Is the neuropathological assessment really a gold-standard to diagnose dementia? YES or NO

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Faculty of Veterinary Medicine
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The dog could be used as a natural animal model for the study of normal aging and human neurodegenerative diseases.
A rude technician in the lab (Utrecht) once said to this man that he had started to ‘Alzheimer’.
Veterinary medicine: species diversity

Human medicine: human being

The “One Health” concept

A cross-sectoral collaboration of the “whole society” to health hazards due to the perspective of uniformity of pathogenesis of diseases
Veterinary medicine: various species - Medicine: one species

**One health > zoonoses** - Zoobiquity is a way of thinking about uniformity of diseases processes.

In animal health we always have a good ‘experimental animal’ to compare, that is the human being.

In medicine for a long time colleagues use experimental animals, most rodents. Alzheimer’s (AD), than > ageing diseases of the brain
-the prevalence of ND, such as AD and PD, increases with age
-accurately understanding the etiopathogenetic mechanisms of these diseases is a crucial step for developing disease-modifying drugs
So its very important to find the suitable animal model for these degenerative diseases in order to

-to increase the knowledge on the pathophysiology of degenerative diseases based on the histopathological lesions and their main symptoms
Global and national organizations remark the rising prevalence of human neurodegenerative diseases.

Comparative gerontology aims to find the most appropriate animal-model, that can be used for the research and the treatment of these diseases.
More than **600 diseases** related with progressive degeneration of the CNS structural components, have been studied in human medicine

Alzheimer's diseases and other dementias
Parkinson’s disease
Huntington’s disease
Prion Diseases
Brain cancer
Degenerative Nerve Diseases
Encephalitis
Epilepsy
Genetic Brain Disorders
Head and Brain Malformations
Multiple Sclerosis
Amyotrophic Lateral Sclerosis
Alzheimer’s (AD), > brain age related diseases

AD is the most common form of dementia and now represents 50%-70% of total dementia cases.

In 2050 the total number of people with AD dementia in the United States is projected to be 13.8 million.
The Degenerative Diseases (DD) of CNS include:

degeneration of neurons and loss of their synapses

degeneration and loss of other cell types of neural parenchyma
Many neurodegenerative diseases of domestic animals are clinically and morphologically very similar to their human counterparts.

Clinical signs

Histopathology

immunohistochemistry for the identification of CNS specific proteins,

PCR
Alzheimer’s disease, Parkinson’s disease, Huntington’s disease

Rodents (Knockout mice), zebra fish, transgenic zebra fish models

Invertebrate animals like *Caenorhabditis elegans* και *Drosophila* are used as animal models for AD and PD because of their short reproductive period and life cycle, many genotypes and because of their known genetic variability

Veterinary Medicine?
Canine
- share the same environment with humans
- diet
- shorter life cycle
- genetic background

Clinical signs
Biochemical parameters
Histopathological lesions
Immunohistochemical findings
Amyloid: amorphous, homogenous, extracellular, glycoproteinaceous complex that has the ability to deposit in the tissues. Rudolf Virchow, second half of 19th century
Electronical microscope: **amyloid fibrils**
(Alan Cohen and Calkins, second half of 20th century)
Amyloid β-protein:

- M.W. 4Da

-produced by proteolysis of β-amyloid protein precursor (βAPP) (neurons, epithelial cells of choroid plexus, smooth muscle cells, microglial cells and macrophages)

- Aβ 39-43 amino-acids
- Aβ40
- Aβ42 και Aβ43 early primitive and senile plaques
- Numerous unbranched fibrils with linear morphology and diameter 7.5-10nm

- The fibrils show the typical structure of β-sheet twist

- The fibrils are made up of two filaments with diameter of 2.5-3.5 each, which are wound one around the other forming an helix shape
Amyloid: Toward terminology clarification
Report from the Nomenclature Committee of the International Society of Amyloidosis

PER WESTERMARK¹, MERRILL D. BENSON², JOEL N. BUXBAUM³, ALAN S. COHEN⁴, BLAS FRANGIONE⁵, SHU-ICHI IKEDA⁶, COLIN L. MASTERS⁷, GIAMPAOLO MERLINI⁸, MARIA J. SARAIVA⁹, & JEAN D. SIPE¹⁰

Table II. Amyloid fibril proteins and their precursors in animals.

