Practical Neurology Paper

'How to do it' (2000 words max)

How to diagnose difficult white matter disorders: An Approach

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Introduction

Neurologists frequently encounter patients with white matter abnormalities on MRI. These patients may present with a variety of symptoms and signs including cognitive deficits, movement and gait disorders, ataxia, and many others. Occasionally the finding is incidental and the patient apparently asymptomatic. Often the diagnosis is straightforward, and an acquired cause is readily identified. However, there is a cohort of patients where despite extensive investigations, no diagnosis is reached. Many of these will have a genetic disorder (i.e. a leukodystrophy) and will require specialist input to advance their diagnosis.

In an effort to improve the care of these patients, we developed the Queen Square Adult Leukodystrophy Group (QSALG), a multidisciplinary team of neurologists, radiologists and metabolic physicians who review the clinical presentations, investigations, and neuroimaging of adults presenting with disorders of white matter throughout the UK and abroad.

In this *how to do it* paper, we describe our approach to these patients, with illustrative cases and tips on avoiding common pitfalls. It is not intended as a comprehensive review, which may be found elsewhere (Ahmed et al., 2014; Lynch et al., 2018; van der Knaap, Schiffmann, Mochel, & Wolf, 2019), but rather a framework that can be applied to such cases. Where specialist advice is needed, the reader is encouraged to contact research groups, or the authors themselves.

When to suspect acquired disease

The most common acquired causes that we see are multiple sclerosis (MS) and acquired small vessel disease (SVD). As common conditions, one may come across uncommon forms of their presentation relatively frequently, and these can raise the possibility of a leukodystrophy. These are important to recognize so specific treatments or risk factor management can be considered.

Neurologists will of course be familiar with the diagnosis MS – both the relapsing and progressive phenotypes. Pitfalls are well described (Brownlee, Hardy, Fazekas, & Miller, 2017). Difficulties can arise with atypical presentations, such as

isolated cognitive involvement, or with end-stage disease, where the white matter changes may be confluent and somewhat symmetrical.

Re-assessment of the history may identify overlooked relapses, and a formal review of any previous imaging may identify earlier diagnostic clues. If uncertainty persists, then spinal MRI involvement, the presence of unmatched CSF oligoclonal bands and abnormal visual evoked potentials can, of course, be very helpful.

Beyond MS, other clues to an acquired cause include a relatively rapid onset and progression (significant deterioration within 6-12 months of onset), systemic features, and cranial enhancement or cervical cord involvement on MRI. Similarly, if patients have previously responded to steroid treatment, an acquired cause is more likely.

In the history, ask about chemotherapy or radiotherapy exposure, as well as drug use. A history of immunosuppression should prompt consideration of progressive multifocal leukoencephalopathy (also rarely seen in the immunocompetent), and all patients should be tested for HIV, syphilis, hepatitis B and C and tuberculosis. Finally, neoplastic causes including gliomatosis and primary CNS lymphoma should be considered. Parenchymal swelling, in particular with gadolinium enhancement, strongly favours an acquired cause (inflammatory or neoplastic), and a brain biopsy might be considered.

We consider the investigation of acquired causes to be *Round 1* investigations (Table 1), and the most critical to avoid missing a treatable cause.

Case 1
A 50-year-old woman was referred with a possible leukodystrophy. She had presented at age 40 with clumsiness of the right hand. She became increasingly unsteady, with subjective cognitive decline and bladder urgency over the next 10 years. There was relatively symmetric and confluent signal change on MRI (Figure 1A-B), however, there was a suggestion of multiple lesions which had coalesced, and distinct lesions were visible in the cerebellum. Review of prior imaging from disease onset confirmed typical demyelinating lesions, and imaging of the spinal cord was recommended. Typical short segment inflammatory lesions were found on STIR/T2 imaging of the spine, with unmatched oligoclonal bands in the CSF (Figure 1B). The patient was diagnosed with primary progressive MS with ongoing radiological activity and started treatment with ocrelizumab.

