Current and Future Diagnostic Means to Better Characterize Osteoarthritis in the Horse—Routine Synovial Fluid Analysis and Synovial Fluid and Serum Markers

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1. Conventional Synovial Fluid Analysis

Examination of the synovial fluid should be a routine procedure in the evaluation of arthritic conditions, as it can provide valuable information in addition to that gained by clinical and radiologic examination.1,2 Conventional analysis will not provide a specific diagnosis; however, it does give an indication of the degree of synovitis and metabolic derangement within the joint. It is more specific in the diagnosis of infective arthritis as this condition causes the parameters of protein and white cell counts to go beyond a level encountered with other inflammatory conditions.

Some basic information on synovial fluid is necessary to appreciate the changes that occur in joint disease. Synovial fluid is a unique tissue fluid. The distribution of electrolytes and most nonelectrolytes between plasma and joint fluid is in accord with the Gibb-Donnan equilibrium concept and indicates that it is mainly a dialysate of plasma with hyaluronan added to it.3 The intercellular space between the synoviocytes in the synovial membrane acts as an important permeability barrier in this filtration process. The source of hyaluronan is the synovial membrane. Studies of the molecular structure of hyaluronan indicate that it is arranged in a random coil with a moderate degree of stiffness. Hyaluronan provides synovial fluid with a number of unique properties. It imparts a high viscosity to the fluid. It acts as a boundary lubricant for the synovial membrane. There is evidence that the hyaluronan in the synovial fluid also influences the further composition of the fluid.4 Data suggest that steric hindrance by hyaluronan may obstruct solute passage through the water surrounding the molecules. In this concept of excluded volume, the size and shape of the molecules presented play an important role. Small molecules are allowed through, whereas large ones, such as fibrinogen, are excluded. With this concept, the quantity and physical state of hyaluronan produced under pathologic conditions may well be the primary determinant of
the nature of the remainder of the contents of the synovial fluid. It has also been suggested that the hyaluronate in the perisynovial connective tissue may be of significance in the exclusion of certain plasma proteins from the synovial fluid. However, the exact mechanism by which permeability changes in disease is not yet well defined.

Hyaluronan is depolymerized in untreated inflammatory arthritis, and this has been considered to be the basis of reduction in viscosity. However, the situation may be more complex than this. There are data to show that hyaluronan has a heterogeneous structure and may have three levels of organization. The viscosity of hyaluronan is apparently dependent on: 1) the length of the polysaccharide chain; 2) the conformation of the chain; and 3) interaction between adjacent chains and other molecules. Therefore, the decrease in viscosity may be due to a change in the overall relationship of hyaluronan and other molecules in addition to simple depolymerization.

Normal values for various synovial fluid parameters in the horse and their changes in joint disease have been documented. These values are quite variable and each laboratory should have its own set of normal values. Most of the synovial fluid parameters provide an indication of the relative amount of synovitis and so follow a spectrum of inflammatory activity within the joint. With the exception of infective arthritis, synovial fluid analysis does not usually furnish a specific diagnosis.

Synovial fluid samples are collected using sterile needles and syringes. All sites for arthrocentesis are prepared aseptically. A recent study confirmed that clipping of hair is not necessary as part of the aseptic protocol. In a study that evaluated skin bacterial flora before and after aseptic preparation of clipped and nonclipped arthrocentesis sites (midcarpal joint and distal interphalangeal joint) in horses, the presence of hair did not appear to inhibit the ability of antiseptics to effectively reduce bacterial flora to an acceptable level for arthrocentesis. Sites for arthrocentesis for various joints have been detailed. Following aspiration of the fluid into a syringe, the fluid is transferred to both plain and EDTA Vacutainer tubes. It should be noted that excess negative pressure on the syringe when attempting to obtain a fluid sample may cause iatrogenic hemorrhage.

The analysis and interpretation of commonly used parameters are discussed below. Each parameter can provide an assessment of the degree of inflammatory process. Defining sharp boundaries for the synovial fluid values in each disease entity is to be discouraged. Cases of osteochondritis dissecans (OCD) and idiopathic synovitis, for instance, produce relatively consistent values; however, the changes in traumatic and infective arthritis may have a wide range.

