Future imaging in dementia

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Abstract

Imaging has made an immense contribution towards supporting the diagnosis of dementias, detecting preclinical and prodromal pathology and allowing disease progression to be objectively tracked. This has led to consensus guidelines for the use of imaging in dementias to be published and a future task will be to validate these guidelines. Additionally, there needs to be standardised approaches over the use of binary thresholds when assigning an abnormality status. Other medical unmet needs include the need for specific imaging markers of: (a) linear tau tangles, TDP-43 and alpha synuclein aggregates; (b) microglial phenotypes that throw light on the activity of these inflammatory cells; (c) activity of intracellular processes which normally act to clear misfolded proteins; (d) epigenetic activity which regulates gene expression. Future imaging studies are predicted to be active in all these areas. Finally, as safer and more effective immunotherapy and other protective strategies against the pathologies of dementias are developed and trialled, imaging will play a major future role in determining the efficacy of neuroprotective treatments and their mechanism of action to be examined.

Introduction

Currently, we have an armamentarium of PET and SPECT imaging agents which can be used to support diagnosis and track progression in subjects with a clinical diagnosis of dementia.

Three ^{18}F PET markers of β -amyloid fibrils (florbetapir - AmyvidTM, flutemetamol -

VizamylTM, florbetaben - NeuroceqTM) and one paired helical tau marker (flortaucipir -

TauvidTM) are now licenced by the FDA for clinical use. Additional PET amyloid (^{18}F -AZD-

4694) and 2nd generation ^{18}F tau markers (MK 6240, PI-2620, RO6958948) are also

available. FDG PET is licenced for discriminating frontotemporal (FTD) from Alzheimer's

dementia (AD) while ioflupane (DaTSCANTM) SPECT is licenced for discriminating

dementia with Lewy bodies (DLB) from AD, only the first showing loss of striatal dopamine

transporter binding. MIBG cardiac scintigraphy also can discriminate DLB from AD cases as

only the former shows loss of myocardial sympathetic innervation. With these imaging

biomarkers it is also possible to detect pathology and dysfunction in prodromal dementia

cases with mild cognitive impairment or REM Sleep Behaviour Disorder (RBD) and in

preclinical cases carrying susceptibility genes.

What has been lacking, however, is an algorithm advising the sequence in which these

various imaging biomarkers should optimally be used to support diagnosis and the validation

of such an approach. Such an algorithm has now been proposed by an interdisciplinary group

of experts comprised of nuclear medicine physicians, radiologists, neurologists, geriatricians,

psychiatrists, clinical and basic neuroscientists and patient-advocates ¹. These experts met at

the European Association of Nuclear Medicine (EANM) Focus Meeting 2 in January 2019,

Cannes, France. The algorithm proposed by these experts chaired by Prof Gael Chetelat has

been accepted for publication by the Lancet Neurology and is shown in the figure below. It

is based on the diagnostic qualities of the various imaging biomarkers and not on tracer

availability across countries or reimbursement considerations. Conclusions drawn by this panel of experts were that:

- PET neuroimaging biomarkers have an important role in the diagnosis workup of neurodegenerative diseases leading to dementia.
- Amyloid PET is most useful to prove or rule out AD and provide specific information on the underlying pathology.
- FDG-PET has great value for differential diagnosis of neurodegenerative diseases, prediction of short term outcome, and staging of neurodegenerative processes.
- Depending on the clinical presentation, specific combinations of biomarkers are of significant added value for early and differential diagnosis.

An important future imaging programme addressing management of dementia will be to form a multicentre consortium to validate this algorithm which currently represents informed personal opinion.

Figure 1

An algorithm advising on the order imaging modalities should be optimally used for classifying dementias. Taken from Chetlat et al. *Lancet Neurology* 2020; 19: 951-962

¹ Exclusion of neoplastic, vascular, and inflammatory changes supporting non-neurodegenerative aetiologies and evaluation of topography of atrophy might inform on the neurodegenerative disease (but FDG-PET might be more sensitive and accurate).

² Whatever is established/available and preferred; always Aβ-PET if CSF is contraindicated or inconclusive. See Table 1 for further details.

³ Age and *APOE* status (when available) may influence the use of FDG-PET even before amyloid-PET especially in individuals with available but inconclusive CSF results. Analyses of FDG-PET images should also take into account comorbidities, i.e. uncontrollable diabetes, brain trauma, chronic ischemia, as well as some medications (e.g. psychotropic drugs or corticosteroids) that might affect the images, since these can alter cerebral metabolism.

