

CASE REPORT

Peculiar Cytological Cerebrospinal Fluid Pattern in a Case of Encephalomyelitis During Anti-Tumor Necrosis Factor- α Therapy

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Received: October 27, 2014 / Published online: March 6, 2015
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ABSTRACT

Introduction: Tumor necrosis factor- α (TNF- α) blocking agents may be associated with neurological adverse events, including demyelinating syndromes, that can be difficult to differentiate from multiple sclerosis (MS) and clinically isolated syndrome (CIS) as neither the clinical nor laboratory distinctive features have been reported. Usually clinicians mainly examine the diagnostic value of immunoglobulin G oligoclonal bands underestimating the value of other cerebrospinal fluid (CSF) parameters (such as CSF cytology).

Case Report: We present a case of a patient who acutely developed mild pyramidal and sensory impairment of lower limbs and urinary hesitancy during treatment with adalimumab, a monoclonal antibody to TNF- α ,

Electronic supplementary material The online version of this article (doi:[10.1007/s40120-015-0027-z](https://doi.org/10.1007/s40120-015-0027-z)) contains supplementary material, which is available to authorized users.

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for psoriatic arthritis. Magnetic resonance imaging demonstrated a widespread area of hyperintense signal extending from C5 to D8 level in T2-weighted images. Two consecutive CSF examinations showed an intense activation of monocyte/macrophage lineage (88% and 90%, respectively) with some giant and binucleated cells that notably decreased five months after TNF- α blocker cessation. We compared the results of CSF examinations of our patient with CSF results of 20 patients with MS and 20 patients with CIS that demonstrated activation of both lymphocytic and monocytic lineage (MS: 48% and 52%, respectively, CIS: 54.5% and 43.5%, respectively) that were very different from the findings in adalimumab-related encephalomyelitis in acute phase (11% and 89%, respectively). CSF cytology in two patients with neuromyelitis optica during the relapse ($n = 3$) showed minor monocyte/macrophage activation (9%) and an increased number of granulocytes (77%).

Conclusion: Prominent activation of monocyte/macrophage lineage with some binucleated giant cells in CSF could be induced by anti-TNF- α treatment. The peculiar CSF pattern, never found in MS, CIS, and NMO,

can help in differential diagnosis and stresses the importance of careful CSF cytology evaluation in the course of demyelinating diseases.

Keywords: Adalimumab; Cerebrospinal fluid; Clinically isolated syndrome; Encephalomyelitis; Multiple sclerosis; Neuromyelitis optica; Psoriatic arthritis; TNF- α blocking agents

INTRODUCTION

Tumor necrosis factor- α (TNF- α) plays an important role in many aspects of immune system development, immune-response regulation, and T cell-mediated tissue injury, and is involved in many pathological conditions [1]. TNF- α inhibition represents a significant advance in the treatment of rheumatoid arthritis, and is approved for the treatment of ankylosing spondylitis and psoriatic arthritis, as well as Crohn's disease and ulcerative colitis [1].

Tumor necrosis factor- α blocking agent adverse events include peripheral and central nervous system (CNS) demyelination [1–8]; however, the role of anti-TNF- α drugs in the development of demyelination has not yet been well defined. It is still a matter of debate whether TNF- α blocking agents could cause de novo nervous system demyelination or unmask latent disease, or whether the use of these drugs and the development of neurological disorders is coincidental [9, 10]. Any clinical, biological, or radiological evaluation can differentiate these conditions that have different prognosis and treatment. Here, we present a case of a patient who acutely developed mild pyramidal and sensory impairment of lower limbs and urinary hesitancy during treatment with

adalimumab, a monoclonal antibody to TNF- α , for psoriatic arthritis.

CASE REPORT

Informed consent was obtained from the patient for being included in the study. A patient came to the authors' attention complaining of gait impairment and lower limb hypoesthesia, rapidly ascending up to the base of the neck, with dysesthesia and urinary hesitation. Symptoms had begun after the third injection of adalimumab, a recombinant human immunoglobulin (Ig) G1 monoclonal antibody that selectively binds TNF- α and blocks its interaction with the p55 and p75 cell surface TNF receptors [1], that was prescribed for psoriatic arthritis. Neurological examination showed mild proximal weakness at the lower limbs and mild hypopallesthesia at the left lower limb.

