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Abstract

Although CSF biomarker testing is incorporated into current NICE guidelines for diagnosis of dementia, it is not widely available for most patients for whom molecular diagnosis could potentially change management. We share our experience of running a clinical cognitive CSF service, and discuss recent developments in laboratory testing including the use of the CSF amyloid-β 42/40 ratio and automated assay platforms. We highlight the importance of collaborative working between clinicians and laboratory staff, of pre-analytical sample handling, and the various factors influencing interpretation of the results in appropriate clinical contexts. We advocate for broadening access to molecular diagnostics by sharing clinical expertise, protocols and interpretation with colleagues working in psychiatry and elderly care, especially when access to CSF may be part of a pathway to disease-modifying treatments for Alzheimer's disease (AD) and other forms of dementia.

Introduction

While CSF has been used in the investigation of dementia for many years, the context of use has changed recently from excluding "treatable conditions" (e.g., neuroinflammation) to detection of the core pathologies underlying specific forms of dementia, using molecular biomarkers. There is a particular emphasis on Alzheimer's disease (AD), where CSF can be used to demonstrate the three main pathologies of AD – the presence of β-amyloid deposition, hyperphosphorylated tau accumulation and neurodegeneration – all of which can also be evaluated using imaging techniques like amyloid and tau positron emission tomography (PET) or MRI for assessing brain volumes/atrophy (Figure 1).

AD is classically associated with a CSF profile of a reduced amyloid-β 1-42/1-40 ratio (Aβ42/40), increased phosphorylated tau (p-tau) and increased total tau (t-tau)[2]. Some limitations of lumbar punctures are that they are more invasive than amyloid PET, they require caution if applying to individuals with coagulopathies, and carry a risk of low CSF pressure headache (about 1 in 10 using atraumatic needles[3]). However, CSF testing allows for a single sample to be assayed for several proteins of interest, as well as cell counts to exclude neuroinflammatory conditions, and is cheaper than amyloid PET. Appropriate use criteria for CSF AD biomarker testing have been published by the Alzheimer's Association[4] (Box 1) and are perhaps more permissive than the recommendations of the NICE guideline NG97 of 2018[5], in which testing is recommended if the diagnosis of dementia is uncertain (according to the National Institute on Aging-Alzheimer's Association 2011 diagnostic guideline[6]), and knowing the dementia subtype would both be useful and change management. Notable differences between these two guidelines therefore are the inclusion of typical AD dementia, mild cognitive impairment (MCI) and some individuals with subjective cognitive decline (SCD) as being appropriate for CSF testing according to the Alzheimer's Association appropriate use criteria.

Aside from providing support for the presence of AD pathology, CSF testing may also be used in non-AD contexts. The first context is in the evaluation of rapidly progressive dementia, in which prion disease is a consideration. Elevation of non-specific biomarkers of neuronal damage in CSF, such as 14-3-3 and S100B proteins, has been superseded by the use of real-time quaking-induced conversion (RT-QuIC) for prion protein, which has significantly greater specificity and slightly better sensitivity for sporadic Creutzfeldt-Jakob disease (sCJD)[7]. The combination of a very

high t-tau (>1400 ng/ml on INNOTEST assays) with a high t-tau to p-tau ratio (>25), in the absence of acute neurological conditions (e.g., stroke or encephalitis), also has >99% specificity for CJD in relation to both AD and other dementias[8].

The second context (not specific to AD) is in considering whether there is evidence for neurodegeneration. In a wide range of processes involving either acute or chronic neuronal damage, neurofilament light chain (NfL) levels are elevated in CSF. Pronounced CSF NfL elevation is seen in prion disease[9]. Compared with AD dementia (fold change 1.9 relative to cognitively normal controls), greater elevations in CSF NfL are seen in certain non-AD dementias such as some types of frontotemporal dementia (FTD; fold change 3.9 relative to healthy controls) and HIV-associated cognitive impairment (fold change 21.4)[10]. However, it is important to note that a normal CSF NfL concentration does not exclude the presence of neurodegeneration.

CSF sampling

We have had a dedicated cognitive CSF clinic at the National Hospital for Neurology and Neurosurgery (NHNN) since 2013. Diagnostic lumbar punctures (LP) are undertaken in a day care setting with an option for research sample donation. Box 2 summarises the standard operating procedure for this clinic, which is broadly in line with the recommendations of a consensus guideline for LP in patients with neurological diseases[11]. Key recommendations to minimise post-LP complications include adequate counselling of patients to allay fears beforehand, use of atraumatic needles, limitation of number of attempts to ≤ 4 and use of passive (drip) CSF withdrawal. Bed rest has not been shown to improve outcomes, so we offer an

opportunity for seated rest. Although the consensus recommendation is for use of ≤ 25G atraumatic needles in the lateral decubitus position, a multicentre feasibility study did not show a significant benefit of using smaller gauge needles[3] and so we use 22G needles and allow for collection in the seated position, to improve the speed of collection especially when larger volumes (e.g., 20 mL) are taken, where the patient has additionally consented to research sample collection, and where measurement of CSF opening pressure is not of clinical concern.

Since 2018, an advanced nurse practitioner (FO'S) has been performing the lumbar punctures at the NHNN cognitive CSF clinic following the development of a local clinical guideline on nurse-performed lumbar puncture (available on request) and appropriate competency-based training. FO'S now runs the clinic independently, with back-up support from a rota of junior doctors if technical difficulties are encountered, and research staff where research samples are also taken.

Pre-analytical handling

We exclusively collect CSF in standardised polypropylene tubes, noting that polystyrene tubes may result in artefactual lowering of the measured Aβ peptide concentrations by up to 30% and of t-tau by up to 15%[12]. We also avoid collecting AD CSF biomarker samples through manometers, as they may reduce measured Aβ peptide concentrations by up to 5%[13].

