Vitamin B-12 deficiency in the setting of nitrous oxide abuse: diagnostic challenges and treatment options in patients presenting with subacute neurological complications

~~Neurological complications due to v~~Vitamin B_{12} deficiency in the setting of nitrous oxide abuse: diagnostic challenges and treatment options in patients presenting with subacute neurological complications~~.~~

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Keywords

Nitrous oxide, vitamin B_{12} deficiency, neurological symptoms, diagnosis, treatment.

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Abstract: 164 words

Article: words

Abbreviations: crea: creatinine, tHCy: homocysteine, MCA: 2-methylcitric acid, MMA: methylmalonic acid; MRI: magnetic resonance imaging; SEP: Somatosensory evoked potentials

Declaration of interest

Funding: this work received no fundings.

Disclosure statement: no conflicts of interest.

Acknowledgement

Professor P. Vermeersch is a senior clinical investigator of the FWO Vlaanderen.

Met opmaak: Lettertype: (Standaard) Times New Roman, 12 pt

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Abstract

It is well recognized that nitrous oxide abuse can lead to vitamin B_{12} deficiency presenting with neurological complications. Nevertheless, establishing this diagnosis can be challenging and treatment guidelines are lacking. In this paper we present a case series of eight patients and discuss the diagnostic challenges and treatment options for vitamin B_{12} deficiency due to nitrous oxide abuse presenting with neurologic complications. Biochemical findings are not always straightforward and complementary testing is often necessary. Magnetic Resonance Imaging (MRI) revealed a longitudinally myelopathy extending over a long segment typically involving the dorsal columns of the cervical cord. To increase the lesion conspicuity, dedicated MRI sequences are needed. In our practice, we recommend the use of T2-weighted images (WI) with fat suppression (FS). Treatment consists of cessation of nitrous oxide abuse and supplementation with intramuscular injections of cobalamin. Because of a lack of treatment guidelines, we also describe the treatment schedule used in our neurology clinic and give a brief overview of treatment options suggested in the literature.

Introduction

The traditional clinical findings associated with severe vitamin B_{12} deficiency are macrocytic anemia and variable neuropsychiatric symptoms. The clinical presentation is, however, often more subtle and many cases are only identified in response to incidental hematological findings. Causes of vitamin B_{12} deficiency include dietary deficiency (e.g., strict vegan diet), pernicious anemia, decreased gastrointestinal absorption (e.g., celiac disease, gastric atrophy), certain drugs (e.g., metformin) and nitrous oxide abuse.

In patients with classical megaloblastic anemia, ~~the presence of a~~ low serum cobalamin level and ~~objective assessment of response in terms of the~~ a rise in hemoglobin ~~concentration~~ after supplementation clearly outlines the treatment pathway. However, most patients do not have such a clear-cut picture. In this case establishing the diagnosis of a vitamin B_{12} deficiency can be challenging. A serum vitamin B_{12} below 200 ng/L is typically considered low and consistent with a deficiency (1). While more than 95% of patients with megaloblastic anemia have a serum vitamin B_{12} below 200 ng/L (1), the sensitivity in patients without overt symptoms is significantly lower (2). This is particularly true for vitamin B_{12} deficiency due to nitrous oxide (3). Vice versa, the significance of low cobalamin levels in patients with non-specific symptoms and without anemia is also uncertain. To improve the specificity and sensitivity, additional testing is often recommended when serum vitamin B_{12} is below 200 ng/L or between 200 ng/L and 300 ng/L. Homocysteine (HCy) is typically elevated both in vitamin B_{12} and folic acid deficiency, while methylmalonic acid (MMA) is only elevated in vitamin B_{12} deficiency. Of note, MMA in plasma is not widely available due to the relatively high cost (1, 3). While measuring HCy and MMA can help in the diagnosis of vitamin B_{12} deficiency, the results of HCy and MMA can also be inconclusive (1).

