Review article

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Amyotrophic lateral sclerosis – looking for pathogenesis and effective therapy

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Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by loss of motor neurons in the spinal cord, brain stem and motor cortex which dramatically reduces life expectancy. ALS occurs either in familial or, more frequently, in sporadic forms. It finally results in death due to respiratory failure that occurs typically 2-5 years after the disease onset. Although the aetiology of ALS remains largely unclear, its heterogeneity suggests that a combination of various factors, including endogenous and/or environmental ones, may be implicated in progressive motor neuron stress that results in the activation of different cell death pathways. Interactions between genetic, environmental, and age-dependent risk factors have been hypothesized to trigger disease onset.

Despite extensive neurobiological, molecular and genetic research, at the beginning of the 21st century ALS still remains one of the most devastating neurodegenerative diseases because of the lack of effective therapeutic strategies. It is a challenge for the clinical and scientific community.

Better understanding of the aetiology of amyotrophic lateral sclerosis is necessary to develop effective treatment of this progressive neurodegenerative disease. This review presents the current state of knowledge in ALS research.

Key words: amyotrophic lateral sclerosis (ALS), aetiology, ALS neuropathology, differential diagnosis, treatment.

Introduction

Amyotrophic lateral sclerosis (ALS), also known as Charcot's disease or in the USA as Lou Gehrig's disease, was first described by Jean-Marie Charcot (1825-1893) and Alexis Joffroy (1844-1908) [18].

ALS is a rapidly progressive, fatal neurological disease. It affects both upper and lower motor neurons (MNs) in the brain and in the spinal cord. ALS belongs to the group of disorders classified as motor neuron diseases, characterized by the gradual degeneration and death of motor neurons. With voluntary muscle action progressively affected, patients in the later stages of the disease become totally paralyzed.

ALS is the most frequent adult-onset motor neuron disorder. The prevalence of the disease is 3-5/100 000. It occurs 10 times less often than multiple sclerosis. The incidence of ALS in Europe is approximately 2 per 100 000 per year. ALS usually strikes in the 5th or 6th decade and its occurrence is more frequent in men than women. In younger patients it occurs with an incidence rate of about 1-3/500 000.

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Three Western Pacific foci of increased incidence of ALS and ALS plus syndromes have been found in Guam, in western New Guinea, and in the Kii peninsula in Japan. Nowadays, in the United States, approximately 20 000 people suffer from amyotrophic lateral sclerosis, and an estimated 5000 new cases are diagnosed each year.

In 90-95% of all ALS cases, the disease occurs randomly as sporadic ALS (SALS) with no clearly associated risk factors and about 5-10% are diagnosed as inherited familial ALS (FALS). The most common inheritance pattern for FALS is autosomal dominant. About 20% of familial ALS cases are the result of an inherited genetic mutation on chromosome 21, in the gene encoding the superoxide dismutase 1 (SOD1) enzyme. However, not all people with a mutated form of SOD1 develop ALS. Clinically, FALS and SALS are basically similar, but the inherited one usually affects younger patients. The prognosis in ALS is terminal and life expectancy of patients is usually 3 to 5 years after diagnosis.

Aetiology

Amyotrophic lateral sclerosis is likely to be a multifactorial and multisystem disease. The pathogenic mechanisms that underlie ALS remain largely unclear, but a large spectrum of aetiological factors have been considered including genetics, autoimmune responses, environmental factors, oxidative stress, glutamate excitotoxicity, mitochondrial damage, defective axonal transport, glial cell pathology, aberrant DNA and RNA metabolism, toxic effect of heavy metals, viral infection, paraneoplastic syndromes, lymphomas, paraproteinaemias and many others.

There have been identified several genes that might be considered as a cause or risk factor for ALS development [4]. Identification of genes causing FALS has been instrumental in understanding the pathogenesis of ALS. Many ALS-related genes play a role in RNA processing pathways which include RNA transcription, removal of noncoding introns by pre-mRNA splicing, editing, transportation, translation, and degradation. The following RNA processing genes are implicated in ALS and other motor neuron diseases: *ANG*, *ELP3*, *FUS/TLS*, *SETX*, *SMN* and *TARDBP* [10]. An important step toward finding the causes of ALS was made in 1993, when it was discovered that mutations in the gene producing the superoxide dismutase 1 (SOD1) enzyme were associated with some cases of familial ALS [12]. More than 100 mutations in the *SOD1* gene have been reported. In the United States, 50% of mutant SOD1 ALS patients have an alanine-to-valine mutation in codon 4 of the gene (A4V mutation); their survival is on average 12 months from disease onset [54].

SOD1 acts as antioxidant and protects the cells from DNA damage caused by the accumulation of free radicals which are produced by cells during normal metabolism. If these highly reactive molecules are not neutralized, they can accumulate and induce damage of DNA and intracellular proteins. However, several reports have demonstrated that toxicity of mutated *SOD1* is not due to the decreased antioxidant activity, but rather to a "gain of unknown toxic function". There has been characterized a molecular pathway by which mutated *SOD1* contributes to the accumulation of malformed proteins with the possibility to form amyloid fibrils inside the endoplasmic reticulum (ER) compartments of motor neurons, which finally results in cell death. This suggests an analogy to other protein misfolding disorders such as prion diseases [19].

