ALZHEIMER - CERTITUDES AND HYPOTHESES

Ioana Cristina Amihăesei[1], Elena Cojocaru[1], O.C. Mungiu[2]
University of Medicine and Pharmacy „Grigore T. Popa" – Iasi
Faculty of Medicine
1. Discipline of Histology
2. Discipline of Pharmacology

ALZHEIMER – CERTITUDES AND HYPOTHESES (Abstract):Alzheimer's disease is a degenerative, progressive and irreversible condition, which affects cognitive functions. It was first described in 1907, by the German physician Alois Alzheimer. Although at the time, it was considered a rare disease, in 2010 in the world were estimated 35.6 million cases of dementia, most of these with the diagnosis of Alzheimer's disease. Typical neuropathologic lesions are represented by the amyloid plaques, neurofibrilar tangles and synapses and neurons losses. It was hypothesized that the amyloid protein has prion-like properties. Even from the first descriptions of the disease, atypical features were observed – the second case described by the physician Alois Alzheimer, had only plaques, the tangles were missing. About 19 % of the healthy old subjects present in the brain the same lesions as Alzheimer's cases, while in 10 % of the cases of disease, in necropsy are present only the plaques or only the tangles. These aspects are even more paradoxical, as the certain diagnosis is established only at necropsy, on anatomopathological lesions. Even so, the international diagnosis criteria, based on clinical aspects, can establish a certain, probable or a possible diagnosis. It exist an early-onset form, as well as a late-onset form of disease (which appears after 80-85 years of life); genes are involved in the genesis of the disease. A lot of money are spent to find an efficient medication in the treatment of the disease (tramiprosate – an amyloid-antagonist, Dimebon, gamma-secretase inhibitors or a vaccine – a synthetic form of the amyloid protein); for the moment the used medication may at its best only to temporary improve the symptoms. Some scientists believe that approx. 30 % of the cases are wrongly diagnosed with Alzheimer, being in fact other forms of dementia, or that we deal with several biologic processes, generating rather an Alzheimer's syndrome, meanwhile others are unsatisfied by a poor diagnosed disease and its popular receipt as a part of the normal aging process. **Keywords**: ALZHEIMER'S DISEASE, DEMENTIA, AMYLOID PLAQUES, NEUROFIBRILAR TANGLES, AMYLOID β PEPTIDES.

EPIDEMIOLOGICAL LANDMARKS, HYPOTHESES AND CONTROVERSIES

Being a degenerative, progressive and irreversible disease, which is dramatically damaging the intellectual functions, the Alzheimer's disease was first described in 1907, by the German physician Alois Alzheimer, being then considered a rare entity. Now is considered an epidemical disease, with major social impact, and which threatening is growing together with the phenomenon of global aging of the human population.

2009 World Alzheimer Report has estimated 35.6 million dementia affected individuals for 2010, and this number was estimated to double at each 20 years, thus resulting 65.7 million in 2030 and 115.4 million in 2050. Large part of this growth is attributed to the increase in number of the dementia affected people in the countries with low and medium incomes/inhabitant. In the same report, the specialists are underlying the under diagnosis of this disease, as well as the stigmatization of the affected people and of their families, all over the world, independent of the size of the country, or the level of wealth. Alzheimer's disease was diagnosed in all the countries, of any culture and race, in which the diagnosis screening was done. The level of awareness of the disease, as a severe medical condition is greatly variable, from one country to another. In low and medium developmental level countries, the degree of awareness is decreased, dementia being still considered a normal estate of the normal aging process (1).

Recognized risk factors are: the advanced age, the genotype Apo E epsilon 4, cranial trauma, cardiovascular risks (smoking, diabetes, high blood pressure, and hyper cholesterolemia), and possible metal exposure, especially to aluminum. The gene for the E apolipoprotein on the 19 chromosome is a protein, which is modulating the transport of the phospholipids, with role in the synaptic remodeling. Apo E epsilon 4 is associated with a high risk for Alzheimer's disease (3).

Classical neuropathological features

- amyloid plaques and neurofibrilar tangles, with synapses and neurons losses.

