

# DOCTORATE (Ph.D) DISSERTATION THESES

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EXAMINATION OF PATHOLOGICAL CHANGES OF THE  
PODOTROCHLEAR REGION BY USING RADIOLOGICAL AND  
HISTOLOGICAL METHODS IN HORSES WITH NAVICULAR  
SYNDROME

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# 1. INTRODUCTION

The navicular syndrome is one of the most common disease in horses between the ages of 4-15 years, mainly affecting the forefeet, causing intermittent lameness and characterized by pathological changes developing in the navicular bone, its ligaments, the deep digital flexor tendon and the navicular bursa. According to different estimations this syndrome accounts for 1/3 of all cases of chronic forelimb lameness. Currently MRI is one of the most up to date technique used for early diagnosis of the syndrome and investigation of its pathogenesis. This diagnostic imaging method has been used so far for the description of the anatomical structures inside the hoof capsule and the identification of pathological alterations in adult horses. Until now no MRI examination has been carried out concerning the tissue-composition of the navicular region in newborn and young foals.

The aims of the investigations discussed in the dissertation were the following:

1. Determine an imaging method suitable for quick examination of the podotrochlear region that allows detailed bone, cartilage and soft tissue visualization.
2. Comparative examination of the usefulness of this method (determined at point 1.) in adult horses and newborn foals.
3. Comparative examination of the MR morphology of the navicular bone between adult horses and newborn foals by using histological and immune-histochemical procedures.
4. Quantitative morphological examination of the navicular bone in adult animals by using MRI.
5. Examination of the degenerative changes of the navicular region by using MRI.

## 2. MATERIAL AND METHODS

48 hooves of 15 half-bred horses (aged between 0 days and 14 years) were examined in 5 phases. The limbs were frozen at -18°C temperature, defrosted at room temperature for 36 h and then subjected to MRI studies in the Institute of Diagnostic Imaging and Radiation Oncology (University of Kaposvár). MRI was performed using a Siemens Magnetom Vision Plus (1,5 T) scanner (Siemens AG, Erlangen, Germany) and the samples were measured in a CP-Helmholtz circular coil. The histological and immune-histochemical examination was performed in the Institute of Human Morphology at the Medical Faculty of Semmelweis University. OSIRIS 3,6 (Univ. Hospital of Geneva) software and SPSS 10.0 statistical analysis software were applied for the quantitative morphological examinations under Excel (Windows 2000 XP).

### Phase 1.

An MRI method was determined on one limb, that presents the anatomical region in good contrast with spin-echo, gradient-echo and inversion recovery sequences in 3 planes (sagittal, transversal and coronal/dorsal).

### Phase 2.

At this stage of the study, the imaging method described in point 1. was evaluated on 16 limbs derived from horses with different age (2 newborn foals, and 3 adults /3-, 6-, 14-year-old/), with special regard to the navicular region of newborn foals.

### Phase 3.

The exact histological composition of regions showing various signal-intensities was determined on 24 limbs (5 newborn foals, younger than 24 hours old, 4 adults /3-, 4-year-old/) with different histological (haematoxiline-eosin, AZAN, Schmorl) and immune-histochemical (type-II collagen) staining methods.

### Phase 4.

The subchondral circumference of the navicular bone was drawn on 18 limbs (animals aged between 3 and 14 years) imaged with T1-weighted SE sequence in sagittal plane on 6-6 slices lateral and medial to the crista sagittalis. The ratio of regions showing high signal

intensity (spongy substance) and low signal intensity (compact substance) was defined in the given volume took the slice thickness into consideration. The results were evaluated by statistical analysis.

#### Phase 5.

At this phase of the study the degenerative alterations were classified and the prevalence of their occurrence was determined in the entire sample (48 limbs of horses aged between 0 and 14 years).

