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Long-term persistence of vaccine-derived aluminum hydroxide is associated with chronic cognitive dysfunction

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ABSTRACT

Macrophagic myofasciitis (MMF) is an emerging condition, characterized by specific muscle lesions assessing long-term persistence of aluminum hydroxide within macrophages at the site of previous immunization. Affected patients mainly complain of arthromyalgias, chronic fatigue, and cognitive difficulties. We designed a comprehensive battery of neuropsychological tests to prospectively delineate MMF-associated cognitive dysfunction (MACD). Compared to control patients with arthritis and chronic pain, MMF patients had pronounced and specific cognitive impairment. MACD mainly affected (i) both visual and verbal memory; (ii) executive functions, including attention, working memory, and planning; and (iii) left ear extinction at dichotic listening test. Cognitive deficits did not correlate with pain, fatigue, depression, or disease duration. Pathophysiological mechanisms underlying MACD remain to be determined. In conclusion, long-term persistence of vaccine-derived aluminum hydroxide within the body assessed by MMF is associated with cognitive dysfunction, not solely due to chronic pain, fatigue and depression.

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1. Introduction

Macrophagic myofasciitis (MMF) is a rare condition characterized by highly specific myopathological alterations at deltoid muscle biopsy, recognized in 1998 (Rare disease #ORPHA592, www.orpha.net) [1], and subsequently shown to assess long-term persistence of vaccine-derived aluminum hydroxide nanoparticles within macrophages at the site of previous intramuscular injections [2]. Clinical manifestations observed in adult patients typically include chronic arthromyalgias and fatigue [3,4], appearing in a delayed fashion after the last aluminum-containing vaccine injection [2]. According to records of the French patient association E3M, 78% of affiliated patients withdrew their professional activity after the onset of clinical manifestations [5], due to these disabling symptoms combined with intellectual disturbance affecting both memory and ability to concentrate. In particular, patients frequently report on difficulties in sustaining their attention on tasks of daily living, such as following a conversation or efficiently allocating their attention resources to different simultaneous stimuli.

Until now, cognitive impairment in MMF patients was downplayed and left out of medical attention, and, indeed, an obvious link between focal intramuscular accumulation of aluminum-loaded macrophages and cerebral dysfunction is missing, despite growing evidence that nanoparticles have the unique capacity to spread throughout the body and cross the blood brain barrier [6]. Notably, there are marked inter-individual variations in the ability to clear out aluminum from the body, and the MMF lesion from the injected muscle [7]. Cognitive complaints of MMF patients are similar to those reported by the multiple sclerosis (MS) [8], and marked fatigue may occur in both conditions [4,9]. In addition, MMF patients suffer from musculoskeletal pain (up to 88%) and chronic fatigue (duration >6 months; up to 93%) [4]. Fatigue was

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found disabling in 87% of patients, most often affecting both physical and mental functioning, and 53% of patients fulfilled criteria for chronic fatigue syndrome (CFS), from either the CDC or Oxford [3]. A case-control study ordered by the French government agency AFSSAPS (Agence Française de Sécurité Sanitaire des Produits de Santé), confirmed a higher rate of fatigue in patients with MMF compared to other neuromuscular disorders [10]. Moreover, impact of fatigue on daily living assessed by Fatigue Impact Scale was higher in MMF patients, and mental fatigue (i.e., cognitive disturbance) was the principal component [10]. As depression and anxiety are known to interact with fatigue on cognitive functioning [11], it appears mandatory to determine whether neuropsychological impairment in MMF patients could be ascribed to a non-specific combination of pain, fatigue, and depression, as previously reported in other chronic pain syndromes [12,13], or reflects a more specific condition.

The present study was designed to characterize the MMF-associated cognitive dysfunction (MACD) and to assess its specificity. It demonstrates that almost all adult MMF patients have a measurable and stereotyped cognitive dysfunction, distinct from non-specific cognitive alterations induced by chronic pain, fatigue or depression.

2. Patients and methods

2.1. Global design of the study

As a preliminary exploration, retrospective analysis of routine neuropsychological evaluation of 22 MMF patients was carried out to set up a comprehensive battery of neuropsychological tests allowing precise delineation of the MACD clinical profile. Then, using these tests, MACD was prospectively evaluated in another series of 25 unselected patients with MMF at muscle biopsy. This was done using a two-step procedure. The first step consisted in a case-control study aiming at determining if MMF patients are more cognitively impaired than control patients with chronic painful disease. The neuropsychological profile of 11 MMF-patients was compared to 11 patients with inflammatory joint disease and chronic pain (controls, CTL). In the second step, 14 additional MMF patients were included, then forming a cohort of 25 patients allowing us to refine the depiction of MACD and to determine if clinical factors may influence MACD severity.

2.2. Participants

The cohort of prospectively evaluated MMF patients included 25 patients with biopsy-proven MMF without taking cognitive complaint into consideration. Histological MMF lesions were defined by focal accumulations of cohesive large aluminum-loaded macrophages in epi-, peri-, or endo-mysium, without formation of epithelioid or multinucleated giant cells (Fig. 1), as previously described (1, 2; for methods, see Supplementary data, E-Appendix 2). Patients were consecutively included, following a two-steps procedure: (1) checking of muscle biopsy findings; (2) exclusion of patients with evidence for previous overt neurological disease that may impair cognitive functioning, such as MS, cerebrovascular disease or degenerative brain disease, from the examination of their medical files. For each patient, clinical evaluation included medical history, symptoms notification, neurological examination, quantification of fatigue by visual analogical scale (VAS; from 0 'no fatigue' to 10 'extreme fatigue'), and depression level evaluation by Montgomery and Asberg depression rating scale (MADRS) and self report centre for epidemiologic studies depression (CES-D) [14]. Neuropsychological evaluations were conducted at the Interventional Neuropsychology platform of Créteil, France (INSERM/U955-Team 01).