The most common neurological complications due to vitamin B_{12} deficiency in the setting of nitrous oxide abuse or 'laughing gas' are numbness, paresthesia, weakness, and gait difficulties (4, 5). These symptoms are caused by subacute combined degeneration and sensorimotor polyneuropathy (3, 4). In clinical practice, we regularly encounter patients with neurologic complications due to functional vitamin B_{12} deficiency caused by nitrous oxide abuse. Given the fact patients are often reluctant to admit nitrous oxide abuse, the diagnosis is often challenging.

In this paper we present clinical, biochemical, and radiological data of eight patients and discuss the ~~diagnostic challenges~~diagnostic testing strategy~~s~~ and treatment options of vitamin B_{12} deficiency in the setting of nitrous oxide abuse. All eight cases were diagnosed within fifteen months of the start of the COVID-19 pandemic. Six of our eight patients ultimately admitted to recreational use of nitrous oxide. Two of our patients denied personal use of nitrous oxide, but may have been affected due to passive exposure to nitrous oxide as bystanders based on the patient interview (6)~~-~~(7). Other causes of vitamin B12 deficiency were excluded. None of the patients followed a strict vegan diet, had pernicious anemia, took a proton pump inhibitor or had celiac disease.

Case 1

A 19-year-old male presented with subacute gait difficulties~~, originated over~~since one week. He reported progressive weakness and sensory disturbances in the lower limbs. He admitted to abusing nitrous oxide frequently for over two years. Physical examination revealed an ataxic gait with ~~normoflexia~~normal reflexes. There was a T6 sensory level with absent sense of vibration in both legs. Romberg's sign was negative.

The patient's blood work revealed a normal full blood count and normal vitamin B_{12} of 220 ng/L [reference range: 187 – 883ng/L], with an elevated HCy level of 59.5 µmol/l

[7.0 – 15.0 µmol/L]. MMA levels and 2-methylcitric acid (MCA) levels in morning urine were elevated, respectively 427 mmol/mol creatinine [≤ 1 mmol/mol crea] and 21 mmol/mol crea [≤ 2 mmol/mol crea]. There were no abnormalities in cerebrospinal fluid (CSF) testing. EMG was not performed, but tibialis SEP (somatosensory evoked potentials) was abnormal. MRI of the cervical spinal cord showed an inverted V-sign on axial FS T2-WI.

He was treated with intramuscular hydroxocobalamin. He regained a normal gait.

Case 2

An 18-year-old male was evaluated in our neurology clinic because of subacute paresthesia in fingers and lower limbs, as well as walking difficulties and balance problems. The cComplaints progressed over three weeks. On physical examination tendon reflexes were weak and ankle reflexes were absent. Superficial as well as deep sensory testing was reduced, and there was an ataxic gait. Romberg's sign was negative. He admitted to abusing nitrous oxide frequently.

Blood examination revealed a normal level of vitamin B_{12} of 482 ng/L and an elevated level of HCy of 39 mmol/L. CSF protein level was slightly increased (47 mg/dL, [15-40mg/dL]) with a normal cytosis. Electromyography (EMG) showed a pattern of axonal morerather than demyelinating neuropathy in the lower limbs. Tibialis SEP was abnormal. MRI of the cervical spine showed an inverted V-sign on axial FS T2-WI. MMA and MCA in morning urine after the start of intramuscular supplementation were normal. He was treated with intramuscular cyanocobalamin and physiotherapy. He regained a normal gait.

Case 3

A 30-year-old male presented to the emergency department with subacute progressive sensory disturbances and subjective weakness in both upper and lower limbs, progressed over one week. Nitrous oxide abuse was denied. He did admit to attending parties where nitrous oxide was inhaled. On examination, there was a hyporeflexia in upper and areflexia in lower limbs. Superficial as well as deep sensory testing were affected. His gait was normal ~~and Romberg's sign negative~~. Romberg's sign was negative.

Blood testing revealed a reduced vitamin B_{12} level of 180 ng/L and an elevated HCy level of 44.6 µmol/L. Morning urine MMA as well as MCA levels were elevated (both 9 mmol/mol crea). CSF showed a minimal elevated protein level of 45 mg/dL with a normal cytosis.

