



Biomarkers in equine bone and joint disorders

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Sammanfattning

Syftet med denna litteraturstudie var att summera forskningen kring användningen av biomarkörer vid ben- och ledsjukdomar hos häst samt att få mera kunskap om hur dessa markörer påverkas av sjukdomarna, träning, ålder, kön och ras. Ben- och ledsjukdomar är en vanlig orsak till minskad prestation hos den atletiska hästen. Osteochondros (OC) är en störning i utvecklingen av ledbrosket och är en vanlig orsak till hältor hos unga hästar vilket leder till minskad atletisk potential. Osteoartrit (OA) karaktäriseras av skador på och förlust av vävnadskomponenter i ledbrosket, tillsammans med minskad ledfunktion. Skadade hästar har en stor ekonomisk påverkan på hästindustrin genom förlust av avelspotential och reducerat marknadsvärde. Att kunna förbygga sjukdomar och reducera smärta är generella mål för djurskyddet inom husdjurskötseln. Därför är det inom detta område välkommet med framsteg i förebyggande, diagnostik, behandling och prognostisering kan biomarkörer vara ett användbart verktyg. Molekyler som är normala produkter och biprodukter från de metaboliska processerna som sker i skelettvävnaderna används ofta som markörer. Biomarkörer är ett bra hjälpmedel för att förutse och påvisa sjukdomar utan att behöva göra ett större ingrepp på hästen. Forskningen har ännu inte kommit så långt att det finns en ”magisk markör” som kan diagnostisera graden av led- eller bensjukdom i en led med 100 % säkerhet. Många biomarkörer har dock potentialen att förutse och urskilja sjukdomar men mera forskning behövs för att öka tillförlitligheten hos dessa markörer.

Abstract

The purpose with this literature study was to summarize the research on biomarkers in equine bone and joint diseases and to get more knowledge about how these markers are affected by the diseases, exercise, age, sex and breed. Bone and joint diseases are a common cause of loss of performance in the athletic horse. Osteochondrosis (OC) is a disease of articular cartilage development and is a common cause of lameness in young horses, leading to decreased athletic potential. Osteoarthritis (OA) is characterized by damage to and loss of articular cartilage matrix components, along with reduced joint function. Disorders in horses have a large economical impact on the horse industry through loss of breeding potential and reduced market value of the affected horses. Disease prevention and pain reduction are general aims of animal welfare within animal husbandry. It would therefore be welcome with any improvement in prevention, diagnosis, treatment and prognostication in these areas and biomarkers may be helpful tools. Molecules that are normal products and byproducts of the metabolic processes that are taking place within the skeletal tissues are often used as markers. Biomarkers are good tools for the prediction and detection of diseases without performing major procedure on the horse. The research is not yet at a stage of having a “magic marker” to diagnose the degree of articular cartilage or bone disease in a single joint with 100% accuracy. Though, many biomarkers have the potential to predict and distinguish the diseases but more research is needed to get higher reliability of these markers.

Introduction

Only part of the skeleton is ossified when the foal is born (van Weeren, 2006). During early fetal development, the primary skeleton is laid down as a cartilaginous structure and so-called ossification centers are formed. Bone can be formed by conversion of cartilage into bone tissue, a process known as endochondral ossification (Sjaastad et al., 2003). The bone formation occurs in osteoid tissue that is produced by the bone forming cells, osteoblasts. Lesions may develop when there is abnormal bone and cartilage formation as a result of defects in the endochondral ossification (Billinghurst et al., 2004).

A disturbance of the endochondral ossification can result in osteochondrosis (OC) (van Weeren, 2006). It occurs when the ossification does not follow a gradual and regular pattern and at places it results in an irregular ossification front with thick cartilage. Osteochondrosis (OC) is in the equine literature the term for lesser defects, when loose fragments not (yet) has been formed. Osteochondritis dissecans (OCD) is the term to use when loose fragments are present. These conditions are possibly affecting the horse's joints and certain joints are particularly prone to develop clinical disease (Wolker, 2007).

