelling evidence to support the inclusion of synovial biopsy and tissue analysis in studies of the cause, pathogenesis, prognosis, and effects of treatment.[65]

Thus, by the early 1990s, several independent and disparate research streams had converged. At the same time, new approaches to treatment, including monoclonal antibody treatment and cytokine blockade, which targeted specific pathogenetic factors in the synovium, were being evaluated in clinical trials.

European Synovitis Study Group

This convergence stimulated several groups of European investigators to convene at the EULAR meeting in Amsterdam in June 1995. The concerns that were foremost at the time included: (*a*) the need to establish acceptable guidelines for training European rheumatologists in arthroscopic techniques and (*b*) to consider collaboration in resolving issues related to tissue selection and preparation, and the methods used to quantify the immunohistochemical features of synovial inflammation. The process developed informally with biannual meetings, and a useful forum has evolved for discussing research protocols and data that incorporated synovial biopsy and tissue analysis. The group is now represented on the EULAR Investigative Rheumatology Committee. In addition, the original European focus has been widened by regular collaboration with like-minded investigators from North America and Australia.

GUIDELINES FOR TRAINING IN ARTHROSCOPY

The need to establish acceptable guidelines for training rheumatologists in arthroscopic techniques has been a priority for the group. After considerable discussion, this task has been completed and a document submitted to ILAR for approval and distribution (Reece R, personal communication). The document, which will be published, identifies minimum requirements for the accreditation of trainers and training centres, and outlines a basic curriculum for acquiring the necessary skills in arthroscopy. In addition, procedures for the assessment and accreditation of trainees are proposed.

TISSUE SELECTION

An important practical question was whether arthroscopic synovial biopsy samples, selected under direct vision, were better than needle biopsy specimens in clinicopathological studies. To answer this question the immunohistological features of synovial tissue samples selected at arthroscopy were compared with samples obtained at the same time by needle biopsy from the suprapatellar pouch of the same joint.[66] The results showed that measurement of most microscopic features of inflammation were similar whether samples were selected under direct vision or obtained blindly by needle biopsy. Moreover, the macroscopic features of inflammation visualised at arthroscopy did not predict the microscopic features. Thus the practical advantages of the closed needle biopsy technique justify its use in many clinicopathological studies. When tissue from specific sites is required or when sample size is important, as in studies which include in vitro experiments, cell separation or analysis of gene expression and protein production, arthroscopic biopsy is a better tissue source.

QUANTIFICATION OF INFLAMMATION IN SYNOVIAL TISSUE SAMPLES

Quantifying the microscopic features of inflammation can be tedious and time consuming. Semiquantitative methods would have the advantages of speed and cost. When semiquantitative and quantitative methods were compared, a cross sectional analysis showed close correlations between the two.[67] However, in some patients with a clinical response to treatment, the semiquantitative method lacked the sensitivity to recognise some biologically relevant changes which were identified by the quantitative method. Therefore, in studies that seek alterations in the immunohistochemical appearance of synovial membrane—for example, during clinical trials, semiquantitative methods may underestimate the degree of change.

Computerised digital image analysis has been applied to aspects of histopathological quantification. It offers possibilities of greater objectivity, reliability, and rapidity than other methods. In an initial study, digital image analysis was successfully applied to the measurement of some features of synovial tissue inflammation, including lining layer thickness and T cell infiltration.[68] These conclusions were independently confirmed and extended in a separate study, which showed strongly positive correlations between measurements of T cell and macrophage infiltration obtained by digital image analysis and two other methods.[69] The results of these two studies support the further development and wider application of digital image analysis in quantifying critical pathological events in the synovium, such as adhesion molecule expression, cytokine and protease production, angiogenesis, and apoptosis.

CLINICOPATHOLOGICAL STUDIES

Pathophysiological studies have been a predominant interest, both within individual groups and in collaborative efforts. Thus studies incorporating synovial biopsy which have emanated from the participating groups and their collaborators have analysed several pathophysiological mechanisms. There has been particular emphasis on studying the pathophysiological events in the synovium of patients with early arthritis, when cell adhesion molecules are upregulated[70] and mononuclear cells, including T and B lymphocytes and macrophages, are diffusely present.[53 56] Proinflammatory cytokines, tissue degrading enzymes, and other mediators of synovial inflammation and matrix degradation are also expressed in abundance in very early arthritis.[71–75] Studies have also examined mechanisms of cell interaction, activation, and apoptosis.[76–78] The observations highlight the need to consider very early introduction of effective treatment that will reduce the tissue damaging effects of persistent synovial pathophysiological activity in several categories of chronic arthritis, particularly in RA.[79]

EVALUATION OF TREATMENT

Another major interest of the group has been the evaluation of pathophysiological changes in synovial tissue obtained from patients receiving new targeted treatment. In some multicentre studies, biopsy samples taken before and after treatment were pooled or exchanged to maximise the numbers studied or to validate results between centres. These studies have facilitated the evaluation of modes of action and the efficacy of several putative therapeutic advances, including treatment with monoclonal anti-T cell antibodies,[80 81] inhibition of the proinflammatory cytokines, tumour necrosis factor α[82 83] and interleukin 1β,[84] and the anti-inflammatory cytokine, interferon β.[85] Initial studies suggest that targeted treatments produce profound effects on cellular infiltration on the synovium,[80–84] associated with inhibition of adhesion molecule expression[82 84] and reduced production of cytokines and chemokines.[83] Treatment with interferon β was also associated with reduced cellular infiltration and collagenase production.[85]

WORK IN PROGRESS

Collaborative protocols that are currently approaching completion are part of the standardisation process for quantifying the features of synovial inflammation, both macroscopically at arthroscopy and microscopically by immunohistochemistry. These protocols involve several centres exchanging video images of inflamed synovium acquired at arthroscopy, and tissue sections for microscopic analysis by conventional methods and, in some centres, by digitalised image analysis. These studies are critical to ensure that optimal technological standards are maintained and to minimise interobserver variation between centres.

Conclusion

Synovial biopsy is now widely practised in arthritis research. Multiple tissue samples can be readily obtained using closed needle biopsy, usually from the suprapatellar pouch. This source may be suitable for many clinicopathological studies. Needle arthroscopy is considerably more expensive, but provides larger samples which can be selected under direct vision. The European Synovitis Study Group was convened five years ago as a forum for discussing, planning, and evaluating studies that may involve arthroscopy, synovial biopsy, or tissue analysis. The group now reports officially to EULAR. The current priorities of the group include studying the pre-erosive phase of destructive arthritis, and evaluating the effects of new treatments on the pathogenetic pathways associated with inflammation and matrix degradation.

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