The Oral Health Study: Feasibility study to reduce oral bacteria and improve periodontal health in those living with mild dementia

Study summary

Bacteria (many from the mouth) have been found by DNA analysis in the post-mortem Alzheimer's disease (AD) brain. As there is also an associated increased risk for AD with periodontitis (gum disease) and poor oral hygiene habits, we wish to see (i) whether it is possible to reduce oral bacteria in order to stabilise gum disease in those living with mild dementia and mild cognitive impairment (MCI) (ii) whether the progression of AD can be slowed by reducing these bacteria.

Aims

- The primary aim is to gain information on compliance, drop-out rate, and the success of bacterial reduction in patients with mild dementia or MCI using the methods outlined below. This information will be used to enable a future application for a NIHR funded Efficacy Pilot study.
- To recruit up to 20 participants with mild dementia [those with a diagnosis of probable AD, mixed dementia with AD or vascular dementia with cerebrovascular amyloid] or MCI and periodontitis.
- To gauge participant compliance in attending dental appointments and following a routine plaque-control programme designed to reduce dental plaque and pathogenic oral bacteria levels over a 2 year period.

Hypothesis

(i) Oral bacteria may influence the pathogenesis of AD by triggering an Aβ response and/or activating microglia in the brain. (ii) Reducing oral bacteria slows the disease trajectory. Methods: Up to 20 people with mild AD will be provided with dental treatment for two years as appropriate to bring them up to dental and oral fitness, if indicated for dental management, suitable antibiotics will be prescribed. Participants will receive tests for oral bacterial levels and cognitive levels at baseline and at approximately six months, and the end of years one and two. Anticipated outcomes for dementia: If we can show that people with mild dementia can attend clinics to receive treatment as well as change their oral hygiene habits this would facilitate an NIHR grant application for an efficacy trial to see if this approach helps to slow AD progression.

Summary

The risk factors for AD need to be discussed in the context of an ageing immune system where antibody production and cell mediated defence (B & T cells, the 'humoral' system) declines. Alongside this the more primitive innate immune system in which cells, including those lining the mouth, produce cytokines such as TNFα and antimicrobials which kill or weaken invading pathogens is maintained and with increasing age predominates. Cytokines such as TNFα are associated with increased levels in inflammation and AD.

Two recent reviews from the applicants explain in detail the correlation between periodontitis and AD [1,2].

Background

Dementia is an umbrella term used to describe a group of symptoms including memory loss, difficulty in thinking and mood changes [3]. These symptoms can be caused by a number of conditions including Alzheimer's disease (AD), vascular dementia, dementia with Lewy bodies and frontotemporal dementia.

In AD, the distinctive neuropathology of neurofibrillary tangles (intracellular fibrillar deposits of hyperphosphorylated tau proteins) and amyloid-Aβ peptides deposited as amyloid plaques [4-5] is found at much higher density than that observed in the brains of age-matched cognitively normal subjects. The hippocampus and cortical brain regions are particularly vulnerable early in the disease trajectory. Cerebrovascular amyloid deposits are found in the small blood vessels of the cortices of around 80% of AD brains [6].