There have recently been identified ubiquitinated inclusions containing pathological forms of TAR DNA-binding protein-43 (TDP-43) in the cytoplasm of motor neurons of patients with sporadic ALS and in some patients with frontotemporal dementia [3,45]. TDP-43 is the RNA processing protein normally found mainly in the nucleus. Shortly after identification of TDP-43 positive inclusions, they were also identified in patients with non-SOD1 FALS [63]. Moreover, the mutations in the gene on chromosome 1 coding for TDP-43 were identified in patients with sporadic and familial forms of ALS. It has been established that mutations in the *TDP-43* gene account for 5% of patients with FALS. TDP-43 inclusions have been found in more than 90% of patients with sporadic ALS, in patients with Guamanian parkinsonism-dementia complex and in patients with familial British dementia [28,56].

Recently, a mutation in another gene for RNA processing protein, fused in sarcoma/translated in liposarcoma (*FUS/TLS*), has been reported. The *FUS* gene, located on chromosome 16, encodes a multifunctional protein thought to be involved in DNA repair and regulation of transcription from DNA to the related compound of RNA. FUS protein belongs to the family of RNA-binding proteins which have been implicated in cellular processes that include regulation of gene expression, maintenance of genomic integrity and mRNA/microRNA processing. Its name

is derived from previous identification of its role in a type of cancer called sarcoma. The FUS pathology has been found in sporadic ALS and in most familial ALS cases [34,66,67,70]. Normally, FUS protein molecules stay in the nucleus and do not clump together. However, when made from mutated *FUS* genes, they are more likely to be located in the cytoplasm, where they tend to clump together. This type of aggregation has been correlated with degeneration of nerve cells in ALS. In fact, the clumps are similar in appearance to those found in another rare form of familial ALS that is caused by mutations in the *TDP43* gene. In patients with *FUS/TLS* mutations cytoplasmic inclusions containing FUS/TLS but not TDP-43 could be found.

All mutations in specific genes have been identified in about 30% of familial ALS cases (10% of all cases). Mutations in both the TAR DNA-binding protein gene (*TDP43*) and the *FUS* gene occur in about 4 to 5% of familial ALS cases. It is probable that patients with the FUS pathology may account for about 80% of all ALS cases. The exceptions to this finding are familial ALS cases associated with a mutation on the *SOD1* gene. In those patients, as well as in the mutant SOD1 transgenic mouse models, evidence of FUS pathology has not been found [22,60]. These observations supported by the results of genetic examinations suggest that disturbances in RNA metabolism are the most important directions in the pathophysiology of the majority of ALS cases.

Considering the aetiology of ALS, one more gene called the *SMN* gene, located on chromosome 5q and coding the survival motor neuron (SMN) protein, should be mentioned. The *SMN* gene exists in two highly homologous copies, telomeric (*SMN1*) and centromeric (*SMN2*). The SMN protein is found throughout the body, with high levels in the spinal cord. It is particularly important for the maintenance of viability of motor neurons located in the spinal cord, the brain stem and motor cortex. The SMN protein plays an important role in processing mRNA and is probably important for the development of MN dendrites and axons. Without mature mRNA, the production of proteins necessary for cell growth and function is disrupted. Motor neurons are particularly sensitive to this abnormality and die prematurely. The main motor neuron disease connected with SMN pathology is spinal muscular atrophy (SMA), caused by mutations in *SMN1* but not *SMN2*. About 95% of individuals with spinal muscular atrophy have mutations in both copies of the *SMN1* gene. As a result, little or no SMN protein is made. There have been identified at least 65 mutations in the *SMN1* gene that cause SMA. It has been investigated whether genetic mechanisms of motor neuron death in SMA might be implicated in neuronal injury in ALS. Some studies suggest that an abnormal number of *SMN1* genes in each cell may be associated with an increased risk of developing amyotrophic lateral sclerosis. Compared to people with two copies of the *SMN1* gene, patients with amyotrophic lateral sclerosis were more likely to have one or three copies of the *SMN1* gene. An abnormal number of *SMN1* genes alters the amount of SMN protein that is produced. It was found that the presence of a homozygous *SMN2* deletion was overrepresented in patients with ALS compared to controls. However, *SMN2* mutation is not pathogenic [68]. Too little or too much SMN protein may impair the function of motor neurons, which increases the risk of developing amyotrophic lateral sclerosis.

Looking for any genetic risk factors of ALS, special attention has been paid to a mutation on chromosome 9p21 as a major cause of FALS in Finland, a country with one of the highest rates of ALS in the world [35]. Many other genes are probably involved in pathogenesis of ALS, as has been reported by different authors [38].

