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## Bovine Tuberculosis in European Bison as Possible Zoonotic Impact in Poland

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### 1. Introduction

Tuberculosis remains the most prevailing disease worldwide. This infectious disease is caused by various strains of mycobacteria – *Mycobacterium tuberculosis*, *M. bovis*, *M. bovis* BCG, *M. microti* and *M. africanum* all known under one name *Mycobacterium tuberculosis* complex. Each of the aforementioned agents is recognized as dangerous both to humans and animals. Bovine tuberculosis (bTB) results from infection by *M. bovis*.

Suspicious of tuberculosis incidents in wildlife of Bieszczady Mountains (Poland, Podkarpackie Province) have a long history, however, first records on the confirmed bTB cases date back 1996 and concern European bison found dead within Brzegi Dolne Forest Inspectorate (Fig. 1, 3, 4) (Żurawski et al.1997). Culling of the European bison for diagnostic purposes, carried out in the period 1997-2001, revealed 13 bTB cases of 18 individuals tested (Welz et al.2006).

### 2. Molecular epidemiology of *M. bovis* isolates

In 2005-2008 in Bieszczady Mountains the next study was conducted. 5 strains of *M. bovis* were isolated – from 2 European bison (of 3 investigated), 2 cows and badger (of 2 investigated).

The whole study covered 215 free – ranging animals: red deer, wild boar, roe deer, a few examples of European bison, badger, wolf and lynx (Fig. 2). All of the animals were examined for mycobacterial infections. The studied material also involved 4 domestic cows. The examination of the collected material resulted in isolation of 14 strains of acid-resistant mycobacteria. The strains were distinguished as follows: 4 strains of *M. tuberculosis* (3 wolfs

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and a cow), 5 strains of *M. bovis* (2 European bison, 2 cows and badger), 4 strains of *M.avium* (4 red deer), and a strain of *M. species* (1 red deer) (Salwa et al. in prep.).

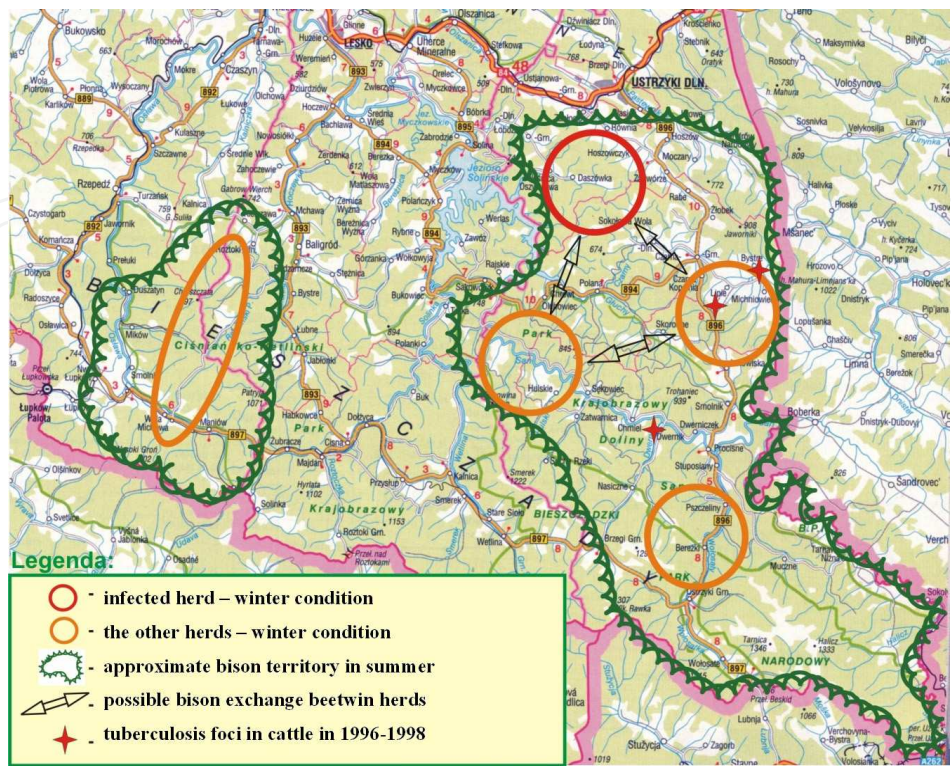


Fig. 1. European bison herds localization in the Bieszczady area in 1996-1998.