Osteoarthritis (OA) is another disorder of the joints, which is characterized by damage to and loss of articular cartilage matrix components, along with reduced joint function (McIlwraith, 2005b). Trauma is suggested to be a common etiological factor in the occurrence of OA (Kidd et al., 2001). This may be a single event trauma, leading to injury in one or more joint structures, or damage due to multiple repetitive traumatic insults, caused by "normal" day to day activities including athletic training and competition.

In competition horses is lameness, or more particularly joint disease, a common cause of loss of performance (Frisbie et al., 2003). Therefore, lameness has also a large economical impact in the horse industry. Bone and joint disorders are also affecting the horse industry through loss of breeding potential and reduced market value of the affected horses (van Weeren, 2006). It would therefore be welcome with any improvement in prevention, diagnosis, treatment or prognostication in this area and biomarkers may be helpful tools (McIlwraith, 2005b).

Historically the term biomarker refers to analyses in biological samples, and any measurement that predicts a disease state or response to a drug can be called a biomarker (Baker, 2005). Discovering biomarkers generally means searching for differences between groups that respond to a treatment and those that do not. Biomarkers can also indicate the presence or likelihood of a particular disease.

The purpose with this literature study is to summarize the research on biomarkers of equine bone and joint diseases and to get more knowledge about how these markers are affected by the diseases, exercise, age, sex and breed.

Bone, cartilage and joints

Bone tissue contains three types of cells, osteoblasts, osteoclasts and osteocytes (Sjaastad et al., 2003). Osteoblasts produce the ground substances of bone tissue, osteoclasts are breaking down the bone substances and osteocytes maintain the bone as a living tissue. Bone consists of an organic part and an inorganic part. Collagen and proteoglycans, synthesized by osteoblasts, forms the organic part of the bone. The inorganic part is primarily made up of calcium phosphate crystals.

The cells producing the components of cartilage matrix are called chondroblasts and then chondrocytes when they are surrounded by the matrix (Junqueira & Carneiro, 2005). The type of cartilage found in the fetal bone model as well as in joints is called hyaline cartilage. The matrix of hyaline cartilage consists of collagen (mainly type II) embedded in proteoglycans (e.g. aggrecan) and glycoproteins.

During endochondral ossification, the cartilaginous tissue is gradually replaced by bone tissue (Sjaastad et al., 2003). The endochondral ossification is initiated from a primary ossification center in the middle of the cartilaginous bone. Later in the ends of the bone, the secondary ossification centers develop. Ossification stops in the distal parts of the bone, before all cartilage has been replaced, and the remaining tissue becomes the joint cartilage.

A synovial joint (figure 1) is a junction between two or more bones and a hyaline cartilage layer covers the articular bone surfaces (Sjaastad et al., 2003). Cartilage is softer and smoother than bone tissue and contains no blood vessels and nerves. Injuries in the cartilage heal slowly, due to the lack of blood vessels. Articular cartilage is insensitive and this may explain why joint lesions could progress far before the patient becomes aware of their existence (Dyce et al., 2002). The function of the articular cartilage is to decrease impact and reduce friction (Björck, 2004). A capsule of dense connective tissue connects the bones of a synovial joint, forming a joint cavity (Sjaastad et al., 2003). The synovial membrane is lining the internal surface of the capsule and secreting a viscous fluid, the synovial fluid.