Because of non-fortuitous association between MMF and MS [4,9], brain MRI including FLAIR, proton-density–weighted or T2-weighted and gadolinium-enhanced T1-weighted MR scans, is systematically performed in routine evaluation of MMF patients in France. In the setting of present study, all MR images were acquired using vendor-supplied sequences approved for clinical use. Patients were scanned either on a 1.5T (AVANTO Siemens) or on a 3T (ACHIEVA Philips) MRI system using an eight-channel head coil. All sequences applied were standard, approved, vendor-supplied pulse sequences. No new experimental sequences were pioneered in this study. Axial 2D, 5 mm thick slices, with a 256 mm^2 FOV, were acquired with the following sequences and parameters: (i) T1-weighted Gradient Echo sequences: TR/TE: 285/6.14, matrix: 512 × 448, nexc: 2 on the 1.5 T system and TR/TE: 252/4.6, 512 × 512 matrix, nexc: 2 on the 3T system; (ii) Turbo spin-echo FLAIR sequences: TR/TI/TE: 9000/2500/99, matrix: 256 × 256, nexc:1, parallel imaging with an acceleration factor:2 on the 1.5 T system and TR/TI/TE: 11000/2800/125, matrix: 512 × 512, nexc:1, parallel imaging with an acceleration factor:2 on the 3T system; (iii) Axial and sagittal Turbo spin-echo T2-weighted sequences: TR/TE: 4000/116, matrix: 512 × 448, nexc:1 on the 1.5 T system and TR/TE:3085/80, matrix: 512 × 512, nexc:1 on the 3T system.

The duration of disease was estimated by calculating for each patient the delay elapsed from last aluminum-adsorbed vaccine administration to the onset of symptoms (Vaccine-Onset Δ), deltoid muscle biopsy (Vaccine-Biopsy Δ), and neuropsychological testing (Vaccine-Test Δ), and from onset of symptoms to neuropsychological testing (Onset-Test Δ). Vaccine-Onset Δ reflects disease course; Onset-Test Δ, the duration of clinical manifestations at time of neuropsychological evaluation; Vaccine-Biopsy Δ, the minimum persistence time of aluminum within the body; and Vaccine-Test Δ, the time elapsed from the last exposure to neuropsychological evaluation.

Controls (CTL) were 11 consecutive patients with a painful chronic inflammatory joint disease, including rheumatoid arthritis ($n = 2$) and ankylosing spondylitis ($n = 9$). They were recruited on the basis of pain level and demographic data, without taking cognitive complaint into consideration. They had pain level $\geqslant 5/10$ at VAS and were similar in age, educational level, fatigue (VAS) and depression (MADRS, CES-D) (Table 1). Written informed consent to participate to the study was obtained from all patients.

2.3. Cognitive assessment

2.3.1. Exploratory analysis

One of us (MC) retrospectively reviewed routine standard neuropsychological evaluations of 22 MMF patients including Rey–Ostereith complex figure (ROCF; 21/22), dichotic listening test (21/22), matrix (21/22) and letter–number sequencing (19/22) subtests of Wechsler adult intelligence scale (WAIS)-III, images arrangement subtest of WAIS-revised (WAIS-R; 18/22), Benton visual retention test (BVRT; 17/22), Grober & Buschke test (16/22), and California verbal learning test (CVLT; 7/22). MMF-patients were impaired in ROCF copy ($p < 0.001$) and recall ($p < 0.001$), and in matrix and letter–number sequencing ($p < 0.05$). These data were suggestive of executive dysfunction with alteration of both verbal working memory and visual memory. Results at dichotic listening test were above pathological threshold for 'words' condition (left ear: $p = 0{,}025$; right ear: $p < 0.001$) and 'sentences' condition in right ear ($p < 0{,}001$; left ear: NS; left ear vs. right ear: $p = 0.001$), such pattern being compatible with inter-hemispheric disconnection [15].