Appearance
Appearance is evaluated by visual inspection at the time of collection. Normal synovial fluid is pale yellow, clear, and free of flocculent debris. Streaks of blood in the aspirate are indicative of hemorrhage occurring with needle puncture. Uniformly diffuse hemorrhage represents an acute traumatic situation, whereas dark yellow or pale amber (xanthochromic) samples represent previous hemorrhage and are most often associated with chronic traumatic arthritis. The presence of opacity and flocculent material in the sample indicates synovitis. This change is variable and, generally, minimal in chronic degenerative joint disease and osteochondritis dissecans but will be more marked in acute synovitis (traumatic or infectious). The intense synovitis associated with infective arthritis results in a serofibrinous to fibrinopurulent sample. The synovial fluid from infected joints is often bloody due to hemorrhage from the severely pathologic synovial membrane.

Volume
The volume of synovial fluid is increased in most cases of active synovitis. A decrease in synovial fluid is present in some cases of chronic degenerative joint disease and may manifest as a “dry joint.” The presence of a truly dry joint can be correlated with fibrotic synovial membrane. It should be emphasized, however, that failure to gain synovial fluid on sampling does not automatically mean that a pathologically dry joint exists. Synovial fluid volume is increased in situations of idiopathic synovial effusion such as bog spavin. Volume increases in cases of osteochondritis dissecans are variable but a marked synovial effusion is characteristic of the disease in tarsocrural (tibiotarsal) joints. Synovial fluid volume is usually increased in cases of infective arthritis, but this depends on the stage of the disease and the amount of fibrin present in the joint.

Clot Formation
Normal synovial fluid does not clot. This property is attributed to a lack of fibrinogen as well as other clotting factors (including prothrombin, factor V, factor VII, and tissue thromboplastin). Pathologic synovial fluid clots, and the size of the clot is roughly proportional to the degree of synovitis. This property may be ascertained by observing the clot tube. Collection of fluid into a clot tube should only be performed after sufficient amounts have been collected in the EDTA tube as the ability to clot is a very nonspecific parameter.

Protein
Protein concentration is usually measured using a refractometer. The differential protein fractions in synovial fluid may be evaluated using paper electrophoresis following treatment of the sample with hyaluronidase and this has been performed on normal equine synovial fluid. For
viscosity of the synovial fluid to the hyaluronan content and it is a measure of the polymerization degree or quality of the polymeric chain. Viscosity measurements may be made by measuring the relative viscosity (RV) at a specific temperature, using a viscosimeter in which the viscosity of the synovial fluid sample is compared to that of distilled water. Although the measurement of relative viscosity has been used to monitor the progression of experimental synovitis in ponies, measurement of RV by an individual clinician using the viscosimeter is tedious and is not routinely used in evaluation of clinical cases. Because the viscosity of synovial fluid varies with shear rate, some authors have advocated the measurement of intrinsic viscosity as a more meaningful determination. We do not measure relative or intrinsic viscosity routinely.

For practical use in the field, a simple estimate of viscosity can be made by watching the fluid drop from the end of the syringe. With normal fluid, the drops usually string out as much as 5 to 7 cm before separating. If the fluid drops from the syringe with the ease of water, viscosity is low. Another test is to place a drop of synovial fluid on the thumb and then touch it with the index finger. Separating the fingers then produces a string 2.5 to 5 cm long before breaking if viscosity is normal. Decreased stringing occurs with decreased viscosity and fluid from an infected joint will not string. These methods are, of course, subjective and are only useful to detect gross changes. However, because the correlation of viscosity and inflammation is not absolute, these techniques are sufficiently approximate, and precise quantitation is inappropriate. A method of measuring relative viscosity using a white blood cell diluting pipette has been developed. The technique is reproducible with 2% accuracy and requires little time. It could be of potential value in monitoring clinical cases of arthritis and their response to therapy.

It is important that a clinician does not place too much significance on the viscosity findings. This parameter does not give a complete picture of the rheologic behavior of synovial fluid and should not be considered a direct quantitative or qualitative estimate of the hyaluronan content. We have also noted that some fluid samples from joints with only mild changes had marked decreases in viscosity. A poor viscosity indicates that inflammation is present, but the clinician should not interpret too far beyond this.