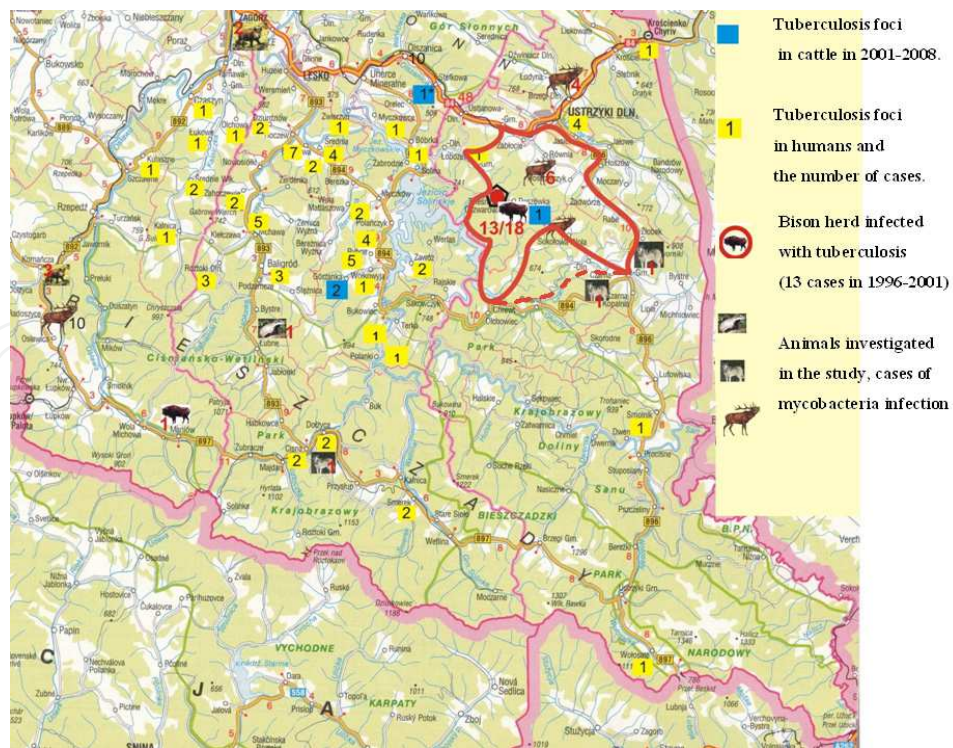


Fig. 2. The localization of tuberculosis foci in animals and humans in the Bieszczady in 2001 – 2008.