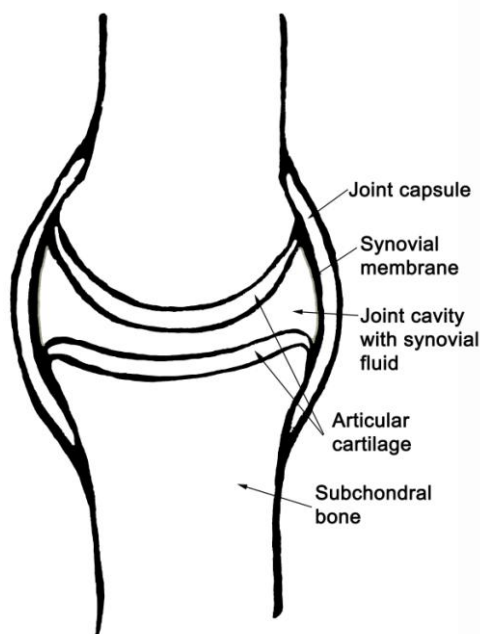


Figure 1. Schematic drawing of a joint.

Biomarkers

Biomarker, biochemical marker and molecular marker are all terms that have been used to describe direct or indirect indicators of abnormal skeletal tissue turnover (McIlwraith, 2005a). Molecules that are normal products and byproducts of the metabolic processes that are taking place within the skeletal tissues are often used as markers (McIlwraith, 2005b). In disease the concentration of these markers may increase or decrease, because changes occur in the balance between the anabolic and catabolic processes within the tissues. In joint disease, when the origin is articular cartilage, menisci, ligament or synovial membrane, these molecules can be released into the synovial fluid. Molecules from osseous tissue will usually be delivered into the bloodstream if the underlying subchondral bone of a joint is involved. The ability to

identify and measure marker molecules in synovial fluid, serum and urine gives the researchers and clinicians a possibility to use them as biomarkers of joint disease.

Biomarkers can be used in different ways (McIlwraith, 2005a):

- To differentiate between affected and non-affected joints/animals, as a diagnostic test.
- To identify joints/animals possible to show rapid development or to predict response to therapy, as a prognostic test.
- To assess the severity, monitor change in disease status or monitor response to therapy, as an evaluative test.

According to the way the biomarkers are detected, they can be subdivided into biochemical and immunological markers (McIlwraith, 2005b). Immunological markers provide the most sensitive way to identify and quantify types and amounts of articular cartilage components. Polyclonal and monoclonal antibodies have been produced against different epitopes on fragments of aggrecan and other matrix molecules that are released from cartilage. Epitopes are areas on the surface of an antigenic molecule against which an immune response is directed. When an antibody to a specific epitope has been produced, the amount of the epitope can be measured using a radio-immunoassay or an ELISA.

Bone and joint diseases

As a consequence of physical disruption of tissues, due to mechanical stress, joint injuries arise (Riggs, 2006). Joints that are being exposed to loads that exceed physiological limits have a higher chance to be damaged, as a consequence of excessive magnitude, excessive number of cycles or abnormal direction of the load. When the mechanical integrity of a tissue has been compromised by concurrent disease, high loads, even within the normal physiological range, may cause injury.

During normal joint metabolism, tissue macromolecules or fragments are released into synovial fluid, and then into blood and urine (Fuller et al., 2001). To detect marker molecules, blood samples from the jugular vein and/or synovial fluid can be collected (Frisbie et al., 2008) and evaluated by biochemical or immunochemical assays (Fuller et al., 2001). The aim of the molecular marker research is to detect changes early and to use minimally invasive sampling, before irreversible disease processes have occurred.

Osteochondrosis and osteochondritis dissecans

Osteochondrosis (OC) is a disease of articular cartilage development (figure 2) and is a common cause of lameness in young horses, leading to decreased athletic potential (Semevolos & Nixon, 2007). OC involves abnormal differentiation and ossification of articular cartilage during development. This results in a weakened cartilage matrix and a following cartilage flap formation within the joint. OC is a multifactorial disease, with nutrition, growth rate, hereditary factors and trauma playing important roles. Young horses with rapid growth rates and factors that lead to rapid growth, including genetic and environmental influences, are predisposed to OC.