<table>
<thead>
<tr>
<th>Amyloid Protein</th>
<th>Precursor</th>
<th>Systemic (S) or Localized (L)</th>
<th>Syndrome or Involved Tissues</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>Immunoglobulin light chain (Apo) serum AA</td>
<td>L</td>
<td>Plasmacytoma</td>
<td>Horse</td>
</tr>
<tr>
<td>AA</td>
<td>Apolipoprotein AI</td>
<td>S</td>
<td>Secondary, reactive</td>
<td>Mouse, guinea pig, cat, dog, cow, duck, etc</td>
</tr>
<tr>
<td>AAPoAI</td>
<td>Apolipoprotein AII</td>
<td>S</td>
<td>Age-related</td>
<td>Dog</td>
</tr>
<tr>
<td>AAPoAII</td>
<td>Apolipoprotein AII</td>
<td>S</td>
<td>Age-related</td>
<td>Mouse</td>
</tr>
<tr>
<td>Aβ</td>
<td>Aβ protein precursor</td>
<td>L</td>
<td>Age-related</td>
<td>Dog, sheep, wolverine</td>
</tr>
<tr>
<td>AβAPP</td>
<td>Islet amyloid polypeptide</td>
<td>L</td>
<td>Islets of Langerhans</td>
<td>Cats, apes, raccoon</td>
</tr>
<tr>
<td>AIns</td>
<td>Insulin</td>
<td>L</td>
<td>Insulinoma</td>
<td>Octodon degus</td>
</tr>
<tr>
<td>ACas</td>
<td>α-S2C casein</td>
<td>L</td>
<td>Mammary gland</td>
<td>Cow</td>
</tr>
</tbody>
</table>
In man more than 30 different major amyloid fibril-protein types with corresponding types of amyloidosis. For animals they have listed 10 types.

An old animal amyloidologist: please, look for similarities and not only for differences >> look for amyloidobiquity!
<table>
<thead>
<tr>
<th>Amyloid protein</th>
<th>Precursor</th>
<th>Systemic (S) or Localized, organ restricted (L)</th>
<th>Syndrome or Involved Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>Immunoglobulin light chain</td>
<td>S, L</td>
<td>Primary Myeloma-associated</td>
</tr>
<tr>
<td>AH</td>
<td>Immunoglobulin heavy chain</td>
<td>S, L</td>
<td>Primary Myeloma-associated</td>
</tr>
<tr>
<td>Aβ2-M</td>
<td>β₂-microglobulin</td>
<td>S</td>
<td>Hemodialysis-associated</td>
</tr>
<tr>
<td>ATTR</td>
<td>Transthyretin</td>
<td>S</td>
<td>Joints, Familial, Senile systemic, Tenosynovium</td>
</tr>
<tr>
<td>AA</td>
<td>(Apo)serum AA</td>
<td>L</td>
<td>Secondary, reactive, Familial</td>
</tr>
<tr>
<td>AApolAI</td>
<td>Apolipoprotein AI</td>
<td>S</td>
<td>Familial Aorta</td>
</tr>
<tr>
<td>AApolAII</td>
<td>Apolipoprotein AII</td>
<td>S</td>
<td>Familial, Sporadic, associated with aging</td>
</tr>
<tr>
<td>AApolAIV</td>
<td>Apolipoprotein AIV</td>
<td>S</td>
<td>Familial, Finnish</td>
</tr>
<tr>
<td>AAGel</td>
<td>Gelsolin</td>
<td>S</td>
<td>Familial</td>
</tr>
<tr>
<td>AALys</td>
<td>Lysozyme</td>
<td>S</td>
<td>Familial</td>
</tr>
<tr>
<td>AAFib</td>
<td>Fibrinogen α-chain</td>
<td>S</td>
<td>Familial</td>
</tr>
<tr>
<td>AACys</td>
<td>Cystatin C</td>
<td>S</td>
<td>Familial</td>
</tr>
<tr>
<td>ABri</td>
<td>ABriPP</td>
<td>S</td>
<td>Familial dementia, British</td>
</tr>
<tr>
<td>ADan*</td>
<td>ADanPP</td>
<td>S</td>
<td>Familial dementia, Danish</td>
</tr>
<tr>
<td>Ab</td>
<td>α₁ protein precursor</td>
<td>L</td>
<td>Alzheimer's disease, aging</td>
</tr>
<tr>
<td>APrP</td>
<td>Prion protein</td>
<td>L</td>
<td>Spongiform encephalopathies</td>
</tr>
<tr>
<td>ACaI</td>
<td>(Pro)calcitonin</td>
<td>L</td>
<td>C-cell thyroid tumors</td>
</tr>
<tr>
<td>ATIAPP</td>
<td>Islet amyloid polypeptide</td>
<td>L</td>
<td>Islets of Langerhans</td>
</tr>
<tr>
<td>AANF</td>
<td>Atrial natriuretic factor</td>
<td>L</td>
<td>Insulinomas</td>
</tr>
<tr>
<td>APro</td>
<td>Prolactin</td>
<td>L</td>
<td>Cardiac arrhythmia, Aging pituitary, Prolactomas</td>
</tr>
<tr>
<td>AIns</td>
<td>Insulin</td>
<td>L</td>
<td>Iatrogenic</td>
</tr>
<tr>
<td>AMed</td>
<td>Lactaderin</td>
<td>L</td>
<td>Senile aortic, media</td>
</tr>
<tr>
<td>AKer</td>
<td>Kerato-epithelin</td>
<td>L</td>
<td>Cornea familial</td>
</tr>
<tr>
<td>AALac</td>
<td>Lactoferrin</td>
<td>L</td>
<td>Cornea Osteotrophic</td>
</tr>
<tr>
<td>Aβ (thb) **</td>
<td>L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Senile Plaques (SP)