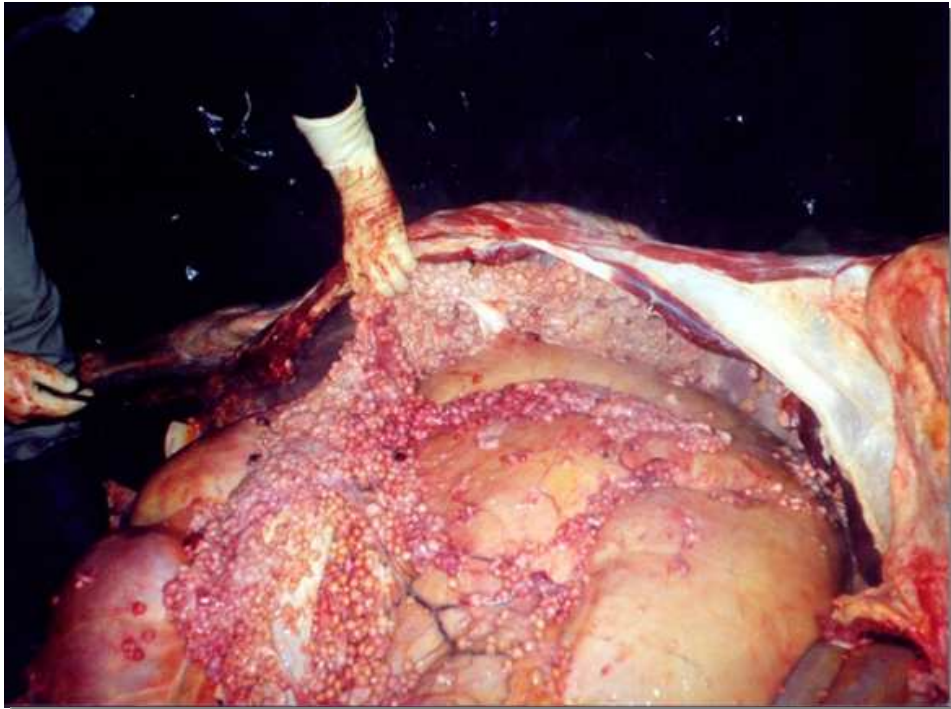


Fig. 3. Lesions characteristic for *M. bovis* infection – European bison, diagnostic cull; February, 1997 (fot. J. Polityński – Brzegi Dolne).

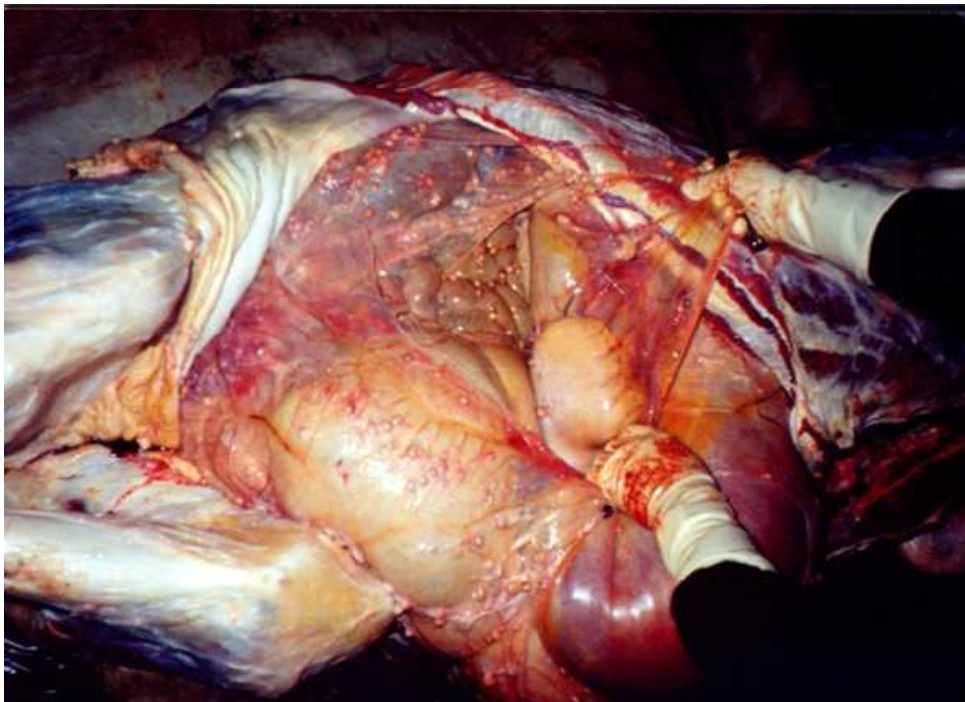


Fig. 4. Lesions characteristic for *M. bovis* infection – European bison, diagnostic cull; February, 1997 (fot. J. Polityński – Brzegi Dolne).

The examination toward the isolation and identification of tuberculosis mycobacteria was carried out by culture technique with the use of biological tests recommended by the Microbiology Unit of State Veterinary Institute in Pulawy. Lymph nodes were



microsectioned and chosen tissue fragments were homogenized. The inoculation was carried out in solid medium according to Lowenstein-Jansen and Stonebrink. Identification of isolated bacteria was carried out by the culture and biochemical methods.

Parallel to bacteriological identification of isolated *Mycobacterium* strains, a confirmation via PCR methods was performed. DNA isolation from examined strains was carried out by the column method using Genomic Mini AX Tissue set (A&A Biotechnology, Gdynia), according to the producer's instructions. The amplification of the selected fragment of region 16S-23 rRNA ITS was carried out utilizing 2 starters complementary to 5' and 3' with the following sequences:

ITS-F1-1 5'- TTG ATC CGA CGA AGT CGT AAC AAG g-3'

Mycom-3 5'- ATG CTC GCA ACC ACT ATC CA-3'

Oligonucleotides were synthesized by using a DNA Synthesizer (Genmed Synthesis, Inc.-USA). After finished reaction, the amplification products were analysed electrophoretically in 2% agarose gel (Sigma, Chemical Co.) with addition of ethydyne bromide. After electrophoresis the gel was photographed with an UV transilluminator (Fotodyne Inc.).

