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ALS, what new 144 years after Charcot?

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ABSTRACT

The most important challenge of ALS remains finding biomarkers. Clinical features remain of key importance in the diagnosis and for follow up. Neurophysiology remains difficult to use in clinical trials. Neuroimaging have some utility for upper motor neuron integrity and function. Among proteins and chemical markers, one of the most promising marker is the level of Nogo in muscle biopsy. In CSF, many candidate proteins have been suggested but their sensitivity and specificity remains disappointing. Recently, -omics technologies have been applied to try to discover biomarkers in ALS, including genomic, proteomic and metabolomic methodologies.

Key words

Amyotrophic lateral sclerosis • Biomarkers • Nogo • MUNE

Introduction

Amyotrophic lateral sclerosis has been formalized as a specific disease by Charcot and pupils after the initial description in 1865 of a patient with muscles spasms that Charcot thought to be related to hysteria. The initial scope of Charcot was to find the anatomopathologic basis of hysteria. The specific feature of this patient was the existence of a permanent muscle contracture looking very close to the contractures observed in hysteric patients during the crisis with paroxysmal spasms. The patient died and at autopsy Charcot observed an involvement of the lateral tracts of the spinal cord, "la sclérose latérale". He clearly isolated the ALS from the other sclerosis: the multiple sclerosis (sclérose en plaques), and the involvement of the posterior fasciculi of the spinal cord (sclérose combinée).

In his book (Leçons du Mardi de la Salpêtrière, 1887-1888), he described the main characteristic clinical features of the disease, a rapid motor involvement of the lower limbs without initial atrophy but with contracture, an amyotrophic involvement of the upper limbs with few contractures, a bulbar involvement.

Clinical diagnosis

The most significant sign of the disease for Charcot was the muscle stiffness, or contracture, that the upper motor neuron signs, if we dramatically summarize the subtlety of Charcot, and latter of Pierre Marie.

Since the initial steps of the description of the disease, the concept of the amyotrophic lateral sclerosis has been modified mainly by increasing the number of possible clinical presentations belonging to the spectrum or nebula of ALS. During this course, I will avoid to use the term of motor neuron disease which appears as a potential source of increasing confusion. It has been recognized that belong to the ALS spectrum, clinical features as:

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– spatially limited motor involvement: of the bulbar region (pure bulbar palsy), scapular belt (flail arm syndrome), lower limb involvement (Pierre Marie and Patrikios);
– a predominant or pure clinical involvement of: the upper motor neurons (primary lateral sclerosis), the lower motor neurons (progressive muscular atrophy), the frontal lobes (FTD with motor neuron).

For each of these clinical presentations, the disease can remain limited to the initial features or it worsens with a progressive evolution toward a typical ALS, as initially described by Charcot.

The extension of the clinical spectrum of the ALS led increasing the difficulty to ascertain the diagnosis, making important the redefinition of the disease. During the El Escorial meeting in 1994 (http://www.wfnals.org/guidelines/1998elescorial/elescorial1998criteria.htm), the accepted definition was *A - the presence of* (A:1) evidence of lower motor neuron (LMN) degeneration by clinical, electrophysiological or neuropathologic examination, (A:2) evidence of upper motor neuron (UMN) degeneration by clinical examination, and (A:3) progressive spread of symptoms or signs within a region or to other regions, as determined by history or examination, together with *B - the absence of* (B:1) electrophysiological and pathological evidence of other disease processes that might explain the signs of LMN and/or UMN degeneration, and (B:2) neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs.

However, this definition remains unsatisfactory and difficult to apply in daily clinic, mainly due to the difficulty in ascertaining the existence of upper motor neurons signs when the initial clinical presentation looks like a progressive muscular atrophy. The difficulty is related to the lack of clear laboratory supporting data which could ascertain for the existence of an upper motor neuron (UMN) involvement, as does the EMG for the lower motor neuron (LMN) involvement.

The clinical features of the lower motor neuron (LMN) are the atrophy, the fasciculations and cramps, the loss of deep tendon reflexes. However, when the clinical diagnosis is difficult as in predominant or pure UMN involvement, EMG is able to provide useful information and to confirm or not the existence of an associated LMN involvement. These criteria are well detailed (http://www.wfnals.org/guidelines/1998elescorial/elescorial1998app3.htm).