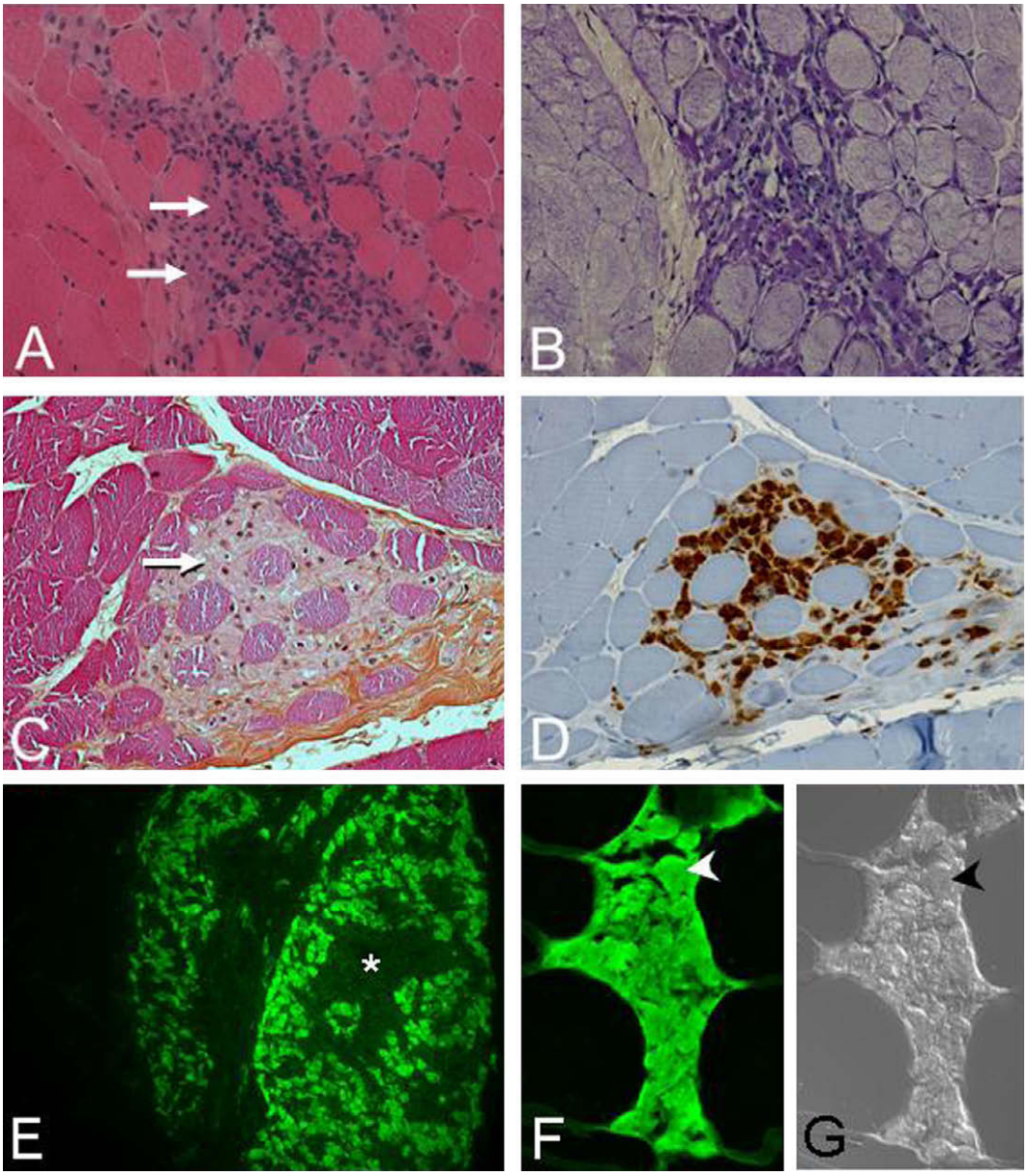

Fig. 1. Macrophagic myofasciitis (MMF) lesions. Typical MMF lesions appearing as focal inflammatory infiltrates in perifascicular endomysium, made of large and basophilic CD68 + macrophages (arrows), intermingled with lymphocytes, and surrounding myofibers (A, C, D). Macrophages were strongly PAS-positive (B). Aluminum staining (Morin) showed marked cytoplasmic fluorescence of MMF macrophages (E) contrasting with the complete negativity of associated lymphocytes (star). The same field observed with fluorescence or differential interference contrast (DIC) microscopy showed that fluorescence originated from large cells with granular cytoplasmic contents (arrowheads), namely aluminum-loaded macrophages. Deltoid muscle biopsy, frozen (A and B) and paraffin (C–G) sections. (A and C): Hematoxylin–eosin; (B): Periodic acid-Schiff (PAS); (D): CD68, immunoperoxidase; (E–G): Morin staining, fluorescence (E and F) and DIC (G).

Table 1
Characteristics of patients at time of neuropsychological evaluation.

	Case-control study			Cohort study
	11 MMF (mean-range)	11 CTL (mean-range)	*p*-Value	25 MMF (11 + 14) (mean-range)
Male/female ratio	2/9	5/6	0.36	7/18
Age (years)	45.5 (29–63)	40.3 (28–59)	0.21	45.3 (29–63)
Educational level (years)	13 (10–17)	12.8 (9–16)	0.87	12.2 (7–17)
MADRS score	10.8 (4–30)	9.9 (6–16)	0.67	13.5 (4–37)
Pre-test fatigue level	4.1 (0.8–7.5)	5.1 (1.9–8)	0.22	4.0 (0.8–8)
Post-test fatigue level	6.1 (4.7–8.8)	6.5 (5–8)	0.59	5.9 (3.5–9.5)