The immune system is thought to be an important factor in the initiation and progression of AD [7], the most common cause of dementia in the elderly. With ageing, the non-specific (innate) part of the immune system, which is also altered due to the aging process predominates so that cytokines play a greater role in infection control [8]. Bacteria from an oral source fail to trigger the symptoms normally associated with frank infection due to immune tolerance by the more targeted humoral and cell-mediated systems [1,2]. In the brain, the production of amyloid β (Aβ) is now thought to be a part of an innate response triggered by low levels of immune-tolerated bacteria [9] with a concomitant release potentially harmful cytokines from microglia. Our hypothesis is that a slow increase of these bacteria over years may ultimately contribute to the cascade of inflammatory reactions in the brain that result in AD pathology. Studies suggest that the source of many of these bacteria may be the mouth and that oral bacteria in particular are able to evade the immune system [1,2]. Furthermore there is a strong association between increased risk for AD and periodontitis (gum disease) and poor oral hygiene [10]. The presence of deep-seated anaerobic bacteria in the largely immune-tolerant gingival margins, poses a threat in at least two ways. Firstly, when released into the blood stream they can provoke a constant production of inflammatory cytokines such as TNFα (tumour necrosis factor-α) or IL1β (interleukin 1β) [11-12] which may increase the permeability of the blood-brain-barrier (BBB). Secondly, bacteria may track up the olfactory bulb and into the brain [13]. There, one of the responses of the innate immune system may be the over-production of the anti-bacterial Aβ which then increases levels of inflammatory cytokines and activates microglia in the brain. This may result in a direct preventative effect on new synapse formation. Synapses are at the interface between neurones and allow communication between them and activation which strengthens connections. Excessive cytokine production and inflammation can result in neuronal damage [14-15].

The mouth is an excellent repository for chronic infection as the oral mucosa generates immune tolerance to bacteria, perhaps explaining how oral bacteria can colonize new locations. It has been suggested that immune silencing may contribute to the links between oral bacteria and increased risk of atherosclerosis and stroke [16-19]

We therefore suggest that the ageing immune system provides an environment where certain oronasal pathogens can flourish. Epidemiological studies show links between oral bacteria

and AD. The North American Nun Study [20] showed that pre-existing tooth loss was shown to carry an odds ratio of 2.2 for developing late-onset AD in nuns. In a study of monozygotic twins (discordant for AD) by the Swedish Twin Registry tooth loss in youth and middle age was associated with increased risk of developing AD in old age. Of the three potentially modifiable risk factors, only tooth loss was statistically significant in the identical twins discordant for dementia [21]. This is corroborated by the recent eighteen year longitudinal USA study showing that individuals who did not brush daily had a 22-65% greater risk of dementia than those who brushed their teeth three times daily [22].

Periodontitis is common in the elderly [23]. Prevalence of dementia increases with age and results in the release of TNFα (raised 25 fold in the cerebrospinal fluid (CSF) of AD patients) [24] thus the mouth provides a plentiful source of immune tolerated bacteria and inflammatory cytokines. The nominal symptoms associated with infections caused by these oral pathogens means that effective treatment may not be sought. The concept of a link between infection and AD is not new, and there are ongoing clinical trials using antibiotics for the treatment of AD [25-26]. However, success is dependent on many factors: the primary source of infection should be ideally identified and modified. Some researchers suggest that Aβ is produced by neurons and glia as an antimicrobial peptide as part of an innate response to infection [27]. It is known that the brain can harbour bacteria without the symptoms normally associated with meningitis or encephalitis. Bacteria mainly from an oral source identified as being associated with AD include A. actinomycetemcomitans, P. gingivalis, and T. Forsythia; additionally T. denticola and B. burgdorferi, have been identified previously in AD brain tissue [28-33].

Emerging techniques able to sensitively detect bacteria in tissues have shown that barriers such as the BBB and placenta do not afford as complete a protection as we had supposed [28,30,34-37]; they may therefore fail to trigger the symptoms we normally associate with pathogens causing meningitis or encephalitis [30].

Dental treatment may result in: (i) Short-term removal of bacteria, in the mouth by means of debriding teeth and gums (the removal of accumulated dental plaque and tartar/calculus) to prevent inflammation and further neuronal degeneration and may also provide time to allow neuronal repair.

We therefore hypothesise that reducing oral bacteria will slow the AD trajectory.
Initially, we wish to conduct a feasibility study over three years including up to 20 mild dementia patients who also have periodontitis, to determine the effect of periodontal stabilisation therapy. If necessary, this may include a single course of antibiotics prescribed by the dentist when plaque control is optimal. This study will allow us to assess compliance and how well people with mild dementia cope with this dental treatment. It will also help us to judge whether reaching and maintaining periodontal health is possible for those with mild dementia. If this study can show that people with mild dementia can engage with this treatment and improve their oral health, this will enable us to apply for funding for a larger trial to assess whether this approach can slow AD symptom progression.