Restriction analysis based on the enzyme *Sau 961* (Fermentas, Lithuania) to digest bacterial DNA, identifying and cutting the DNA chain in the region of a specific nucleotide sequence. The electrophoresis was carried out in polyacrylamide gel electrophoresis apparatus filled with TAE buffer. The electrophoresis was carried out at voltage 100V/3 hours.

Sequencing was carried out using an automatic sequencer ABI PRISM 3100 Avant Genetic Analyzer, Applied Biosystem. The analysis involved a chosen area of the 16S-23 ITS region of all strains included in the examination. The obtained DNA sequences were then subjected to comparative analysis and subsequently a phylogenetic tree image was created by the Tree View program (version: 1.6.6).

The following strains were used for comparative examination: *M. tuberculosis* isolated from humans (strain PBG/28) and *M. bovis* isolated from a cow (strain DG/358-9). The strains were supplied by the Tuberculosis Diagnostic Pomeranian Center of Infectious Diseases and Tuberculosis in Gdansk, Poland and from the Tuberculosis Diagnostic Laboratory Veterinary Hygiene Institute in Bydgoszcz, Poland.

Among the compared *M. bovis* strains, the highest affinity was displayed by strain derived from two European bison. Sequence comparison of the analyzed fragment 16S-23S rDNA revealed that it was homologous except for only two nucleotides. What is important, the animals came from two herds living in different regions of Bieszczady. A high genetic differentiation was demonstrated comparing nucleotide sequences of *M. bovis* strains isolated from the badger and cattle. These evidence indicate that *M. bovis* spread within the Bieszczady region is represented by different variants (Salwa et al. in prep.). Consistent with this observation, studies of others authors have shown DNA diversity of *M. bovis*. (Skuce et al. 1996, Sechi et al.1999)

The result of the aforementioned study suggest that tuberculosis had been transmitted to European bison from the cattle when grazing the same pastures. The role of badgers in the spreading of *M. bovis* in Bieszczady Mountains is not yet fully recognized. It is necessary to underline that *M. bovis* was not isolated from deer and boars in Bieszczady Mountains.



### 3. Discussion

According to the report listing the local zoonotic disease in Bieszczady found during 1960-2008, in the sixties tuberculosis was common in cattle of Bieszczady. Although in the seventies the disease was believed to be eradicated, some isolated cases of tuberculosis positive cows were being found regularly every year thereafter. The report pointed at Daszówka as the location with the highest density of tuberculosis loci (Fig. 2).

A natural reservoir of *M. bovis* can be both a livestock, cattle in particular, and wildlife. Mutual contacts of animals from these two groups, taking place at pastures and meadows, facilitate an interspecies transmission of mycobacteria. Long term observations point at farm animals and humans as a primary source for Mycobacterium infections (Pavlik et al. 2005, Tessaro et al. 1990). Free-ranging animals might in turn become a reservoir of bTB and a secondary source of infection for other free-ranging animals or livestock. Infections may have a direct or indirect character. There are also many routes of *M. bovis* spreading (via air, with feces, urine, etc.). In England Courtenay et al. (2006) studied the route of *M. bovis* transmission and found out that infections of cattle had an indirect character and took place on the pastures via water and soil contaminated with urine of infected badger. A further support for this finding came from DNA comparative analysis of *M. bovis* strains isolated from cattle and badger that revealed a genetic similarity (Cheeseman et al. 1981, Costello et al. 2006). What is important, in Great Britain badgers are the main wildlife reservoir for bTB and there are different badger control strategies for reducing bTB in cattle (ex. culling, vaccination) (defra.gov.uk). During 1992-2001 cases of tuberculosis were reported in buffaloes of the Kruger National Park, South Africa (De Vos et al. 2001, Miller et al. 1997). Further epidemiological and molecular studies pointed at wild felids to play an important role in spreading the bacilli (De Vos et al. 2001).

