Argyrophilic Grain Disease: Case Report of the First Two Cases in the Czech Republic and Review of the Literature

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Summary

Argyrophilic grain disease (AgD) is a relatively newly described neurodegenerative disease with late-onset dementia. Morphologically it is characterized by the presence of abundant spindle-shaped argyrophilic grains (ArG) in neuronal processes and coiled bodies in oligodendrocytes. ArG consist of abnormally hyperphosphorylated form of tau protein. AgD is a substrate of at least 5% of all dementia cases with increasing incidence in the old age. Here we report the cases of a 91-year-old woman and an 83-year-old man clinically diagnosed with dementia. Neuropathological, histochemical and immunohistochemical examination of the brain tissue show the changes to be compatible with a definite diagnosis of AgD. This is the first description of two cases of AgD in the Czech Republic.

Key words: argyrophilic grain disease – dementia – tau protein

Souhrn

Nemoc s argyrofilními zrny: kazuistické sdělení prvních dvou případů diagnostikovaných v ČR a přehled literatury

Nemoc (demence) s argyrofilními zrny (AgD) patří mezi neurodegenerativní onemocnění spojená s hromaděním patologické hyperfosforylované formy tau proteinu. Klinicky se obvykle projevuje jako demence v pozdním sénium a její diagnostika je do současné doby možná pouze posmrtným neuropatologickým vyšetřením mozkové tkáně. Neuropatologické vyšetření mozkové tkáně 83letého muže a 91leté ženy s klinicky diagnostikovanou demencí bez bližší specifikace bylo doplněno impregnací solemi stříbra a imunohistochemickým průkazem tau proteinu monoklonálními protilátkami proti jeho hyperfosforylované formě. Morfologický obraz AgD je charakterizován přítomností početných argyrofilních zrn v neuropilu a specifickými perinukleárními inkluzemi v subkortikálních oligodendrogliích v oblasti spánkových laloků. V obou vyšetřovaných případech byla splněna diagnostická kritéria definitivní AgD. Jedná se o první popis tohoto dle literárních údajů relativně častého neurodegenerativního onemocnění v České republice.

Klíčová slova: nemoc s argyrofilními zrny – demence – tau protein

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In 1987 Braakęs group described for the first time argyrophilic grains (ArG) in brains from patients with late-onset dementia (2). In 1989 the same authors found ArG as the main histopathological change in 28 brains in an autopsy study of 80 demented subjects (3). This observation suggests a very high incidence of silver grains in the brains of old demented patients. ArG represented the unique pathological finding in 10 of these cases. In the 18 remaining cases ArG were associated with Alzheimer type changes (3). With these findings the term dementia with argyrophilic grains, or argyrophilic grain disease (AgD) started to be used. Recent clinicopathological studies of demented people showed that AgD is a frequent, neuropathologically well-defined entity from the group of neurodegenerative diseases called tauopathies (28-30). The frequency of the AgD related neuropathological changes in the brains of elderly people vary from 5% to 23% (6, 9, 10). A cognitive decline was reported in the variation from 20% of AgD cases in Braak's series to 43.2% published by Saito and coworkers (22). In a more recent study by Knopman et al., ArG were found in 31% of brains from cognitively normal elderly subjects (18). Concluded from relevant data, what is significant for this time is that: (I) the presence

of ArG in a human brain is not associated with dementia in all cases, (II) ArG are not physiological age-related changes of the human brain, and (III) AgD is a morphological substrate of at least 5% of neurodegenerative dementia cases in advanced age.

Materials and methods

Patients

A 91-year-old polymorbid woman who died from heart failure and an 83-year-old polymorbid man who died from terminal bronchopneumonia were subjects of autopsies. Cognitive impairment clinically diagnosed as "dementia AD type" in the first case, and with clinical diagnosis dementia caused by "AS cerebri" without any detailed specifications raised suspicion of the involvement by Alzheimer disease (AD). Unfortunatelly, relevant evaluations of cognitive status were not performed before the death. The brains showed only mild diffuse cortical atrophy; in gross examination there was no atrophy of the hippocampus formation or the amygdaloid complex, only a small internal hydrocephalus was seen. The brain weight was 1130g and 1250g, respectively.

Neuropathology

The brains were investigated after a 3-week-long fixation in 10% buffered formaldehyde following a standardised protocol based on the modified Spielmayer scheme. The formalin-fixed tissue blocks were routinely processed and embedded into paraffin. Routine hematoxyline and eosin staining was performed followed by the histochemical method using luxol fast blue for detailed examination of myelin and impregnation by silver salts. For silver staining, the method AgNOR was routinely used, and after that complemented by the Gallyas impregnation method.