CSF AD biomarker testing in the UK is currently carried out at a single UKAS-accredited laboratory at the NHNN. We recommend that all external samples which may incur a transport delay of > 2 hours are first processed in the referring hospital's

laboratory. This involves centrifugation of CSF for 5 minutes at room temperature at 1750 *g* and aliquoting into 2 mL polypropylene aliquot tubes, ensuring a minimum volume of 0.5 mL per tube (one per test requested). The aliquots must then be frozen directly at -80°C on the same day of LP, and transported on dry ice to the NHNN laboratory. Information about the tests offered, sample requirements, costs and turn-around times can be found in the laboratory's user handbook.[14]

CSF AD biomarker assays

Until recently we used CE-marked INNOTEST enzyme-linked immunosorbent assays (ELISAs) for Aβ 1-42, total tau (t-tau) and tau phosphorylated at serine 181 (p-tau181). However, we switched to the automated Lumipulse platform (Fujirebio) in March 2020, to allow for testing of Aβ1-42, Aβ1-40, t-tau and p-tau181 using CE-marked assays, the main advantages including:

- an improvement in analytical sensitivity, as the CSF Aβ42/40 ratio improves classification of clinical AD vs non-AD dementias and improve detection of cerebral fibrillar amyloid deposition (as defined by amyloid PET) compared to CSF Aβ42 alone[15]; this is likely to be because use of CSF Aβ40 concentration corrects for inter-individual variability in the overall rate of Aβ peptide production.
- reduction in susceptibility of results to variations in pre-analytical handling: normalising to Aβ40 levels at least partly mitigates against the artificial lowering of Aβ42 through adsorption to sample tube surfaces[12], across multiple freeze-thaw cycles[16] and through repeated tube transfers[17];
- reduction of manual error introduced when doing ELISAs/reduction in need for highly trained operators;

- increased throughput and reduced turnaround time (results in 10 as opposed to 25 days);
- potential for harmonisation of cut-points or interpretation with other centres using the same automated platform calibrated against certified reference material.

Determining cut-points for clinical use is not straightforward; some challenges include accurate definitions of case/control status (noting that some healthy individuals will have preclinical disease) and balancing sensitivity (favouring minimising false negatives) with specificity (minimising false positives). We undertook a validation of CSF Aβ42/40 ratio testing on the Lumipulse platform in relation to both the INNOTEST platform and to amyloid PET data, resulting in a cut-point of 0.065 which gives 95% sensitivity and 89% specificity for identifying symptomatic individuals previously defined as having AD-like CSF on INNOTEST, and 85% sensitivity but up to 96% specificity for identifying asymptomatic amyloid PET-positive individuals using 18-F florbetapir PET scans[18]. The t-tau and p-tau assay cut-points we use are from the manufacturer but also validated in local samples.

CSF neurofilament light chain (NfL)

Since 2019, the NHNN laboratory has offered CSF NfL testing using the CE-marked UmanDiagnostics sandwich ELISA, which allows measurement of CSF NfL over a wide range of values encountered in normal physiology and pathological states (100 to 10 000 pg/ml, although the assay's dilution linearity allows higher values to be quantified reliably if needed). Figure 2 shows the values obtained by measuring CSF

NfL at the NHNN lab in the first 300 CSF samples since the assay was adopted; as NfL increases with age, the normal ranges we use are age-specific (by decade), taken from a group of >350 normal controls in an international multi-centre study[19].

Interpreting biomarker results

While the NIA-AA research framework for AD biomarkers conceptualises a spectrum of A/T/N (amyloid, tau and neurodegeneration) biomarkers on which all individuals may be defined independently of clinical status; for example an individual with A+T-N- would be classified as having “Alzheimer’s pathologic change”, in routine clinical practice, and as advocated by the latest International Working Group (IWG) consensus on *clinical* diagnosis of Alzheimer’s disease[20], we believe it is essential to interpret AD CSF biomarkers in the clinical context. Biomarkers should be used to lend support for, or against, a clinical formulation of the patient’s presentation, and not on their own to make a diagnosis of dementia or any sub-type. This is because currently available biomarkers do not reliably predict clinical progression in an individual, and there are problems of interpretation of values near binary cut-points, and issues of classifying individuals with evidence of AD along with other pathologies.

In our own practice, after having first requested the test only in the appropriate clinical context (see Introduction and Box 1), we employ a staged approach as shown in *Figure 2:* Results from the first 300 samples measured at the NHNN laboratory for CSF neurofilament light chain (NfL).

Where available, age-adjusted norms (black horizontal lines) are shown from an independent cohort of 359 healthy controls from Yilmaz *et al*. Note: usually the upper limit of quantification is 10 000 pg/mL, however where CSF NfL has been quantified as >10 000 pg/ml, the sample has been re-tested after further dilution.