Null Hypotheses

- It will not be possible to maintain dental compliance in patients with mild dementia.
- It will not be possible to reduce the numbers of pathogenic bacteria in these patients.
- It will not be possible to improve periodontal health in these patients.

Aims

To gain information on compliance, drop-out rate, and bacterial reduction success in patients with mild dementia, which will be used to enable a future NIHR funded Efficacy Pilot study.

Objectives

- To recruit up to 20 participants with mild dementia [those with a diagnosis of probable AD, mixed dementia with AD or vascular dementia with cerebrovascular amyloid] and periodontitis.
- To enable participant compliance in attending dental appointments and effectively adhering to a routine plaque control programme designed to reduce dental plaque levels.
- To reduce and maintain reduced levels of pathogenic oral bacteria over a 2 year period in up to 20 patients with mild dementia.
- To improve the periodontal health of participants with mild dementia.
- To analyse the bacterial profiles as participants progress through the study.
- To compare the rates of cognitive decline of the cohort of participants with the predicted rates of cognitive decline for those with mild dementia for additional observational data at this point.

Methodology

Participants

We aim to follow a cohort of 20 participants with mild dementia and periodontitis for a period of 2 years during periodontal stabilisation and maintenance therapy. As this is a feasibility study it is not possible to carry out a formal sample size calculation. However, it is expected that some participants may find compliance challenging due to increasing cognitive impairment and possibly other co-existent medical conditions. This may result in those participants withdrawing from the trial.

Up to 20 potential participants with mild dementia will be recruited from the following Participant Identification Centres (PICs):

(i) Cognitive Disorders clinic in Bristol
North Bristol NHS Trust
Contacts: Consultant Dr Elizabeth Coulthard (Director of the Dementia Health Integration Team and member of this research team) and Helen Lewis (NBT Lead Research Nurse)

(ii) Bristol Dementia Wellbeing Service
Partnership between the Alzheimer's society and North Devon Trust
Contact: Paula Shears (Support Services Manager at Alzheimer's Society)

(iii) Avon and Wiltshire Mental Health Partnership NHS Trust

Contact: Hannah Antoniades (Research & Development Manager)

(iv) Avon Primary Care Research Partnership
Contact; Rachel Avery (Research and Development Governance Manager)

(v) Research Institute for Care of Older People (RICE). (Contact: Helen Horn)

Other sites:
Alzheimer's Society Community Groups (contact: Paula Shears; Support Services Manager at Alzheimer's Society)
St Monica's Trust (contact: Jacqui Ramus)
The Bristol Brains for Dementia Research project (contact: Dr Laura Palmer)
Dementia Health Integration Team (HIT) Network (contact: Dr Hayley Dash Clinical Studies Officer – Dementia HiT coordinator, Avon & Wiltshire Mental Health Partnership NHS Trust)
Join Dementia Research (contact: Charlotte Procter, Research Associate and Project Officer for Join Dementia Research, Avon & Wiltshire Mental Health Partnership NHS Trust)
The Carer's and Alzheimer's Society (contact: Peter Conway)

For all the PIC sites:
The research team will provide leaflets and posters where possible outlining the study which will have contact details for the research team. The PICs will be asked to put up the posters and display the leaflets in clinic waiting areas.

In addition to providing leaflets and posters for the Cognitive Disorders Clinic in Bristol (North Bristol NHS Trust), members of the research team, most frequently the psychology assistant will attend the clinics so that they are on hand to explain the study to any interested participants, and provide information leaflets and more details if requested. The consultant Dr Elizabeth Coulthard or an associate on the clinic will identify those patients who may be suitable for inclusion in the study, in some cases these patients may have already expressed an interest in another trial, but have been ineligible for it.