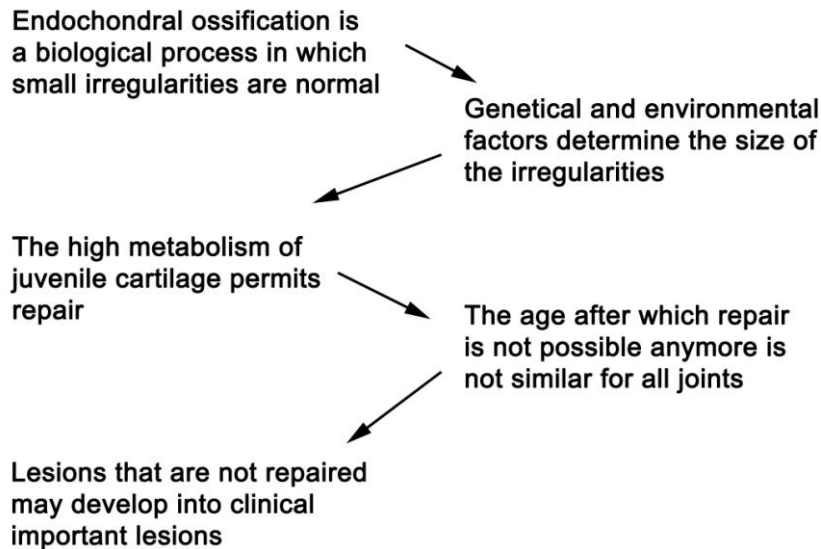


Figure 2. The proposed mechanism of OC (modified from van Weeren, 2006).

An OC lesion with loose fragments is called osteochondritis dissecans (OCD) (Wright & Minshall, 2005). Lesions considered as OC may include osteochondral fragments (chip fractures), chondral fragments, osteochondral flaps, chondral flaps and degenerative subchondral or epiphyseal spongiosa bone. The majority of OCD lesions are found on the non-loaded margins of high motion joints. OC can only develop while there is active endochondral ossification, and most OCD lesions therefore develop before nine months of age, although many lesions will appear and resolve spontaneously before this time. Certain OCD lesions that are apparent at nine months may subsequently continue to heal.

Bone markers have been found usable in diagnosis of OC, as well as in predicting progression and monitoring response to treatment (McIlwraith, 2005a). The markers may also be important in assessing bone remodeling in training and identifying abnormalities in the bone of exercising horses before they progress into potentially severe injuries, such as fractures.

Osteoarthritis

Osteoarthritis (OA) is a disease process of synovial joints and symbolized by cartilage degeneration, subchondral bone sclerosis, osteophyte formation, varying degrees of synovial inflammation and periarticular tissue fibrosis (Kidd et al., 2001). OA in humans is not readily comparable to that of horses, because of differences in the biomechanics of locomotion and the fact that the age of affected horses is often much lower than of humans with similar OA changes. Repetitive high-speed activity is a well recognized risk factor for the development of OA (Riggs, 2006). The pathological effects of the trauma associated with high-impact loads, which may ultimately lead to OA, are variable and occur in all tissues of the joints. Some of these effects are immediate while others are expressed over time.

The ideal biomarker(s) in OA should (McIlwraith, 2005b):

- Detect joint damage at an earlier stage than conventional methods.
- Provide information on disease activity and progressive joint damage.
- Predict future disorder and cause of disease.

Significant differences in the concentration of biomarkers between OA joints and normal joints indicate that there are differences in the metabolism of the joint tissue (Fuller et al., 2001). These markers have clinical potential in the assessment of OA in the horse, particularly where comparable samples are available.

Biomarkers of changes in bone and joint metabolism

All parts of articulating joints participate in load transmission, and failure of the bone, articular cartilage, muscles, ligaments/tendons or nerves of a joint may lead to exercise-induced damage (Billinghurst et al., 2003). The ability to detect these changes at an early stage would potentially enhance the ability to form exercise programs on an individual basis, thereby avoiding irreversible damage to joint structures. One such diagnostic tool is to evaluate body fluid levels of byproducts of skeletal tissue metabolism.