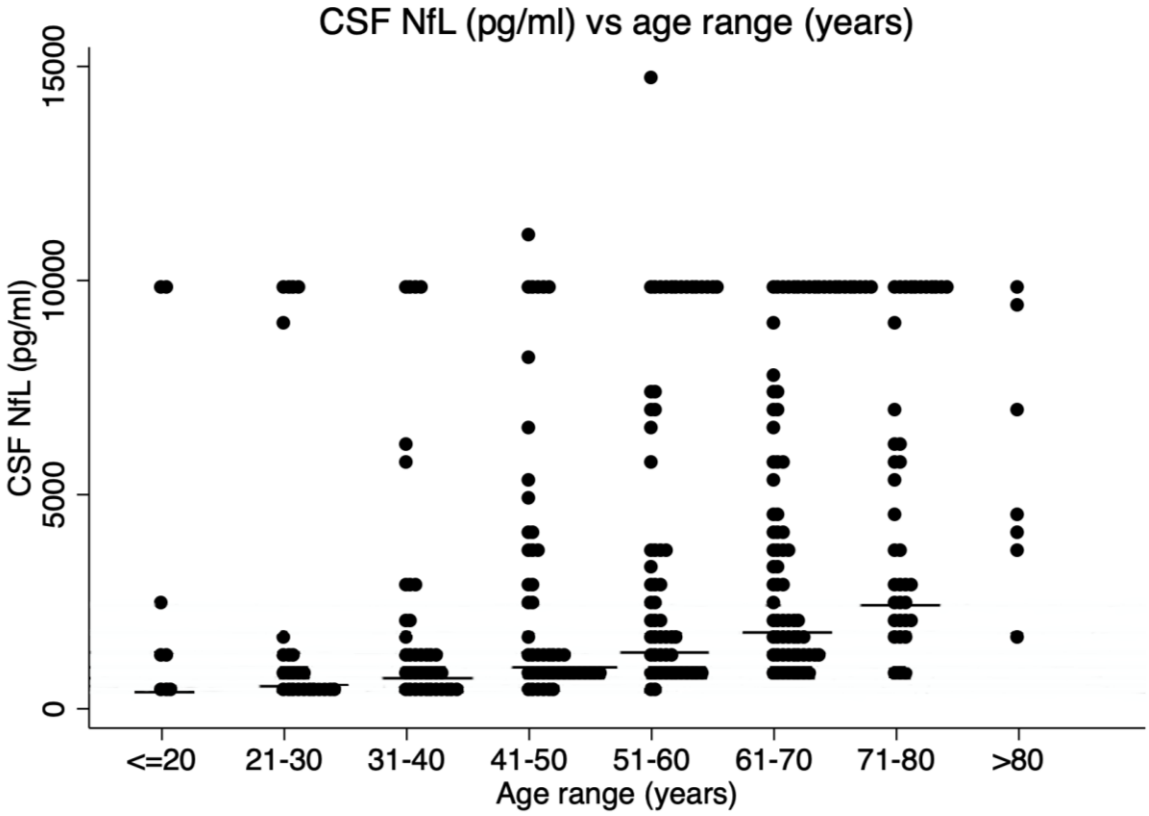
CSF NfL (pg/ml) vs age range (years)
CSF NfL (pg/ml)
0
5000
10000
15000
<=20
21-30
31-40
41-50
51-60
61-70
71-80
>80
Age range (years)

Figure *3*. Here, a low CSF Aβ42/40 ratio is required, but not sufficient for, making a diagnosis of clinical Alzheimer's disease. This is because a proportion of individuals with normal cognition will have amyloid deposition (about 10% at age 50 years to 16%, 23% and 33% at ages 60, 70 and 80 years respectively[21]). We currently do not routinely request t-tau, as in the AD context it gives similar information to p-tau. However, in situations where prion disease is a consideration, a very high t-tau (e.g., above the upper limit of quantification of the assay) with an elevated t-tau to p-tau ratio would be supportive of a diagnosis of prion disease that could then later be confirmed with other more specific biomarkers like CSF RT-QuIC for prion protein or characteristic diffusion weighted MR imaging changes.

Some illustrative case studies in which CSF contributed to the clinical diagnosis are discussed in Box 3, with accompanying Figure 4.

The setting of cut-points for interpretation of CSF biomarkers, and harmonisation of reporting to patients and clinicians, is a topic of active work. Currently the Alzheimer's Association has an external quality control programme for CSF Aβ42 in which the NHNN laboratory participates, allowing for monitoring of performance relative to other laboratories performing this test globally, as well as assessing drift of results over time. Certified reference materials and methods are available for CSF Aβ42[22,23]. The development of similar materials and methods for Aβ40, t-tau, p-tau181 and NfL is underway.

A recent survey of 40 laboratories across 15 countries indicates cut-points are more similar across centres (but still not exactly the same) when automated assays are

used, and this may in part relate to differences in pre-analytical conditions but also to differences in the populations served. This study proposed a harmonisation of reporting of CSF AD biomarkers according to the eight different combinations that may be derived when the axes of amyloid, t-tau and p-tau are interpreted using centre-specific binary cut-points. Other methods of reporting used mostly in Europe include the PLM score[24], derived simply as the number of these three biomarkers that is abnormal, and the Erlangen score, which incorporates the concept of border zones to account for the possible influence of assay imprecision on the interpretation[25,26.]

The future

The majority of patients assessed in “memory clinics” or dementia services in the UK are under the care of physicians for the elderly, or psychiatrists. A recent report by Alzheimer’s Research UK and the Royal College of Psychiatrists[27] details that many barriers need to be overcome in order for these services to be ready to deliver disease-modifying treatments, which have become particularly topical given the Federal Drug Administration’s recent accelerated approval of one anti-Aβ antibody for therapeutic use in MCI-AD or mild AD dementia and the pending applications for approval of this and other similar agents in the USA and Europe. Deployment of molecular biomarkers such as CSF testing at scale in these services currently is limited by physical infrastructure, availability of clinical expertise, and clinical commissioning. Through better multidisciplinary working, neurologists and allied professionals in neurology services will have a role in supporting our colleagues in these areas of significant unmet need, by sharing our clinical experience and our pathways to accessing these investigations.

Blood measurement of NfL on the Single molecule array (Simoa) platform is set to enter the clinical testing schedule in the UK soon via the NHNN laboratory, and this will be followed by technical validation and application of plasma phospho-tau. While blood biomarkers may in due course have sufficient sensitivity and specificity to be used alone, currently the most likely scenario is for these to be used to pre-screen individuals before proceeding to CSF examination or amyloid PET.