Deposition of amyloid in the walls of the cerebral vessels (CAA)

Neurofibrillary tangles (NFTs)
Do we have AD in the dog?

Human Alzheimer-patients > loss of memory,
and
# senile plaques with Aβ-amyloid
# loss of cholinergic extensions
# neurofibrillary tangles (tau) within neurons
# role of neurons during the plaque-formation
COGNITIVE DYSFUNCTION
Translation of nerve impulses that are transmitted to the brain and their feedback
CLINICAL SIGNS OF DEMENTIA SYNDROME

<table>
<thead>
<tr>
<th>Disorientation</th>
<th>Inability of orientation in a familiar space</th>
</tr>
</thead>
<tbody>
<tr>
<td>Social inclusion</td>
<td>Difficulty of coexistence with other animals and people of familiar background</td>
</tr>
<tr>
<td>Sleep/wake cycle alterations</td>
<td>Aimless wandering at nighttime</td>
</tr>
<tr>
<td>Mobility issues</td>
<td>Anxiety or lethargy, repeated aimless moves</td>
</tr>
<tr>
<td>Training</td>
<td>Urination/defecation in inappropriate places</td>
</tr>
<tr>
<td>Other symptoms</td>
<td>Constant barking/miowing, appetite disorders, disinterest of self-catering</td>
</tr>
</tbody>
</table>
Statistical facts (Osella et al., 2007)

Almost half of elderly aged canines demonstrate for various time periods one of the dementia syndrome’s symptoms
Gross lesions

- brain atrophy
- thickening of leptomeninges
- widening of sulci and narrowing of gyri
- ventricular enlargement
- haemorrhages
Histopathological findings

- fibrosis of leptomeninges and choroid plexus
- gliosis
- satelitosis and neuronophagia
- neuronal degeneration
- lipofuscin storage
- white matter vacuolation
- polyglucosan bodies (PGB) - Lafora bodies
- spheroids
Pathogenesis of β-amyloidosis-SP (Selkoe 1998)

- mutations of genes that encode the composition of AβPP, result to the raise of Aβ42 or/and Aβ40

- concentration of the insoluble form of Aβ42 peptide in brain interstitial fluid

- diffuse plaques (combined with proteoglycans)

- in these plaques are deposited Aβ40 peptide and other inflammation mediators-proteins – activation of microglial cells results to neuronophagy, astrocytes hyperactivity, release of cytokines
- Aβ is responsible for the damage of neurons near to the plaques. Free radicals are responsible for the lesions in other parts of neuropil - (oxidative stress).