Participants will also be recruited through Everyone Included (Avon and Wiltshire Mental Health Partnership NHS Trust - listed as a PIC site above). Everyone included has reviewed the study and the letter of invitation that will be sent to their service users. Once ethical approval, and approval from AWP is granted, Everyone Included will review their patient records and send a letter of invitation to service users who they judge may fulfil the criteria of this study. Service users who are interested in participating in the study will be asked to respond to Everyone Included who will pass on the details of interested parties.
We will contact those who have expressed an interest in taking part in the study, and provide them with an information leaflet (or poster, if as an email attachment) containing more details of the study (new document - text for initial response to EI participants version 1, 13th April 2016).

The Dementia Health Integration Team (HIT) Network holds the details of individuals who have expressed an interest in taking part in research. The research team will request contact details for those who appear to fit the eligibility criteria and will send them a letter of invitation and a leaflet outlining the study.

The Bristol Brains for Dementia Research project holds a list of donors who have expressed an interest in taking part in further research studies. Dr Laura Palmer, lead for the Bristol Brains for Dementia Research project will identify participants who may be eligible for our study and will send them a letter/email of invitation and a leaflet outlining the study. If only phone contact details are available first contact will be by phone where a brief overview of the study will be given and if requested, further information sent to the potential participant. Dr Palmer will forward details of any participants who confirm that they are interested in taking part in our study to us.

Join Dementia Research (JDR) s an on-line self-registration service that enables volunteer with memory problems or dementia, and carers of those with memory problems or dementia to register an interest in taking part in research. The JDR on-line service and all associated documentation, methods of contacting volunteers and handling their data have been reviewed by a specially convened HRA committee. Following JDR procedures a lay summary agreed with JDR will be made available to those on the JDR website. As agreed with JDR, we will contact those on the register who have dementia to confirm whether or not they are likely to have periodontal disease. Contact will be made in line with the volunteers preferred methods of contact. If those contacted express an interest in taking part in the study we will send them a leaflet and patient information sheet about the study and arrange a time to contact them again to see if they are interested in taking part.

The research team will provide leaflets and posters to Alzheimer's community groups in and around Bristol; these will outline the study and will contain contact details for the research team. Research team members will also attend a number of drop-in sessions/cafes as agreed with the community groups including the Alzheimer's Society, Bristol Dementia Wellbeing Service and St Monica's Trust where they will explain the study in more detail and be available to answer questions. To aid explanation, a short presentation will be prepared and given in an informal manner to those attending drop in/café sessions. In addition, where possible the research team will post a brief description of the study and their contact details on these community group websites and where appropriate, facebook and twitter pages.

Interested potential participants and their carer/friends will be given a leaflet which provides information relating to the study. Where a member of the research team is available at a venue they will be able to answer questions relating to the study. Included on the leaflet will be contact details for the research recruitment team to enable individuals to register their interest.
After participant or carer/friend has initiated contact with the research team (either through direct contact if the researcher is on hand, or by telephone / email) potential participants will be asked a series of questions to assess likely eligibility so as to prevent unnecessary travel for those who it can be quickly ascertained are ineligible for the study. Participants will also be provided with the participant information sheets (one for the patient and the other for the carer/friend) and will be invited to attend a screening appointment at the Bristol Dental Hospital.

Referral from General Dental Practices (GDPs)
We will invite GDPs within 10 miles of the Bristol Dental Hospital to approach patients who fulfil the inclusion criteria of the study (a diagnosis of AD or MCI and a degree of periodontitis).

The research team will provide leaflets and posters to the GDPs who will pass them on to patients interested in the study. The GDPs or participant, will complete a simple referral form, giving their permission to receive further information about the study. This will be returned to the study team via a SAE or secure email and will allow us to contact them with more information (patient information sheet) to see if they would like to take part and will be invited to attend a screening appointment at the Bristol Dental Hospital.