Attempts to identify molecules that can serve as early markers of metabolic abnormalities in specific tissues of the body have been done by many investigators (Billinghurst et al., 2004). Such biomarkers are continually being characterized for bone and cartilage. For osteochondrotic cartilage from horses, alternations in the expression and distribution of components of the extracellular matrix of these tissues and the enzymes that degrade them have been reported. Concentrations of these biomarkers can be monitored through minimally invasive means in body fluids, such as blood, synovial fluid and urine. Biomarkers of bone degradation and synthesis could be valuable in determining the stage of bone disease, predicting fracture, and monitoring the role of the bone in joint disease (McIlwraith, 2005b).

Osteocalcin

Osteocalcin is a small, noncollagenous protein mainly synthesized by osteoblasts and believed to be associated with mineralization of newly formed osteoid (Billinghurst et al., 2003). Osteocalcin is a putative biomarker of bone formation and mineralization (Billinghurst et al., 2004). Concentrations of serum osteocalcin showed significant correlation with severity of OC in foals during the first year after birth. The foals with naturally developing OC had increased serum concentration of osteocalcin. Measuring the concentration of osteocalcin during the foals first few weeks after birth may have potential value for the prediction of risk for OC development (Donabedian et al., 2008).

Matrix metalloproteinase

Matrix metalloproteinases (MMPs) are candidate biomarkers for physiological and pathological tissue remodeling because of their key role in articular cartilage homeostasis (Brama et al., 2004). Disruption of the collagenous architecture is thought to be central in chronic degenerative diseases such as OA, and the collagenases form an interesting subset of the MMPs. Collagenases are enzymes of the MMP family and are capable of cleaving intact fibrillar collagen, e.g. MMP-1 is one MMP with collagenase activity. Extremely high MMP-1 activity levels were observed in synovial fluid of fetal metacarpophalangeal joints. After birth these levels declined gradually and the decline coincided with the cessation of tissue turnover and growth in mature animals. In synovial fluid of OA metacarpophalangeal joints from mature horses, MMP-1 activity was significantly higher than in the synovial fluid of age-matched healthy metacarpophalangeal joints. Exercise had no significant influence on the MMP-1 activity. MMP-1 activity may be a useful tool in diagnostic, therapeutic or prognostic studies on horses with suspected OA.

Bone-specific alkaline phosphatase

Bone-specific alkaline phosphatase (BAP) is an isoform of alkaline phosphatase and plays an important role in bone formation (McIlwraith, 2005b). In synovial fluid of active equine OA joints, the BAP concentrations were increased compared with normal joints (Fuller et al., 2001). The positive correlation between synovial fluid BAP and articular cartilage damage demonstrated a link between changes in bone and articular cartilage in OA. A correlation between cartilage damage and marker levels validates the use of synovial fluid BAP in OA assessment. In serum of horses with OC, BAP concentrations were significantly lower than in serum of normal horses (Trumble et al., 2008). On the other hand, synovial fluid levels of BAP were significantly higher in horses with OC injury than in healthy horses.

Collagenase cleavage neopeptide

A major component of articular cartilage is type II collagen. In OA, degradation of type II collagen is increased (Poole et al., 2002). Collagenase cleavage neopeptide (C2C) is a degradation product of type II collagen and can be found in synovial fluid. In Thoroughbred racehorses with OC injury, increased synovial fluid content of C2C was shown compared to both rested and exercised horses. Analysis of C2C in synovial fluid may be a useful tool for assessment of joint injury (Trumble et al., 2009).