Mucinous Precipitate Quality
Mucinous precipitate quality (MPQ) is evaluated by adding 0.5 ml of synovial fluid to 2 ml of 2% acetic acid and mixing it rapidly with a glass rod. The precipitate formed (mucin clot) appears to be a salt of anionic hyaluronate and protein made cationic by acidification. When the MCP or mucin clot is normal, a tightropy mass forms in a clear solution and this conventionally is called a “good” mucin. A softer mass with some shreds in solution constitutes a “fair” mucin, while a “poor” result shows shreds and small soft masses in a turbid solution. Fluids that produce only a few clump flecks of mucin suspended in a cloudy solution are classified as “very poor.” In general, the more inflamed the joint the worse the mucin clot. Typically, a good to fair mucin clot is associated with traumatic and degenerative arthritic conditions, whereas infected joints have very poor mucin tests (due to bacterial enzymes degrading mucin). However, the correlation is a loose one as poor mucin clots have been observed in the presence of only mild inflammation.

Cytologic Examination
Cells are best preserved when collected in EDTA vials. Total white blood cell (WBC) counts may be performed on synovial fluid using hemocytometers. It is essential to use a physiologic saline diluent and not the usual white cell diluent containing acetic acid, for the latter precipitates the hyaluronate-protein complex. Red blood cells may be preferentially lysed by hypotonic saline. Smears for differential cell counts are made in the standard way for periph-
eral blood smears with minor modifications. If the white cell count is elevated, the smear is made directly from the synovial fluid. Otherwise, the sample should be centrifuged and the sediment resuspended in 0.5 ml of supernatant after which a smear is made. The smears are air dried and stained with Wright’s stain or new methylene blue.

Erythrocytes are not considered normal constituents of synovial fluid. Their presence in small numbers is usually attributed to contamination of the sample at the time of arthrocentesis. The erythrocyte count may vary greatly and is dependent on the amount of contamination during arthrocentesis. Hyperemia in an inflamed synovial membrane will increase the tendency for bleeding. Because of this marked variation the red cell count does not usually offer useful information.

The white cell count of normal equine synovial fluid has been reported by different workers as 167 ± 21 and 87 cells/mm³ respectively. Neutrophils, lymphocytes, and large mononuclear cells are observed, but the percentage of neutrophils is generally less than 10%. Quantitative and qualitative changes in the leukocytes can provide an indication of the magnitude of synovial membrane inflammation. Because of the wide range observed in some diseases, one should be cautious about grouping types of effusion and matching them to disease. However, some generalizations are appropriate.

Idiopathic synovitis (bog spavin) and osteochondritis dissecans will generally have white cell counts less than 1000 cells/mm³. Although these situations have been classified as noninflammatory effusions, histologic examination of the synovial membrane from cases of OCD has revealed inflammatory changes.

In traumatic arthritis and osteoarthritis, the cell count may vary tremendously depending upon the amount of active synovitis present. The cell counts for human cases of degenerative joint disease are typically low. Synovitis seems to be a more prominent feature of equine degenerative joint disease. Consequently, counts of 5,000–10,000 cells/mm³ may be encountered. In severe inflammatory effusions the proportion of neutrophils is usually increased.

Cases of infective arthritis have the highest white cell counts. In general, cell counts over 50,000/mm³ are indicative of infection and counts over 100,000 are virtually pathognomonic. Published figures for cases of infective arthritis are 105,775 ± 25,525 (59,250–178,000). Neutrophils are the predominant cells. One may observe toxic change in the neutrophils, but commonly they are healthy in appearance. Bacteria are not commonly seen on smear examination.

Synovial fluid samples that have cytologic changes typical for infective arthritis will commonly culture negatively. Factors involved in this are felt to be the presence of antibiotics, sequestration of the bacteria in the synovial membrane, and the normal bactericidal ability of synovial fluid. Treatment of an infected joint is an emergency and the use of cytologic examination of synovial fluid is useful for a rapid diagnosis. In instances in which only a drop or two of synovial fluid are available, a simple smear examination will often provide useful information. In some cases there may be an indication for more than a simple bacteriologic examination. Both Chlamydia and Mycoplasma have also been associated with polyarthritis conditions in foals.