### **3. RESULTS**

#### Phase 1.

The determined imaging method consisted of the following 4 sequences: T1 spin echo (sagittal plane), T2\*-weighted GE (sagittal plane), T1 inversion recovery (sagittal plane), T2 turbo spin echo (transversal and dorsal plane).

#### Phase 2.

The optimal imaging method that gives contrasted images of anatomical structures of the navicular region in adult horses was used in foals with the following results: The image obtained with the T1-weighted sequence is not as ideally contrasted as in adult horses because the fluid compartment is not as markedly differentiated from the cartilaginous tissue in foals. Using the T2\*-weighted GE sequence none, or only a limited image can be obtained of the fluid compartment of the coffin joint and the podotrochlear bursa, unlike in adult horses. In foals the T1 inversion recovery sequence gives optimal contrasted images of the fluid compartments as well as the actively growing areas containing hyaline cartilage of the navicular bone, the short pastern and the coffin bone. The T2 turbo spin echo sequence improved the contrast between the synovia and the surface of the hyaline cartilage compared to the T1-weighted sequence.

#### Phase 3.

The results of the MR morphological examinations between or within the different layers of the tissues of the navicular bone were determined by histological and immunohistochemical staining methods were the following: the borders of the different layers -

stained with various histological procedures and the contrast of the MR image made from the same slice - in sagittal sections of the navicular bone showed different but typical signal intensities. Using T1-weighted spin echo sequence in foals, the hyaline cartilage can be differentiated from the subchondral region, as well as the areas of the subchondral region containing substantial amounts of bone matrix can be differentiated from the regions still containing substantial amounts of cartilage matrix. The border-line between the zone of degeneration and the zone of proliferation in the hyaline cartilage is visualized with good contrast in foals with the T2\*-weighted GE sequence. Also in foals, the T1 inversion recovery sequence allows differentiation of the superficial, mature cartilage from the zone of proliferation and the zone of degeneration. In adult horses, the exact differences between the tissue layers were proved histological provided optimal MRI contrast of them.

#### Phase 4.

The compact/spongy substance ratio was determined by the morphometrical examination of the navicular bone in adult horses. The ratio is between 1.16 and 1.74. In the cases of navicular bones without degenerative changes it is lower than 1.41 and higher than the mentioned average value in the opposite case, although the difference between the two groups is not significant.

#### Phase 5.

No pathological changes were found in the navicular region of newborn foals and foals not older than 1 week. In the case of adults aged between 3 and 14 years the two most common lesions were: 1. high signal intensity areas in the subchondral bone plate under the facies flexoria indicating bone necrosis, bone fibrosis or edema (33.3%); 2. thinning of the subchondral bone plate under the flexor surface (33.3%). No lesions were found in the examined region of 10 limbs.

## 4. CONCLUSIONS

### Phase 1.

The following factors must be taken into consideration regarding the MRI examination of the navicular region in the horse:

1. duration of the examination
2. fat-suppressed inversion recovery sequence with a short acquisition time should be used for the examination of the cartilage surface
3. fat-suppressed T2-weighted sequence should be used for imaging the changes of fluid content of the tissues during acute pathological alterations.

With the determined MRI method the examination takes 15.17 minutes. The method corresponds to the above mentioned criteria and enables quick examination of limbs on live animals with good contrast quality.

### Phase 2.

The water content of growing tissue in newborn animals differs significantly from the water content in adult animals. This has a negative influence on the examination of the navicular bone with T2-weighted sequences (it is not possible to differentiate the hyaline cartilage from the synovia in the joint). The T1 inversion recovery sequence that often used in adult animals for orthopedic examinations, proved to be informative in foals also, as it optimizes the contrast between the articular surface and the synovial fluid while the different layers of growing bone and cartilage can be distinguished. The navicular bursa of newborn animals is not represented on any of the images as a result of its very small amount of fluid content. The results suggest that an MRI method should be determined, that gives us the possibility to make in vivo examination of the navicular region in young horses as preventive screening.