Carboxypropeptide of type II collagen

Carboxypropeptide of type II collagen (CPII) is a marker of type II collagen synthesis (Billinghurst et al., 2004). During their first year of life, foals with OC showed higher CPII serum levels than healthy foals. It appears that CPII is a consistent indicator for foals that have or will develop OC (van de Lest et al., 2004). Young (less than 24 months) horses with OCD lesions showed significantly higher CPII synovial fluid levels than age-matched non-affected horses (Lavery et al., 2000). No significant correlations were observed between OCD-affected mature (24 months or older) horses and non-affected horses. Another study showed increased levels of CPII in both synovial fluid and serum of horses with osteochondral fragmentation and also in the synovial fluid and sera of horses with OC (McIlwraith, 2005a). A direct relationship between the levels of CPII and the severity of disease was also shown.

CS-846

Chondroitin sulphate is a component of cartilage proteoglycans. CS-846 is a chondroitin sulphate epitope that is normally found in fetal and OA cartilage and is almost absent in healthy, mature articular cartilage (McIlwraith, 2005b). CS-846 gradually disappears from cartilage with aging, but reappears in joints with OA (McIlwraith, 2005a). Compared with control horses, horses with osteochondral fragmentation had significantly higher synovial fluid and serum levels of this epitope. In a study of horses with induced OA, the CS-846 levels in synovial fluid increased significantly compared with control horses (Frisbie et al., 2003). CS-846 seemed to be very useful for early differentiation between normal and OA joints. Using a combination of CS-846 and CPII concentration allowed correct classification of osteochondral changes in 79% of cases (McIlwraith, 2005b).

Cartilage oligomeric matrix protein

Cartilage oligomeric matrix protein (COMP) is an abundant noncollagenous protein constituent of articular cartilage (McIlwraith, 2005b). Thoroughbreds with an osteochondral fracture had an increased concentration of synovial fluid COMP, compared to Standardbred

trotters with osteochondral fractures and Thoroughbred and Standardbred trotters with OA (Skiöldebrand et al., 2005). Only intact COMP was present in the synovial fluid from a joint with osteochondral fractures, whereas, fragmented COMP was more prominent in synovial fluid from a joint with OA. Synovial fluid COMP may be a useful marker for carpal joint osteochondral fragments. Increased concentration of synovial fluid COMP is correlated to length of time post injury, suggesting that COMP may also be a suitable marker for longitudinal studies to evaluate its role in joint healing.

Interleukin-6

The development of equine OA is probably influenced by cytokine production in synovial membranes and osteochondral fragments (Ley et al., 2009). In the development of intra-articular lesions, the pro-inflammatory cytokine interleukin-6 (IL-6) may be of great importance (Ley et al., 2007). Horses with chip fractures showed significantly higher IL-6 levels compared with horses without chip fractures. A dramatic increase of IL-6 in synovial fluid indicates the presence of osteochondral fragmentation, even though low or undetectable levels of IL-6 do not exclude chip fractures. The high synovial fluid levels of IL-6 in horses with chip fractures indicate that IL-6 may be of importance in the development of joint lesions seen in OA.

The influence of age, sex and breed

Carboxyterminal telopeptide of type I collagen (ICTP) is a marker of bone resorption (Jackson et al., 2003) and carboxyterminal propeptide of type I collagen (PICP) is a marker of bone formation (Jackson et al., 1996). Plasma concentrations of osteocalcin, PICP and ICTP have been shown to decrease with age (Vervuert et al., 2007). The reductions were more pronounced in late-born foals than in early-born foals, whereas sex had no influence on the change in bone markers. Changes in the marker concentrations were similar for foals with OC and healthy foals.

Serum ICTP (McIlwraith, 2005a) and osteocalcin concentrations may differ between breeds, as a study of healthy Thoroughbred and Quarter horse foals showed that Thoroughbred foals had higher levels of osteocalcin than Quarter horse foals (Reller et al., 2003). No difference was shown between genders.