Gas-liquid chromatography has been useful in providing a specific etiologic diagnosis in septic arthritis. Preliminary work in the horse has identified specific fatty acid peaks identified for certain bacteria but this technique has not achieved routine use.

It should be noted that a “gray” zone exists between traumatic arthritis with a high white cell count and infective arthritis with a low white cell count. White cell counts of up to 50,000 have been recorded in cases of traumatic arthritis in humans, but these cases have been differentiated from infectious arthritis by the presence of fat droplets in the synovial fluid. It was theorized that the synovial fluid leukocytosis was secondary to lipid droplet phagocytosis. Lipid globules, present intra- and extracellularly in the synovial fluid and in the upper fatty layer following centrifugation of hemorrhagic synovial fluid, are indicative of a traumatic arthritis. Although infected joints typically have a high white cell count, there seem to be some situations that are atypical. We have encountered a few cases of seemingly “latent” septic arthritis. Initially the fluid evaluation was more typical of traumatic arthritis but septic disease became evident a short time later. Fortunately, these cases are rare. At the same time it should be noted that it is quite possible for nonseptic synovitis to develop into septic synovitis. In polyarthritis in foals, the count may sometimes be considerably less than 50,000/mm³.

**Enzymes**

In general, there is a close correlation between the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AAAT), and lactic dehydrogenase (LDH) in synovial fluids and the clinical severity of joint disease. The proportionate increase of enzyme activity with the degree of synovitis has been demonstrated experimentally in the equine midcarpal (intercarpal) joint. However, specificity of enzyme levels enabling separation of one diagnostic entity from another has not been demonstrated.

It has been suggested that the increased enzyme activity in the joint fluid may result from one of several mechanisms. These include: 1) the release of enzymes from leukocytes; 2) the release of enzymes from necrotic or inflamed synovial tissue; and 3) production and release of increased amounts of enzymes by altered synovial tissue. A positive correlation observed between the number of leuko-
cytes in the field and the enzyme levels is indirect evidence for the first possibility.

Rejno reported that LDH isoenzyme levels in equine synovial fluid were useful for differentiating whether articular cartilage damage was present or not.24 He reported that LDH4 and LDH5 were present in high amounts in articular cartilage, and an increase in these isoenzymes was the most characteristic feature in synovial joint fluid samples from joints with cartilage damage. However, in a more recent study at Colorado State University, we have found the relationship to be less clear. High levels of each isoenzyme were produced with synovial membrane inflammation. Cartilage has a much lower level of all isoenzymes of LDH and consequently lesions of the articular cartilage do not make a significant contribution to the overall LDH elevation.25

Particle Analysis
The foregoing parameters generally provide an indication of the degree of synovial inflammation in a joint but do not provide an assessment of the degree of cartilage damage. Attempts have been made to assess this by using LDH isoenzymes and also microscopic examination of metachromatically stained sediment after centrifugation.9 However, the sediment technique has not received common usage.

2. Synovial Fluid and Serum Markers
As mentioned in the previous section, conventional synovial fluid analysis will not define the degree of articular cartilage damage but merely the degree of synovitis. Previous attempts at techniques such as synovial sediment analysis have not solved the problem. Over the past decade, researchers have developed biochemical and immunologic markers to identify and quantitate breakdown products of the articular cartilage. The principle of markers is that because cartilage degradation involves disruption of the collagen framework as well as loss of proteoglycan, breakdown products of type II collagen and proteoglycan fragments are liberated in increased concentrations into the synovial fluid and ultimately the serum.26 Recently, some of these markers have been looked at with naturally occurring joint disease in the horse.27 Further details on the principles and use of synovial markers has been extensively described in a textbook chapter.26

Biochemical Markers
Biochemical markers for identification of proteoglycan (PG) fragments and glycosaminoglycans (GAGs) and synovial fluid include the dimethyl methylene blue (DMMB) assay using conjugation of 1,9-dimethyl methylene blue to GAGs and comparing the spectrophotometric absorption with that of a chondroitin sulfate (CS) standard. The DMMB assay identifies all GAGs present in synovial fluid regardless of origin. Significantly higher levels were found in horses with OCD and traumatic arthritis. However, this test is not very specific.