Immunohistochemistry

Tissue slices (3 μm) were deparaffinized and transferred into the water solution of tris buffered saline (TBS). Consequently they were boiled in citrate buffer (pH 7.6) 3x5 minutes in a microwave oven. Endogenous peroxidase was blocked with 0.05 mg of natrium acid and 5 ml of hydrogen peroxide in 50 ml of demineralized water. Non-specific positivity was blocked with 150 μl of rabbit serum in 10 ml of TBS for 30 minutes.

The sections were incubated overnight at 4°C with the rabbit polyclonal primary anti-human tau protein antibody (A 0024 DakoCytomation, Glostrup, Denmark) diluted 1:500 and mouse monoclonal anti-human PHF-Tau antibody (Clone AT8, MN1020 Pierce Biotechnology, Rockford, USA) diluted 1:1000 in 5% fetal bovine serum in TBS. The detection of immunostaining was performed using the Envision® kit, and diaminobenzidine was used as a chromogen. Specimens incubated with secondary antibody only and with nonspecific isotype-matched primary antibodies were used as a control of specificity. Mayer's hematoxylin was used as a nuclear counterstain.

Results

Neuropathological investigation of the brain tissue in both cases showed only very slight features of AD pathology. Only very few neurofibrillary lesions (NFL) and isolated primitive senile plaques were found in the hippocampal regions, no developed senile plaques were found (figure 1). Neocortical regions showed only a very smooth degree of the numeric neuronal atrophy and slight isomorphic astrogliosis. Morphological criteria of definite AD were definitely not filled. There were no morphological hallmarks of the other type of "organic" causes of dementia (e.g. Lewy body disease, fronto-temporal dementia, Binswanger disease, Wernicke encephalopathy etc.).

Dominant neuropathological changes were represented by numerous ArG in the hippocampal region detected in routine paraffin embedded tissue by use of conventional silver staining methods (figures 1 and 2). The origin of ArG was confirmed immunohistochemically by a positive reaction using both types of antibodies against tau protein (figure 3). Silver staining and immunohistochemical detection showed the second most important feature for this kind of tauopathy called coiled bodies. Coiled bodies are conspicuous, curvaceous, whip-like and often branched oligodendroglial inclusions, which are located in close vicinity to the cell nucleus (figure 4).

Discussion

The neuropathological criteria for diagnosis of AgD summarised in Table 1 were fullfilled in both cases (11, 23, 29, 30). Argyrophilic grains represent the most important histopathological hallmark. These oval, spindle-shaped, drumstick or comma-like lesions were detected in routine paraffin embedded tissue by use of conventional silver staining methods (AgNOR and Gallyas silver staining) and confirmed after using both

antibodies against tau protein (figure 2 and 3). The highest density of ArG was found close to the transentorhinal-entorhinal border; ArG were abundantly found in the external and internal pyramidal layers of the first Ammon's horn sector (CA1) (figure 1) (8, 19, 23, 26). In other parts, significant decrease of density of ArG was seen. Argyrophilic grains are easily distinguished from the neurofibrillary lesions of Alzheimer disease and it is clear that ArG are distinct lesions among tauopathies (25, 28).

Silver staining and immunohistochemical detection showed the second most important feature for this kind of tauopathy. It is considered that coiled bodies make one of the essential histopathological hallmarks of AgD, and these structures were found as well. Coiled bodies are not specific findings for AgD and they can be found in a variety of tauopathies, e.g. progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease (PiD), Alzheimer disease (AD) and frontotemporal dementia and parkinsonism linked to chromosome 17, associated with tau gene mutations (FTDP-17) (7, 13). In AgD, however, coiled bodies represent a frequent finding in the white matter close to cortical areas and subcortical nuclei full of ArG (20, 29).

The main reason that AgD is still questioned as to whether it represents a distinct disease entity is the observation that most, if not all, reported AgD cases show changes associated with the AD-type pathology especially NFL (5, 12, 24, 27). Some authors have regarded AgD as a