### Phase 3.

The navicular bone, showing appositional growth, is composed of small volumes of different molecular structures next to each other (cartilage: GAG level, level of mineralization; bone: hydroxyl-apatite, fluorine, magnesium, sodium, osteocalcin, syaloprotein; bone marrow cavity: vascular components, fat cells). This and the fluid content

are the cause of the significant inhomogeneity of the different anatomical regions. The most informative images of the borders of anatomical regions within the cartilage of the navicular bone could be obtained with T2\*-weighted gradient echo sequences and the T1 inversion recovery sequences. The different areas of the subchondral region were also visualized with good contrast with T1-weighted SE, T2\*-weighted gradient echo and T1 inversion recovery sequences in newborn foals. It is much easier to differentiate the mature, homogeneously structured tissues of adult animals on MRI images. This suggests that it is necessary to describe the in vivo MRI morphology of regions of the developing navicular bone of foals aged between 1 and 12 months.

#### Phase 4.

When using an imaging method for the diagnosis of the navicular syndrome, researchers have always tried to find a numerical correlation between the ratio of different anatomical structures matching each other and the clinical form of the disease or the absence of symptoms. It is a difficult task, since it is not only the comparison of one dependent factor with an independent one but trying to find how the reactions of different tissues are related to various endogenous (originating from the organism itself) and exogenous (originating from outside the organism) factors. In adult animals, the difference between the two groups was not significant, regarding the compact and spongy substance ratio in the subchondral region and the degenerative lesions or their absence in the navicular bone. This means that MRI quantification is not relevant in this context.

#### Phase 5.

While examining the degenerative changes in horses, we obtained images of such lesions (fine rupture between the lateral and medial part of the deep flexor tendon, asymmetry of the two parts of the deep flexor tendon, moderate subchondral necrosis), that would not have been possible before by other diagnostic imaging techniques. The hooves were collected randomly neither the clinical symptoms nor their absence was taken into account. In this sense, the percentage (55.5%) of negative limbs (no pathological alterations) is not depressing. But taking into consideration that 3 animals of these 4 are aged between 3 and 4 years, which is the lower age limit of the disease, the results are not so encouraging. As the number of samples is not representative, it is important that conclusions are drawn carefully. On the contrary, the 100% MRI negativity of the animals aged between 0 and 7 days is favorable.

In conclusion: MRI imaging is suitable for the detailed examination of both developing and mature tissues. It is possible to differentiate the phases of normal tissue growth (cartilage, bone) and to localize and determine the progress of small lesions in regions that are difficult to examine with other imaging methods.



## 5. NEW SCIENTIFIC RESULTS

1. We have found that the T2-weighted sequences used for orthopedic examinations in adult horses, is of little diagnostic value in the perinatal period, because of the active endochondral ossification and the specific anatomy of the hoof. The T1 inversion recovery sequences provided the best contrast of the different tissues in young animals.
2. The MR morphology of the navicular bone containing large amounts of hyaline cartilage has been described in newborn foals. We came to the conclusion, that MRI is suitable for the precise visualization of the borders of the different layers in the hyaline cartilage of the navicular bone that are formed during bone growth, and also for the differentiation and analization of the various stages of ossification in the subchondral region.
3. The comparative histological and MRI examinations gave important datas for providing algorithms to skeletal tissue examination. These datas can be used not just for veterinary researches, but useful for any other investigations dealing with the formerly mentioned tissue even in the human studies.
4. We found that the T1-weighted spin echo sequence in the sagittal plane is suitable in adult horses for the MR quantification of the subchondral region (calculating the ratio of high and low signal intensity areas), but there is no significant difference between the two groups.