- granulovacuolardegenerescence of the hypocampus and deposition of amyloid in the vessels, are usually present as well.

- the amyloid plaques are made of 40-43 aminoacidsoligopeptides, derived through proteolysis from a much larger precursor amyloid protein fraction – APP – amyloid precursor protein. The precursor protein is coded by a single gene and is made of several sequential forms, fractions of the plasma membrane integral proteins type I. The amyloid is made of 28 aminoacids of the big extracellular aminoterminal domain of the APP and of the first 12-15 aminoacids of the intramembranary region.

- the associated microtubular tau protein, under a hyperphosphorylated, modified form, is the major protein component of the spiralated filaments in the structure of the neurotubules, thus in the structure of the neurofibrilar tangles. The normal tau protein is not glycosilated, while in the brain of the Alzheimer's patients, this protein appears under a glycosilated form. Although the abnormal phosphorylation is leading to the inhibition in the assembly of the microtubules, the glycosilation is mantaining the helical structure. Normal tau protein is involved in the axonic growth. The abnormal phosphorylated form of the tau protein is forming the spiralated filaments pairs of the neurotubules, and it appears in the neurons of the hippocampus, medial temporal lobe, parietotemporal region and in the associative frontal cortex, leading to the death of the interneurons. These zones are affected by the loss of the synapses, with the injury of the synaptic passages of the neurotransmitters (2).

- mutations at the level of three proteins – presenilin 1, presenilin 2 and the precursor protein of the β amyloid, were identified in the familial form of the Alzheimer's disease. These mutations are increasing the extracellular concentration of the β amyloid and are enhancing its brain deposition (3).

ETIOPATHOGENIC HYPOTHESES

1. Although the hypothesis which is stating that the β amyloid peptides (Aβ) are the primary causal agents of the Alzheimer's disease is supported by the large majority of the researchers, a large number of data is showing that it is rather unlikely, the Aβ peptides to be the single etiologic factor of the disease.

2. There are proofs which support the fact that factors which are unrelated to amyloidosis, including genetical factors, are important in the pathogenesis – defects of the endolysosomal metabolism, the alteration of the intracellular signal cascades, damaging in the releasement of the neurotransmitters, are all contributing to the appearance of the synaptic dysfunctions and/or neurodegenerescence and hence to the dementia of the Alzheimer's disease (4).

3. A hypothesis is launching the possibility of the development of the neurofibrilar tangles, as a result of the age-related dysfunction in the function and viability of the microglial cells. The neurodegenerescence would be from this point of view, the result of a decline of the immune functions, rather than a hyperactivity of of the immune system (5). The death of the cholinergic neurons of the Meynert basal nucleus is resulting into the deficiency of acetylcholine, characteristic for the disease; a transmitter involved in the memory process (7). The loss of the serotoninergic neurons of the median zone of the raffe and of the adrenergic neurons of the locus coeruleus is determining the serotonin and the norepinephrine deficits. The exact role of the inflammation in the pathogenesis of the disease is still controversial; although studies which evidentiate the microglial activation do exist (as a marker of the brain immune response), prednisone, as well as NSAID (non steroid alantin inflammatory drugs) were ineffective in the treatment of the disease (6).

4. New studies have even proposed a prionic hypothesis in the pathogenesis of the Alzheimer's disease. It was noticed that the peripheral deposits of beta-amyloid, from different tissues, others than the brain, may finally reach „to infect" the brain. Injection of the amyloid-containing brain tissue, obtained from old mice, in the abdomen of younger animals, had led several months later to the detection of the amyloid in the brain of younger injected animals. The studies which used transgenic mice (genetically induced to produce Aβ human amyloid) had led to the prion-like qualities of the amyloid protein hypothesis. Prions show protein structure and are transmissible, as it is happening in the Creutzfeld-Jakob disease. Yet, there are no proofs that the Alzheimer's disease or the brain amyloid angiopathia are transmissible (between mammals or humans) in the same way as the prionic diseases (8).

DIAGNOSIS – DISEASE OR SYNDROME ?