Despite numerous potential genetic abnormalities that might be considered as a cause or risk factor for ALS development, it is still unknown which genes may play a critical role in ALS aetiopathogenesis, and in which way.

Despite gene mutations in ALS, mitochondrial dysfunction is likely to be an important point of multiple pathways underlying the ALS pathogenesis and course. Mitochondria have been known to be an early target in ALS pathology and contribute to disease progression. Morphological and functional defects in mitochondria were found in both human patients and ALS mice overexpressing mutant SOD1. The SOD1 mutation was found to be preferentially associated with impairment of mitochondrial function. Recent studies suggest that axonal transport of mitochondria along microtubules is disrupted in ALS. These results also illustrate the critical importance of maintaining proper mitochondrial function in axons and neuromuscular junctions, supporting the emerging "dying-back" axonopathy model of ALS [5,57].

Excitotoxicity is undoubtedly one of the most accepted pathogenetic mechanisms of neuronal pathology in ALS. Glutamate-induced excitotoxicity is responsible for neuronal death in acute neurological

conditions as well as in chronic neurodegeneration and probably plays a role in motor neuron disorders [55]. It has been found that, compared to healthy people, ALS patients exhibit higher levels of glutamate in the serum and spinal fluid. Laboratory studies have demonstrated that neurons begin to die when they are exposed over a long period to excessive amounts of glutamate. The motor neuron death seems to be related to a high glutamate-induced calcium influx in combination with a low calcium buffering capacity. Excitotoxicity remains an intriguing pathological pathway that could explain the motor neuronal death and indicates the disrupted role of surrounding non-neuronal cells in ALS [11,21].

The role of neuroinflammation in amyotrophic lateral sclerosis (ALS) is unclear but it is usually taken into consideration in MNs pathology. It has recently been shown in an animal model of ALS that the balance between neurotoxic and neuroprotective agents plays a key modulating role in the course of progression and clinical symptoms of the disease [26,29].

Autoimmune responses have been suggested as one possible cause of the development of ALS. The presence of auto-antibodies in CSF of ALS patients against cellular proteins of the spinal cord has been found, but their real role is still not defined. Whether they appear as a secondary immunological consequence of neuronal death or alternatively accelerate the course of neuronal degeneration is unclear [47].

Alterations of brain cholesterol homeostasis have recently been considered as one of the possible cofactors in many neurodegenerative disorders, including ALS. The liver X receptor beta (LXRbeta) is involved in lipogenesis and cholesterol metabolism. The results suggest that LXRbeta may inhibit neuroinflammation and maintain cholesterol homeostasis [9]. It has been suggested that statins, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, commonly used in the therapy of cardiovascular diseases, may induce amyotrophic lateral sclerosis (ALS) in some patients. The hypothesis is that statins may induce or aggravate ALS by impairing liver X receptor (LXR) signalling. Statins inhibit the synthesis of endogenous LXR agonists, oxysterols, and decrease the expression of LXR target genes in many cells; they increase the concentration of plant sterols in plasma and tissues, partially by impairing LXR-dependent signalling, which results in augmented intestinal absorption and impaired biliary excretion of plant sterols. Mice lacking LXRbeta exhibit ALS-like phenotype [7].

Considering possible ALS causes, environmental factors such as exposure to toxic or infectious agents have been studied. In Guamanian ALS, the cyanobacterial neurotoxin beta-N-methylamino-l-alanine (BMAA), one of the dietary compounds found in the seed of the cycad *Cycas cirinalis*, a tropical plant, was thought to be a risk factor of the disease. BMAA was used to make flour and it was a major dietary component during the 1950s and the early 1960s, when this type of ALS had an exceptionally high incidence [6,49]. Some other environmental factors were also suspected, e.g. smoking. Active smokers face approximately double risk of developing ALS compared to people who never smoked, whereas former smokers face an intermediate risk [24]. Furthermore, even an established risk factor is not automatically a cause.

Histopathological findings

The neuropathological findings obtained from human ALS autopsy cases usually represent the terminal stage. That is why it is difficult to clarify how and why ALS motor neurons are impaired at each clinical stage of the disease. The essential pathological features of ALS in the terminal stage of all clinical forms are simillar and include motor neuron loss accompanied by presence of characteristic inclusions in the cell cytoplasm, dendrites and axons [31]. Pathological findings in the investigated cases of autopsy-confirmed ALS reveal various morphological changes including atrophy of the motor cortex, loss of MNs in the brain stem and anterior horns of the spinal cord, degeneration of corticospinal tracts, accumulation of lipofuscin, marked chromatolysis, gliosis, numerous macrophages, spheroids, Lewy-body-like inclusions, numerous polyglucosan bodies, Bunina bodies, hyaline inclusions, skein and other ubiquitin positive inclusions. An accumulation of lipofuscin was noted in almost all cases including lower and upper motor neurons. The number of macrophages varied markedly between ALS cases. It has not been definitely decided which of the many kinds of pathological changes represent the main pathological process and which are secondary [1]. Human ALS is classified into two major subtypes, sporadic ALS (SALS) and familial ALS (FALS), that generally exhibit similar histopathology.