2.3.2. Tests used for prospective analysis

From these results a comprehensive neuropsychological battery was designed for prospective assessment. Three subtests of WAIS-R were chosen to assess general cognitive functioning: 'Block design' evaluating visuo-perceptual abilities; 'vocabulary' which gives an estimate of pre-morbid level and semantic knowledge;

and 'pictures arrangement', evaluating planning. An estimate of current non-verbal reasoning abilities was provided by the 'matrix' subtest from WAIS III. Verbal memory was assessed for short-term function trough the 'digit span' while long term episodic memory used the CVLT. Short-term visual memory was assessed by the 'Corsi block tapping test' and the BVRT, and long-term visual memory by ROCF recall three minutes after the copy. The testing of executive function included the trail making test A and B, and the verbal fluency tasks (letter P and animals in 2 min) for cognitive flexibility; The Zazzo's cancellation test for attention; the Stroop test for inhibition abilities; the ROCF copy and Images arrangement from the WAIS-R for planning; and the letter–number sequencing from the WAIS III and the Brown–Petersen test for working memory. Language abilities were evaluated by the vocabulary subtest of WAIS-R, verbal fluencies, and a picture naming using the 15-item Boston naming test. Ideomotor, ideational and dynamic praxis were systematically explored using a standardized protocol of the neuropsychological unit. Briefly, 13 gestures were imitated with hands, three symbolical gestures, five pantomimes, three mimed postural gestures and two dynamic gestures sequencing. Dichotic listening test was used to detect an inter-hemispheric disconnection syndrome. References of used tests are quoted in E-Appendix 1 (Supplemental data).

2.3.3. Achievement of neuropsychological profiles

To facilitate visualization of MACD specificities, we used composite scores, each score evaluating a specific domain of cognitive functioning and combining the more relevant tests by domain, while avoiding the classification of a test in several domains. For each score, we averaged the percentage of success rate obtained at tests forming the score, 100 representing the reference value. In the case-control study, 100 represented the mean results in controls. In the cohort study, 100 represented the normal value according to published norms. Ten specific domains were defined: attention, working memory, planning, inhibition, flexibility, visual memory, verbal memory, visuoperceptual organization, language, and praxis. *Attention* composite score corresponded to combination of the three Zazzo's Cancellation Task conditions. A *planning* score was issued from the ROCF copy and the Pictures Arrangement; an *inhibition* score from the interference part of Stroop task; a *flexibility* score from the part B of the trail making test; A *working memory* score was computed from backward span, letter–number sequencing and the four conditions of the Brown–Petersen task; a *visual memory* score computed from the Corsi span, the Benton VRT and the ROCF delayed recall; a *verbal memory* score from forward digit span and all the CVLT scores; a *visuoperceptual organization* score from ROCF copy, WAIS bloc design and matrix; a *language* score from WAIS vocabulary, Boston naming test score and verbal fluencies and a *praxis* score. To allow immediate visualization of neuropsychological profiles, results were represented in radar graphs.

2.4. Statistical analyses

Results were expressed as mean ± standard deviation. For each test, *Z*-scores were calculated in order to place obtained values in relation to pathological (−1.6SD) threshold. For each patient, *Z* was calculated from the formula $Z = x - m/\sigma$, x was being the patient's score; m, the predicted score; and σ, the standard deviation. Pathological threshold was defined according to Pointrenaud (1995) (Supplemental data, E-Appendix 1). *Z*-scores were used in correlation analysis with fatigue and depression levels, except for dichotic listening and Zazzo's cancellation test since no standard deviations are available in these tests. Mean performances of patients were compared to mean expected normative scores (paired *t*-test). Mean scores of both MMF patients and CTL were compared using Mann–Whitney test. Correlations were assessed by Spearman *r* test. Statistical analyses were carried out using GraphPad InStat® 3.05 software. Only a p-value < 0.05 was considered significant for all univariate analyses, which compensated multiple comparisons.

3. Results

3.1. MMF patients: medical history and clinical features (Table 1)

Prospectively evaluated MMF patients had chronic fatigue lasting more than 6 months (25/25, 100%), myalgias (24/25, 96%), arthralgias (21/25, 84%), cognitive complaints (17/25, 68%), and mood disorders (13/25, 52%). Delay (median; range) elapsed from first symptoms to neuropsychological evaluation was 69.2 months (72; 30–133). The quantification of fatigue by visual analogical scale (VAS) showed heterogeneity of both baseline fatigue level before examination (median: 4.0; range: 0.8–8), and fatigue level after neuropsychological evaluation (median: 5.9; range: 3.5–9.5). Thirteen patients (52%) reached depression threshold according either MADRS or CES-D scale (MADRS: 40%; CES-D: 48%), only one patient being found severely depressed at both scales.

Delay (median; range) elapsed from last vaccine injection to first symptoms was 20.6 months (6; 0–96), to biopsy 67.6 months (72; 12–144), and to neuropsychological evaluation 89.8 months (89; 30.5–165). None of the patients had physical neurological signs suggestive of CNS involvement at time of muscle biopsy. Brain MRI was normal in 16/25 (60%) or showed non-specific brain supratentorial white matter T2-weighted hyperintensities in seven patients, small and punctuate in six, nodular in one. Two patients had other MRI changes including the sequel of a single small cerebellar ischemic lesion in one, and a silent carotid aneurysm in the other one. Patient #12 unexplainably died during sleep at age 37. Autopsy was denied.