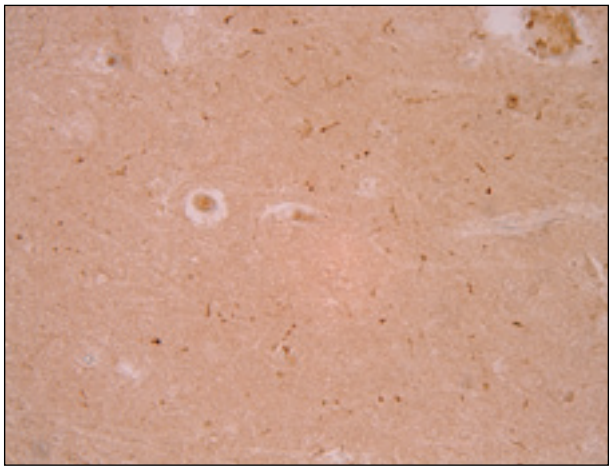

Fig. 1. Diffuse argyrophilic grains (ArG) in the neuropil of hippocampal sector CA1. Note the total absence of senile plaques and neurofibrillary tangles. AgNOR silver impregnation method, original magnification 100x

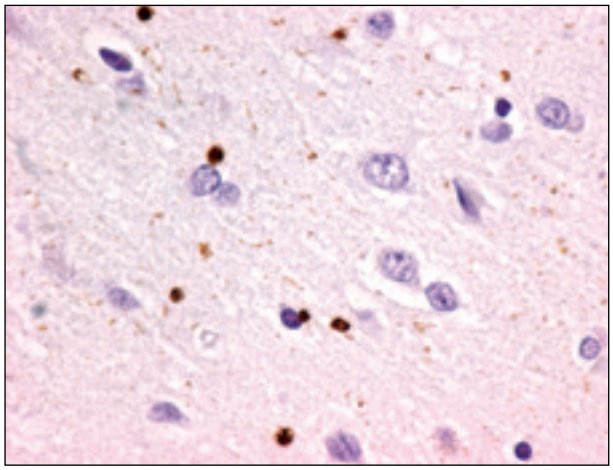

Fig. 3. Abundant AT8-immunoreactive argyrophilic grains in hippocampal sector CA1, original magnification 400x

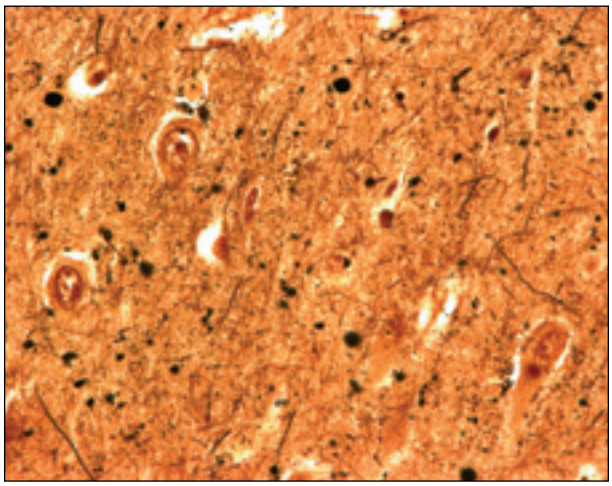

Fig. 2. Details of the morphology of diffuse argyrophilic grains (ArG) in the neuropil of hippocampal sector CA1. Gallyas silver impregnation method, original magnification 400x

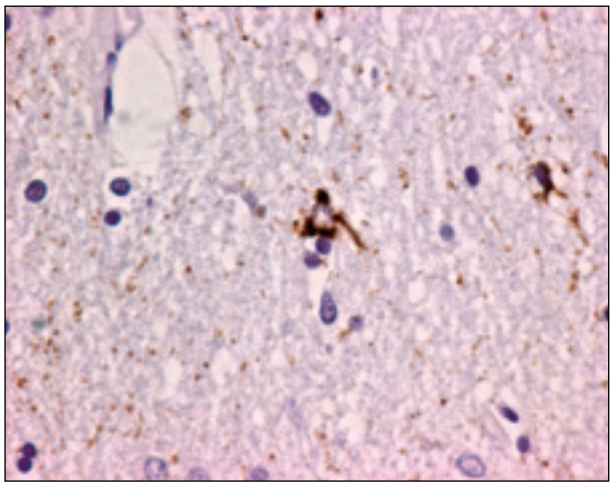

Fig. 4. AT8-immunostained coiled body in the oligodendroglial element in the subcortical white matter close to the cortex. Original magnification 400x

variant of AD. However, new biochemical data show that AgD is a unique entity different from AD (12, 25, 33). Biochemical analysis of tau protein pathology started using a system of classification of tauopathies which is now widely accepted. Based on these studies, the following subtypes are distinguished according to their pathological tau profile: class I (as in AD; Tau 60, 64, 69, minor 74; 3R and 4R tau isoforms), class II (as in PSP and CBD; Tau 64, 69, minor 74; predominantly 4R isoforms), class III (as in PiD; Tau 60, 64, minor 69; mainly 3R isoforms), class IV (as in NFL of myotonic dystrophy), and class 0 (as reported in some sporadic and inherited cases of frontotemporal dementia). Recent biochemical analysis of AgD cases revealed that AgD is a class II (4R) tauopathy, similar to PSP and CBD but distinct from AD and PiD. Using monoclonal antibodies specific to tau isoforms with four (4R) or three (3R) repeats in the microtubule-binding domain confirmed that ArG are immunostained with 4R, but not with 3R antibodies.