An essential histopathological feature of ALS is loss of both upper and lower motor neurons, especially the large cells of the anterior horn throughout the length of the spinal cord (Fig. 1A). The oculomotor, trochlear and

abducens nuclei controlling eye movements, as well as Onufrowicz's nucleus, are usually spared. Degeneration of the corticospinal tracts in the anterior and lateral columns of the spinal cord are particularly evident, especially in the lower spinal cord segments. This supports the hypothesis of a dying back degeneration of axons. In the degenerated primary motor tracts, there is marked loss of large myelinated fibres in association with variable astrocytic gliosis. Destruction of these fibres is usually associated with the appearance of lipid-laden macrophages demonstrated by immunohistochemical studies with expression of ferritin (Fig. 1B). In addition, the striated muscles demonstrate denervation atrophy, i.e., neurogenic muscle atrophy. The surviving motor neurons often reveal cytoplasmic shrinkage and accumulation of lipofuscin granules.

The cytopathology of the affected motor neurons is characterized by the following intracytoplasmic inclusions, which are thought to be the pathognomonic features for ALS:

1. Bunina bodies – small eosinophilic granular inclusions in the anterior horn cells, observed within the cytoplasm or dendrites. Ultrastructurally, Bunina bodies consist of electron-dense amorphous material that contains tubules or vesicles. These specific structures were originally described by the Russian pathologist Bunina in 1962 [14]. Although Bunina bodies might not be histologically detected in all cases of ALS, these structures themselves are currently considered a specific histopathological hallmark of ALS.
2. Skein-like inclusions (SLIs) and round hyaline inclusions (RHIs), negative for filaments or Lewy-like bodies, which represent the intracytoplasmic filamentous structures visible in H&E preparations (Fig. 1C). In more aggregated forms, SLIs show dense collections of filaments formed in spherical structures. Various forms of pale, usually round eosinophilic inclusions could be detected within the neuropil of the anterior horn, some of which might be related to spheroids (Fig. 1D-F). Immunohistochemically, SLIs and RHIs are positive for ubiquitin or TDP43 (Fig. 2A-B).

The histopathology of FALS is usually identical to SALS, and frequently contains Bunina bodies. It might also show degeneration of the middle zone of the posterior column, Clarke nuclei and posterior spinocerebellar tracts. In certain long-surviving FALS patients with SOD1 gene mutations neuronal LBHIs and astrocytic hyaline inclusions (Ast-His) are characteristic intracytoplasmic structures. Another characteristic pathological feature of patients with SOD1-mutated FALS is slight or mild involvement of the corticospinal tract, in contrast to severe degeneration of the lower motor neurons.

Neuronal injury in various pathological conditions is usually associated with marked gliosis, which has long been considered as a non-specific response of glial cells to different noxious factors, known as "reactive astrogliosis" [61]. Widespread astrogliosis with marked immunoreactivity for glial fibrillary acidic protein (GFAP) is commonly observed in amyotrophic lateral sclerosis patients. Transgenic mice expressing SOD1 gene mutation are thought to provide the most suitable animal model of human ALS. The essential cytopathological features of SOD1 transgenic mice consist of motor neuronal loss with astrogliosis and the presence of SOD1-positive inclusions [31]. It has been documented that abnormalities in cytoskeletal proteins in astrocytes are characteristic features of both human sporadic ALS cases and a transgenic rat model of FALS [52]. Considering the neuronal-glial connections it could be suggested that astroglial cells are directly involved in the process of MN degeneration dependent on glutamate excitotoxicity. Marked changes in astrocytes were documented in an *in vitro* ALS model of slow glutamate excitotoxicity [40].

There is increasing evidence that both programmed cell death and so-called "autophagic cell death" participate in cell degeneration in different pathological conditions. Our ultrastructural studies performed on organotypic cultures of rat lumbar spinal cord chronically exposed to specific glutamate uptake blockers DL-threo-b-hydroxyaspartate (THA) and L-trans-pyrrolidine-2,4-dicarboxylate (PDC) documented different modes of cell death, including necrotic, apoptotic and/or autophagic degeneration [39] accompanied by distinct glial changes [40]. Special emphasis is placed on the role of autophagy, which seems to prevail as a protein clearing system over other multienzyme pathways within motor neurons. The evidence which links an altered autophagy to the onset of motor neuron death proposes that this biochemical pathway might represent a final common mechanism underlying both inherited and sporadic forms of ALS [50].