As clinical trials move into earlier stages including the preclinical and MCI stages of disease, it would be ideal to be able to predict individualised risks for conversion to dementia. In research cohorts outside the UK, algorithms have been derived incorporating CSF biomarkers[28] or plasma biomarkers[29] with demographic factors, cognitive testing and imaging findings to predict conversion of MCI to AD dementia. Further work is needed to validate this type of approach in the UK National Health Service.

Efforts are ongoing to discover and validate molecular biomarkers for other dementias and related disorders, such as specific tau strains in non-AD tauopathies like progressive supranuclear palsy, α-synculein in dementia with Lewy bodies, and TAR DNA-binding protein 43 (TDP-43) in frontotemporal dementias. The most advanced assay in this regard is an RT-QuIC-like assay for α-synuclein seeds. In time it may become possible to index the relative contributions of different coexisting molecular pathologies to a patient's symptoms or likelihood of progression to dementia, and provide evidence for targeted therapies in a more personalised way.

Key points

- CSF testing for dementia biomarkers predominantly is focused on identifying the presence of Alzheimer's disease (AD), with current guidelines/appropriate use criteria indicating testing should be carried out where knowing the molecular diagnosis will change management.
- The typical AD CSF profile is a low Aβ42/40 ratio, elevated p-tau181 and t-tau; elevated NFL may also be seen.
- CSF AD biomarker testing has the advantage of being cheaper than amyloid PET but commissioning of services must incorporate the relevant clinical expertise in procedures, logistical requirements for sample transport and processing, and robust processes for interpretation and feedback of results to requesting clinicians and patients. Developments such as nurse led lumbar puncture clinics and automated platforms for CSF testing will need to be complemented by improved multidisciplinary work between neurology, psychiatry and elderly care services.

Ethics statement

Patient consent for publication: not required.

Data sharing statement

Data from this publication will be made available on reasonable request.

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Contributors

AK and JMS conceived of and drafted the main manuscript. All authors contributed significantly to the content of the manuscript and reviewed the drafts and provided feedback.

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Competing interests

RP is co-director of the NfL consortium (an industry-funded research consortium) and has given educational lectures sponsored by GE Healthcare. JDR has served on a Medical Advisory Board and had a consultancy agreement with Alector, Arkuda Therapeutics, Wave Life Sciences, and Prevail Therapeutics, and had a consultancy agreement also with UCB, AC Immune, Astex Pharmaceuticals, Biogen, Takeda and Eisai. CJM has been an advisor to IONIS, Biogen, Lilly and Roche; is on the international steering committee for aducanumab, and has lectured for Biogen. NCF's research group has received payment for consultancy or for conducting studies from Biogen, Eli Lilly Research Laboratories, GE Healthcare, and Roche, but NCF receives no personal compensation for the aforementioned activities. JMS has received research funding from Avid Radiopharmaceuticals (a wholly owned subsidiary of Eli Lilly), has consulted for Roche Pharmaceuticals, Biogen, Merck, and

Eli Lilly, given educational lectures sponsored by GE Healthcare, Eli Lilly, and Biogen, and serves on a Data Safety Monitoring Committee for Axon Neuroscience SE. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

All other authors report no competing interests.

Box 1: Clinical indications for appropriate use of lumbar puncture and CSF testing in the diagnosis of Alzheimer's disease, as detailed by the Alzheimer's Association appropriate use criteria. Reproduced with minor reformatting from Shaw et al.[4] with the publisher's permission. *Indications that would be considered appropriate according to the current NICE guideline.

Abbreviations: AD, Alzheimer's disease; *APOE*, apolipoprotein E gene; MCI, mild cognitive impairment; NICE, National Institute for Health and Care Excellence; REM, rapid eye movement; SCD, subjective cognitive decline

Clinical indication	**Alzheimer's Association appropriate use criteria**
Meeting core clinical criteria for probable AD dementia with typical age of onset	**Appropriate**
Symptoms suggesting possible AD dementia*	
Dementia with onset age below 65 years*	
MCI with onset age below 65 years	
Persistent, progressing or unexplained MCI	
SCD but considered to be at high risk for AD (persistent decline in memory rather than other cognitive domains; onset in the last 5 years; age at onset >60 years; worries associated with SCD; feeling of worse performance than others of the same age group; confirmation of cognitive decline by an informant; carriage of *APOE* ε4)	
Dominant symptom of change in behaviour, where AD diagnosis is being considered*	
Determination of disease severity in patients who have already received a diagnosis of AD	**Inappropriate**
SCD not considered to be at high risk of AD	
Symptoms of REM sleep behaviour disorder	
Carriers of autosomal dominant AD mutations, with or without symptoms	
In lieu of genotyping for suspected carriers of autosomal dominant AD mutations	
Carriers of *APOE* ε4 with no cognitive impairment	
Cognitively unimpaired on objective testing and no SCD but considered as high risk due to family history of AD	
Cognitively unimpaired on objective testing, no SCD, no expressed concern about developing AD and no condition suggesting high risk	

Box 2: Standard operating procedure for cognitive CSF clinic at NHNN, including clinical and research sampling of CSF and blood

Referral information
Demographic and contact details
Need for coordination with other tests
Not likely to require radiological guidance for lumbar puncture
Specify whether CSF opening pressure needed (not routinely done)
Arrangements for antiplatelet and anticoagulation medication cessation if relevant
Full blood count and coagulation profile not required unless has known history of thrombocytopenia or coagulopathy

Pre-clinic telephone call (if not already addressed in clinic from which patient was referred/if patient requests additional call)
Address concerns/questions
Ask if willing to receive research information
Arrange to send lumbar puncture information leaflet by post/e-mail, along with research information leaflet if relevant
Participant not instructed to fast but to drink copious fluids on the day if not contraindicated
Confirm not on antiplatelets or anticoagulants for the proposed appointment date or has a plan for cessation and recommencement (following the ABN 2018 guideline)[1]