The patient underwent brain and spinal magnetic resonance imaging (MRI): T2-weighted images showed a widespread area of hyperintense signal extending from C5 to D8 level and a clinically silent brain lesion adjacent to the left trigonum (Fig. 1). Spinal cord diameters were slightly increased, mostly at C5–C7 levels (Fig. 1). Both brain and spinal cord lesions were hyperintense on T1-weighted images after contrast enhancement.

The patient underwent serological examinations (immuno-rheumatological, thrombophilic, virological screening, and venereal disease research laboratory tests) that did not show any abnormalities. Immuno-rheumatological tests included: erythrocyte sedimentation rate, C3, C4, rheumatoid factor, IgG, IgA, IgM, antinuclear antibodies, extractable nuclear antibodies, anti-neutrophil cytoplasmic antibodies, anti-double-stranded DNA antibodies, anti-tissue antibodies,

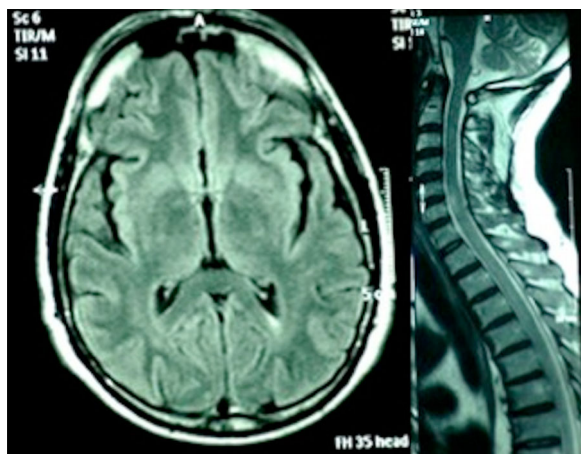


Fig. 1 Brain and spinal magnetic resonance imaging. T2-weighted images showed a widespread area of hyperintense signal extending from C5 to D8 level and a clinically silent brain lesion adjacent to left trigonum. Spinal cord diameters were slightly increased, mostly at C5–C7 levels

anti-endomysium antibodies, anti-transglutaminase antibodies, anti-gliadin antibodies, and anti-liver–kidney microsomal antibodies. Thrombophilic tests included: anti-beta 2 glycoprotein 1 antibodies, anti-cardiolipin antibodies, lupus anticoagulant antibodies, activated protein C resistance, protein C, protein S total, and omocysteinemia. Virological screening and venereal disease research laboratory tests included: anti-HIV, anti-hepatitis A virus (HAV), anti-hepatitis C virus (HCV), anti-hepatitis B virus (HBV) antibodies, hepatitis B surface antigen (HbsAg), venereal disease research laboratory test, anti-toxoplasma antibodies (IgM, IgG), anti-cytomegalovirus (CMV) antibodies (IgM, IgG), anti-viral-capsid antigen (VCA) antibodies (IgM, IgG), anti-herpes simplex virus (HSV) 1 and 2 antibodies (IgM, IgG), and anti-varicella zoster virus (VZV) antibodies (IgM, IgG).

Two consecutive CSF examinations were performed: glucose and protein levels, and IgG index were in normal ranges, whereas cell count (45 and 43 cells/ μ L, respectively) and blood–CSF

barrier permeability index (11.3 and 10.5, respectively) were increased. Oligoclonal bands were negative in both samples. A great number of activated cells of monocyte/macrophage lineage were present (88% and 90%, respectively), often forming large clusters. Prominent nucleoli were present in the majority of the clustered cells (Fig. 2a). Moreover, some huge cells with irregular cellular membrane and eosinophilic, vacuolated cytoplasm were detected. Several cells showed a big kidney-shaped nucleus; notably a few cells presented two nuclei (Fig. 2b).