EMG showed a mild axonal neuropathy. Tibialis SEP was not performed. Magnetic Resonance Imaging (MRI) revealed bilateral increased T2-signal intensity in the dorsal columns C3 through C5, with an inverted V-sign on axial FS T2-WI ~~weighted images (WI) with fat suppression (FS)~~ (figure 1). There was no enhancement after administration of gadolinium-based contrast agent.

The patient was treated with intramuscular cyanocobalamin. He was lost to follow-up.

Case 4

A 19-year-old woman presented with a one-week history of progressive numbness and paresthesia in upper and lower limbs. She said she did not use nitrous oxide herself, but did attend gatherings where nitrous oxide was used. Physical examination revealed an ataxic gait with absent deep tendon reflexes. Superficial as well as deep sensory testing were affected. Romberg's sign was positive.

The patient's blood work showed a normal B_{12} level of 330 ng/L, with an elevated HCy level of 25.9 µmol/L. MMA and MCA were elevated in morning urine (31 mmol/mol

crea and 5 mmol/mol crea, respectively). Further testing was negative. There were no abnormalities in CSF testing. EMG showed a mild axonal neuropathy. Tibialis SEP was not performed. MRI of the cervical spine revealed a hyperintense signal of the dorsal columns extending from C2 to C6 with an inverted V-sign on axial FS T2-WI (figures 2A and C). There was no enhancement after administration of gadolinium-based contrast agent.

The patient was treated with intramuscular cyanocobalamin and physiotherapy, with slow recovery of gait.

Case 5

An 18-year-old woman presented with subacute numbness and paresthesia in the lower limbs which extended to the upper limbs. Complaints progressed over six weeks. She admitted abusing large amounts of nitrous oxide during several months. Physical examination revealed an ataxic gait with weak deep tendon reflexes in upper and lower limbs. Sensory testing was affected ~~in~~ more in th~~n~~e lower ~~more~~ than in upper limbs.

Laboratory workup showed a normal vitamin B_{12} of 280 ng/L with an elevated HCy of 32.4 µmol/L. MMA in morning urine was elevated (10 mmol/mol crea). Tibialis SEP was abnormal. EMG showed a pronounced axonal sensorimotor polyneuropathy and an inverted V-sign on axial FS T2-WI was seen.

She was treated with intramuscular cyanocobalamin. An improvement of her gait was observed over weeks.

Case 6

A 22-year-old male presented because of progressive numbness in upper and lower limbs for three months. He admitted to infrequent abuse of nitrous oxide. Neurological examination was normal~~.~~

Examination of his blood showed a normal vitamin B_{12} of 280 ng/L with an elevated HCy of 48.5 µmol/L. ~~-~~MMA and MCA were both elevated (4 and 3 mmol/mol creatinine, respectively). EMG nor tibialis SEP were performed. MRI of the cervical spine revealed a doubtful inverted V-sign on the axial FS T2-WI because of artefacts.

He was treated with intramuscular cyanocobalamin. On ~~and lost to~~ follow-up, sensory complaints had dissapeared ~~afterwards~~.

Case 7

A 24-year-old male consulted because of subacute progressive sensibility ~~disorders~~ loss in upper and lower limbs since a few ~~over~~ days. He admitted abusing large amounts of nitrous oxide. Physical examination revealed absent ankle reflexes and an ataxic gait. Superficial as well as deep sensory testing were affected. Romberg's sign was positive.

Laboratory testing revealed a normal vitamin B_{12} (274 ng/L) but a clearly elevated HCy (65.3 µmol/L), MMA (82 mmol/mol crea) and MCA (7 mmol/mol crea). MRI of the cervical spine was normal and CSF testing showed no abnormalities, but EMG showed an axonal-demyelinating polyneuropathy. Tibialis SEP was normal.

The differential diagnosis of polyneuropathy secondary to nitrous oxide abuse and acute motor-sensory axonal neuropathy (AMSAN) was made. Because of a major functional loss due to the symptoms, treatment was started without delay with both intravenous immunoglobulin for five days and intramuscular cyanocobalamin before ~~the~~ all the biochemical results were known, followed by in-hospital rehabilitation. He was lost to follow-up.