The clinical features of an UMN are mainly the inversion of the cutaneous plantar response, the muscle contracture and the brisk deep tendon reflexes. The Babinski sign, even if nearly constant, could be delayed by more than one year after the first signs (Meininger et al., 1995). In our hands, the most consistent feature, but probably the most difficult to test, is the evaluation of the contracture, which can be pyramidal and/or extra pyramidal (Desai and Swash, 1999). Attempts have been made to evaluate the UMN involvement using electrophysiological methods, such as the magnetic stimulation of the cortico spinal tract (CST). This method allows to analyze various parameters, but there is a consensus in the literature to propose as the most significant one: the resting motor threshold (RMT), the motor evoked potential (MEP) and the cortical silent period (CSP). It was suggested that the magnetic stimulation of the CST provides arguments for a cortical excitability (Zanette et al., 2002). They also conclude that the alteration of different cortical inhibitory functions seems to take place with disease progression, rather than being the primary event in the pathogenesis of ALS. They considered the impaired inhibition as being due to both depletion of specific subpopulations of intracortical GABAergic neurons and mechanisms involved in motor cortex reorganization following progressive neuronal loss. It was also shown (Attarian et al., 2006) that MEP gain and CSP duration decreased during the course of the disease. They also concluded that MEP gain improved detection of the CST dysfunction and CSP duration seems to reflect the alteration of activation of inhibitory networks, spinal and/or cortical. For these authors (Attarian et al., 2007), the triple stimulation method (TST method) seems to provide a very useful tool for analyzing the degree of UMN involvement (abnormalities were found in 55.6% of patients). They also concluded that the silent period (CSP) was useful to detect ALS (in 47.6% of patients). However, these methods remains rather limited in their use since they require trained personnel to be used.

Clinical and neurophysiological markers

The most commonly used clinical marker is the analysis of muscle strength assuming that muscle strength is directly related to the number of motor neurons (Kent Braun et al., 1996). However, this assumption is not 100% true since a lot of parameters can interfere with muscle strength, such as fatigue, stiffness and emotion. When the patient is depressed, the performance of the patient will be less than when the patient is in a good condition.

Another clinical marker is the ALSFRS-R scale (Cedarbaum et al., 1999), which is a functional scale. It has been largely used during the last five years both in clinical trials and in daily clinic to assess the functional status of the patients. It has been claimed that ALSFRS-R is predictive for survival and muscle strength. However, if there is a correlation between these parameters, the relation is far to be 100%.

This statement raises the questions of the two types of end points in clinical trials, i.e., function/survival. Common sense suggests that survival is related to respiratory function meaning that survival is directly related to respiratory muscles function. However, it is clear from most recent trials that there is no clear correlation between function and survival. This point was raised in view of the results obtained in a recent trial conducted in Europe and trying to demonstrate an efficacy of pentoxifyllin in ALS patients (Meininger et al., 2006). In this trial, we observed a worsening effect of the compound as compared to placebo on the survival end point, but no effect on function. Similar differences were observed in previous trials and discussed in our paper. This finding leads to propose that both end points have to be evaluated in future clinical trials meaning a potential increase of the number of patients. The only way to avoid such an increase is to find surrogate markers for both endpoints.

Electrophysiological methods allow counting the number of motor numbers which is very sensitive to disease progression and correlated with muscle strength (Shefner, 2001).

But the major question about this method is the difficulty to obtain a clear intra and interrater reliability. Composite electrophysiological end points have been proposed, but at the moment none have been accepted as surrogate markers in any clinical trials.

Neuroimaging markers

Neuroimaging has been largely developed since the last five years. The most common finding of the conventional MRI (Kassubeck et al., 2005) remains a certain degree of atrophy with a very asymmetrical distribution in the cortex (right primary motor cortex and left medial frontal gyrus) associated with an atrophy of the the corpus callosum, the cerebellum and the occipital lobes. Similar results with regard to an asymmetrical repartition of the atrophy were reported by others (Elis et al., 2001; Grosskreutz et al., 2006). A subcortical atrophy was also reported with a relation with a cognitive impairment (Abrahams et al., 2005). Modifications of the signal of the CST have been reported but they lack of sensitivity particularly when the UMN remains weak. Comparing ALS patients and controls, Graham et al., (2004) reported that T2 motor cortex hypointensity and corona radiate hyperintensity offered a sensitivity of less than 20% but a specificity of 100%. They also analyzed the fractional anisotropy in the same population. They showed that the reduction of FA in the posterior limb of the internal capsule offered a sensitivity of 95% to separate ALS patients from controls but a specificity of 71%.