Polymerase chain reaction (PCR) for HSV-1, HSV-2, VZV, and CMV was negative both in serum and CSF. Tuberculosis reactivation and meningeal carcinomatosis were excluded by X-ray of the chest, colonoscopy, serum neoplastic markers evaluation, and appropriate CSF examination. Neoplastic markers evaluation included alpha-fetoprotein, prostate-specific antigen, carcinoembryonic antigen, cancer antigen 19.9, cancer antigen 125, and cancer antigen 15.3. Peripheral blood lymphocyte typing was normal.

The patient underwent high-dose intravenous steroid therapy for 5 days with regression of dysesthesia. CSF examination was performed two more times, the last one, 5 months later: cell count progressively decreased (7 cells/ μ L), with the reduction of monocyte/macrophage lineage (47%), but rare giant cells were still present at the last examination. Blood–CSF barrier permeability index was slightly increased (9.6). During the follow-up, a test for anti-aquaporin antibodies was performed that did not reveal their presence in serum.

Neuroradiological follow-up did not show any spinal abnormality 4 months after symptoms onset, when both spinal diameter and signal intensity returned normal. Six months after onset of disease, the patient only

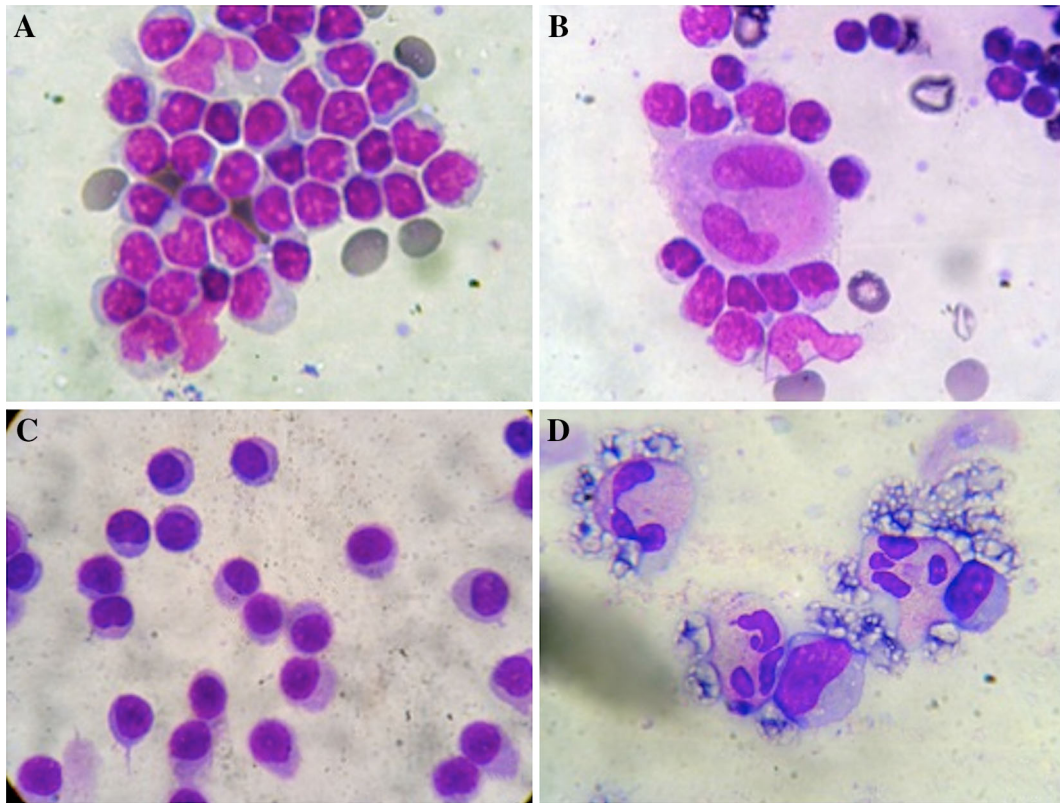


Fig. 2 CSF cytological analysis. **a** A large cluster of activated lymphomonocyte, with prominent nucleoli. **b** A giant cell, showing two big kidney-shaped nuclei, with irregular cellular membrane and eosinophilic, vacuolated

cytoplasm. **c** CSF examination during relapse of multiple sclerosis shows activation of lymphocytic lineage. **d** CSF examination during acute phase of neuromyelitis optica shows high levels of granulocytes. *CSF* Cerebrospinal fluid

complained of paraesthesia at foot level and very mild urinary hesitation. After 7 years of follow-up, the patient only complains of very mild dysesthesia; repeated brain and spinal MRI examinations were normal.