Clinical symptoms

At the onset of ALS, the symptoms may be so slight that they are frequently overlooked. The dis-

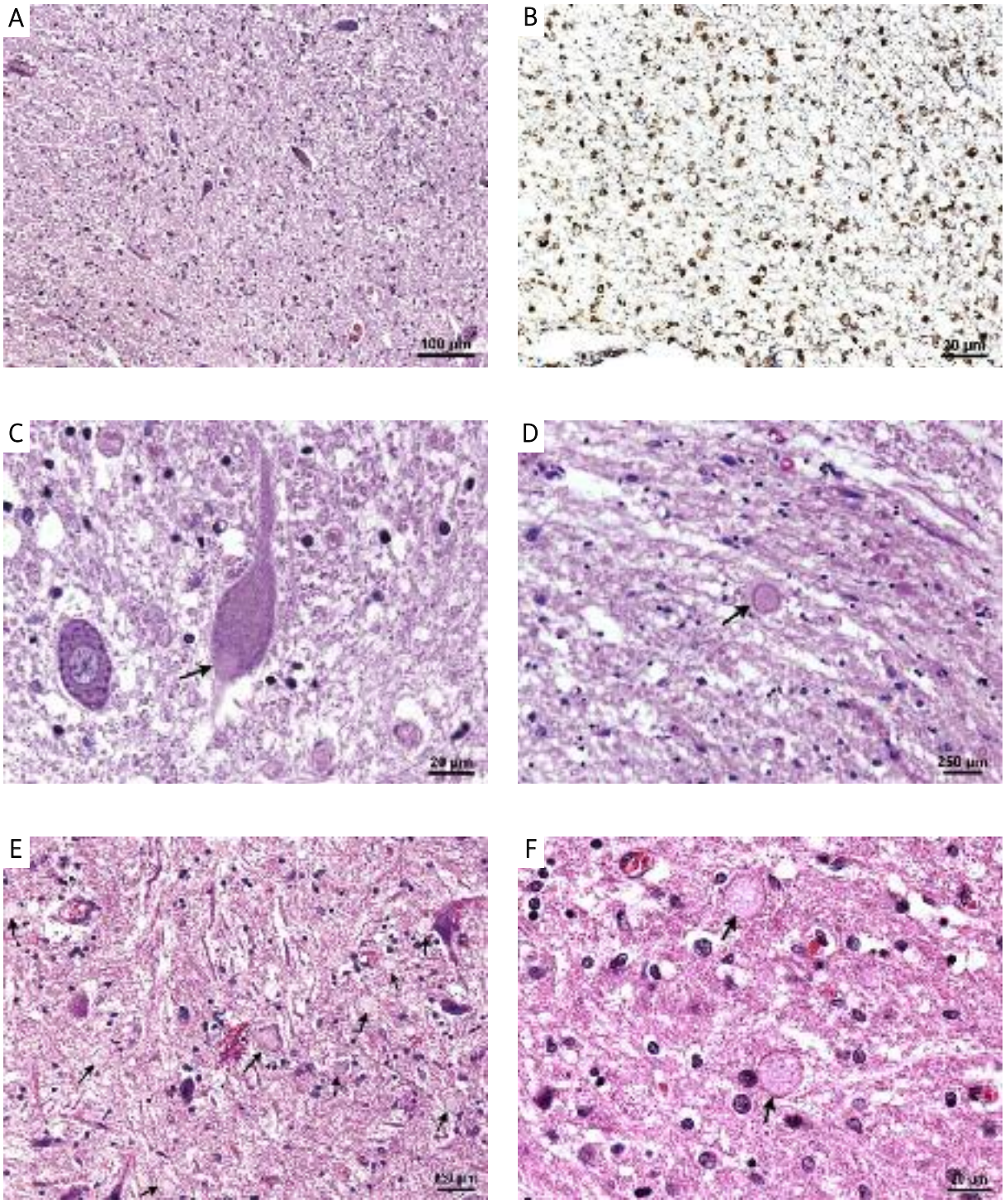

Fig. 1. Neuropathological findings in human ALS (courtesy of Dr Dorota Dziewulska, IMDiK PAN). **A)** Loss of motor neurons in anterior horn of spinal cord, H&E. **B)** Accumulation of macrophages in destroyed corticospinal tract, ferritin immunoexpression. **C)** Round hyaline inclusion in the cytoplasm of a spinal motor neuron (arrow), H&E. **D)** Round hyaline inclusion (arrow) in the spinal anterior horn, H&E; **E)** Numerous eosinophilic inclusions (arrows) within neuropil of spinal anterior horn, H&E; **F)** Round, slightly granular eosinophilic inclusions (arrows) in the spinal anterior horn related to spheroids, H&E.