3.2. Case-control study: evidence for specific cognitive dysfunction in association with MMF

MMF patients' cognition tests were more severely impaired than control patients with similar levels of pain, fatigue, and depression (Fig. 2; Supplemental data, E-Table 1). Actual intellectual abilities assessed by abstract reasoning (Matrix WAIS subtest), were found similar in MMF patients and controls, but test-by-test

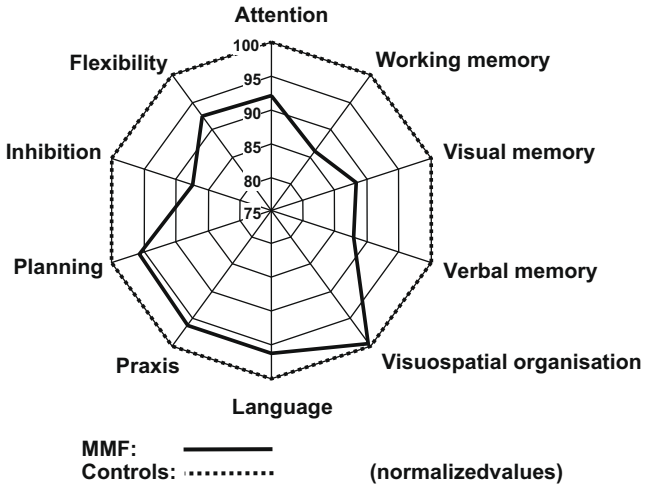

Fig. 2. Comparison of neuropsycological profiles in macrophagic myofasciitis (MMF, $n = 11$) and arthritic (CTL, $n = 11$) patients. Composite scores are shown in percentage (100 indicates value obtained in CTLs).

analysis showed distinctive cognitive alterations in MMF patients. Executive functions analysis revealed deficits in working memory as assessed by Brown–Petersen test ($p < 0.02$ for 5 s delay), and in inhibitory control, as assessed by Stroop interference part ($p < 0.05$). Memory tests showed that MMF patients were more severely impaired than controls in visual immediate memory, as assessed by Benton VRT ($p = 0.001$). Conversely, neither short-term verbal memory nor long-term visual and verbal memories were affected. MMF patients and controls had similar results for language, praxis and visual gnosia functions, except for the Boston naming test where MMF patients named worse than controls ($p < 0.02$). Finally, dichotic listening test was found impaired in MMF patients for the sentence repetition part of the task and for the left ear (left ear, MMF vs. controls: $p < 0.05$; MMF, left ear vs. right ear: $p < 0.05$), but not in the other test conditions (word repetition) (Fig. 3). Composite scores-based neuropsychological profiles (Fig. 2) further emphasized the discrepancies between MMF and arthritic patients, with decoupling, mainly affecting executive functions.

3.3. Cohort study: characterization of MMF-associated cognitive dysfunction (MACD)

Patient-per-patient analysis showed that at least one test gave results below the pathological threshold in all patients (100%), at least three tests in 18 (72%), at least five tests in four (16%). They displayed poor performance in immediate memory on both verbal and visual material (digit forward span, $p < 0.01$; Corsi block tapping test, $p < 0.001$ and Benton visual retention test (BVRT), $p = 0.06$). While long term episodic memory seemed to be spared as assessed by normal scores at California verbal learning test (CVLT), MMF patients presented impairment of visual long-term memory with a pathological recall in the Rey–Ostereith complex figure (ROCF) ($p < 0.01$). Language, praxis and visuo-spatial abilities were spared. Attentional deficit, which patients commonly complain of, largely contributed to the poor performances at the digit span (forward, $p < 0.01$ and backward, $p < 0.001$), the Corsi BTT ($p < 0.001$), the Zazzo's Cancellation Tasks ($p < 0.001$ for the three parts) the trail making tests A and B ($p < 0.05$) and finally the 0, 5, 10 and 20 s-delay part of the Brown–Petersen test ($p < 0.05$). Working memory was especially altered in the backward digit span and the Brown–Petersen test. A comparison between WAIS subtests revealed tendency for weaker scores in letter–number sequencing than vocabulary tasks ($p = 0.06$), suggesting working memory deficiency, whereas the standard score was not pathological according to the WAIS scale. Consistently with a classical dysexecutive syndrome, MMF patients displayed pathological performances on the copy of the ROCF ($p < 0.01$) mainly because of a disorganized structure construction, suggesting planning alteration at least in the visual construction (Fig. 4). Performance at the verbal fluency tasks was not significantly altered. Finally, the dichotic verbal auditory test confirmed results previously observed in both the exploratory and the case-control studies: MMF patients performed poorly only in the repetition of sentences displayed in the left ear (not different from pathological threshold, $p = 0.45$) and were normally above the pathological threshold (significant difference) in the other conditions. This left ear extinction in MMF patients could reflect either inter-hemispheric disconnexion or alterations in right primary auditory areas. (Figs. 5 and 6; Supplemental data: E-Table 2).

Composite scores, defining the normative scores as 100, displayed deficits in working memory, visual memory and visuoperceptual organization and, to a lesser extent, in planning, while remaining functions were spared (Fig. 6).

Cognitive impairment did not correlate with neither fatigue level (visual analogical scale) nor depression (MADRS and CES-D scores) except in a few circumstances (Spearman r test): the 0 s-delay part of the Brown–Petersen and the ROCF copy with fatigue level ($p = 0.03$ and $p = 0.02$, respectively). These findings indicated that fatigue had only a marginal impact on results, and that neither fatigue nor depression could be responsible for cognitive dysfunction.