Immunologic Markers
Immunologic markers appear to provide the most sensitive means to identify and quantitate types and amounts of articular cartilage components. Polyclonal and, more recently, monoclonal antibodies have been produced against various epitopes on fragments of aggrecan and other molecules that are released from the cartilage. An epitope is an area on the surface of an antigenic molecule against which an immune response is directed. In serum and synovial fluid markers, the epitopes that have been used more frequently are present on fragments of PGs liberated from both normal and degenerating articular cartilage. Epitopes have been identified in a number of areas of the PG monomer including the CS and keratan sulfate (KS) side chains, the hyaluronic acid binding region (G1), the CS attachment region, the CS rich region and the G3 globular domain, and link protein. Once an antibody to a specific epitope has been produced, the amount of epitope can be measured using a radioimmunoassay or an ELISA. Antibodies have been produced specific for “native” CS GAG chains as well as those that require predigestion or “neoepitopes.” In our laboratory, we evaluated CS epitope (846) as well as a KS epitope. The mean value of the CS-846 epitope in joints with osteochondral fragmentation was significantly elevated compared to control joints. KS fragments in the synovial fluid did not show a significant difference between the two groups.27

Antibodies to the C-propeptide of type II collagen (CPII) have been developed and in work in our laboratory in the horse, it was found that there was a significant increase in CPII levels in joints with fragmentation compared to those of controls.27 More recently a specific assay has been used to recognize an epitope in type II collagen fragments by Billinghurst and we are currently evaluating that in our orthopaedic research laboratory.28,29 Fig. 1 illustrates the principle behind detecting a marker of collagen degradation.

Markers of Cartilage Degradation and Synthesis
These will be extremely valuable in determining the stage of articular disease, predicting disease progression, monitoring therapy, or assessing the efficacy of therapeutic agents. Research continues in pursuit of new epitopes and the development of more specific antibodies to identify articular cartilage-specific products for the study of joint metabolism and disease. Metalloprotease (MMP), tissue inhibitors of metalloproteinases (TIMP), and cytokine levels are considered as indirect markers of joint disease. Although these are useful in a research situation, it is considered that they are of minimal value in assessing the amount of articular cartilage damage or defining the status of the joint.30
Markers of Bone Degradation and Synthesis

It is hoped that markers of bone degradation and synthesis will be valuable in determining the stage of subchondral and diaphyseal bone disease in predicting fracture as well as of course monitoring the role of the bone in joint disease. Until now, studies involving bone markers have looked at changes with age,31 exercise,32 and OCD.7,33 Individual predictability of bone markers for disease is currently lagging behind articular cartilage markers as a monitor of joint disease. The usefulness of these individual markers will now be discussed.

Biomarkers of Anabolic Processes

The carboxypropeptide of type II collagen (CPII) is a useful measure of the anabolic process of type II collagen synthesis. This marker was examined in the synovial fluid and serum of horses with osteochondral fragmentation of the carpus. Although CPII concentrations were not significantly higher in joints with osteochondral fragmentation, their levels were significantly higher in the serum of horses with osteochondral fragmentation. It appears that increased synthesis of type II procollagen is a systemic reaction to osteochondral fragmentation.27

Chondroitin sulfate (CS), a major GAG of aggregan, is proving to be a useful biomarker for aggregan synthesis. An epitope called CS-846, that is normally found in fetal and osteoarthritic (OA) cartilages and is almost absent in healthy adult articular cartilage, has been measured in a number of species. Levels are increased in synovial fluid in humans following an injury and in cases of primary OA compared to the fluid of normal joints. Serum levels are also elevated in joint disease but to a lesser extent than synovial fluid levels.34,35 Other CS epitopes such as 3-B-3 and 7-D-4 have also shown to be useful in cartilage injury in both animal models and human clinical cases. A negative concentration of 3-B-3 detected in synovial fluid decreased significantly with arthroscopic evidence of cartilage damage in human knees.36 Increased levels of 7-D-4 were identified in synovial fluid from injured knees of human patients compared to the contralateral normal knee.37 In a study in horses with osteochondral damage of the knee, the synovial fluid epitope 846 (CS-846) was significantly higher in joints with osteochondral fragmentation than in infected joints and serum levels were also significantly higher.27 Discriminate analysis using a combination of serum CS-846 and CPII concentrations allowed for 79% of horses to be correctly classified as having osteochondral change.