Case 2
A 65 year-old woman was referred to the neurogenetic clinic after negative *NOTCH3* testing. She was a retired nurse who presented with parasthesiae in both feet. She was found to have a mild axonal neuropathy, lymphopaenia and diffuse white matter MRI changes, felt to represent small vessel disease. Three years later, she had developed cognitive difficulties, as well as pyramidal signs in her legs. There was significant progression of the MRI appearances (Figure 1C-D) with prominent involvement of the external capsules and anterior temporal lobes. CSF revealed a raised protein of 1.39g/ml and matched serum and CSF oligoclonal

bands. She underwent Round 1 investigations which identified her to be HIV positive. She showed clinical and radiological improvement with anti-retroviral treatment.

Case 3
A 31 year old woman had a background of mild intellectual impairment, longstanding unilateral deafness, short stature and diabetes. Nine months prior to review, she developed disinhibited behavior. Five months later she developed episodic vomiting, and over the next 3 months she became more withdrawn with abnormal behaviour. She subsequently presented in status epilepticus, requiring intubation and ITU support. She had frontal and pyramidal signs, and MRI demonstrated asymmetric bifrontal T2 signal abnormalities with trans-callosal extension, significant cortical and subcortical swelling, and small foci of enhancement (Figure 1E-G). CSF was acellular with raised protein (0.8g/ml), matched CSF and serum oligoclonal bands, and normal cytology.

Her background features and longer prodromal symptoms raised the possibility of a genetic disorder, but overall the rapid decline over 3 months and imaging features favoured an acquired cause. A frontal biopsy demonstrated a diffusely infiltrating glioma, WHO grade IV.

When to suspect severe small vessel disease

The most common diagnosis reached by the Adult Leukodsytrophy Group on externally referred patients is severe small vessel disease (SVD), representing 16% of our diagnoses.

Patients with acquired SVD typically have few symptoms or signs, and may be asymptomatic. They are usually older (>60 years) with multiple vascular risk factors (for example, diabetes, hypertension, smoking, dyslipidaemia, renal disease). MRI typically demonstrates patchy T2/FLAIR signal changes in the periventricular and deep white matter, which become confluent over time with involvement of the pons, thalamus and basal ganglia. Lacunar infarcts and deep microhaemorrhages are supportive; *cord imaging is normal* (Figure 1H-J). In severe small vessel disease, there may be involvement of the internal and external capsules, as well as the anterior temporal lobes, reinforcing that anterior temporal lobe signal change is not pathognomic of monogenic small vessel diseases (e.g. CADASIL). Conversely, when a patient is young (e.g. <40y), without typical vascular risk factors and/or in the presence of a suggestive family history, a monogenic SVD should be considered (case 4).

Case 4
A 32-year-old woman born of consanguineous parents was referred with a 3-year history of slowly progressive gait disturbance, headaches and subcortical cognitive dysfunction. MRI demonstrated patchy T2 periventricular high signal and evidence of mature striato-capsular and pontine infarcts with scattered microhaemorrhages in the posterior fossa, thalami and basal ganglia. There was subtle anterior temporal lobe involvement.

The imaging was in keeping with a vascular leukoencephalopathy. Given the young age of onset and absence of vascular risks, a monogenic small vessel disease was suspected. Single gene testing for *NOTCH3* had already been undertaken and was negative. Single gene sequencing revealed a homozygous loss of function mutation in *HTRA1*, confirming a diagnosis of Cerebral Autosomal Recessive Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CARASIL).

How we interpret metabolic tests

Once acquired causes have been excluded, we request metabolic investigations in all patients (*Round 2* investigations – table 1). This includes: very long chain fatty acids (X-linked adrenoleukodystrophy / adrenomyeloneuropathy, X-ALD/AMN); white cell enzyme activity (specifically galactocerebrosidase (Krabbe disease) and arylsulfatase A (metachromatic leukodystrophy, MLD)); plasma amino acid profile and total homocysteine (homocystinuria and methylenetetrahydrofolate reductase deficiency, MTHFRD); plasma sterols and urinary bile alcohols (cerebrotendinous xanthomatosis, CTX). These tests are relatively inexpensive (~£200), accessible and results can currently be obtained much more quickly than genetic panels. Together, they will exclude >90% of all metabolic leukoencephalopathies seen in adults and can be invaluable in interpreting variants identified by genetic testing.