The prominent monocyte/macrophage lineage activation found in CSF of this case prompted us to compare CSF cytology examinations of our patients with those of multiple sclerosis (MS), clinically isolated syndrome (CIS), and neuromyelitis optica (NMO). The last 20 CSF cytological examinations of MS and CIS and 3 cases of NMO were compared with the 3 lumbar punctures performed in our patient (Table 1).

Cerebrospinal fluid analysis demonstrated the same degree of activation of both lymphocytic and monocytic lineage in MS and CIS group (MS: 48% and 52%, respectively, CIS: 54.5% and 43.5%, respectively; Fig. 2c), that was quite different from the findings in adalimumab-related encephalomyelitis in acute phase (11% and 89%, respectively), characterized by prominent activation of monocyte/macrophage lineage. There was no statistically significant difference in CSF parameters between patients with MS in relapse and remission (analyses were carried out using R version 3.1.1). CSF cytology in two patients with NMO during the relapse ($n = 3$)

Table 1 Results of CSF cytology in a patient with adalimumab-related encephalomyelitis in patients with CIS, MS, and NMO

CNS disorder	WBC, cells/ μ L	Lymphocytes, %	Monocytes, %	Macrophages, %	Monocytes + macrophage, %	Granulocytes, %
CIS ($n = 20$)	9 (5–45)	54.5 (24–95)	43 (5–65)	3 (1–20)	43.5 (5–68)	3 (1–10)
MS ($n = 20$)	14 (5–44)	48 (33–90)	52 (10–67)	0 (0–0)	52 (10–67)	5 (5–5)
Relapse in NMO ($n = 3$)	220 (2–500)	1 (0–35)	9 (3–44)	10 (6–14)	9 (4–23)	77 (21–90)
Remission in NMO ($n = 2$)	27 (8–46)	49.5 (48–51)	44.5 (41–48)	3 (3–3)	46 (44–48)	4.5 (4–5)
Adalimumab-related encephalomyelitis ^a	44 (43–45)	11 (10–12)	5 (5–5)	80 (80–80)	89 (88–90)	0 (0–0)
Adalimumab-related encephalomyelitis ^b	7	53	30	17	47	0

All values are presented as median (range)
 Range: minimum and maximum values

CIS clinically isolated syndrome, CNS central nervous system, CSF cerebrospinal fluid, MS multiple sclerosis, NMO neuromyelitis optica, WBC white blood cells

^a Two consecutive examinations of the same patient during the acute phase

^b CSF examination 5 months after therapy cessation

showed minor monocyte/macrophage activation (9%) and increased number of granulocytes (77%; Fig. 2d).

DISCUSSION

Tumor necrosis factor- α , secreted by microglia and macrophages, has a critical role in demyelination [11]. A clinical trial in 1999 showed a worsening of the course in patients with MS during anti-TNF- α treatment [12]. Subsequently, central or peripheral nervous system demyelination was recognized as a well-known adverse event of anti-TNF- α therapy [1–8]. In 2013, the Food and Drug Administration Adverse Event Reporting System documented 772 cases of demyelinating diseases related to TNF- α antagonists, including 167 cases related to adalimumab [10].