The diagnosis is established upon the clinical criteria stated by the National Institute of Neurological and Communicative Disorders and the Alzheimer's Disease and Related Diseases Association, in the USA. It is estimated that these criteria are establishing the diagnosis, with an accuracy of 90 %. On the basis of these criteria, the diagnosis is certain, probable, or possible, but the certitude of the diagnosis is obtained only through necroptic examination of the anatomo-pathologic lesions. Nevertheless, were signaled cases of subjects with minor memory troubles, but who had

typical Alzheimer's disease lesions, in necropsy.

The major clinical manifestation is represented by the memory loss, first for the recent events, but afterwards for the past events, as well. Language troubles are first manifesting by the search of the right words, to name different objects, evolving into communication disability, limited vocabulary, nominal aphasia and difficulties of understanding.Apraxia is developing (the inability to perform simple daily activities, such as combing the hair), acalculia (the inability to perform simple arithmetic calculations) visual and spatial disorientation (disorientation in what concerns the position in space of the body).

The mood troubles are including depression, anxiety, apathy, which may appear early in the development of the disease, while in the advanced stages, hallucinations are frequent, together with psychotic symptoms; aggressiveness and sexual behavioral troubles are most unpleasant for the caring persons. In the advanced or terminal stages, is appearing the frontal lobe syndrome, and the sphincters incontinence, myoclonus, convulsions, up to the vegetative estate, when all cognitive activities are disappearing (10).

Ten cardinal symptoms
of the Alzheimer's disease

- decline of the rational thinking
- difficulties in accomplishing complicate tasks
- language troubles
- time and spatial disorientation
- decline of the abstract thinking
- loss of the recent memory affecting daily tasks
- personality changes
  mood and behavior troubles
- changes in perception of the reality
  loss of the initiative

MRI examination is revealing the progressive atrophy of the hippocampus, which is an important zone of the memory processes (10). Dosages of the Aβ peptide and of the tau protein, in the cerebrospinal fluid, are considered biomarkers for the Alzheimer's disease, being estimated that their use, will increase the accuracy of the diagnosis. Decrease of the levels of the Aβ peptide and increase of the tau protein, in the cerebrospinal fluid, are the proof of the evolution of the disease, being the consequence of the deposition of the amyloid peptide into the brain, and of the excretion of tau protein by the neurons which undergo degenerescence (11).

Anatomopathologic lesions, which represent the certitude diagnosis, might be present in healthy old-aged subjects (19 % among these), as well as in subjects who developed another type of dementia. In the necroptic examination, in about 10 % of the cases of Alzheimer's disease are present only the amyloid plaques (otherwise, the second case described by Alois Alzheimer had only plaques in necropsy), or only the neurofibrilar tangles.

The book written by dr. Peter Whitehouse and Daniel George, „The Myth of Alzheimer's: What You Aren't Being Told about Today's Most Dreaded Diagnosis" (2010, http//booksandideas.com) is putting the question whether the concept itself of Alzheimer's disease is clear enough, and if there are not rather several different pathologic processes, since not even A. Alzheimer was not sure that he had described something different from the senile dementia. Dr. Whitehouse is considering that too often, this is a label, in the same way like that of dementia, and that it must be reevaluated in what measure the

Alzheimer's lesions are not a part of an individual aging process.

Early onset and late onset Alzheimer's concepts appeared as a consequence of the fact that, the majority of the Alzheimer's disease diagnoses are now established in patients over 85 years old, while the cases described by Alzheimer were relatively young – 51, respectively 54 years old. Even in the early onset forms, it is difficult to state that the pathologic process is really distinct, from that one in the senile dementia.

Based on the similarity of the brain lesions, in the sixth decade of the XX century, especially under the pressure of the Anglo-Saxon specialists, the concepts of Alzheimer's disease and that of senile dementia were unified. This contributed to the perception of the Alzheimer's disease as a separate medical condition, since the term of senile dementia had such deep negative connotations. There are differences in the symptoms of the two types of Alzheimer's disease – in young patients, the troubles of language, gesture, recognition of faces/objects are predominating, while in the case of old-aged patients, memory troubles are clearly predominating. There are studies which conclude that approximately 30 % of the Alzheimer diagnosed patients do have in fact, another type of dementia.