Clinic day
History and Investigation review:
Review of recent neuroimaging (within last 6 months) to confirm no contraindications (tonsillar descent/large mass lesion)
OR
Confirm no history of features of raised intracranial pressure AND no papilloedema on funduscopy
Confirm no allergies (latex, plasters, lignocaine)

Consent
Formal written consent on hospital's general procedure consent form 1, OR if patient is deemed to lack capacity to consent, document consultee discussion on consent form 4
Indication: Diagnostic
Risks: Common - Headache (10%), back pain (25%); rare – failure of procedure, bleeding, infection, nerve damage; very rare – hearing loss, double vision, need for a blood patch to treat prolonged post LP headache (<1 in 500)
Separate consent for research sample donation (CSF and blood)

Lumbar puncture
Second member of staff acts as an assistant/chaperone
CSF collected between 08:00 and 12:00
Patient in either lateral decubitus position, or seated with lumbar forward flexion (when CSF opening pressure not required)
Intervertebral space between L2/3 to L5/S1 located by palpation
Skin preparation as per local clinical guidelines (with chlorhexidine 2%/ethanol 70%, allowed to dry fully before proceeding
Technique: aseptic (sterile gloves, standard personal protective equipment including plastic apron)
Local anaesthesia: lignocaine 2%, maximum 3 mg/kg
Spinal needle: atraumatic 22G; no more than 4 passes
Method of collection: drip without suction
If manometer used, contents are emptied into routine CSF analysis container and manometer is removed before collection for AD CSF biomarkers
Primary containers for clinical CSF collection: Polypropylene screw top yellow cap 25 mL (Sarstedt 63.9922.254)
Volume collected for clinical sample, each into different tubes: Routine tests (cell counts, protein, glucose, microscopy culture and sensitivities) 1 mL; AD CSF biomarkers 2 mL, virology (if needed) 1 mL, cytology (if needed) 5 mL
Primary containers for research CSF collection: Polypropylene screw top clear cap 10 mL (Sarstedt 62.610.018)
Volume collected for research sample (if relevant): maximum 15 mL
Needle bevel re-inserted before withdrawal
Dry adhesive dressing applied to site
Patient assisted to supine position

Paired venous blood sample
Taken immediately after lumbar puncture, tourniquet used
Venepuncture location: upper limb peripheral vein
Needle: 21G or 23G butterfly needle with BD Vacutainer adaptor
Blood collection tubes (clinical), BD: 1 x SST serum (gold top) 4 mL, 1 x fluoride oxalate (grey top) 2 mL
Blood collection tubes (research), BD: Up to 1 x Lithium heparin plasma 5 mL, Up to 4 x SST serum 4 mL, up to 4 x EDTA plasma 5 mL
Dry adhesive plaster applied to site

After-care
Patient transfers to reclining chair, is offered a beverage and rests for 1 hour before going home; advised to avoid bending/straining for the rest of the day; plaster removed following day. Offer patient-initiated telephone follow-up post-lumbar puncture: document any symptoms and arrange further follow-up if needed (*e.g.* to monitor post LP headache and arrange blood patch if persistent); organise radiologically guided lumbar puncture if procedure failed

Box 3: Case studies

Patient 1
A 61-year-old woman presented with a four-year history of cognitive decline starting with reduced motivation, followed by word finding difficulty and mispronunciation. No ritualistic/obsessive behaviours were noted and memory was not a significant complaint. She had a past history of depression and anxiety but denied feeling low or anxious currently. She took mirtazapine, propranolol and folic acid. Her mother was diagnosed with Alzheimer's disease in her 50's and died a few years later. She retired prior to symptom onset, and drove a car on familiar routes with no concerns from family. She was an ex-smoker and drank minimal alcohol.

On examination she turned frequently to her son for reassurance. She was not orientated to time (day, date, month or year) or location (town or country). She was able to register three items but not to recall after a delay. Her reading was slow with frequent pauses and some mispronunciations. Spontaneous speech was fluent but with word-finding pauses.

On neuropsychological testing, single word and phrase repetition was satisfactory; naming was impaired. Single word comprehension was intact but sentence comprehension impaired. Visuo-perceptual processing was impaired but simple visual detection was intact. Speed was very slow and there was executive dysfunction.

MRI brain scan (Figure 4D) did not show significant atrophy or vascular burden but a FDG PET scan (Figure 4E) revealed hypometabolism in the temporoparietal region with involvement of the precuneus bilaterally, more pronounced on the left side, and also minimal FDG hypometabolism in the left frontal lobe.

CSF parameters were WCC 1/µL (0-5), RBC 1/µL, protein 0.24 g/L (0.13-0.45), albumin quotient 3.02 (<7.2), glucose 3.3 mM (2.2-4.2), plasma glucose 5.2 mM (3.9-5.8), oligoclonal bands negative in CSF and serum.

CSF dementia biomarkers were Aβ42/40 ratio 0.036 (≥0.065), p-tau181 216 pg/mL (≤57), t-tau 1041 pg/mL (146-595) and NfL 2892 pg/mL (<1781).

She was given a diagnosis of Alzheimer's disease (AD) dementia, logopenic variant, and advised that she would need to stop driving and inform the DVLA of her diagnosis. She was offered treatment with a cholinesterase inhibitor referred for speech and language theerapy and, in view of her family history, genetic testing for rare monogenetic causes of AD.