Unless experienced in requesting these tests, we would recommend contacting a metabolic laboratory in advance to ensure appropriate sampling and handling, as spurious results can be obtained if guidance is not followed. In particular, delays in sample processing will lead to falsely low enzyme activity results, and difficulties in interpreting amino acid profiles. These tests therefore require planning and co-ordination between the neurologist, patient and laboratory to ensure valid results are obtained, and careful interpretation to avoid misdiagnosis.

If abnormally low enzyme activity results are reported despite adequate sample handling, one should consider the possibility of a pseudo-deficiency allele. Pseudo-deficiency alleles are variants present in the normal population, which lead to low levels of enzymatic activity, but not levels low enough to cause disease. This is most commonly seen with arylsulfatase A, where ~2% of the population carry a pseudo-deficiency allele. If low enzyme activity results are reported, but not as low as typically found in the relevant disease, pseudo-deficiency should be considered and discussed with the laboratory. Confirmatory genetic testing for pseudo-deficiency alleles is available.

Case 5

A 28-year-old male was referred following an abnormal MRI scan. He had a background of adrenal failure diagnosed at 6 years of age, and mild subcortical dysfunction on cognitive testing. T2 imaging demonstrated confluent and symmetrical increased signal in the subcortical white matter of the frontal lobes, sparing the U-fibres and extending into the genu of the corpus callosum. T1 post-contrast sequences demonstrated enhancement in the left frontal lobe and genu

(Figure 2A-B). A very long chain fatty acid profile revealed raised C24:22 and C26:22 ratios (below).

VLCFAs	Patient results umol/L	Reference range umol/L
C22	81.4	30.5-97.7
C24	97.4	24.2-65.9
C26	3.89	0.15-0.91
C24/C22 ratio	1.20	0-0.96
C26/C22 ratio	0.048	0-0.022

Targeted single gene testing of *ABCD1* revealed a hemizygous missense variant in exon 1. The patient was diagnosed with X-linked ALD/AMN, with active cerebral demyelination based upon the post-contrast enhancement. Most patients present with involvement of the splenium and parieto-occipital white matter, but this less common frontal form is well recognized. Allogenic haematopoietic stem cell transplantation may have a role in selected cases (Kühl et al., 2017).

Case 6

A 38 year old woman was referred following a seizure and abnormal MRI. She had normal development milestones and no significant previous medical history. Her parents were consanguineous. The family reported a 4 year history of cognitive decline. She stopped working due to this, and subsequently developed episodes of incontinence, focal seizures, increasingly child-like behavior and disinhibition. MRI demonstrated symmetrical confluent periventricular T2 signal abnormality with frontal predominance and volume loss (Figure 2C). Nerve conduction studies demonstrated a generalized demyelinating peripheral neuropathy. Arylsulfatase A activity was very low (4nmol/hr/mg, NR 22-103), and targeted single gene sequencing confirmed a diagnosis of metachromatic leukodystrophy (MLD).

How we do genetic testing

Only once the results of Round 1 and 2 investigations have been sent and reviewed do we proceed to genetic testing. The potential implications should be discussed with the patient and their family in advance. We interrogate the phenotype both clinically and radiologically, to see if we can identify a likely single gene, or group of disorders (e.g. genes causing hypomyelination). Although the clinical and radiological appearance of many leukodystrophies is overlapping, there are useful signs that we use to guide our testing. Clinically, we have found the presence of endocrine abnormalities, in particular adrenal failure (X-ALD/AMN), ovarian failure (vanishing white matter disease, AARS2-related leukodystrophy) or hypogonadism (Gordon Holmes Syndrome) to be very useful. Parkinsonism or ataxia may suggest mutations in the CSF1R or *CLCN2* genes, respectively. Neuropathy may be either demyelinating (MLD) or axonal (X-ALD/AMN, CTX).