There are voices who state that we should talk about the „Alzheimer's syndrome", which would include several types of diseases (12, 13).

THERAPY, BETWEEN FAILURES AND PROMISES

Among the factors which decrease the risk for Alzheimer's disease, with some degree of efficiency is signaled – the brain stimulation (examples – chess, puzzles) and regular physical exercise (14, 15, 16). The therapy is targeting on one hand to reduce the risk of development of the disease, by using food supplements, NSAID (such as aspirin, ibuprofen, which unfortunately in several studies, did not prove to be efficient) and the treatment of the acetylcholine deficit, by administration of cholinesterase inhibitors and antagonists of the receptor N-methyl-d –aspartate (NMDA) on the other hand, and experimental medication, hoped to be a real revolution in the therapy of the disease.

Some studies investigated the real prevention efficiency of group B vitamins, of high doses of E and C vitamins, of the choline, omega 3 fatty acids (DHA), of the statines and NSAID; unfortunately, the results were not those which were expected (17,18,19).

Cholinesterase inhibitors (Donepezil, Rivastigmine tartrate, Galantamine) and the antagonists of the receptor – NMDA - (Memantine – Namenda) are determining only a temporary improvement of the cognitive functions, and of the behavior (20).

From the recently experimented medications, Tramiprosate, an oral administration, small molecule antagonist of the amyloid, is considered promising; this is linking the soluble amyloid β peptide (Aβ) thus interfering with the cascade effect of the amyloid, its deposition, and with the brain toxic effects of the peptide. The signaled side effects were mild – mouth dryness, slight depression and increase of the sweat secretion (21).

Dimebon is another small molecule, with oral administration, which was commercialized in Russia in the 80^{s}, for its antihistaminic properties and was dropped off, as other superior antihistaminic agents were

developed. 70 % of the Alzheimer's patients who took Dimebon for one year, have improved their estate, or at least have stabilized their Alzheimer's symptoms (22).

Gamma-secretase inhibitors – LY 450 139, were developed by the Eli Lilly company as a modifier of the disease evolution; the medication inhibit the gamma-secretase, enzyme involved in the formation of the Aβ peptide. From 51 patients who received a 100/140 mg daily dose, for 6-12 weeks, in 43 cases who finished the trial, was registered the marked decrease of the blood Aβ levels (58.2% from those who received 100 mg/day and 64.6 % from those who received 140 mg/day). Minor side effects, such as rash, fatigability and sleepiness appeared (23).

A new approach in the Alzheimer's disease was to furnish an alternative source of energy to the glucose-deprived neurons. Ketasyn (AC-1202) is providing ketonic bodies to the glucose-deprived neurons; they can be metabolized, even when glucose cannot be. AC-1202 is converted into ketonic bodies (beta-hydroxibutirate) as a glucose-alternative for the energy-compromised neurons. Good results were obtained in patients who had not the E4 variety of the apolipoprotein gene (ApoE epsilon 4) which appears in half of the Alzheimer's disease patients. AC-1202 has significantly improved the memory and the cognitive capacity in negative ApoE 4 patients. The subjects who took AC-1202 for 9 months have registered a very small progress of the disease. The side effects were minimal; sometimes appeared gastro-intestinal troubles (24).

In the case of the vaccine AN 1792, which uses a synthetically form of the amyloid's protein, some patients developed an aseptic meningo encephalitis, reason for which the trial was stopped. In 59 patients, anti Aβ antibodies had detectable levels after 4.5 years; it was observed a more reduced dependence on the caring personnel and a slower decline of the memory tests. Among the vaccinated patients who developed antibodies, 75% did not need institutionalization, being able to remain at home, compared to only 53% in the placebo group. The necroptic studies of the deceased cases, after the finalization of the trial, showed reduced deposition of amyloid in the brain. Now are conducted experiments to elaborate safer vaccines. It is unknown why not all the vaccinated cases responded to the vaccine; probably the particular features of the aging process and the individual variability of the immune system are making the difference (24).