Efforts to look for the aspects of fibers have been largely developed with the diffusion MRI technique. In ALS, there is an increase of the mean diffusivity (MD) with a decrease of the fractional anisotropy (FA), of the CST mainly in its posterior part (Winhammar et al., 2005). It seems to be a consensus to correlate MD with the disease duration, FA with disease severity (Wang et al., 2006), but these results have been disputed (Ciccarelli et al., 2006). DTI based color maps have been proposed to measure the volume of the CST and the organization of its fibers (Wang and Melhem, 2005).

Attempts to correlate the findings of DTI with UMN involvement remained rather weak. The most important modifications have been observed in the cerebral peduncle with a correlation between FA and the extent of UMN signs (Hong et al., 2004). Modifications of the CST seem to be detectable before the appearance of the clinical signs (Sach et al., 2004). Various methods have been proposed to quantify the degree of involvement of the CST (Schimrigk et al., 2007). They proposed that the best region to be analyzed was the caudal half of the

internal capsule with a negative correlation between FA and disease duration, but no correlation with the clinical functional scales as the ALSFRS (ALS functional rating scale). Other attempts have been made to correlate DTI with the ALSFRS (Thivard et al., 2007). A reduced FA was observed in the CST (bilaterally), the left insular and ventrolateral premotor cortex, the right parietal cortex and the thalamus with a correlation with the ALSFRS. An increased MD was seen in the motor cortex (bilaterally), the ventrolateral premotor cortex, the hippocampus and the right superior temporal gyrus, but without correlation with the ALSFRS. VBM analysis showed no change of the white matter and a widespread decrease of the grey matter volume in regions exhibiting MD abnormalities. These results suggest that FA is related to axonal degeneration and is a good marker of disease severity; MD is related to brain atrophy with an extension of the lesions in the non motor regions.

Only one publication reported an analysis of the spinal cord using DTI (Valsasina et al., 2007). In the spinal cord, ALS patients have a lower FA, a lower surface, and this method seems promising to quantify the degree of spinal cord injury.

Functional MRI (fMRI) provides also a useful tool to analyze the degree of cortical involvement. During a simple motor task (Konrad et al., 2006), there is an increased activation of the supplementary motor area and of subcortical regions (cerebellum, midbrain and diencephalon). However, using a similar technique, another group (Tessitore et al., 2006) showed more complex results with an increased activation of the left putamen, a decreased activation of the left sensorimotor cortex, of the lateral premotor region, supplementary motor area and right posterior parietal cortex. When UMN signs are prominent, there is an increase activation of the anterior cingulate cortex and right caudate.

Spectro MRI (sMRI) has been a matter of disappointment. If most studies conclude that there is a decrease of concentration of NAA, NAA/Cho and NAA/ creatine (Wang and Melhem, 2005), there is no consensus about their specificity and there relation with the degree of involvement of the UMN. Another publication showed that the NAA/Ins ratio was correlated with the functional scales, with a 93% specificity and a d71% sensitivity (Kalra et al., 2006).

Due the weakness of each technique when used alone, composite approaches have been proposed. Wang et al. (2006) suggested that the predictivity of the NAA/Cr ratio was increased when it was coupled with the FA. Mitsumoto et al. (2007) suggested combining both the (1)H MR spectroscopy imaging measures for the primary motor cortex NAA and NAA/Cr with the central motor conduction time to the tibialis anterior (composite marker).