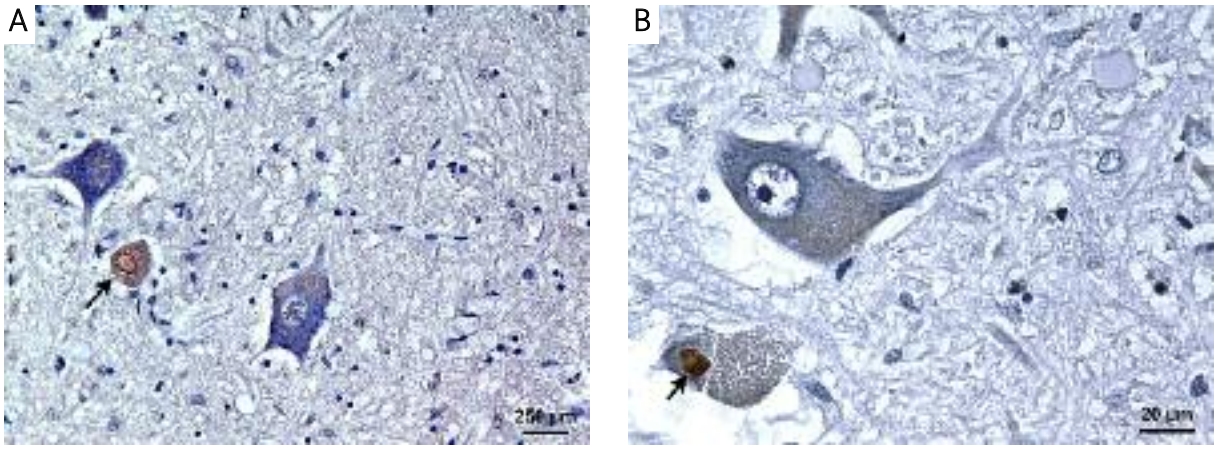

Fig. 2. Immunohistochemical staining with TDP43 (courtesy of Dr Dorota Dziewulska, IMDiK PAN). **A)** Round bodies with strong ring-like immunoreactivity of TDP43 in completely degenerated spinal motor neuron (arrow). **B)** Strong TDP43 expression in degenerated motor neuron (arrow).

ease is characterized by progressive muscle weakness in hands, arms or legs and the muscles responsible for speech, swallowing or breathing. Muscle weakness is a hallmark initial sign in ALS, occurring in approximately 60% of patients. The hands and feet may be affected first, causing difficulty in lifting, walking or using the hands for the activities of daily living such as dressing, washing and buttoning clothes. The muscle weakness is accompanied by fasciculation and cramping of muscles, especially those in the hands and feet. In more advanced stages, the patients experience progressive muscle weakness and paralysis. Shortness of breath and difficulty in breathing and swallowing usually appear. However, not all ALS patients demonstrate the same clinical symptoms or the same sequences and pattern of disease progression. When the breathing muscles become affected, ultimately, the patient will need permanent ventilatory support.

Since ALS attacks predominantly motor neurons, the sense of sight, touch, hearing, taste and smell are not affected. For many people muscles of the eyes and bladder are generally not involved. The disease does not impair an individual's mind, personality, intelligence or memory.

In neurological examination the following are usually observed: Babinski's sign, hyperactive tendon reflexes, impaired speech (dysarthria), impaired swallowing (dysphagia), increased muscle tone and spasticity, rapidly alternating muscle contractions and relaxations (clonus). Twitching may occur in the tongue and in affected limbs. The patient may experience muscle pain and muscle cramps, and greater difficulty swallowing saliva and liquids than solid food. ALS patients often experience fear, anxiety, and depression [48,62].

Classification

The term classic amyotrophic lateral sclerosis is reserved for the form of disease that involves upper and lower motor neurons. If only lower motor neurons are involved, the disease is called progressive muscular atrophy (PMA). When only upper motor neurons are involved, the disease is called primary lateral sclerosis (PLS). Occasionally, the disease is restricted to bulbar muscles, and called progressive bulbar palsy (PBP). Most patients with initial involvement of bulbar muscles evolve to classic ALS.

The World Federation of Neurology (WFN) has developed an algorithm that combines the clinical findings and, in some cases, electrophysiological findings, to express the degree of ALS development at the time of the examination (the El Escorial criteria) [13], revised some years later and still valid [43,53].

For the diagnosis of ALS, the WFN criteria require all of the following: evidence of upper motor neuron findings, evidence of lower motor neuron findings, evidence of progression (within the site of onset and beyond the site of onset). Alternative causes for this presentation and findings need to be excluded. For the purpose of applying the WFN criteria, 4 regions or levels of the body are recognized: bulbar (muscles of the face, mouth, and throat), cervical (muscles of the

back of the head and the neck, shoulders, and upper back, and the upper extremities), thoracic (muscles of the chest and abdomen and the middle portion of the spinal muscles), lumbosacral (muscles of the lower back, groin, and lower extremities).

There are the following distinguished qualifying terms for ALS diagnosis up to Revised El Escorial diagnostic criteria for ALS:

- Definite ALS – lower and upper motor neuron signs involving three regions.
- Probable ALS – lower and upper motor neuron signs involving two regions.
- Laboratory-supported probable ALS – lower and upper motor neuron sign involving one region or upper motor neuron signs in one or more regions with electromyographic evidence of acute denervation in two or more limbs.
- Possible ALS – lower motor neuron and upper motor neuron signs involving one region.
- Suspected ALS – lower motor neuron signs only in one or more regions or upper motor neuron signs only in one or more regions. When it is a pure lower motor neuron syndrome, wherein the diagnosis of ALS could not be regarded. This category is deleted from the Revised El Escorial Criteria for the Diagnosis of ALS.