Patient 2
A 68-year-old woman presented with a two-year history of deterioration in driving ability, vision problems, emotional blunting, reduced social interaction, increased eating, word finding problems and frequent falls. Her mood had become low since failing a road driving test that identified problems with attention, information processing and vehicle placement. Her medical history included hypercholesterolaemia and urinary urgency and she took a statin, omeprazole, calcium-vitamin D supplements and mirabegron. She lived alone and was independent in daily activities, as well as being a carer for her mother who was diagnosed with dementia at age 84. Her father had late onset ataxia and died at age 81. She had two siblings and two daughters who were all well. She was an ex-smoker of 40 pack years and drank 1 bottle of wine per week.

On examination she was intermittently tearful and had frequent pauses in spontaneous speech. She scored 27/30 on the mini-mental state examination, losing two marks for orientation and one for recall. Bedside cognitive testing showed impaired letter fluency (9 "C" words in one minute) but intact naming, repetition and comprehension. There was patchy difficulty with memory and arithmetic. She gave concrete explanations for proverbs and had mild difficulty with the Stroop test. Physical examination showed square wave jerks in primary gaze, and "round the houses" vertical eye movements. There was left arm bradykinesia with no rigidity, no ataxia or tremor, and reflexes were symmetrical with down-going plantars.

On neuropsychological testing, expressive language was slightly lacking in grammar but she was able to use sentences. She made occasional semantic and phonemic errors but single word and phrase repetition were intact. Single word comprehension was good for concrete but poor for abstract nouns and also for grammar. Tests of executive function showed significantly impaired response inhibition (Stroop), impaired letter and category fluency, and inflated cognitive estimates, with mildly slowed processing speed. Visuoperceptual functions were acceptable but visuospatial processing (AMIPB figure copy < 5th centile) was impaired.

MRI brain scan showed mild generalized parenchymal volume loss without lobar preference or asymmetry. The choroidal fissures were prominent but hippocampal volumes appeared preserved. (Figure 4C, D and E). A mild burden of white matter microangiopathy was noted without evidence of recent infarct (Figure 4F).

CSF parameters were WCC 1/µL (0-5), RBC 2/µL, protein 0.48 g/L (0.13-0.45), albumin quotient 7.35 (<7.2), glucose 3.2 mM (2.2-4.2), plasma glucose 5.0 mM (3.9-5.8), oligoclonal bands negative in CSF and serum.

CSF dementia biomarkers were Aβ42/40 ratio 0.054 (≥0.065), p-tau181 46 pg/mL (≤57), and NfL 4318 pg/mL (<1781).

She was diagnosed with progressive supranuclear palsy-frontotemporal dementia (PSP-FTD) and given speech therapy input with an 8-week course in communication partner training, as well as signposted to Rare Dementia Support and the PSP Association.

Figure 1: Biomarker correlates of pathologies of amyloid, tau and neurodegeneration in Alzheimer's disease.

A: Immunostaining for amyloid-β (clone 6F3D, DAKI, M0872) shows frequent parenchymal deposits in the neocortex, including many plaques with central amyloid cores (black arrows).

B*:* Immunostaining for hyperphosphorylated tau (clone AT8, Thermo MN1020) shows a dense meshwork of neuropil threads in the neocortex with neuritic plaques (white arrow) and frequent neurofibrillary tangles (black arrow).

C: Coronal section at the level of the lateral geniculate nucleus from a patient with no known neurological disease shows mild dilation of the frontal and temporal horns of the lateral ventricle but no other macroscopically visible pathology.

D: Coronal section from a patient with Alzheimer's disease shows prominent dilatation of the frontal and temporal horns of the lateral ventricle and enlarged insular space (black asterisks). Cortical ribbon shows widespread thinning and blurred outlines between the cortical grey and white matter. The white matter volume is significantly reduced and the corpus callosum is thin (white asterisks). The hippocampus is severely atrophic (black arrow).

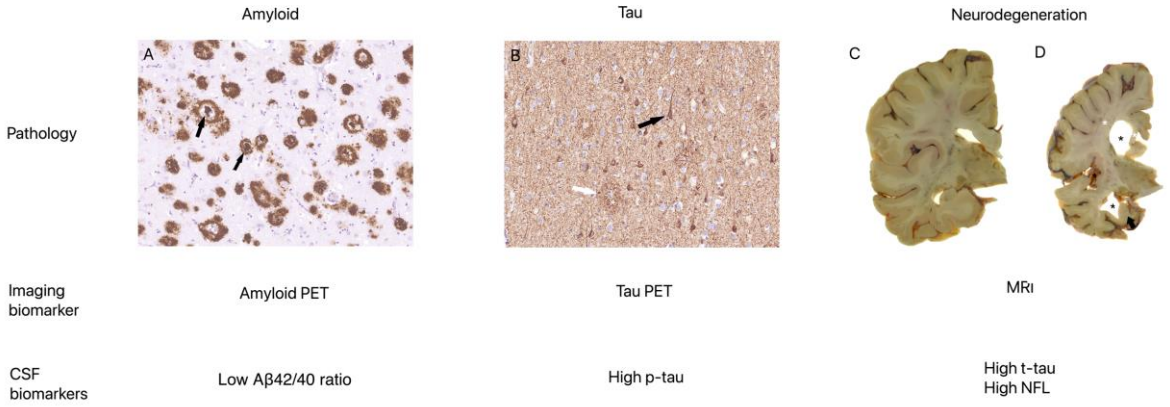


Figure 2*:* Results from the first 300 samples measured at the NHNN laboratory for CSF neurofilament light chain (NfL).

Where available, age-adjusted norms (black horizontal lines) are shown from an independent cohort of 359 healthy controls from Yilmaz *et al*. Note: usually the upper limit of quantification is 10 000 pg/mL, however where CSF NfL has been quantified as >10 000 pg/ml, the sample has been re-tested after further dilution.