## **6. PROPOSALS**

### **Practical benefits of the results**

Unfortunately nowadays certain diseases (navicular syndrome, DOD (developmental orthopedic disease), OCD (osteochondritis dissecans), CVM (cervical vertebral malformation) etc.) are affecting sport horses (Hanoverian, Holsteiner etc.) at a very high percentage (5-30%). This is due to the increased inbreeding, aimed at achieving the uniformity of the animals' appearance and improving competition abilities. It is very expensive to raise a racehorse of a good genetical background. The later a lesion that negatively affects the race-career of a horse is recognized in life, the bigger amount of money can be lost. Regarding the horse as an individual, often the excessive training stress causes these hidden pathological changes or constitutional weaknesses to hinder the horse being ridden as a hobby horse. These unfavorable conditions lead to the deterioration of the horse, development of constant pain, and a quick worsening of its life quality. Advanced societies lay great emphasis on prophylaxis in both human- and companion animal medicine. This study sets the principles of a screening method –probably being used routinely in a few years - which helps eliminate the problems described above. The current improvement of MRI appliances in the USA gives us confidence in the future.

### **Further proposals for research**

1. Examination of the in vivo applicability of the developed MRI method in 1-6 month old foals.
2. Examination of in vivo MR morphology of the anatomical regions of the navicular bone in 1-12 month old foals.
3. MRI examination of alterations in the navicular region of foals (1-6 months old) raised under different conditions (nutrition, exercise, shoeing).
4. Radiographic and histological examination of the navicular region in animals suffering from a disease of the DOD group.
5. The connection between the MR morphology of the navicular region at a young age and the performance of the same horse at a mature age.

6. Examinations of the connection between the MR morphology of the cartilaginous elements in the navicular bone and the vitality of the newborn foal.
7. Applicability of CAD (computer aided diagnosis) in the MRI examination of the navicular region in adult and immature horses.

## **7. PUBLICATIONS CONCERNING THE SUBJECT OF THE DISSERTATION**

### **Articles in foreign languages**

**Á. Hevesi**, R. Garamvölgyi, P. Bogner, I. Repa: Examination of the navicular region of the horse by using magnetic resonance imaging. Methodical study. Acta Agraria Kaposvariensis, (2004) Vol 8 No 1, 1-10.

**A. Hevesi**, Ch. Stanek, R Garamvolgyi, Zs. Petrasi, P. Bogner and I. Repa: Comparison of the Navicular Region of the Newborn Foals and Adult Horses by Magnetic Resonance Imaging. J. Vet. Med. A (2004) 51. 143-149.

### **Articles in hungarian language**

**Hevesi Á.**, Garamvölgyi R., Petrási Zs., Lőrincz B., Bogner P., Repa I.: The correlation between the degenerative changes in the subchondral bone volume and the quantitative MR morphology in the navicular bone of the horse. HVJ (2005) 127. (9.) 515-522.

### **Full conference papers in proceedings**

**Hevesi Á.**, Bogner P., Repa I.: Examination of the anatomical structures in the navicular region of the horse by using magnetic resonance imaging. IX. Congress of Hungarian Equine Veterinary Association Budapest, (2001) Congress proceedings page 50.

**Hevesi Á.**, Bogner P., Repa I.: Applicability of MRI sequences used for the distal limb examination in adult horses compare to foals. XI. Congress of Hungarian Equine Veterinary Association Budapest, (2003) Congress proceedings page 32-35.

### **Abstracts in proceedings**

**Hevesi Ákos**, Garamvölgyi Rita, Bogner Péter Ph.D., Repa Imre Ph.D.: Imaging possibilities of the anatomical structures in the navicular region by using magnetic resonance imaging. Academic Report (2003) Book of clinical section page 14.

### **Lectures**

**Hevesi Á.**, Kis J., Boa L.: Developmental orthopaedic disease (DOD) in young horses. 1<sup>st</sup> Animal Health Day, Kaposvár (2004) 21<sup>st</sup> october.

### **Documentary publications in Hungarian**

**Hevesi Á.**: The hoof formed by nature. Equestrian Life (1999), 10. 18-19.