Effects of exercise

Training may affect the serum concentration of bone markers. A study of 2-year-old Thoroughbreds showed that treadmill-exercised horses had significantly lower serum osteocalcin and ICTP concentration than walk-exercised control horses (Jackson et al., 2003). Because biochemical bone markers are a direct measure of bone cell activity, this finding indicates that cells in bones of growing horses are sensitive to changes in their mechanical environment. The concentrations of serum and synovial fluid levels of CS846, CPII and osteocalcin were significantly higher in OA-affected horses compared to healthy exercised horses (Frisbie et al., 2008).

Lack of exercise in early-born foals leads to retardation of bone maturation, reflected by a more prolonged decrease in bone marker concentrations (Vervuert et al., 2007). The risk for musculoskeletal injury could be increased by delayed bone metabolism. Routine analysis of bone markers that reflect bone turnover appears to be useful for monitoring bone metabolism in growing foals in the first months after birth.

Discussion and conclusion

Disease prevention and pain reduction are general aims of animal welfare within animal husbandry. In addition, losses to the equine industry because of bone and joint disorders are huge (van Weeren, 2006). These do not only include direct losses caused by the costs of treatment and the time lost to rehabilitation; another factor is indirect losses, which may be even more important. Indirect losses consist of a large reduction of breeding potential because of the exclusion of affected stallions from breeding and the reduction in market value of good equine athletes when they show signs of bone and joint disorders. These large economic losses, and the impairment of many horses that have to undergo surgery, turns prevention into an interesting and urging item.

The ability to identify early stages of progressively debilitating diseases before permanent and irreparable damage has developed is a goal of researchers investigating molecular markers of skeletal metabolism (Billinghurst et al., 2004). Early diagnosis of bone disease is the key for the prevention of injuries (McIlwraith, 2005a). It is recognized that early disease in the subchondral bone is contributing to the development of osteochondral fractures. The ability to recognize these changes early could potentially prevent many osteochondral fractures, as well as more severe fractures. The possibility to use biomarkers to predict disorders would at an early stage distinguish the horses with potential to develop and get injuries. These predictions would potentially increase the ability to form exercise programs on individual basis and thereby avoiding damage to bone and joint structures.

Routine sampling and assaying of biomarkers may allow the identification and monitoring of potentially unfavorable effects of exercise on skeletal development in animals (Billinghurst *et al.*, 2003). It is useful to find serum biomarkers in the horse that will differentiate OA from exercise alone (Frisbie et al., 2008). When the horses are in active training this knowledge can be of major value in screening clinical cases for OA. Potential markers to distinguish OA from exercise effects on the skeleton are CS846, CPII and osteocalcin. These markers have showed significantly higher levels in OA-affected horses compared with exercised horses.

Biomarkers may also be used to identify horses that are at risk for the development of OC (van Weeren, 2006). For these horses, environmental conditions may then be manipulated to reduce the risk of OC. Osteocalcin is a possible, very early, marker for an increased risk of OC and this finding is a very promising development. Such early markers may help to identify horses that are at risk and then allow the establishment of customized programs for these horses that aim at the optimal reduction of environmental risk for OC.

The research is not yet at a stage of having a “magic marker” to diagnose the degree of articular cartilage or bone disease in a single joint with 100% accuracy (McIlwraith, 2005b). Though, much progress has been made. The researchers want to find markers that could detect joint damage at an earlier stage than conventional methods, provide information on disease activity and progressive joint damage and predict future risk of disease. A lot of marker molecules have already been used successfully and an increasing number are coming onto the market in the form of ready-to-use kits. For a good evaluation of the condition of the cartilage and other tissues of the joint, a combination of the determination of selected markers with other diagnostic techniques, such as arthroscopy and/or MRI, seems most promising.

In conclusion, biomarkers are good tools for the prediction and detection of diseases without any bigger procedure on the horse. Many biomarkers have the potential to predict and distinguish the diseases but more research is needed to get higher accuracy of these markers.

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