Case 8

A 23-year-old male presented with subacute progressive numbness and paresthesia in upper and lower limbs and gait problems, progressive since a couple of weeks. He admitted abusing nitrous oxide. Physical examination revealed weak deep tendon reflexes and absent ankle reflexes as well as an ataxic gait. Superficial as well as deep sensory testing were affected. Romberg's sign was positive.

Laboratory workup showed a normal vitamin B_{12} (247 ng/l) and an elevated HCy (54.5 µmol/L), MMA (43 mmol/mol crea) and MCA (3 mmol/mol crea). EMG revealed an axonal sensorimotor polyneuropathy and MRI of the cervical spine showed an inverted V-sign on the axial FS T2-WI. Tibialis SEP was abnormal.

He was treated with intramuscular cyanocobalamin and his gait improved gradually.

Discussion

Nitrous oxide was first used as an inhaled anesthetic in 1844. The first description of nitrous oxide as a recreational drug dates already from the end of the nineteenth century (8). The inhalation of nitrous oxide causes a euphoric feeling but lasts for only one minute. It has gained popularity as a recreational drug in recent years and even more so during corona 'lockdown parties'. It is still legal in Belgium, although efforts are being made to change legislation, especially for minors. The eight patients we present in this case series were seen in our regional hospital in a 15-month period during the COVID-19 pandemic. It is well known that nitrous oxide use can cause secondary functional vitamin B_{12} deficiency. Sometimes cyanocobalamin supplementation tablets are even sold alongside nitrous oxide.

From the patient's perspective the most important clinical neurological consequence that persuades him or her to seek medical attention are gait problems. All our patients had a ~~subacute~~recent onset of complaints, ranging from a couple of days to weeks. ~~Long-term~~ ~~n~~Neuropsychiatric symptoms, which often only present after ~~long~~prolonged ~~term~~ vitamin B12 deficiency, might not develop within ~~present at this~~ a couple of weeks~~interval~~. Vitamin B_{12} deficiency causes toxic myelopathy and is specifically pernicious to the posterior columns of the spinal cord causing sensory ataxia. In cases one, two, four, five, seven and eight the sensory ataxia was the most important clinical sign with a broad-based gait and positive Romberg's sign. All six of them admitted the recreational use of nitrous oxide. However, the patient of case four used it only once, but mentioned the frequent exposure to other people abusing it. We previously described the possible toxic effect in innocent bystanders earlier (6, 7).

Vitamin B_{12} metabolism

The two most important locations for the uptake of vitamin B_{12} (cobalamin) are the stomach and terminal ileum. Intrinsic factor, produced by the parietal cells in the stomach, is essential for adequate absorption in the terminal ileum. One of the most common causes of vitamin B_{12} deficiency in older patients is autoimmune gastritis with a lack of intrinsic factor (pernicious anemia). It can be detected by testing the serum of the patient for parietal cell antibodies and intrinsic factor antibodies which are present in respectively 80% to 90% and 50% to 60% of patients with pernicious anemia (9). Another significant cause of vitamin B_{12} deficiency is a disease of the terminal ileum like Crohn disease.

Vitamin B_{12} in plasma is bound to two types of proteins: holotranscobalamin (30%) and holohaptocorrin (70%). Holotranscobalamin is the active form of cobalamin and is the most important due to its essential role in the uptake of vitamin B_{12} into the cells and tissues. ~~The analysis of vitamin B_{12} bound to transcobalamin, however, is not routinely performed in laboratories due to the higher cost~~ (10)~~.~~

Cobalamin has two major forms that are used as co-factor in two metabolic pathways: adenosyl cobalamin and methyl cobalamin. Adenosyl cobalamin plays a major role in converting methylmalonyl-CoA to Succinyl-CoA, while methyl cobalamin assists in converting homocysteine to methionine (11).

Nitrous oxide oxidates the cobalt ion in cobalamin and irreversibly inactivates the enzyme methionine synthetase. This impairs the production of methionine from homocysteine (12). Methionine is used in the synthesis of thymine, in turn an essential nucleobase for DNA. In case of vitamin B_{12} depletion there will be a problem in the DNA-synthesis which will cause megaloblastic anemia. As another result of the lack of methionine,

myelin proteins can't be methylated and thus cause demyelination within the central and peripheral nervous systems (11).