Brain MRI abnormalities were not correlated with cognitive damage. The mean number of pathological tests was 3.2 (range 1–8) in patients with normal brain MRI and 3.7 (1–7) in patients with abnormal brain MRI (NS). Moreover, we were unable to find a single test correlated with brain MRI abnormalities. In particular,

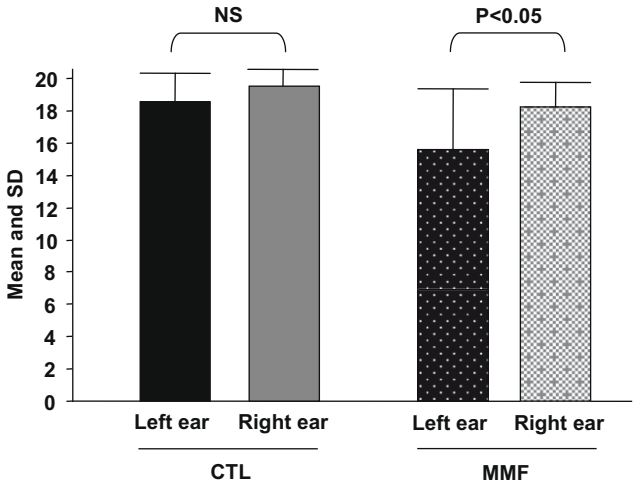

Fig. 3. Dichotic listening test, 'sentences' condition. Results in CTL (left panel; black: left ear; grey: right ear) and MMF patients (right panel; ultramarine: left blue; light blue: right ear). In MMF patients, the significant difference observed between left ear and right ear indicates inter-hemispheric dysconnexion.

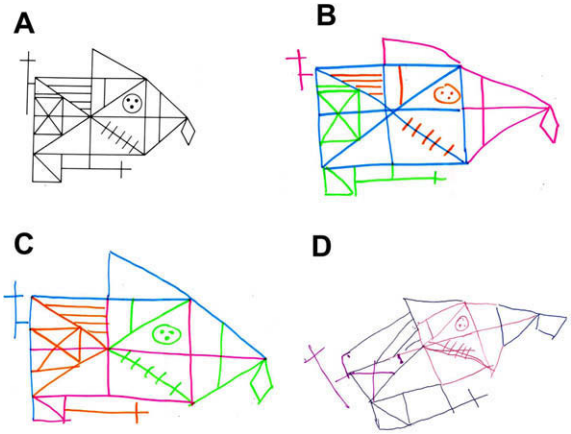

Fig. 4. The Rey–Ostereith complex figure (ROCF). In the administration of the test, subjects are shown the figure (A) and asked to copy it while it is in full view (ROCF Copy). Next the figure is taken away, and after a designated time period (three-mindelay for differed recall), subjects are asked to reproduce the figure from memory (ROCF Memory). Sample ROCF Copy drawings from healthy control (B) and MMF patients (C and D). In order to follow the drawing process, four different colored pens were successively used. (B) Normal copy of ROCF (Type I), starting from larger components to ending by smaller ones, i.e. from frame (blue) to details (red); color order (from 1 to 4): blue/pink/green/red. (C and D) Type IV abnormal results. Here, patients had planning disorder and used a piecemeal strategy evidenced by the different colors, leading either to almost accurate reproduction (C) or to deconstructed drawing (D); color order (from 1 to 4): C, blue/pink/green/red; D, blue/red/black/purple.