Inclusion criteria

- Mild dementia, such as sporadic early-stage AD, mild early stage familial AD, mixed dementia (most commonly AD and vascular dementia) and vascular dementia associated with cerebral amyloid angiopathy i.e. dementias associated with evidence of influence with bacterial infections such as periodontitis [1,2] or MCI.
- Cognitive scores that demonstrate the patient is likely to have the capacity to consent.
- Moderate to severe periodontitis.
- ≥2 sextants score 4 on Basic Periodontal Examination (BPE) or a score on BPE, combined with other visible factors, which indicates a degree of periodontitis which the Study Dentist deems in need of treatment.
- Minimum of 6 remaining teeth.
- A carer/friend who will consent to take part in the study in this capacity and attend appointments with the participant living with mild dementia.

Exclusion criteria

- Insufficient capacity to consent
- Allergy to essential oil mouthwash.
- Tobacco smoking.
- Uncontrolled systemic disease.
- Uncontrolled dental disease other than periodontitis.
- Pregnancy or breast feeding.
- Severe renal impairment (GFR≤30ml/min)
- Those scoring ≥3 on the American Society of Anaesthesiologists (ASA) Physical Status Classification System.
- Those currently enrolled in another study whose inclusion/exclusion criteria state that while on the study they cannot enrol in new studies such as the Oral Health Study.

To determine whether potential participants are eligible for inclusion in the study they will undergo screening by a clinical psychologist. They will also undergo a dental assessment including a visual oral health assessment and a basic periodontal examination.

Consent

Consent to take part in the study

All those who have expressed an interest in taking part in the study will have received a patient information sheet prior to screening. At the initial screening visit, the study will also be explained verbally to each potential participant and their carer/friend on a case by case basis by one of the clinical investigators. The implications of taking part in the study will be explained and both the patient and carer/friend will have the opportunity to ask any questions which will be answered as fully as possible by the clinical investigator. If the patient and/or their

carer/friend would like more time to decide if they would like to take part in the study the clinical investigator will arrange a new time to contact them by phone to confirm whether or not they are interested in taking part, and to arrange a new screening visit. Consent to communicate with the patient's General Dental Practitioner (GDP) will be gained and their GDP will be informed in writing of the patient's interest/participation in the study.

The clinical investigator will assess the patient's competence to consent. This will include the patient's capacity to consent, their ability to retain information long enough to make a decision, their ability to understand what the decision relates to, the consequences of not making a decision and the benefits, risks and inconveniences of participating in the research for them. The patient's ability to communicate their decision will also be assessed. Autonomous informed and written consent will be recorded where applicable at this visit. This consent process will be completed prior to screening patients for inclusion in the study.

Where the patient's competence to consent is in doubt, every effort will be made to maximise the patient's capacity using simple language, leaflets and drawings. Consent will only be gained if the dentist and clinical psychologist believe the patient is competent to consent. Only patients who have the capacity to consent for themselves will be included in the study.

The carer/friend will be involved in all aspects of the consent process and similarly be asked to consent to their support of the patient in the clinical trial. Support will include reminding and aiding the patient in appointment attendances, home-care routine and compliance with antibacterial protocols (described in Oral Care Complete 5th Oct2015 version 1.0). Should the carer/friend or patient wish to discuss any matters alone with the clinician this will be arranged.

Clinical Consent for Dental Treatment

Following a detailed dental assessment if supplementary dental treatment is required this will be explained, options for treatment will be discussed and informed clinical consent using standard NHS forms will be gained prior to commencing any supplementary dental treatment i.e. non-periodontal dental treatment required to improve oral health and reduce the bacterial load. Verbal consent for dental treatment will be confirmed in a similar manner at the beginning of each dental appointment. If during treatment the participant makes an audible sound that indicates they do not wish to proceed (e.g. moan, groan), or a behavioural sign (e.g. withdrawal, closed mouth, refusal to enter dental chair) treatment will cease. Further participation / withdrawal from the trial will then be discussed in more detail with the patient and carer/friend.