We can assume this is not the only pathophysiological mechanism of nitrous oxide because not all patients develop subacute combined degeneration. Two cases were described with sensorimotor peripheral neuropathy and a history of nitrous oxide abuse without imaging evidence of myelopathy (11). This is also true for our patient of case seven. It seems not every biochemical mechanism of nitrous oxide abuse is known to this date (9).

Biochemical analyses

In all our cases, except for case three, normal cobalamin levels were measured. However, this does not exclude a functional vitamin B_{12} deficiency. Even when the serum level of vitamin B_{12} is normal, it is still possible that there is a functional depletion of vitamin B_{12} in the body tissues. The analysis of active vitamin B_{12} (holotranscobalamin) is considered a more ~~reliable~~sensitive test for vitamin B_{12} deficiency, especially as an early marker~~.~~ (13). Holotranscobamaine was, however, normal in the 4 patients in whom this was tested (see Table 1).~~Our laboratory recently started performing this analysis. Not unimportant, there is also the recurring problem of variability in cut-off values depending on the laboratory where this active vitamin B_{12} is analyzed. If the result is in the indeterminate 'grey zone',~~

~~confirmatory testing is needed in order to determine its clinical relevance.~~

~~Therefore~~To rule out a possible vitamin B12 deficiency, fasting HCy in plasma and MMA and ~~methylcitrate~~ MCA in urine must be ~~analyzed~~ determined (14). Alternatively, MMA can also be measured in plasma, which is more practical and less time-consuming, but this test is not reimbursed in Belgium. The elevated fasting plasma HCy and the ~~organic~~

Met opmerkingen [PV1]: Ik zou dit weglaten want dit geld took voor gewoon B12.

~~acids~~increased MMA in urine ~~is~~ are the result of accumulation because they cannot be converted into methionine or Succinyl-CoA, respectively, due to a functional depletion of cobalamin. We noted that both HCy level in plasma and MMA ~~and methylcitrate~~ in urine was~~ere~~ elevated in all patients, except for the second patient ~~o~~where MMA ~~and methylcitrate~~ in urine ~~were~~ was only measured after treatment was started.

Of note, a mild to moderate increase of HCy and MMA can also be caused by a reduced renal function, as both are excreted in urine. In addition, HCy can also be ~~-~~elevated in case of depletion of folic acid or vitamin B_6 (10).

While vitamin B_{12} depletion can cause megaloblastic anemia, patients with neurological complications secondary to functional vitamin B_{12} deficiency have a normal MCV in 25% of the cases. A normal MCV therefore can't be used as an exclusion criterium (1).

Given the detrimental neurological complications of vitamin B_{12} deficiency, the clinical picture plays a crucial role in the diagnosis and the treatment should not be delayed in case of doubtful biochemical findings (1).

Imaging

The clinical picture of our patients can largely be explained by spinal cord demyelination. On MRI, ~~this~~ the demyelin~~is~~ation due to vitamin B12 deficiency typically involves the dorsal columns of the spinal cord bilaterally over a long segment extending over three or more cervical segments usually extending from C2 to C6. ~~These MRI findings are specific to vitamin B_{12} deficiency, not to a specific reason of this deficiency.~~ In other demyelinating diseases, like multiple sclerosis, the hyperintense lesions are shorter and transverse myelitis is differentiated by predominant localisation in the anterior and lateral columns of the spinal cord with even longer involvement (15).

Met opmerkingen [PV2]: MCA is formed when C3 is incorporated instead of C2 in the citric acid cycle in case of accumulation of of propionyl-CoA (also in propionic academia upstream from methylmalonyl mutase).

Met opmerkingen [PV3]: Zou MCA weglaten voor eenvoud

The lesion conspicuity can be increased by using dedicated sequences for demyelination. In addition to routine sagittal T1- and T2 sequences (16) and T2* weighed spoiled gradient echo sequence (GRE), we recommend using sagittal and axial images with fat suppression. Axial images should be prescribed on the areas of abnormal signal seen on the sagittal images. Involvement of the dorsal columns results in the inverted V-sign, resembling inverted rabbit ears on axial images (17, 18).