Biomarkers of Catabolic Processes

Measuring the degradation of type II collagen with markers is proving to be of benefit in monitoring OA as well as OCD in the horse. Antibodies have been developed to identify type II collagen fragments that have been cleaved and/or denatured, exposing previously inaccessible regions (neoepitopes) of the molecule (Fig. 1). Using these types of antibodies, significant elevations in levels of degraded type II collagen have been demonstrated in synovial fluid and serum samples from horses, dogs, and rabbits with experimental OA.28,38 One of the authors (RCB) developed the COL2-3/4C short immunoassay for detecting collagenase-cleaved collagen fragments. We have used this assay in our laboratory for monitoring collagenase induced collagen degradation in vitro to measure the inhibitory effects of a synthetic MMP inhibitor on IL-1 induced degradation of equine articular cartilage explants.39 More recently we have developed a collagen degradation immunoassay that is specific for type II collagen degradation and is equine-specific.29 This antibody used in this assay is designated as 234CEQ. In a recent study of skeletal markers in developmental orthopedic disease of the horse, we have been able to show a combination of significantly higher levels of CPII, higher levels of COL2-3/4C short (which is also a biomarker for type I as well as type II degradation) and lower levels of 234CEQ in foals with high osteo-

Fig. 1. Location of 234CEQ neoepitope of equine type II collagen.

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chondrosis (OC) scores. This study suggests that there is increased collagen turnover in OC and by measuring the serum levels of specific biomarkers of collagen metabolism, we can identify foals with OC. An earlier study in cases of osteoarthritis found that there were significantly higher levels of CPII and lower amounts of CS-846 and KS epitopes in affected compared to normal joints.

Keratan sulfate (KS) is one of the GAG molecules found in proteoglycan molecules of aggrecan that have been evaluated repeatedly as an indicator of cartilage turnover. Based on work evaluating synovial fluid and serum KS concentrations in normal horses and horses with osteochondral fragmentation we conclude that this marker has very little actual or potential value in the horse. In dogs, a specific KS epitope (5-D-4) has also been of limited value in experimental and clinical cases of cruciate ligament damage.

Cartilage oligomeric protein (COMP) is an abundant noncollagenous protein constituent of articular cartilage. It was once thought to be cartilage specific but recently has also been localized in tendons, menisci and synovium. It has shown promise as a prognosticator of rapid joint destruction in humans with levels in both serum and synovial fluid being increased in patients with OA compared to patients without joint disease. Initial work assessing a COMP assay in serum and synovial fluids from both normal horses with various forms of joint disease has been promising. In that study, synovial fluid levels of COMP were shown to be lower in diseased joints compared to normal controls and serum COMP levels were also significantly lower. Other equine researchers looking at more specific lesions such as those found in the third carpal bone in the midcarpal joint have observed significantly lower COMP levels in affected compared to normal joints.

Individual Skeletal Biomarkers of Bone Metabolism in Joint Disease

Specific markers of both anabolic and catabolic cascades exist for bone as well as cartilage. Their inter-relationships in both normal and diseased states as well as changes with exercise are not yet definitively known but work is progressing in this area. Most of the biomarkers of bone metabolism center around changes associated with type I collagen, which is the most abundant protein of bone matrix but assays for noncollagenous proteins have also been developed.

Biomarkers of Anabolic Processes

During normal type I collagen synthesis, as with type II collagen, cleavage of carboxy and amino terminal propeptides (PICP and PINP respectively) of the procollagen molecule occurs and these cleaved propeptide fragments can be exploited as markers reflective of bone formation. These two markers have been of limited use in human patients with rheumatoid arthritis (RA) and metabolic bone disease due in part to the extraosseous location of type I collagen in such tissues as tendons, ligaments and skin. PICP has been measured in the horse and levels decrease significantly with age and increase with exercise when compared to nonexercised control horses.