There are numerous studies which investigate the prophylactic and curing potential of plants, used in the traditional medicine (chinese, indian, european) in Alzheimer's disease. *Ginkgo biloba* has the property to neutralize the neurotoxic effect of the amyloid peptides, largely attributed to its antioxidant action. Galantamine, an alkaloid of the Amarylidaceae family was used in the southern Russia and in Bulgaria in the treatment of the poliomyelitis; its properties of acetylcholinesterase inhibitor were signaled in the fifties and consequent studies had led to the commercialization of Galantamine. Different components from *Panax ginseng, Lyciumbarbarum, Uncaria rhyncophylla, Yokukansan* and a lot of other plants, used in the oriental traditional medicine, as well as *Salvia officinalis, Crocus sativus* (saffron), *Mellisaofficinalis*, used in the european traditional medicine, proved useful in the therapy of the memory troubles and in the improvement of the cognitive dysfunctions of Alzheimer's

disease (25) .

While some researchers are deploring the insufficient financing of the research efforts in the Alzheimer's disease (for example the winner of The Nobel Prize for medicine for 2010, Robert G. Edwards), others, like dr. Peter Whitehouse, believe that in fact Alzheimer's disease is overestimated and should be considered an aspect of the aging process.

REFERENCES

1. Alzheimer's Disease International, *World Alzheimer Report 2009* – Scientific Group, Institute of Psychiatry, King's College London – dr. C.P. Ferri, R. Sousa, E. Albanese, W.S. Ribeiro, M. Honyashiki, Editors prof. M. Prince, Inst. Of Psychiatry king's College London (chapter 1-2), Jim Jackson (chapter 3-4).
2. Ji C, Song C, Zuo P – The mechanism of Memory Impairment Induced by Aβ Chronic Administration Involves Imbalance Between Cytokines and Neurotrophins in the Rat Hippocampus, *Curr AlzheimerRess*, jan. 2011 (Epub ahead of print).
3. Rocchi A, Pellegrini S, Siciliano C, Murri L – Causative and susceptibility genes for Alzheimer's disease: a review, *Brain Res Bull* 2003; 61: 1-24.
4. Pimplikar SW, Nixon RA, Robakis NK, Shen J, Tsai LH **–** Amyloid-independent Mechanismsin Alzheimer Disease Pathogenesis, *J of Neurosciences* 2010; 30(45): 14946-14954.
5. Jones RW – Inflammation and Alzheimer's disease, *Lancet* 2001, 358: 436-437.
6. Streit JW, Xue QS **–** The Brain's Aging Immune System, *Aging and Disease* 2010; 1(3): 254-261.
7. Hyman BT, Tanzi R – Molecular Epidemilogy of Alzheimer's Disease, *N Engl J Med* 1995; 333: 1283-1284
8. Pizza V, Agresta A, D'Acunto CW, Testa M, Capasso A – Neuroinflammation and Ageing: Current Theories and an Overview of Data, *Rev Recent Clin Trials* 2011 (Epub ahead of print).
9. Paricak-Vance MA, Grubber J, Bailey LR, Hodges D – Identification of novel genes in late-onset Alzheimer's disease, *ExpGerontol* 2000; 35: 1343-1352
10. Tripoliti E, Fotiadis ID, Argyropoulou M– Diagnosis of Alzheimer's Disease using fMRI Data and Modifications of Random Forests Algorithm, *World Congress of medical Physicsa and BiomedicalEngineering*2009; 25(2): 754-757.
11. Choi YS, Choe LH, Lee KH – Recent Cerebrospinal Fluid Biomarker Studies of Alzheimer's Disease, *Expert Rev, Proteomics* 2010; 7(6): 919-929.
12. Whitehouse P, George D – The Myth of Alzheimer's: What You Aren't Told about Today's Most Dreaded Diagnosis, 2010, *http//booksandideas.com*.
13. Mahoney AEJ– Age- or stage-appropriate? Recreation and the relevance of Piaget's theory in dementia care, *Am J Alzheimer's Dis Other Demen* 2003; 18: 24-28.
14. Tarraga L Modinos G, Espinosa A, Diego S– A randomized pilot study to assess the efficacy of an interactive, multimedia tool of cognitive stimulation in Alzheimer's disease, *J Neurol Neurosurg Psychiatry* 2006; 77: 1116-1121.
15. Larson EB et al – Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older, *Annals of Internal medicine* 2006; 144(2): 73-81.
16. Szeckely CA et al– Nonsteroidal anti-inflammatory for the prevention of Alzheimer's disease: a systematic review, *Neuroepidemiology* 2004; 23(4): 159-169.
17. Boothby LA, Doering PL – Vitamin C and vitamin E for Alzheimer's disease, *Annals ofpharmacotherapy* 2005; 39(12): 2073-2080.
18. Lorett A, Badia MC, Mora NJ, Pallardo FV– Vitamin E paradox in Alzheimer's disease: it does not prevent loss of gognition and may even be detrimental, *J Alzheimer's Dis* 2010; 19 (1): 27-30