Furthermore, in case of high clinical suspicion of demyelination, it is of utmost importance to inform the radiologist appropriately, and specifically ask for demyelination and/or fat suppression when planning cervical MRI imaging. Not using the best sequence, with fat suppression, may result in failure to detect pathological signs. Figures 2B and C show an axial T2* weighed spoiled gradient echo sequence and FS T2-WI respectively on the same level and in the same patient. The lesion is clearly better visible on the FS T2-WI.

Electrophysiological testing

Peri~~f~~pheral neuropathy on EMG and prolonged SEP's are common in vitamin B12 deficiency. This is especially true when there are neurological complaints, and might even correlate with the disease severity and prognosis (19)~~(ref puri et al 2005)~~. Even in asymptomatic – or subclinical - patients with vitamin B12 deficiency, somatosensory evoked potentials might be abnormal (20) ~~(ref Gökçe Çokal et al 2016).~~

~~In our patient group,~~The six ~~out of eight~~ patients who had an EMG~~;~~ all ~~of them were~~had an abnormal result. ~~In four of them,~~EMG showed~~-~~ mostly axonal damage ~~was seen,~~ in four of the patients and ~~in two out of the six, there was a~~ mixed axonal-demyelinating ~~image~~damage in two patients. This ~~seems to correspond~~results are in line with~~to~~ previous

studies ~~ly described populations~~ (19, 20) ~~(ref ref puri et al 2005 and ref Gökçe Çokal et al 2016).~~

In five patients, somatosensory evoked potentials were performed.~~,~~ ~~I~~n all but one, tibialis SEP was abnormal. This also corresponds to previous reports (20).

The electrophysiological test had no added value ~~In~~for the clinical ~~differential~~ diagnosis in our patients ~~however, these results did not really make a difference~~. It is possible that results of electrophysiological testing are ~~is~~ correlated with disease severity and prognosis (19) ~~(ref Puri et al)~~, but this was not the topic of our research.

Treatment

The treatment of choice in patients with vitamin B_{12} deficiency caused by nitrous oxide abuse is suppletion by intramuscular injections of cyanocobalamin of 1000 µg, besides of course the cessation of nitrous oxide abuse. There is no clear-cut guideline available in literature about frequency and duration of this treatment. In our hospital the following scheme was used: one injection every day for one week, followed by one injection every week for one month and finally one injection every month for five months. Thus, a total treatment duration of six months.

Another common approach is intramuscular cyanocobalamin 1000 µg daily for a minimum of five days followed by a four-week regimen of 1000 µg intramuscular weekly (21). Oral administration of cyanocobalamin has proved to be as effective as intramuscular injections at a dose of 1000 to 2000 µg daily for one to two weeks, followed by maintenance of 1000 µg daily (21).

~~A recent case report also suggested the combination of intramuscular vitamin B_{12} injections 500 µg per day for 19 days and intravenous Methylprednisolone 500 mg per day for five days, followed by oral vitamin B_{12} 500 µg per day and methylprednisolone 20 mg per day. No duration for oral treatment was mentioned in the study. Methylprednisolone was added because of the subacute combined degeneration due to demyelination in a patient with vitamin B_{12} deficiency secondary to nitrous oxide abuse. Other possible causes of demyelination were excluded thanks to extensive analyses on serum and CSF. There was a complete recovery, clinical and radiological, in this patient in only eight months (22).~~

In literature a methionine supplementation has also been suggested as a possible treatment option, but methionine is not standard available at hospital dispensaries and the methionine supplementation will only correct the lack of methionine (21).