Osteocalcin is a small noncollagenous protein that has been associated with bone assembly and turnover. This marker has been studied in serum and synovial fluid samples from human patients with OA and levels have been shown to correlate to bone scan findings and markers of cartilage metabolism. Altered absolute levels are seen in samples from OA compared to normal individuals.

Based on the finding of higher osteocalcin levels in the serum than synovial fluid, osteocalcin in joint fluids may be derived from the general circulation rather than local production and this is important in evaluating osteocalcin as a marker of local joint disease. Osteocalcin levels have been measured in the horse and appear to vary with age and with the administration of corticosteroids. Although the effect of gender on osteocalcin levels is still unclear, general anesthesia has been shown to affect circulating osteocalcin levels for four days. Based on work in our laboratory, it does not appear that osteocalcin is useful in detecting early subchondral bone disease in the exercising horse.

Bone-specific alkaline phosphatase (BAP) is one of the isoforms of alkaline phosphatase that is expressed at high levels in the cell surface of the bone-forming osteoblasts, playing an important role in bone formation. In a recently published cross-sectional study, a correlation was found between synovial fluid levels of BAP, the 5D4 KS epitope and total GAG, as well as between all three biomarkers in the amount of arthroscopically defined joint damage as determined using a previously described grading system. It was also considered that the significant positive correlation between BAP and arthroscopically assessed cartilage damage supported a putative role for subchondral bone metabolism in equine OA. Onset of race training will also increase BAP concentrations in the serum.

Biomarkers of Catabolic Processes

The release of a fragment of the type I collagen non-helical telopeptide (ICTP), which includes a collagen cross-linking region, has been evaluated as a marker of bone resorption in human arthritis. Current thoughts suggest that it may be a general indicator of bone turnover. Levels of ICTP have been measured in the horse in relation to age, exercise, and breed differences but have not been shown to be of value in determining pathologic processes.

A relative new set of antibodies recognizing type I collagen C-telopeptides (CTX) has proven useful as a marker of specific bone resorption based on clinical data from cases of human joint arthritides. CTX levels in humans with RA were positively correlated with indices of disease activity in joint de-

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struction although it was noted that this marker was influenced by the administration of steroids. Preliminary work in our laboratory has shown this marker to be present in equine serum, although the usefulness of this marker in pathologic processes is still not defined.

Bone sialoprotein (BSP) is found only in bone of mature humans and levels are 7-fold higher at the interface of cartilage and bone compared to other locations in bone. Serum levels are significantly elevated in human patients with clinical OA and those with bone scans consistent with OA.35,36 No published studies have been reported on the detection of equine BSP, although development of such an assay has been undertaken through collaboration between our laboratory and the Department of Medical and Physiological Chemistry at Lund University. It is hoped that an equine-specific BSP antibody will prove useful in the identification of subchondral bone change in equine joint disease.

3. The Future
Publications so far on the use of biomarkers for the diagnosis of monitoring equine joint disease have been previously discussed. Confounding effects that may influence the interpretation of marker levels have yet to be fully characterized. For example, because the liver and kidneys can play an important role in the metabolism and clearance of many biomarkers, the function of these organs must be assessed whenever fluid levels of biomarkers are being measured. Other factors that have been shown to influence biomarker levels are circadian rhythms, intestinal peristalsis, physical activity, age, breed, diet, and sex as well as surgery and general anesthesia. Collection and storage of samples is also critical in assessing biomarker levels.

In October 2000, an industry sponsored panel of equine and human researchers convened in Northampton, England, to assess the state of molecular biomarker research of bone and cartilage metabolism in the horse and a report of this meeting and abstracts are forthcoming in Equine Veterinary Education and the Equine Veterinary Journal. This group met to try to unite the field in practical areas such as sample collection, the best set of biomarkers to assay and optimization of collaborative efforts.

The use of biomarkers for the detection of joint disease in the horse is relatively new and extremely exciting in its potential. However, one must be careful that as with any other emerging field, initial enthusiasm of the technologies not be translated into unrealistic goals. It is unlikely that one marker will be the “magic bullet” but with careful examination of a series of markers in light of specific disease processes, the use of markers for the diagnosis and monitoring of joint disease is not unrealistic.

References