⁴ FDG-PET can be performed before DaT-SPECT or mIBG, particularly if the cortical involvement of neurodegeneration is in the diagnostic focus.

Standardising imaging approaches

While the above algorithm provides a guide for the sequence in which imaging biomarkers could be used for differential diagnosis of imaging biomarkers, it does not address issues such as use of binary thresholds for defining abnormality. Most FDG, amyloid, and tau PET scans are reported abnormal or normal on the basis of visual reads but agreement between experts can vary depending on the tracer being used and the population being studied. A potential way around this problem is to use quantitative approaches. In trials of amyloid PET sensitivity and specificity for detecting pathology in cohorts of clinically probable AD, MCI and elderly normals it is usual to select an SUVR or DVR threshold based on young normal

and AD distributions of cortical uptake. A difficulty is that current amyloid tracers bind primarily to beta sheeted fibrils and signals can only be confidently detected when individual subjects have advanced A β deposition equivalent to Thal stage 3-5 at pathology. The use of an SUVR threshold of 1.4 or higher at baseline will miss the developing pathology in some MCI or normal cases where tracer uptake is high normal but subsequently shows a rise over the two next years unless follow up is included ^{2,3}. This is important as these are the very cases one needs to target in future trials of neuroprotective therapies. It is also now clear that cerebrospinal fluid (CSF) studies can detect falls in A β -42 levels in elderly normal subjects ahead of amyloid PET changes ⁴. What is needed in the future are either tracers that bind to A β oligomers ahead of fibrillar formation - such benzothiazoles exist but have low affinity - or an acceptance that cases of high normal levels of current amyloid tracer uptake on current thresholding need to have a follow up scan for an accurate diagnosis.

The threshold problem becomes worse with the first generation tau imaging agent ¹⁸F-flortaucipir PET. Here there is a less clear separation of Alzheimer and elderly amyloid negative normal ranges but a young normal range suggests that an SUVR threshold above 1.3 is abnormal ⁵. As a rule, cortical tau tangles are only seen when β -amyloid plaques are also present ⁶. The tracer shows off-target binding to melanin and to metal containing proteins in the basal ganglia and also has an affinity for TDP-43. ¹⁸F-flortaucipir binds primarily to paired helical hyperphosphorylated tau fibrils (PHF) and, while increased pallidal uptake has been reported in 4R linear tauopathies such as PSP and CBD, little binding is evident on post-mortem examination. Second generation tau PET tracers MK-6240 and PI-2620 are now available with higher binding affinity for PHFs and early trials suggest they show low off target binding in early trials ^{7,8}. Alzheimer cases were clearly discriminated from healthy normals by both MK-6240 and PI-2620 PET at 90' post injection producing cortical SUVRs of ≥ 2 in AD vs ≤ 1.5 in HCs. There is still controversy over

whether these newer radioligands bind specifically to 4R linear tau tangles or rather bind to associated metalloproteins in the basal ganglia.

A need for specific linear tau, TDP-43, and alpha synuclein biomarkers

While PET markers of the mixed 3R and 4R fibrils in paired helical tau tangles now exist, these tracers bind only weakly if at all to 3R tau in the Pick bodies of frontotemporal dementia and the 4R linear tangles in dementias associated with progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). The only tracers to date that have given a high signal in both PHFs and 3R and 4R tau fibrils are ^{11}C -PBB3 and its analogue ^{18}F -PM-PBB3^{9,10}. ^{11}C -PBB3 photo-isomerises in the presence of visual and ultraviolet light so has to be synthesised and used under red-light conditions. Another issue is a metabolite of ^{11}C -PBB3 also crosses the blood-brain barrier increasing the background signal. It is unclear whether ^{18}F -PM-PBB3 has more user friendly properties than ^{11}C -PBB3.

TDP-43 aggregates are found in frontotemporal and semantic dementias associated with C9ORF72 and progranulin gene mutations and are also found in idiopathic disease. The main differential diagnosis is FTD due to tau-containing Pick bodies. ^{18}F -THK 5117 was originally developed as a PHF tau marker but also binds to both TDP-43 and alpha synuclein (ASyn) aggregates. Unfortunately, it also binds to monoamine oxidases and so has been dropped as a PET marker. Flortaucipir was originally developed as a tau ligand but was reported to bind to lateral temporal areas in cases of semantic dementia where TDP-43 aggregates would be expected¹¹. In another study, involving both bvFTD C9ORF72 mutation subjects and semantic variant primary progressive aphasia (svPPA) cases, increased temporal flortaucipir binding was again seen in svPPA¹². A non-significant rise in inferior frontal flortaucipir uptake was present in bvFTD C9ORF72. Post mortem ^3H -flortaucipir autoradiography, however, was unable to confirm tracer affinity for brain TDP-43 aggregates in C9ORF72