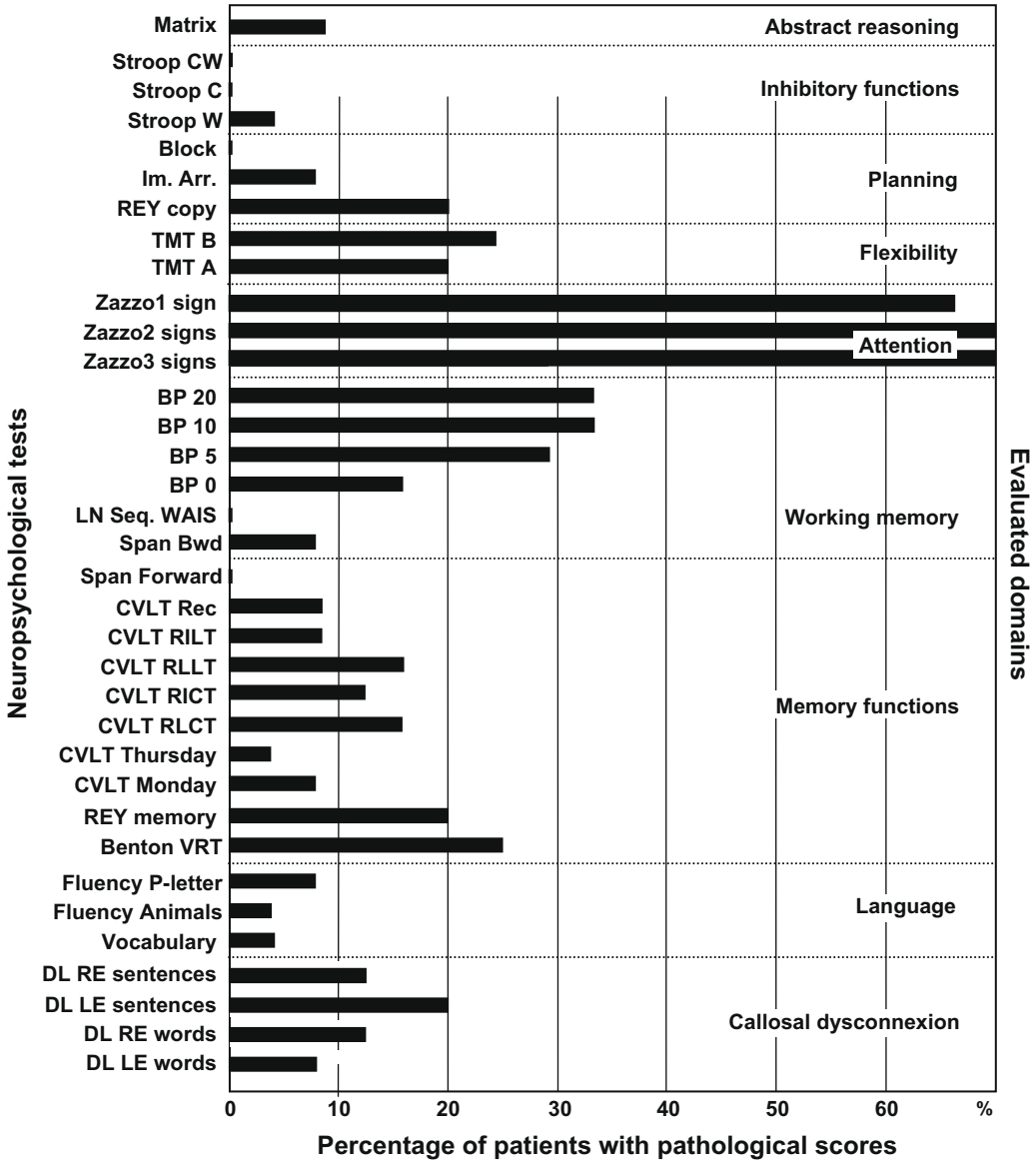

Fig. 5. Macrophagic myofasciitis patients ($n = 25$): neuropsychological profiles with composite scores in percentage.

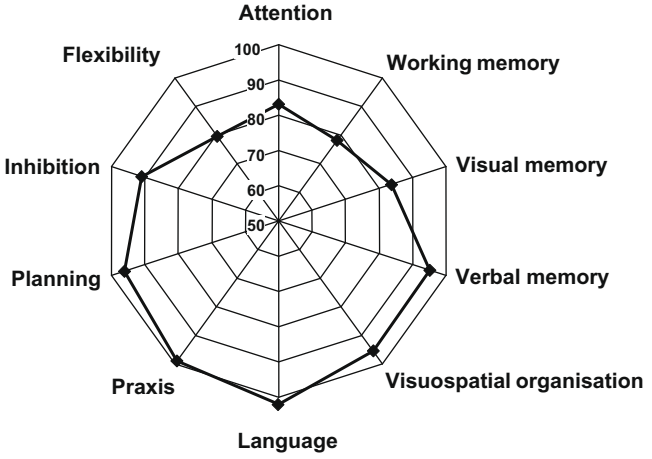

Fig. 6. Neuropsychological evaluation of MMF patients ($n = 25$): Percentages of pathological scores for each test distributed according to main evaluated domains.

among the five patients with impaired dichotic listening, four had normal brain MRI, and one had unspecific small punctuate hypersignals.

To evaluate if duration of the pathological process correlates with the severity of cognitive impairment, we compared temporal parameters (Vaccine-Onset Δ, Vaccine-Biopsy Δ, Vaccine-Test Δ, Onset-Test Δ) with results obtained at the three most sensitive tests, i.e. Brown–Petersen, ROCF and Dichotic Listening, and with the total number of tests reaching pathological threshold per patient. None of the temporal parameters correlated with cognitive performance.

4. Discussion

This study shows that almost all MMF patients exhibit characteristic deficits at neuropsychological testing, consistent with cognitive complaint, and largely unrelated to pain, fatigue or depression.

MMF patients had a normal general intellectual level according mean scores obtained at WAIS subtests. However, analysis subtest

by subtest showed that patients performed better at vocabulary subtest, which estimates pre-morbid intellectual ability [16], than at letter–number sequencing subtest, which depends on current functioning. Such a discrepancy unmasks alteration of actual intellectual functioning in MMF patients, hidden by the seemingly normal results [17–19].

Ad hoc neuropsychological battery provided precise depiction of MMF-associated cognitive dysfunction (MACD). Dysexecutive syndrome was the prominent feature of MACD, as attested by marked impairment of working memory, planning, flexibility, attention and inhibition. Both short-term and delayed visual memory and short-term verbal memory were also altered, while verbal episodic long-term memory and fluencies appeared preserved. Finally, dichotic listening test revealed inter-hemispheric disconnection.

Neuropsychological alterations have been reported in patients with chronic diffuse pain or depression [20]. In particular, patients with chronic widespread musculoskeletal pain compared to pain-free patients were shown to have attention deficit regardless of diagnosis [21]. The case-control study allowed us to point out and withdraw non-specific factors that may impact cognition, including depression, pain, fatigue and educational level. Compared to controls, MMF patients had similar general intellectual functioning (WAIS), but exhibited significant deficits in executive functions, and at dichotic listening for the left ear in "sentences" condition. Consistently, the cohort-study showed a similar pattern at dichotic listening. Taken together, data indicate that MMF patients have a specific cognitive dysfunction combining impaired visual memory, dysexecutive syndrome and alteration of dichotic listening. Such a pattern is highly suggestive of cortico-subcortical organic damages, in fronto-parieto-thalamo-striatal areas, mainly affecting the dorsolateral prefrontal cortex [22,23]. Disturbances at dichotic listening most often indicate deep white matter damages. Conventional MRI failed to reveal structural brain changes underlying MACD. Accordingly, we believe that further studies dealing with MACD should use diffuse tensor imaging that may reveal diffuse axonal injury or changes of white matter microstructural integrity, and 3D-MRI that allows measurement of cortical thickness and detection of atrophy.