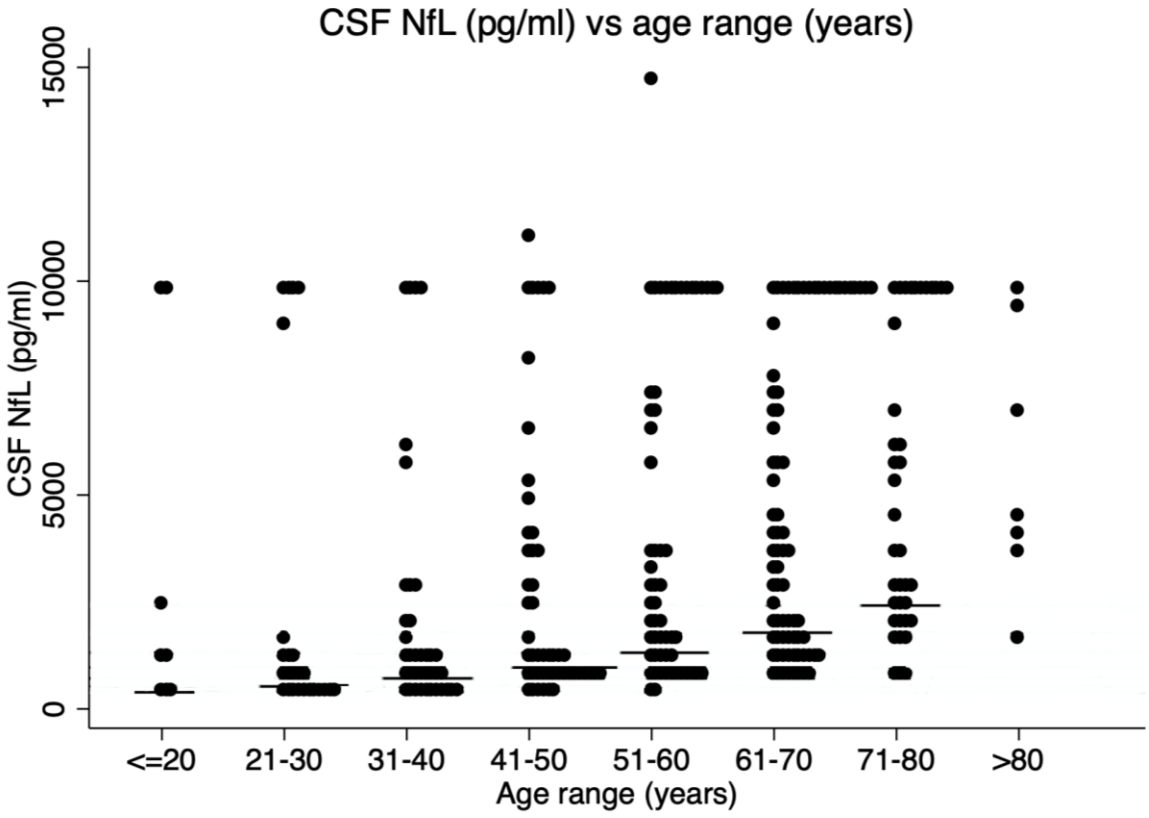


Figure 3: Flow diagram for interpretation of CSF AD biomarkers requested in the context of a possible Alzheimer's disease phenotype