mutation carriers. In the absence of a specific TDP-43 ligand it will remain difficult to discriminate idiopathic FTD due to TDP-43 pathology from that due to 3R tau Pick bodies. Alpha synuclein (ASyn) aggregates in neurones and neurites are the pathological hallmark of dementia with Lewy body dementia and Parkinson's disease. ASyn aggregates in glia are characteristically seen in multiple system atrophy (MSA). Like TDP-43, small molecules exist which will bind non-specifically to ASyn aggregates. Thioflavin T dye binds to ASyn fibrils in vitro and its neutral benzoxazole analogue, the PET tracer ^{11}C -BF-227, was reported to be taken up by MSA white matter oligodendroglia, the cortex, basal ganglia, and substantia nigra ¹³. The specific : background signal, however, was very low. Additionally, BF-227 binds more avidly to amyloid plaques ¹⁴ and, as these are prevalent in DLB and to a lesser extent in PD dementia, this limits its use as an ASyn marker. A series of other polyaromatic fluorescent probes have been trialled as ASyn aggregate markers but have either failed to cross the blood brain barrier, showed non-specific binding, or were also β -amyloid plaque markers ¹⁵. The phenylpyrazole analog anle138b is another example of the last category. The hunt continues for small molecules that show selective ASyn aggregate binding using high throughput screening. A major issue is that, unlike β -amyloid which exists mainly in an extracellular fibrillar form in Alzheimer cases, ASyn exists mainly as a range of intracellular oligomeric forms all with different binding properties for ligands.

Inflammatory markers in dementias

Dementias are associated with intrinsic brain inflammation in association with the aberrant aggregation of proteins that causes the neurodegeneration. This inflammation takes the form of microglial activation and in AD the microglia surround both extracellular deposits of β -amyloid fibrils and dying tau tangle containing neurones. The mitochondria of activated microglia express translocator protein (TSPO) which has a binding site for isoquinolines.

This initially led to the development of the isoquinoline ^{11}C -PK11195 PET inflammation marker but a drawback is its low specific signal. A number of 2nd generation TSPO PET markers followed including ^{11}C -PBR28, ^{18}F -DPA714, and ^{18}F -FEPPA ¹⁶. Unfortunately, the newer tracers have the problem that their binding is influenced by the TSPO polymorphism expressed and subjects fall into high, mixed, and non-binder groupings ¹⁷. It is usual practice to triage the non-binders and combine the mixed and high binders. This greatly reduces power to detect change in dementias.

Activated microglia adopted different phenotypes which can broadly be classified into M1-type cytotoxic and M2-type protective groupings ¹⁸. M1 cells release cytokines while M2 cells are phagocytic, clearing dead cells and remodelling synapses, and release growth factors. Both M1 and M2 cells express TSPO and current PET inflammation markers are unable to discriminate between them. Attempts have been made to find phenotype specific markers of activated microglia. M2 cells express cannabinoid CB2 receptors which are not normally present in intact brain. The ligand ^{11}C -NE40 was developed as a PET CB2 marker but has low brain penetration and has not proved useful ^{18,19}. Hopefully in the future specific M1 or M2 PET ligands will be discovered.

Ligands that are less influenced by the TSPO polymorphism expressed have been developed, such as flutriciclamide (GE180). This tracer, however, while fine in rodents, has a relatively low extraction across the blood-brain barrier in humans. GE180 PET has, however, been successfully used to detect inflammation in multiple sclerosis ²⁰. A different approach may be to develop ligands to purinergic receptors as activated microglia express P2X7 sites which contain ATP-gated ion channels. A number of ^{11}C and ^{18}F P2X7 ligands including [11C]SMW139, 11C-JNJ-54173717, and 18F-JNJ-64413739 are now available for trial ²¹. These ligands, however, do not discriminate between M1 and M2 microglial phenotypes so

it is likely in the future that the search for ligands to target other microglial aites will continue.