PET and SPECT neuroimaging provided some insight on the UMN involvement. PET studies used Flumazenil of markers to quantify the loss of neurons (Lloyd et al., 2000). In ALS patients, they observed significant decreases in the prefrontal cortex bilaterally, parietal cortex bilaterally, visual association cortex bilaterally and left motor/premotor cortex, the left ventrolateral and dorsolateral prefrontal cortex, Broca's area and the right temporal and right visual association cortex. Comparing SOD mutated patients with sporadic one, Turner et al. (2005a) observed differences in the distribution of reduced cortical [11C]flumazenil binding suggesting that this might reflect differences in cortical neuronal vulnerability. Using another marker to analyze the serotoninergic sites, Turner et al., (2005b) observed a marked reduction (21%) in both the global cortical and raphe BP of [11C]-WAY100635 in ALS patients, with regional variations in the VOI analysis that ranged from 16% to 29% decrease compared with the control group, and trends to greater reductions in those with bulbar involvement. Inflammation has been recognized as a major physio pathological mechanism of neurodegeneration in ALS. Using a marker of microglial activation, Turner et al., (2004) found a significantly increased binding in motor cortex, pons, dorsolateral prefrontal cortex and thalamus, with significant correlation between binding in the motor cortex and the burden of upper motor neuron signs clinically. These findings indicate that cerebral microglial activation can be detected in vivo. However, all these studies need to be confirmed and quantified in the view of using PET to assess for the UMN involvement.

SPECT can be also useful but data remains rather scarce until now. Correlating perfusion abnormalities with functional scales (ALSFRS), Haber et al., (2007) showed that a correlation was seen with the whole score and with the lower limbs and bulbar scores, but not with the upper limbs score.

Biological markers

The usefulness of biological markers for the diagnosis of ALS and / or for the evaluation of the extent of the neuronal or axonal injury remains rather disappointing.

To assess for the evaluation of axonal injury, various proteins have been tested. Until now, it appears that the most convincing results were obtained with the neurofilaments proteins, either heavy (Brettscheneider et al., 2006) or light (Zetterberg et al., 2007) chains. Heavy chains marker (NfHS135) is increased 5 fold in ALS with a correlation with the rate of evolution. Light chain dosage showed a 10 fold increase with a correlation with disease duration. Other markers such as tau (Jimenez-Jimenez et al., 2005) or S100 (Sussmuth et al., 2003) did not prove to be significantly modified both in CSF and plasma.

Markers for inflammation have been largely analyzed with regard to the importance of this mechanism in the neurodegenerating process. The most convincing at the moment is MCP1 (monocyte chemoattractant protein). Most studies (Simpson et al., 2004; Baron et al., 2005; Zhang et al., 2006 and Nagata et al., 2007) showed an increase in the serum and/or the CSF with a correlation with the limb function. Among the other markers of inflammation, there is a lack of consensus. Most of cytokines seems normal in ALS patients including TNF alpha (Holmoy et al., 2006). But this result is disputed and another group reported an increase of this cytokine with the IL6 which is so important in the neurodegeneration (Moreau et al., 2005). Prostaglandins E2 is increased in serum and CSF in 85% of ALS patients but without correlations with the clinical parameters (Almer et al., 2002).

Glutamate is another important compound in the neurodegenerative process. Most studies (Spreux Varoquaux et al., 2002) reported an increase of glutamate in the CSF in approximately 2/3 of the patients with a correlation with the severity of motor impairment. However, there was no change of the glutamate concentration with riluzole, a compound with an antiglutamate activity.

Detection of growth factors in body fluid appeared also to be controversial with conflicting results in the different reports. VEGF which is one of the most analyzed with regard to its possible role in the disease was diversely modified. For Ilzecka (2004), VEGF levels are increased in the CSF with a correlation with the spinal form of the disease and with the duration of the disease. However, Devos et al., (2004) showed that VEGF levels in CSF were decreased, in relation with hypoxemia. The same group reported an increase of erythropoietin in CSF which seemed to be specific of ALS patients (Just et al., 2007). This increase was disputed by Brettschneider et al., (2007) who reported a decrease in CSF.

Other studies devoted to growth factors remain more anecdotic. The most convincing study reported an increase of GNDF (and not BDNF) in CSF in 80% of ALS patients (Grunstrom et al., 2000).

IgF1 was largely analyzed in relation with its possible interaction with new therapeutic avenues. However, once again results remain conflicting. Pirttila et al. (2004) found no change in CSF; Billic et al.,(2006) found a decrease in CSF of both IgF1 and insulin; Morselli et al. (2006) found no change in serum but a bad response to GHRH stimulation in 72% of ALS patients.