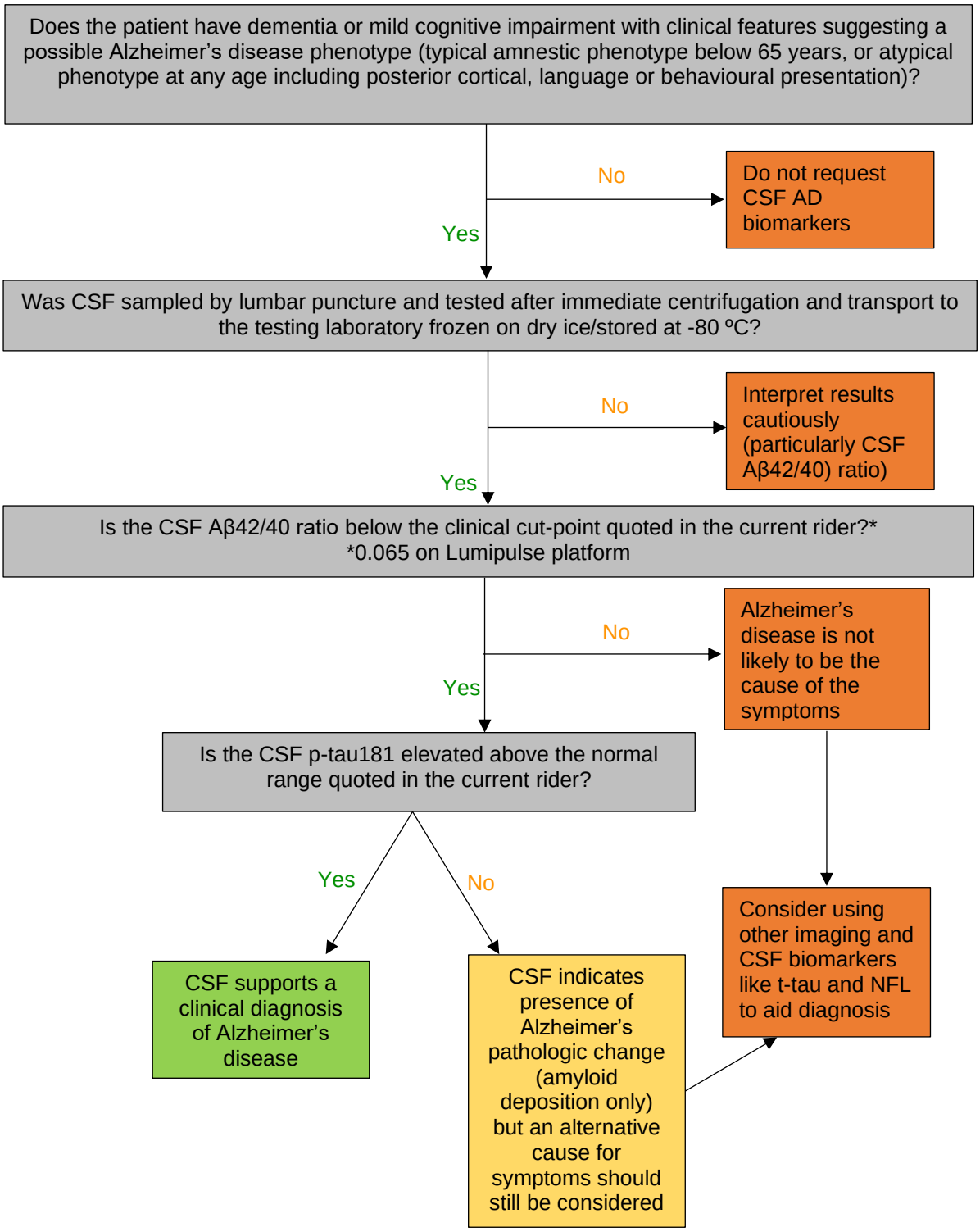
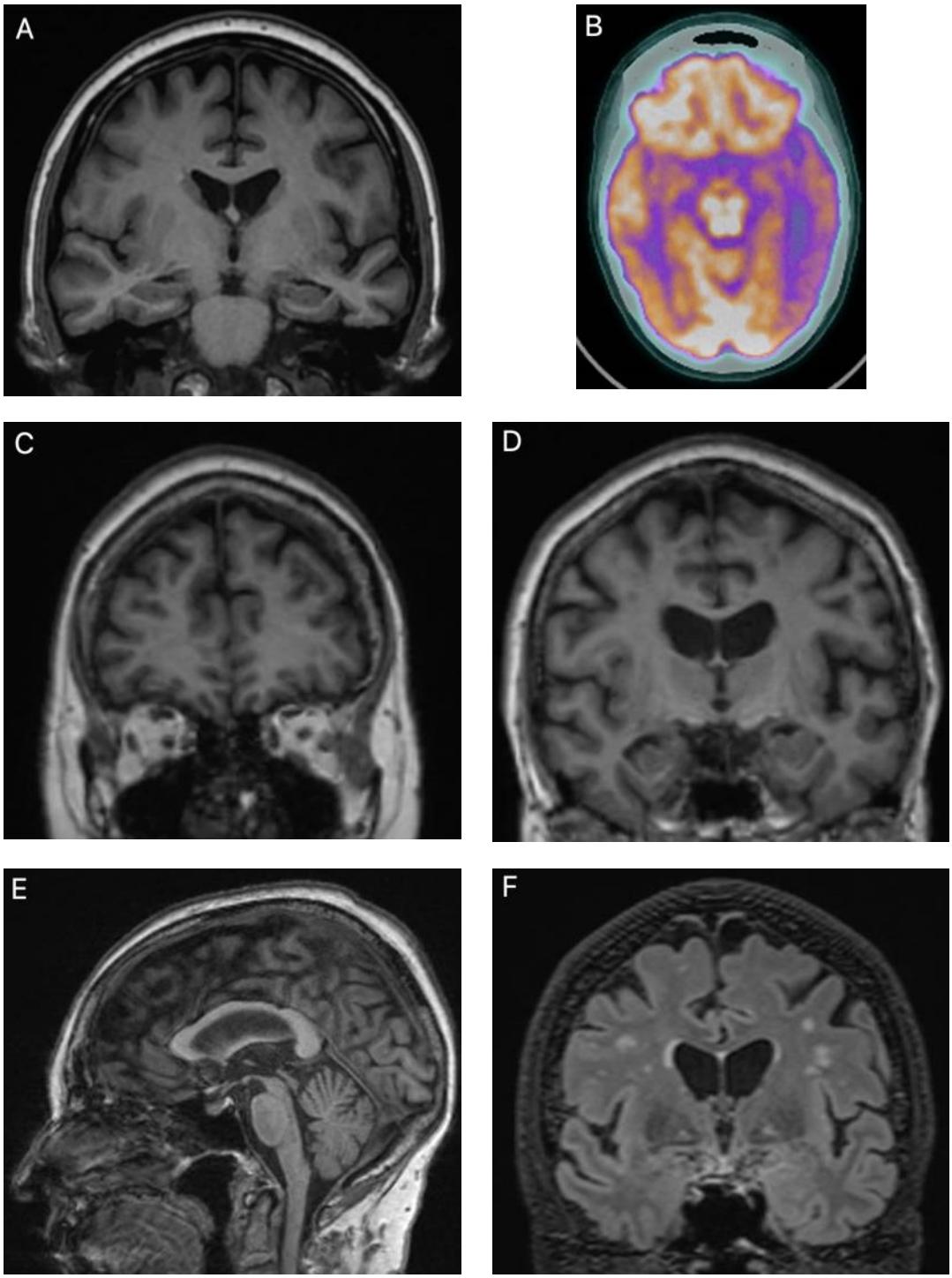


Figure 4: MRI brain scans from the two patient case studies.

A: Patient 1, MR T1 coronal MPR at mid-hippocampal level B: Patient 1, FDG PET axial fused C: Patient 2, MR T1 coronal MPR at orbitofrontal level D: Patient 2, MR T1 coronal MPR at mid-hippocampal level E: Patient 2, MR T1 sagittal MPR at mid-brain level F: Patient 2, MR FLAIR coronal



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