In presented cases only a very small amount of typical features of AD pathology were found during neuropathological examination of investigated brains. Only very few NFL and isolated primitive senile plaques were located in the hippocampus, no developed senile plaques were found (figure 1), and criteria of AD were definitely not filled (4, 21).

AgD shows a significant correlation with advancing age but ArG might or might not be associated with a cognitive decline. Both of our cases were diagnosed with significant cognitive decline but without serious psychological investigation. In this time, only very little is known about the clinical presentation of AgD patients, especially the early symptoms. In the original paper by Braak and Braak, all demented patients were reported to suffer from adult-onset progressive mental deterioration (2). During the course of the disease, patients became anxious, restless, often depressed and emotionally shallow (6, 15, 16). Many of them showed incontinence and cachexia. Quite detailed clinical data have been provided by Itagaki *et al.* and Yamada *et al.* (17, 31) yet it is uncertain whether their patients represent 'typical' AgD cases, because post-mortem examination of the brains revealed, in addition to ArG, extensive subcortical pathology (astrocytic gliosis in striatum, amygdala, thalamus, substantia nigra).

Although AgD shares pathological, biochemical and probably also genetic similarities with PSP and CBD, there are significant differences between the clinical presentation of these disorders. In contrast to PSP and CBD, motor symptoms, eye movement abnormalities and parkinsonism are not characteristic clinical features in AgD patients. Therefore, AgD patients should normally not be misdiagnosed as suffering from PSP or CBD, respectively. AgD, however, might be difficult or nearly impossible to distinguish clinically from early AD or other 'limbic' forms of dementia, among them "senile dementia with neurofibrillary tangles" (14, 32), the localized type of AD observed in old patients, and hippocampal sclerosis (1).

Tab. 1. Argyrophilic grain disease (AgD): neuropathological and biochemical features

1. **Core lesions of AgD**
   - 1.1. Essential for diagnosis
     - Argyrophilic grains (Gallyas-positive, Tau-positive)
   - 1.2. Consistent features but not essential for diagnosis
     - Coiled bodies (Gallyas-positive, Tau-positive)
     - Abundant non-argyrophilic (Gallyas-negative), tau-positive limbic projection neurons ('pretangle' neurons).
   - 1.3. Biochemical tau profile of AgD
     - Tau doublet at 64 and 69 kDa. Pathological tau aggregates mainly made of four-repeat tau isoforms
2. **AgD associated lesions – frequent findings but not essential for diagnosis**
   - Ballooned neurons (Gallyas-negative. Tau- and B-crystallin-positive. Present in amygdala and layers V and VI of basal temporal neocortical areas)
   - Non-argyrophilic (Gallyas-negative), tau-positive astrocytes (present in amygdala and entorhinal and transentorhinal cortices)
   - Associated lesions of the Alzheimer-type
   - Neurofibrillary lesions (Braak stages I-III; frequent finding)
   - Senile plaques (few, mainly diffuse type; two thirds of cases)
3. **AgD associated lesions – atypical findings**
   - Superficial laminar spongiosis (layers II-III of basal temporal neocortical areas)
   - Cortical and subcortical gliosis (entorhinal and transentorhinal cortices, posterior parahippocampal gyrus)

Adapted from Tolnay *et al.* (30)

Conclusion

It has been elucidated that AgD is a dementing disorder of old age more frequent than first believed. Morphological, immunohistochemical, biochemical and genetic studies strongly support the view that AgD is a unique entity, separate from AD. More precise epidemiological studies should be done for determination of true incidence of AgD in the population especially in the Czech Republic, from where we present the first two cases of this disease. However, it might be difficult to clinically

diagnose AgD, especially in a differential diagnostic procedure in dementia of the AD type. AgD takes place in the neurodegenerative disorders called tauopathies but further neuropathological, biochemical and genetic studies will be needed to define AgD in the group of 4R tauopathies (as PSP and CBD). Moreover, there is an urgent need for a precise clinical definition of AgD, what the clinical criteria for its diagnosis are, and how to distinguish it from AD.

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