Tumor necrosis factor- α blocking agents could cause de novo nervous system demyelination or unmask latent disease, or the use of these drugs and the development of neurological disorders could be coincidental. Neither clinical nor laboratory distinctive features have been reported to differentiate demyelinating diseases related to TNF- α antagonists from other CNS demyelinating diseases. In fact, brain and spinal MRI usually show areas of hyperintensities in T2-weighted images with or without gadolinium enhancement on T1-weighted images that are not helpful in differentiation between demyelination due to anti-TNF- α treatment and underlying MS and CIS. CSF examination received very little attention and any distinctive CSF pattern was not reported to distinguish between CIS/MS and TNF- α induced CNS lesions. A detailed CSF examination was reported only in few cases and mainly focused on oligoclonal bands, protein concentration

and IgG index [3, 4, 7]. To the best of our knowledge, CSF cytology has not been reported.

In our case, CSF cytology showed a pattern that was different from CIS, MS, and NMO, being characterized by a great number of activated cells of monocyte/macrophage lineage with large kidney-shaped nucleus (88% and 90%, respectively, in two subsequent CSF analyses during the acute phase), often forming large clusters with lymphocytes; moreover, a few cells were binucleated. This pattern was clearly different from both CIS and MS that showed a similar increased percentage and activation of both lymphocyte and monocyte lineages. NMO is characterized, during acute phase of the disease, by the presence of granulocytes [13]. Moreover, in any of the 42 cases of CIS, MS, and NMO we found binucleated giant cells as in the adalimumab-related CNS inflammation.

The presence of binucleated cells in CSF can suggest a neoplastic origin and prompt testing for cellular expression or molecular tumoral markers. In the present case, the binucleated cells were not been assessed in regard to expression markers or molecular biology because the patient had two lesions, one in the spinal cord and one in the brain; bifocal lesions are very rarely of tumoral origin [14]. Besides, neoplastic origin for the binucleated cells was excluded by the negativity of the examinations performed to find a primary tumor, by the progressive reduction of the giant cells in the last lumbar puncture performed 5 months after the cessation of adalimumab, and by the absence of any neoplasia during 7 years of follow-up. A non-oncological origin of binucleated cells in CSF is the fusion of monocyte/macrophages, a well-known phenomenon occurring during granulomatous and herpetic infection [15], that in our patient were excluded by negativity of both viral PCR

detection and tuberculosis tests. In theory, the presence of binucleated cells would also require bone-marrow assessment, but this was not performed as CNS tumors in the course of anti-TNF therapy have never been reported [11, 16].

The mechanism causing the peculiar CSF pattern in our patient, that is a prominent activation of monocyte lineage, with increased number of macrophages and their fusion into binucleated cells during adalimumab treatment, is unknown; however, some data suggest an involvement of the CD36 receptor expressed on monocytes and a direct role of the monoclonal anti-TNF- α antibody [15, 17]. In fact it has been demonstrated that, while TNF- α inhibits, on the contrary, adalimumab increases CD36 membrane expression on human monocytes [17] and CD36 can cause macrophages fusion [15].

The 7 years of follow-up without a second CNS inflammatory involvement, the absence of oligoclonal bands in CSF, the progressive attenuation of the symptoms, and the normal spinal cord and brain MRI performed during the follow-up strongly suggest that the CNS lesions were a single acute episode caused by the treatment with adalimumab, that did not unmask a preclinical demyelinating disease and was not triggered MS.

CONCLUSION

Our case indicates that CSF cytological examination must be included in the study of CNS adverse events occurring in anti-TNF- α -treated patients. Further CSF cytological examinations in patients with similar clinical conditions will establish if the finding of prominent activation of monocyte/macrophage lineage with binucleated CSF cells is a hallmark of this clinical condition, allowing for the distinction

between a demyelinating chronic disease and a single inflammatory episode, remitting with the withdrawal of the anti-TNF- α treatment.

ACKNOWLEDGMENTS

No funding or sponsorship was received for this study or publication of this article. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval for the version to be published.

Conflict of interest. Yana Motuzova, Alessia Di Sapio, Marco Capobianco, Arianna Sala, Fabiana Marnetto, Simona Malucchi, and Antonio Bertolotto declare no conflict of interest.

Compliance with ethics guidelines. Informed consent was obtained from the patient for being included in the study.

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