Recently, a group of experts proposed to revise the WFN criteria further, primarily by giving equal weight to clinical and electrophysiological evidence of denervation [17].

Diagnostic tests and differential diagnosis

Clinical diagnosis of ALS is based on signs of progressive upper and lower motor neuron dysfunction supported by exclusion of other neurological conditions with similar symptoms. Currently, there is no single diagnostic test or procedure that might be useful to confirm or exclude the diagnosis of ALS. A comprehensive diagnostic schedule includes mainly electrodiagnostic tests: electromyography (EMG) and nerve conduction velocity (NCV). To confirm the diagnosis of ALS, the EMG changes ought to be established on three levels with fasciculation on asymptomatic level. In ALS, the speed of the nerves is usually slowed, and compound muscle action potentials (CMAPs) may be decreased. A consensus meeting was held in 2008, to determine the best use and interpretation of electrophysiological data in the diagnosis of ALS. The utility of needle EMG and nerve conduction studies was stressed. It is recommended that electrophysiological evidence for chronic neurogenic denervation should be taken as equivalent to clinical data supporting the involvement of individual muscles. In addition, in the context of a suspected clinical diagnosis of ALS, fasciculation potentials should be taken as equivalent to fibrillation potentials and positive sharp waves in recognizing denervation. These changes allow one to build the category of probable laboratory-supported ALS based on the interpretation of electrophysiological data in the modified El Escorial diagnostic criteria for ALS [17].

Other tests may be performed to exclude many neurological disorders that can mimic ALS. Magnetic resonance imaging (MRI scan) may be used to rule out any spinal cord or brainstem disease. Blood tests may detect abnormal proteins or hormone levels or the presence of heavy metals such as lead associated with other neurological diseases. An analysis of the cerebrospinal fluid ought to be done to establish genetic abnormalities (e.g., viral, autoimmune, neurotoxic).

Certain neurological conditions should be particularly taken into consideration in differential diagnosis of ALS, such as: cervical myelopathy, tumours of the cervical spine, syringomyelia or polyneuropathies. In the course of these diseases some other symptoms not typical for ALS such as paraesthesias and sphincter function disabilities may occur. In such cases MRI might be performed to clear the diagnosis. The diagnostic process should include repeated clinical examinations to evaluate disease progression, repeated electrophysiological and/or neuroimaging examinations to exclude other structural disorders and laboratory analysis to exclude other disorders or support the diagnosis of so-called ALS-plus syndromes (with extra-pyramidal features or dementia), ALS-mimic syndromes (including the post-poliomyelitis syndrome; multifocal motor neuropathy with or without conduction block; endocrinopathies, especially hyperparathyroid or hyperthyroid states; lead intoxication; infections; and paraneoplastic syndromes), or ALS with laboratory abnormalities of uncertain significance.

According to known gene mutations in some ALS cases, prenatal genetic testing technology for the SOD1 mutation exists. The right message on this subject in the right way should be sent to people asking for the preclinical possibility of ALS diagnosis. Presymptomatic testing for FALS seems difficult

because little information can be given to the patient regarding the responsibility of the mutation in the disease, age of onset, and disease trends. The majority of families with FALS (80%) will not have a change in their SOD1 gene and therefore a normal SOD1 genetic test is not informative in a family where an SOD1 change has not been identified. At this time there is no genetic testing to offer to non-SOD1 families. Therefore, the determination that an individual has FALS is typically based on family history rather than a genetic test. It is possible to have prenatal testing or genetic counselling to access the risk of passing ALS along to children [20,23,65]. A family history of ALS does not mean that the children will develop the disease. Not everyone who has the genetic marker will develop symptoms of the disease, and not every child born to a family with a history of ALS will even have the genetic marker. Genetic testing should only be performed after clinical examination and in cases with a proven or uncertain family history of ALS. Because five different parts of the SOD1 gene need to be looked at, the testing usually takes about 2-3 months.

There have been recently identified individual proteins from blood plasma and CSF that are thought to be biomarkers for ALS, although many of these proteins are not unique to this disease. Validation of these findings is necessary to achieve the real role of the proteins as diagnostic biomarkers or disease progression factors. Protein biomarkers specific to ALS could point to another target for drug therapy [33].

Treatment and prognosis

Despite recent advances in understanding of the pathophysiology of ALS, there is no effective cure for this disease. In the course of disease progression patients gradually lose their ability to walk, talk and breath.