Markers of oxidative stress are modified in ALS patients, but most studies need to be confirmed. Among the most interesting potential compound, the 8OH2dG seemed to be very convincing with an increase in all fluids including urines (Bogdanov et al., 2000). However, no further studies were conducted with this marker.

Lipid metabolism is altered in ALS patients and has some relations with the disease process. ApoE is present in the serum. We have shown that in the serum ApoE is increased in ALS patients and this increase is related to the rate of evolution (Fig. 4), the age of patients and survival time (Lacomblez et al., 2002). There is approximately 8 or 10 months difference in the mean survival time between patients with high or normal ApoE values. We also demonstrated that the frequency of hyperlipidemia is two-fold higher in ALS patients than in control subjects (Dupuis et al., 2008). As a result, steatosis of the liver was more pronounced in ALS patients than in Parkinson's disease patients. Correlation studies demonstrated that bearing an abnormally elevated LDL/HDL ratio significantly increased survival by more than 12 months.

New approaches have been recently proposed using metabolomic (Bowser et al., 2006) or proteomic (Ranganathan et al., 2005).

Another approach has been proposed analyzing specific proteins in tissue biopsy. In the muscle, it was proposed (Dupuis et al., 2002) that Nogo A, which belongs to the reticulon family of proteins, has a specific pattern of expression. Comparing a cohort of patients with a pure LMN syndrom, Pradat et al. (2007) proposed that expression of Nogo A could offer a marker of ALS at the earlier stages of the disease.
At the moment, even if we have no marker for diagnosis or prognosis, important steps have been made since the initial description of the disease in various fields, neurophysiology, neuroimaging and biological analysis of body fluids and/or biopsy tissues.

Conclusions

140 years after the initial description by Charcot of ALS, major steps have been made. For many years ALS has been considered as a harmful disease for which nothing could be done and about which nothing was known. Important walls have been broken.
Genetic and molecular biology allowed a better understanding of the basic mechanisms leading to the motor neurons injury and death. We start getting new methodological approaches which will lead to new markers. However, mute zones remain and important efforts are still necessary.
International cooperation allowed clarifying the standard of care of ALS patients with a significant impact on the quality of life of both patients and relatives. However, efforts have to be made to improve the criteria for nutritional and respiratory supports and to convince governments of the specificity of the disease and the necessity of dedicated centres.
Even if the activity of riluzole remains disappointing, this drug remains the only one drug tested in neurodegenerative diseases which demonstrated a clear effect on physiopathogenic mechanisms. In spite of numerous negative trials, we have to sustain our efforts and strengthen the methodological approaches of therapeutic trials both at preclinical and clinical levels.

Summary

Since the initial description of amyotrophic lateral sclerosis (ALS) by J.M. Charcot, the most important challenge of the disease remains finding markers. The importance of treating patients as soon as possible by the most adapted care solutions and by compounds active on the disease process is the most important target of neurologists in charge of ALS patients. Biomarkers may be genetic, epigenetic, protein, metabolic, neurophysiologic, clinical or imaging. Genetic markers are mainly used within specific families, and account for only a small subset of ALS patients. Clinical features remain of key importance in the diagnosis and for follow up. Neurophysiology remains difficult to use in clinical trials in spite of many attempts to demonstrate that techniques like MUNE or composite index can be useful as a marker of progression.
Results of neuroimaging such as magnetic resonance spectroscopy, diffusion tensor imaging, SPECT or PET may have some utility as biomarkers for upper motor neuron integrity and function. Their usefulness in approaching the quantisation of cell death is not actually clearly demonstrated.
Among proteins and chemical markers, one of the most promising marker is the level of Nogo in muscle biopsy samples which allows both for a marker of disease progression and for an early diagnosis of the disease. In CSF, many candidate proteins have been suggested such tau proteins, levels of glutamate transporters, macrophage-derived cytokines, but their sensitivity and specificity remains disappointing.
Recently, -omics technologies have been applied to try to discover biomarkers in ALS, including genomic, proteomic and metabolomic methodologies. Even if such methods appear to be interesting, many works remain to be done to demonstrate that this methodology has some interest in ALS.

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