- Thaker et al. 2003, claim that oxidative damages cause alterations in kinases and phosphatases activity that leads to

- hyperphosphorylation of tau, NFTs
CAA (Rensink et al 2003)

- Aβ found in vessels is originated mainly from neurons, smooth muscle cells, pericytes and blood.

- Blood: damage of blood-brain barrier due to hypertension, atherosclerosis, trauma.

- Neurons-vessels through extracellular brain fluid drainage-smooth muscle cells or around them-destruction of those and endothelial-destruction of blood-brain barrier-deposition of larger amount of Aβ.
Immunochemistry investigation of the brain of aged dogs. I. Detection of neurofibrillary tangles and of 4-hydroxynonenal protein, an oxidative damage product, in senile plaques.

Papaioannou N¹, Tooten PC, van Ederen AM, Bohl JR, Rofina J, Tsangaris T, Gruys E.

Author information

Research Report

Cognitive disturbances in old dogs suffering from the canine counterpart of Alzheimer’s disease

J.E. Rofina¹,*, A.M. van Ederen¹, M.J.M. Toussaint¹, M. Secrèv⁶, A. van der Spek¹, I. van der Meer¹, F.J.C.M. Van Eerdenburg¹,², E. Gruys¹

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²Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands
Biomarkers in Alzheimer’s Disease

-Cerebrospinal fluid (CFS) levels of Aβ40, Aβ42, total tau and phosphorylated tau have diagnostic values in AD

-the level of inflammatory marker YKL-40 is elevated in cerebrospinal fluid from patients with AD and not PD

-young to middle-aged dogs with high amyloid-β levels in cerebrospinal fluid are impaired on learning in standard cognition tests
Serum amyloid P component

- plasma
- neurons
- axons
- microglial cells
4-Hydroxy-2-nonenal

- non-well circumscribed Aβ antigenic material
- diffuse plaques
- capillary wall areas
- arterial wall amyloid stained either negative or weakly positive and was considered inconclusive
- perivascular cells representing mononuclear phagocytes
- neuronal cytoplasmic bodies
Pathology of the Aging Brain in Domestic and Laboratory Animals, and Animal Models of Human Neurodegenerative Diseases

S. A. Youssef, M. T. Capucchio, J. E. Rofina, J. K. Chambers, K. Uchida, H. Nakayama, and E. Head

Alzheimer disease research in the 21st century: past and current failures, new perspectives and funding priorities

Francesca Pistollato, Elan L. Ohayon, Ann Lam, Gillian R. Langley, Thomas J. Novak, David Pamies, George Perry, Eugenia Trushina, Robin S.B. Williams, Alex E. Roher, Thomas Hartung, Stevan Harnad, Neal Barnard, Martha Clare Morris, Mei-Chun Lai, Ryan Merkley, and P. Charukeshi Chandrasekera
Is Your Pet Showing Signs of Alzheimer's Disease?
Are canines the **ONLY** subject for the study of AD in human beings?

OR

After using the question above we answer as vets that is there the NEED for the EARLY diagnosis of this cognitive dysfunction of our friends?

In conclusion, the dogma of the one health is the top of modern medicine and the way of the approach of clinical conditions and how they are involved is identical between humans and animals.
The dog is natural spontaneous animal model for the study of normal aging and human neurodegenerative diseases
I would like to thank my colleagues

Dr. Anestakis Doxakis, MD, Ph. D
Ioanna Stylianaki, DVM
Dimitris Dimzas, DVM

Prof. Dr Erik Gruys
Peter Tooten, Technician, Utrecht, The Netherlands
A. M. van Ederen, Technician, Utrecht, The Netherlands
Thank you very much for the attention!