Although 86% of patients with myeloneuropathy treated with cyanocobalamin have some recovery, only 14% fully recover 1 to 84 weeks after diagnosis. (12)

When there is suspicion of a hereditary cobalamin C/D deficiency, for example in patients with a remarkably high level of MMA or MCA~~methylcitrate,~~ or a confirmed MMACHC gene mutation, treatment consists of intramuscular injections of hydroxocobalamin. Because of the relative high levels of MMA and ~~methylcitrate~~ MCA in case one, we treated this patient with hydroxocobalamin in anticipation of the results of his genetic testing for a MMACHC gene mutation. Afterwards this returned to be negative. Intramuscular injections of cyanocobalamin in patients with a hereditary C/D deficiency are not useful, because cyanocobalamin is an inactive form that must be activated and converted by cobalamin reductase, an enzyme produced by the MMACHC gene. (23)

Conclusion

There is an increasing number of patients in clinical practice with neurological symptoms due to vitamin B_{12} deficiency in the setting of nitrous oxide abuse. Diagnosis is not always straightforward. Clinical examination and patient interview, including possible nitrous oxide exposure, is essential to avoid a missed diagnosis of nitrous oxide abuse. With respect to biochemical testing, vitamin B_{12} level alone is often not sensitive enough in the setting of nitrous oxide use. Homocysteine level in serum and urine organic acid analysis~~s~~ (MMA and ~~methylcitrate~~ MCA) ~~in urine~~ are useful, although not with perfect specificity. The use of dedicated MR sequences, such as T2-WI in combination with fat-suppressed techniques, is useful to increase lesion detection and precise evaluation of the extent of the lesion. Treatment should be started as soon as possible. Prognosis is variable, but correct and timely treatment are essential.

Figure legend

Figure 1: inverted V-sign on axial FS T2-WI in the patient of case 3.

Figure 2: hyperintense signal of the dorsal columns extending from C2 to C6 on sagittal T2-WI (A) and inverted V-sign on axial FS T2-WI (C) in the patient of case 4.

Figure 2 (B and C): axial T2* weighed spoiled gradient echo sequence (B) and FS T2-WI (C) respectively on the same level in the patient of case 4.

Abbreviations: FS, fat suppression; WI, weighted image.

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Table 1: clinical, biochemical, and radiological characteristics of our patients.

Case	M/F	Symptoms			Biochemical results								In
		numbness and paresthesia	hypo- to areflexia	ataxic gait	vitamin B_{12} (187-883 ng/L)	Active vitamin B_{12} (20-134 pmol/L)	HCy (7.0-15.0 µmol/L)	MMA (≤1 mmol/ mol crea)	MCA (< *or* = 2 mmol/ mol crea)	Hb (g/dL)	RBC (10*6/µL)	MCV (84-96 fL)	M sequen sup
1	M	T6 level and lower limbs	no	yes	220	NM	59.5	427	21	15.2	4.58	96	invert
2	M	upper and lower limbs	yes	yes	482	NM	39.0	<10*	0not detectable <1*	16.8	5.49	86	inve sign
3	M	upper and lower limbs	yes	no	180	NM	44.6	9	9	16.5	5.06	92	invert
4	F	upper and lower limbs	areflexia	yes	330	NM	25.9	31	5	13.9	5.24	87	invert
5	F	upper and lower limbs	yes	yes	280	36,4	32.4	10	1	13.7	4.12	97	inverted V-sign
6	M	upper and lower limbs	no	no	280	23,7	48.5	4	3	15.3	5.04	93	doubtful inverted V-sign
7	M	upper and lower limbs	yes	yes	274	65,5	65.3	82	7	13.7	4.09	98	normal
8	M	upper and lower limbs	yes	yes	247	32,2	54.5	43	3	16.3	4.99	92	inverted V-sign

** results after start of treatment*

Abbreviations: M, male; F, female; NM, not measured; HCy, homocysteine; MMA, methylmalonic acid; MCA, 2-methylcitric acid; Hb, hemoglobin; RBC, red blood cell count; MCV, mean corpuscular volume; MRI, magnetic resonance imaging.

~~Reference values: Vitamin B_{12}: 187-883 ng/L; HCy: 7.0-15.0 µmol/L; MMA: ≤ 1 mmol/mol; MCA: < or = 2 mmol/mol; MCV: 84-96 fL~~

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Methods: Vitamin B12 and holotranscobalamin were determined with Alinity (Abbott), HCy with ACL Top 500 (Werfen), and MMA and MCA with gas chromatography mass spectrometry.

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