Tuberculosis can have a chronic or rapidly progressive course. In free-ranging animals it is very often diagnosed after the onset of the typical clinical signs. This fact favours a prolonged period of the disease transmission.

The implementation of numerous cattle tuberculosis control programs have efficiently decreased the number of mycobacteria - infected animals to the level below 0.5%. Hence there are now countries, including Poland, regarded as free from bTB (WHO Report 2006). The presence of the disease in free-ranging wildlife in the Bieszczady region is a considerable threat to cattle and can lead to the loss of officially bTB free status of the region.

Epidemiological situation of Great Britain and New Zealand clearly demonstrates that the natural reservoir of *M. bovis* circulating in wildlife impedes the efficient eradication of tuberculosis in farm animals, especially in cattle. Numerous studies have concluded that the prevalence of tuberculosis in free-ranging animals is related to cases of tuberculosis in cattle (Bengis et al. 2002, De Lisle et al. 2001, Gallagher et al. 2000, Mathews 2006, Nishi et al. 2006).

According to the study bTB is endemic in the herds of free-ranging European bison of Bieszczady Mountains. The transmission of *M. bovis* probably originated from the infected cattle. Despite the actions taken in order to eliminate infections, new cases of bTB in European bison are still being reported. Last two cases are quite recent (2011) and they legitimate further studies on the spread of tuberculosis also in many wildlife species, also from the orders Carnivora (wolf - *Canis lupus*, red fox - *Vulpes vulpes*, badger - *Meles meles*) and Rodentia.

*M. bovis* has been isolated from many free-ranging animals worldwide (Aranaz et al. 2004, Delahay et al. 2007). First world reports on tuberculosis in wildlife related to the kudu



antelope in South Africa (Griffith 1929). Since then the disease have been observed in other species inhabiting different countries for example: the red deer, elks and coyotes from Texas (Perumaalla et al. 1996), red deer, elks, lynxes and hares from Spain (Aranaz et al. 2004), the African buffalo from Southern Africa (De Vos et al. 2001, Michel et al. 2006) and the bison from Canada (Schmit et al. 2002). Tuberculosis was also found in the baboon, lion, panther and cheetah (Michel et al. 2006). In Poland tuberculosis in wildlife was described for the first time in roe deer in the Gdańsk district (Czarnowski 1956). Later research showed quite frequent occurrence of *Mycobacterium* infection in the zoo animals (Dąbrowski 1974, Żurawski et al. 1980)

Tuberculosis, one of the oldest recognized disease in humans and animals, still remains a serious health hazard. It is estimated that one third of the human world population is infected with TB. The number of the strains showing a multi-drug resistance is still increasing (Augustynowicz-Kopeć et al. 2009). Human tuberculosis due to *M. bovis* has become very rare in countries with pasteurized milk and bovine tuberculosis eradication programs. In the region of Bieszczady Mountains there had been over 100 outbreaks of tuberculosis noted in humans over last 20 years. *Mycobacterium tuberculosis* complex was described as a causative agent of infections. Further procedures of mycobacteria identification were not performed. Cases of infections with *M. bovis* in cattle are also more numerous here as compared to any other regions of Poland. For this reason, there was a time when the bovine tuberculin tests were performed on some area every year (normally when the area is considered a tuberculosis free, the law stipulates a three-year interval between obligatory tuberculin tests). The analysis of epidemiological background of tuberculosis in the region revealed that the role of *M. bovis* in spreading the disease among people is underestimated.