Most patients with MMF fulfill criteria for chronic fatigue syndrome (CFS) [3], a complex multidimensional condition in which fatigue can manifest in multiple forms including mental fatigue [24]. One important symptom domain in CFS is cognitive dysfunction, 50–80% of CFS patients reporting cognitive difficulties. Although neurocognitive symptoms may considerably contribute to social and occupational dysfunction, their genuineness is still debated in this setting. Most often reported cognitive disorders in CFS refer to memory, concentration/attention, and information-processing speed [24–27]. Notably, the impairment of visuomotor skills ascribed to working memory deficits in CFS patients [28,29], is reminiscent of that found in MMF patients. We have previously proposed MMF histological lesions as a marker of a particular subset of CFS. So far, the prevalence of biopsy-proven MMF among CFS patients remains undetermined. Considering the extensive use of aluminum hydroxide-containing vaccines worldwide, it seems plausible that a number of MMF patients could have been enrolled in previous studies evaluating cognitive functioning in CFS.

The pathophysiology of neurocognitive impairment in MMF is unclear. Current knowledge indicates that MMF-type macrophagic infiltrates are found only at the site of previous intramuscular injection of aluminum hydroxide-containing vaccines, but, to our knowledge, brain pathological examination has never been performed in MMF. Notably, various types of inflammatory processes may occur, remote from the specific MMF lesion, in the setting of associated MS and other autoimmune disorders [4,9]. Neurocognitive dysfunction in MS notably resembles that found in MMF since domains commonly impaired in MS are episodic memory, attention/concentration, processing speed, verbal fluency and executive functions such as concept formation, abstract reasoning, planning, monitoring, and visual perception [8]. Cognitive deficit is frequent in MS, ranging from 43% to 72% of cases [8], and can occur early in the course of MS [30], without clear correlation with physical disability or disease duration, and sometimes as an isolated feature [31]. However, the rate of cognitive dysfunction is out of proportion to that of overt MS in MMF (9% in our series [4]). Notably, MMF patients with overt MS were deliberately excluded from the studied cohort to avoid bias.

Neurocognitive disorders similar to that found in MMF may also be observed in various pathological conditions unrelated to MS. The impairment of attention, concentration and working memory has been also reported in patients chronically infected by HCV or HIV, independently of depression, fatigue, or drug abuse, and assigned to viral neuroinvasion through particle-loaded mononuclear cells of the monocyte/macrophage lineage, used as 'Trojan horse' allowing viral penetration into the brain [32]. The local virus-induced release of cytokines and nitric oxide is thought to result in neurocognitive dysfunction [32]. Indeed, cytokines released by virally infected macrophages could induce synaptodendritic injury, thus affecting interneuronal communication and axoplasmic flow [33]. Higher cognitive functions critically depend on a most complex synaptodendritic network, which damage results in deficiencies in cognitive skills and behavior, as previously shown in HIV-associated cognitive dysfunction [33]. Notably severity of cognitive impairment in our patients did not correlate with disease duration, a finding more consistent with synaptodendritic injury than with neuronal loss, as previously reported in MS [31]. In summary, our results provide support to an emerging concept linking CFS, MS and HIV-related brain dysfunction [34].

Exposure to aluminum may also induce chronic alterations of visual memory, working memory and attention/concentration, as previously reported in hemodialyzed patients [35], metal inert gas welders [36], and people accidentally exposed to drinking aluminum sulphate-contaminated water [37]. In addition, an increased body burden of aluminum has been reported in both CFS patients [38] and MS patients [39], and more recently in a single patient with MMF [40]. In animals, absorption of aluminum from i.m. injected aluminum hydroxide is significant and may eventually be complete [41] that supposes entry in bloodstream and potential distribution to the whole body. Intramuscular injection of aluminum hydroxide adjuvant to rabbits was followed by entry of aluminum in brain [42], which may persist in brain tissue for a long time [43]. Aluminum is highly neurotoxic (see review in [44]). Parenteral administration of aluminum hydroxide suspension to mice was shown to induce behavioral and motor deficits, and increased neuronal apoptosis in brain [45]. In addition, aluminum opens the blood brain barrier [46] and increases endothelial adhesion of circulating leukocytes [47]. Taken together, these data may suggest that MACD could be, at least in part, ascribed to aluminum toxicity, although further studies are clearly needed to substantiate this hypothesis.

In conclusion, this work is the first firm demonstration that cognitive dysfunction is a central feature in MMF, this dysfunction being much more frequent and severe than suspected by routine neurological evaluation. Instead of being a non-specific bystander effect of pain, fatigue or depression, MACD seems to reflect an underlying organic, inflammatory or toxic, brain involvement.

5. Abbreviations

AFSSAPS	agence Française de sécurité sanitaire des produits de santé
AS	ankylosing spondylitis
BVRT	Benton visual retention test
CDC	centers for disease control
CES-D	centre for epidemiologic studies depression
CFS	chronic fatigue syndrome
CVLT	California verbal learning test
i.m	intramuscular
MACD	MMF-associated cognitive dysfunction
MADRS	Montgomery and Asberg depression rating scale
MMF	macrophagic myofasciitis
MRI	magnetic resonance imaging
MS	multiple sclerosis
NS	non-significant
RA	rheumatoid arthritis
ROCF	Rey–Ostereith complex figure
VAS	visual analogical scale
WAIS	Wechsler adult intelligence scale

Disclosure

The authors report no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jinorgbio.2009.08.005.

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