Only one agent (riluzole) that slows the progression of ALS has been approved by the FDA so far [8,11]. Riluzole is believed to reduce motor neuron damage by decreasing the release of glutamate. It does not reverse the damage of motor neurons that has already occurred, and people taking it must be monitored for liver damage and other possible side effects including dizziness, elevated liver enzymes, reduced leukocytes in the blood (granulocytopenia) and weakness (asthenia) [8,36]. There have been performed many clinical trials with different neuroprotective agents, such as: Memantine, Tamoxifen, Ceftriaxone, Creatine, Myotrophin®, Celebrex, Neurodex, Oxandrolone, CoQ10, Topiramate, Xaliproden, Indinavir, Minocycline, Buspirone and others [25,51]. None of them has got a recommendation for treatment in ALS patients.

Other treatments are designed to relieve symptoms and improve the quality of life. Different drugs are available to help individuals with pain, depression, sleep disturbances, and constipation. Baclofen or tizanidine may relieve spasticity. Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen or naproxen may relieve general discomfort. Tramadol is often prescribed for pain relief. Patients with amyotrophic lateral sclerosis may eventually consider forms of mechanical ventilation (respirators).

The hypothesis is proposed that statins may induce or aggravate ALS by impairing liver X receptor (LXR) signalling. If this hypothesis is confirmed, LXR agonists could be used together with statins in patients predisposed to develop ALS or in those known to have the disorder [7].

From the neuroprotective point of view there have been many agents investigated both *in vitro* and *in vivo*, and their role in the treatment of ALS has been discussed.

Citicoline, also known as CDP-choline (cytidine-5-diphosphocholine), is an endogenous nucleoside that exhibits neuroprotective abilities in certain central nervous system (CNS) injury models [15,71]. The neuroprotective properties of CDP-choline seem to be related to its action on glutamate-mediated cell death. Citicoline might decrease the extracellular level of glutamate by inhibition of neuronal glutamate efflux and increased astrocytic glutamate uptake. It has been suggested that the neuroprotective effect of this compound is related to inhibition of the glutamate-induced apoptotic pathway of cell injury [41]. Erythropoietin (EPO) is suggested to be one of the compounds that might play a potential neuroprotective role in ALS. There are many data showing that EPO helps neurons exposed to damaging agents to survive. It is especially involved in the apoptotic mechanism of cell death. As a neuroprotective agent, erythropoietin has many functions: antagonizing glutamate cytotoxic action, enhancing antioxidant enzyme expression, reducing the free radical production rate and affecting neurotransmitter release. EPO exerts a neuroprotective effect in the investigated model of chronic excitotoxicity mainly through pre-

vention of apoptotic neuronal changes. Our results as well as previous data [27,37] suggest that EPO may be a promising therapeutic drug in various neurological diseases, including ALS [44].

The therapies stimulating the protective and regenerative effect of the immune system in humans, i.e. application of intravenous immunoglobulins and experimental treatment with vaccination, minocycline, antibodies, and neurotrophic factors [30] have shown promise in ALS. Consequently, several immunosuppressive and immunomodulatory therapies have been tried in ALS, generally with no particular success [16].

Because of the absolute lack of effective drug treatment in ALS, attention has been given to stem cell transplantation. A positive effect has been observed in several animal models. The replacement of lost motor neurons is the main, but is not the only mechanism of action in ALS therapy [32,46,69]. Preliminary stem cell transplantation trials have been performed in ALS patients to see if the invasive injection into the spinal cord was safe for the patient with hope to slow down the progression of the disease, but the results are conflicting. The stem cells do not generate motor neurons but they protect the still-functioning ones [42,58,59].

Physical therapy is an important part of treatment and helps relieve cramping and muscular pain. Passive stretching helps avoid permanent contraction of muscles that may cause joint problems. Other ALS treatments are designed to relieve symptoms and improve a person's quality of life. People with ALS who have difficulty speaking may benefit from working with a speech therapist. As the disease progresses, speech therapists can help people develop ways for responding to yes-or-no questions with their eyes or by other nonverbal means. They can also recommend aids such as speech synthesizers and computer-based communication systems. Weakness of muscles that assist in breathing calls for nocturnal ventilator assistance. For long-term use, a tracheostomy should be performed with ventilation support which helps breathing and prolongs survival, although it does not affect the progression of ALS.

According to current knowledge, in clinical practice, all ALS/MND (motor neuron disease) patients respond similarly to potential treatment. There was no statistical evidence for a different response to treatment in patients with familial ALS/MND compared to those with sporadic ALS/MND. It seems probable that in future, patients with specific genetic mutations classified into subgroups might be treated with gene-directed therapies.

Although the mean survival time with ALS is three to five years, about 10% of people with the condition will survive for ten or more years. In a small number of people, ALS is known to remit or stop its progression, though there is no scientific understanding as to how and why this happens. There are medically documented cases of people in whom ALS stops progressing or progresses at a very slow rate.

Multidisciplinary teams of healthcare professionals ought to be organized to provide supportive care, including physicians, pharmacists, physical, occupational, and speech therapists, nutritionists, social workers, and home care and hospice nurses [2,64].

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