19. Raina P, Santaguida P, Ismaila A– Effectiveness of cholinesterase inhibitors and memantine for treating dementia: evidence review for a clinical practice guideline, *Ann Intern Med* 2008; 148: 379-397.
20. Knopman DS, DeKosky ST, Folstein M– Practice parameter: Diagnosis of dementia (en evidence based review); Report of the Quality Standards Subcommitte of the American Academy of Neurology, *Neurology* 2001; 56: 1154-1166.
21. Eastman P – New Alzheimer's Disease Therapies Move Forward, Despite some setbacks, *NeurologyToday* 2007; 7(13): 12-15.
22. Wang J, Feruzzi MG, Varghese M, Qian X, Cheng A– Preclinical study of dimebon on beta-amyloid-mediated neuropathology in Alzheimer's disease, *MolNeurodegener* 2011; 19 (6): 1-7.
23. Pratt KG, Zhu P, Watari H, Cook DG– A novel role for (gamma)-secretase: Selective Regulation of Spontaneous Neurotransmitter Release from Hippocampal Neurons, *J Neurisci* 2011; 31(3): 1023-31.
24. Morley JE – Alzheimer's disease: future treatments, *J Am Med DirAssoc* 2011; 12(1): 1-7.
25. Eckert GP – Traditional used plants against cognitive decline and Alzheimer disease, *Frontiers inPharmacology* 2010; 1(138): 1-10.

NEWS

MOLECULAR EPIDEMIOLOGICAL SURVEILLANCE FOR TUBERCULOSIS BY WHOLE GENOME SEQUENCING

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis* characterized by a high rate of incidence and mortality worldwide. Although the World Health Organization (WHO) established a global program for controlling and monitoring tuberculosis, the decline in the number of cases has been slow. Thus, the TB control strategies have targeted the development of molecular techniques to monitor disease transmission. Genotyping methods analyzed a small part of the bacterial genome for specific identification of strains, but they may not be able to determine the origin a tuberculosis outbreak or how it spread in population. A longitudinal molecular epidemiological study conducted by Niemann and collaborators showed that whole genome sequencing (WGS) provides more information about transmission chain of TB than classical genotyping methods: IS6110 RFLP (restriction fragment length polymorphism) and 24-locus MIRU-VNTR (mycobacterial interspersed repetitive unit–variable number of tandem repeats). The authors a study demonstrates that WGS is able to characterize clinical isolates with high discriminatory power, at strain level. Using WGS method, the researchers have estimated that the *M. tuberculosis* has a time-dependent genomic variation with a rate at 0.4 mutations per genome per year and with a doubling time of 22 hours, or 400 generations per year. Based on the results of the study, the same authors concluded that, in the context of decreasing sequencing costs, WSG can be an effective method for TB surveillance by identification of outbreak-related transmission chains (Roetzer A, Diel R, Kohl TA et al. Whole Genome Sequencing versus Traditional Genotyping for Investigation of a Mycobacterium tuberculosis Outbreak: A Longitudinal Molecular Epidemiological Study. *PLoS Medicine*, 2013;10:e100138 DOI: 10.1371/journal. pmed.1001387).

Cătălina Luncă