Markers of intracellular processes

FDG and F-dopa PET are both markers of nerve terminal metabolism imaging hexokinase and aromatic aminoacid decarboxylase (AADC) activity, respectively. Most PET tracers, however, target cell surface receptors. It is thought that aberrant intracellular protein aggregation results in part from either a failure of lysosomal or proteosomal clearance of misfolded proteins and so imaging markers of these processes are an unmet need. Mutations of the glucocerebrosidase (GBA) gene increase the risk of Parkinson's disease and Lewy body dementia due to a failure of ASyn clearance. Inhibitors of the related enzyme, GCase, are now available which could potentially be labelled with PET isotopes to measure its activity²². Inhibitors of proteosome function are also known, such as lactacystin which blocks peptidase function on the 20S component. Lactacystin has been used to produce rodent models of Parkinson's disease. It may be possible to synthesise a PET tracer analogue of lactacystin that would allow direct measurement of proteosome activity in dementias. Gamma and beta secretases are responsible for β -amyloid production from amyloid precursor protein. Ligands for both these secretases have been developed by Pharma as putative neuroprotective agents. These could be labelled with PET isotopes to measure secretase activity. Glycogen synthase kinase 3 phosphorylates tau to form P-tau. Inhibitors of kinases are plentiful though tend to be non-specific. It may be possible to find and label a specific GSK-3 inhibitor in the future providing a PET marker of rates of tau hyperphosphorylation and tangle formation.

The ligand, ¹⁸F-BCPP-EF, binds to mitochondrial complex 1 (MC1) and its uptake reflects respiratory activity in the brain²³ - its uptake has been modelled in non-human primates²⁴.

¹⁸F-BCPP-EF PET is now being trialled in both Alzheimer's dementia and Parkinson's disease and initial findings have found MC1 activity to be decreased in association cortical areas in AD patients (A Mansur et al. J Nucl Med 2018; 59 Supp 1: 1709). This suggests that mitochondrial stimulants such as ubiquinone may have a therapeutic role to play in dementias.

Epigenetic modification of genes, such as methylation and glycosylation, can influence control of their expression. Histone deacetylases (HDAC) are known to regulate gene function by silencing their expression. In Alzheimer's and other neurodegenerative diseases it has been suggested that HDAC inhibitors may have beneficial effects by restoring gene expression and improving cognition. Recently HDAC-1/II class inhibitors that can be labelled with PET isotopes have become available, such as ¹⁸F-FAHA and ¹¹C-martinostat. ¹⁸F-FAHA shows relatively low brain uptake but in vivo non-human primate studies have shown that ¹¹C-martinostat should be a useful tracer - it showed an averaged nondisplaceable volume of distribution (VND) of 8.6 ± 3.7 mL/cm³ which was 99% blocked with the cold compound ²⁵. The level of HDAC activity in Alzheimer's and other neurodegenerations can now in principal be assessed with ¹¹C-martinostat PET.

Monitoring therapeutic efficacy with PET

There have been a number of studies where PET has been used to study drug mechanisms of action, their dose occupancy profiles, and efficacy in Alzheimer's dementia. For example, it has been shown that the acetylcholinesterase inhibitor donepezil only partially blocks the activity of this enzyme at clinically tolerable doses ²⁶ but it still raises cortical glucose metabolism after its acute administration ²⁷.

There have been several trials of immunotherapy as an anti-amyloid strategy in both established Alzheimer's disease and β -amyloid positive mild cognitive impairment (MCI)

cases. These have established that the β -amyloid load can be reduced with infusions of antibodies though at the expense of causing areas of vasogenic edema (ARIA)^{28,29}. In nearly all of these trials no significant cognitive improvement in subjects was evident. An exception was a phase 2 aducanumab infusion trial where amyloid load was reduced in a dose dependent manner and the MMSE and Sum of Boxes scores both improved³⁰. Again areas of vasogenic edema and microbleeds were seen at higher doses. A re-analysis of the following phase 3 trial was announced by the sponsors to have reproduced the phase 2 findings, which is encouraging, but they will need to be reproduced in further cohorts. There is currently a view that β -amyloid immunotherapy should be targeted at early mild cognitive impairment cases and elderly healthy normals who are β -amyloid positive rather than at established AD where irreversible neuronal loss has already occurred. The A4 trial is currently running where 1000 elderly healthy normals who showed β -amyloid positivity on florbetapir PET are receiving infusions of solanezumab or placebo. They will be followed for at least five years to determine whether reducing their amyloid load is protective against future cognitive decline³¹.