Bovine tuberculosis can only be eradicated by controlling *M. bovis* infection in both wildlife and domestic animals. Otherwise cattle herds can be continually re-infected. The persistence of bovine tuberculosis in wildlife and the subsequent spread to farmed animals has stimulated research into new methods for controlling the disease in wildlife as well as cattle. A promising option for control of *M. bovis* infection in wildlife in the longer term is the development of a tuberculosis vaccine for wildlife and oral bait vaccines are the most practical means of delivering vaccines to wildlife. *M. bovis* bacilli Calmette-Guerin (BCG) is the world's most widely used human vaccine (intradermal vaccination). BCG is a live attenuated strain of *M. bovis* and was originally derived from a cow with tuberculous mastitis (Aldwell et al. 2003). Oral BCG vaccination of wildlife reservoirs of bTB is being extensively researched for application in many countries, including New Zealand (brush-tail possum), Great Britain, Ireland (badger), United States (white-tailed deer) and South Africa (African buffalo) (Nol et al. 2008). Nol et al. 2008. indicate that oral BCG Danish 1331 is effective in protecting white-tailed deer against disease caused by experimental *M. bovis* infection. Orally vaccinated deer had fewer tuberculosis lesion. The results of the present experiment indicate that white-tailed deer can be vaccinated orally using BCG incorporated in a lipid-formulated bait. This oral bait has successfully induced protection against *M. bovis* and *M. tuberculosis* infection in a number of species, including laboratory mice (Aldwell et al., 2003), brush-tail possum (*Trichosurus vulpecula*; Aldwell et al., 2003a) and domestic cattle (Buddle et al., 2005). White-tailed deer are a good candidate for oral vaccination programs to control bovine tuberculosis in the field (Nol et al. 2008). Trial with oral BCG vaccination in



European bison in Bieszczady Mountains should also be undertaken (evaluation of vaccine efficacy by microbiological and molecular examination – samples from characteristic pathological lesions; gamma interferon and lymphocyte proliferation tests, ELISA – blood samples). Oral administration of vaccines has a number of advantages including ease of administration, low cost, and the avoidance of needles. Furthermore, oral immunization more effectively targets the mucosal immune response. Oral bait vaccines have been successfully used to prevent rabies in foxes and other wildlife carriers (Schneider 1995).

It is necessary to remind that the European bison (*Bison bison*) is the largest terrestrial mammal in Europe (Fig. 5.). Long since being exterminated, it has been eventually returned to the wild. Considering health threats to European bison, the fact that those alive today descent from just 12 individuals (5 males and 7 females) survived in zoos and animal parks should be taken into account. Limited genetic variability characterizes all the European bison living now. That may lead to the appearance of genetic defects, less-flexibility and tolerance to unfavorable environmental changes, or a lowering of resistance and consequent vulnerability to disease. The threats to the European bison not only concern infectious disease, but also genetically derived problems such as testicular anomalies and environmental limitation like lack of opportunity for migration between isolated populations, limitations on food resources, competition with other ungulates (Krasińska et al. 2007). There are 5 free-ranging population of European bison in Poland, four in the east of the country and one in the north-west. The total number of bison present in the wild is about 750 individuals. The largest population is in Białowieża Forest, about 370 individuals. Bieszczady Mountains are the only place in Poland where bison of the Białowieża-Caucasian Line are present, about 300 individuals. Unfortunately, a serious threat to this population is posed by the tuberculosis.



Fig. 5. European bison (*Bison bison*) is the largest terrestrial mammal in Europe.



Bieszczady Mountains is one of the most valuable Polish mainstays for many species. It houses about 230 species of vertebrates, including protected animals as European bison, the wolf, brown bear, lynx and badger.

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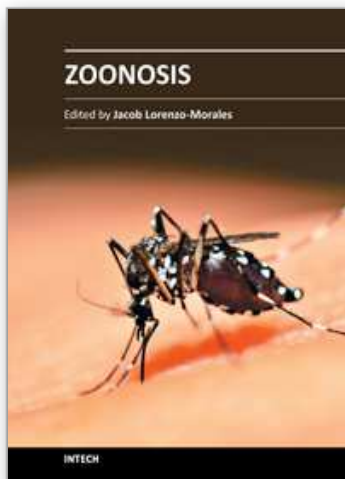


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## **Zoonosis**

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Zoonotic diseases are mainly caused by bacterial, viral or parasitic agents although "unconventional agents" such as prions could also be involved in causing zoonotic diseases. Many of the zoonotic diseases are a public health concern but also affect the production of food of animal origin thus they could cause problems in international trade of animal-origin goods. A major factor contributing to the emergence of new zoonotic pathogens in human populations is increased contact between humans and animals. This book provides an insight on zoonosis and both authors and the editor hope that the work compiled in it would help to raise awareness and interest in this field. It should also help researchers, clinicians and other readers in their research and clinical usage.

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