Vanishing white matter disease (Figure 2D-F), *CSF1R* (Figure 2 G-I) and AARS2-related leukodystrophies have relatively specific imaging appearances. Specific signs, such as hypomyelination or prominent posterior fossa abnormalities, may suggest groups of disorders. A more comprehensive guide to clinical genetic correlation may be found in our recent JNNP review (Lynch et al., 2018).

Single gene testing remains important for confirming a metabolic leukodystrophy suspected from the presentation and Round 2 investigations. Beyond this role, however, and despite rigorous phenotyping, we no longer recommend single gene sequencing. Genetic panel testing can usually be performed with a similar cost and turnaround time, with the advantage of sequencing multiple genes simultaneously, which is useful in heterogeneous disorders. Genetic laboratories usually use a generic sequencing panel which sequences thousands of genes, but restrict their analysis to relevant genes. This is to prevent the clinician being overwhelmed by variants not relevant to the patient's presentation. Genetic panels have their limitations, which are important to be aware of (Box 1).

As many genes are sequenced simultaneously by panels, many variants are identified. Most will be harmless polymorphisms, not relevant to the patient or their disorder. Determining which are not clinically significant and which are pathogenic can, however, be difficult. Variants that cause rare diseases should themselves be exceedingly rare in the population, and usually lead to a change in protein function or expression. Very often, the laboratory will report 'variants of uncertain significance', where there is not enough evidence from the literature, population databases or prediction tools to decide if the variant is benign or pathogenic. These require experience to interpret, in particular to determine whether the genotype matches the phenotype radiologically and clinically (Box 2).

Case 7

A 45 year old woman was referred due to cognitive decline. She had no significant previous medical or family history. She presented with a 2 year history of increasing difficulty with tasks at work, culminating in her being made redundant. She subsequently developed episodes of incontinence, had difficulty following conversations and latterly required assistance in activities of daily living. She had marked frontal and subcortical cognitive deficits, a parkinsonian gait and broken saccades.

Nerve conduction studies were normal. MRI demonstrated symmetrical periventricular T2 signal abnormality with a frontal predominance and volume loss. The external capsule and temporal poles were spared, and there were no microhaemorrhages. Arylsulfatase A activity was moderately reduced, but not as low as seen in MLD. Following discussion with the laboratory, genetic analysis confirmed the presence of an arylsulfatase A pseudo-deficiency allele. NOTCH3 sequencing identified a heterozygous mutation, previously reported as pathogenic. The patient and family were informed of a CADASIL diagnosis. Following re-assessment of the phenotype and genotype, the NOTCH3 variant was found to be an uncommon polymorphism, unlikely to be causative of the phenotype, which was also not typical for a monogenic SVD. Further genetic analysis revealed a pathogenic variant in the CSF1R gene, confirming a diagnosis of Hereditary Diffuse Leukoencephalopathy with axonal Spheroids (HDLS).

Conclusions

Prominent MRI white matter changes are commonly encountered by general neurologists, and in many cases the diagnosis is straightforward. However, when a diagnosis is not apparent, there is often uncertainty as to how best to investigate, particularly when access to specialist tests and expert clinical and radiological

opinions is not readily available. In such cases, we suggest the following a structured approach.

Firstly, patients should undergo a detailed assessment for acquired causes (Round 1). Further neuroimaging (including longitudinal and retrospective review of prior images when available) of both brain and cervical spine with gadolinium can be particularly helpful, as can CSF examination. Round 2 tests for metabolic disorders should be performed following discussion with a metabolic laboratory, and carefully planned to ensure appropriate sampling, handling and transport. Where a genetic diagnosis is considered, it is usually expedious to request genetic panel testing, which is becoming more widely available. Clinicians should be aware of the high rate of incidental findings, potential limitations of genetic panels, difficulties interpreting heterozygous variants in recessive disorders, and consider whether any genetic results fit with the phenotype. For this reason, we advocate seeking specialist advice once genetic testing is considered. This may be through a regional neurogenetics or clinical genetics service. Clinicians are welcome to refer cases to be reviewed remotely or in person by the Queen Square Adult Leukodystrophy Group.

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