Vaccines against β -amyloid fibrils are also in development. An initial vaccine AN 1792 directed against A β -42 proved effective in removing amyloid plaques but led to encephalitis in 6% of the 375 Alzheimer subjects. The extent of the reduced plaque load at eventual post-mortems over the following two decades correlated with levels of anti-AN1792 antibody response in the blood³². New vaccines such as ACI-24 and a DNA amyloid β -42 trimer vaccine are currently under development against A β -42 which are designed to produce antibodies without activating B cells³³. Ideally, these trials will use serial amyloid PET to monitor vaccine efficacy in reducing β -amyloid load so that this can be correlated with the cognitive response.

Two vaccines and six antibodies targeting different tau epitopes are currently in trial to remove hyperphosphorylated tau in an attempt to reduce the neurofibrillary tangle (NFT) load in Alzheimer and PSP patients ³⁴. AC-Immune are trialling ACI-35.030 as their fore runner, ACI-35, produced an antibody response against P-tau. Another active tau vaccine in trial involves AADvac1 which is a synthetic peptide derived from a tau protein sequence. The efficacy of these vaccines and antibodies in Alzheimer patients are being monitored with tau PET. It is unclear whether the mechanisms of action of these different immunotherapies are overlapping or completely separate or which PET tau tracer provides the optimal signal for an outcome measure. Currently only PBB3 ligands appear to show strong binding to 4R linear tau tangles but these tracers are not easy to synthesise or kinetically model.

Alpha synuclein immunotherapy (antibodies and vaccines) are also in trial sponsored by AFFIRIS, Prothena, and Biogen ³⁵. The AFFIRIS vaccine is directed against a synthetic ASyn epitope. In the absence of a specific PET ASyn imaging marker, the efficacy of these agents in removing ASyn in PD can only be judged via its prevention or reversal of dopaminergic dysfunction. Dopamine terminal dysfunction can be measured with F-dopa or VMAT2 PET or with DAT PET or SPECT. In dementia with Lewy bodies or PD dementia more widespread cortical involvement is present and loss of cholinergic function accompanies dopaminergic loss. General terminal function could be assessed with ¹¹C-UCB-J or ¹⁸F-UCB-H PET, markers of the SV2A protein found in all presynaptic terminals. Cholinergic function could also be followed with FEOBV or F-donepezil PET, markers of vesicular transporter and acetylcholine esterase availability.

Use of hybrid PET-MRI

Hybrid PET-MRI systems are now commercially available with 3 tesla magnets and these cameras enable simultaneous acquisition of MRI sequences and PET radiotracer detection ³⁶.

This allows structural and functional images to be precisely co-aligned so cortical thinning in dementia can be matched to equivalent areas of metabolic change or amyloid / tau tangle deposition. Structural and functional brain connectivity measured with DTI and BOLD fMRI can also be correlated with the load of Alzheimer and vascular pathology present or changes in cholinergic and other neurotransmitters all done at a single visit. Hybrid 7 tesla cameras are now being produced which can resolve individual cortical layers and the subregions of nuclei allowing their metabolism to be measured.

Conclusions

This review has examined the current state of play in dementia nuclear imaging and indicated future possible ways of filling medical unmet needs. This includes a need for PET or SPECT markers of alpha synuclein and TDP-43 aggregates. A difficulty is that PET radiochemists are constrained to discover small brain permeable molecules with fast uptake and washout to use as imaging biomarkers due to the short lived isotopes with which they are labelled to keep dosimetry reasonable. A possible future way forward could be to manufacture antibodies or peptide ligands specifically targeting particular aberrantly aggregated proteins and provide them with a mechanism to cross the blood-brain barrier. This could involve tagging them with a molecule such as transferrin which is transported across the BBB by a carrier ³⁷. Another possibility is the design of lipophilic cage compounds which contain the peptides or antibodies and then break up and release them after crossing the BBB ³⁸. A third direction could be to synthesise aptamers which are circular strands of RNA that bind to the peptide or antibody and are transported into the brain. Whether these mechanisms can transport enough ¹⁸F-labelled peptide within two half lives (220 minutes) of the isotope to give a good brain signal remains to be determined. An alternative is to label peptides with ⁸⁹Zirconium with its three day half life. However, because of the high dosimetry involved it

may only be possible to use ^{89}Zr -peptides in cancer studies where this is less of a concern.

Without progress in the facilitation of peptide transport into the brain within a few hours we will continue to be reliant on small molecule imaging markers. Trying to find small molecule ligands that not only bind selectively to aberrant protein oligomers and aggregates but also have all the other properties required for a good PET tracer is likely to be like finding a needle in a haystack.

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