Loss of Capacity during the study

Capacity to consent will be assessed at each clinical appointment. Where the patient's capacity to consent is reduced, every effort will be made to maximise their capacity using simple language and visual diagrams. If the patient's ability to consent remains in question the dentist will seek the advice of the clinical psychologist. Where the capacity to consent has been lost the aim of periodontal and oral care will shift from stabilisation to palliative care. Palliative care will be better delivered in the community to correspond with other lifestyle

changes likely to be required when capacity to consent is lost. Where palliative care is indicated a letter will be written to the patient's GDP advising them of this.

Withdrawal from the study
The participants and carers/friends will be able to withdraw from the study at any time. If a participant decides to withdraw, data gathered until the point of withdrawal will be retained and analysed.

Dental Assessments & Intervention
The proposed dental interventions and psychological assessments for the participants who are living with dementia are summarised in the flow chart in Figure 1. This will take place in Bristol Dental Hospital. Appointment reminders will be sent prior to scheduled appointments. At the **initial assessment** the dentist will:

- explain the study
- answer any questions the participant may have about the study
- gain informed consent to take part in the study from participants and project partners
- take a medical history
- assess eligibility by completing suitable oral health screening assessments

If the patient is eligible to participate in the study the dentist will:

- collect a saliva sample to enable analysis of oral bacteria and human DNA for Single Nucleoptide Polymorphisms (SNP) as described below. The participant will be given a mouthful of mouthwash to swish around their mouth, and they will be asked to expectorate (spit) into a pot.
- take intraoral radiographs if these are clinically justified.
- provide an electric toothbrush and toothpaste and demonstrate how to use it effectively.
- give the participant a leaflet with instructions about tooth-brushing to take home (Toothbrushing instructions 16th October 2015 version 1).
- make arrangements for the next dental visit.

At the following appointment a **full dental assessment** will be completed and **treatment plan** formulated. The purpose of this visit is to determine exactly what treatment will be required to stabilise dental disease.
The dentist will:

- ask the participant and their project partner how easy it has been for them to use the electric brush effectively
- take a detailed medical and dental history
- complete special investigations, as necessary to determine in detail the health of the teeth and surrounding gingivae
- collect a saliva sample
- design a treatment plan and explain to the participant and their project partner what this will entail

- provide mouth wash and interdental aids to aid plaque control
- give advice on when to use the dental products
- if indicated clinically the dentist may complete a supragingival debridement (professional cleaning above gum line)
- make arrangements for the next appointment

Further **dental stabilisation** visits will include as necessary:

- an update of medical history
- further plaque control advice (described in Oral Care Complete 5th Oct2015 version 1.0)
- full mouth debridement (using ultrasonic and manual instrumentation techniques), if clinically indicated, antibiotics may be prescribed in conjunction to physical techniques
- supplemental primary dental care to stabilise active dental disease.
- collection of saliva samples

The details and timings of this treatment will be clarified during the treatment planning process. Where necessary local anaesthetic will be administered.

Supportive therapy

Following the stabilisation of dental disease, 3 monthly monitoring and maintenance visits will be required. These visits will include:

- an update of medical history
- appropriate oral health assessment using relevant special investigations
- maintenance debridement
- supportive advice to help maintain/improve oral health
- if necessary arrange return to active treatment
- collection of a saliva sample
- arrangements for future appointments, and at the end of the study advise the participant how to seek ongoing dental care

With the exception of the collection of saliva samples all other aspects of the dental care pathway are delivered as part of the routine management for the stabilisation of dental disease.

Bacterial Load Determination

Plaque scores will be used to determine a crude measure of bacterial quantity. Bacterial load in saliva will be analysed and polymerase chain reactions (PCR) using bacterial DNA from saliva samples and bacteria gene sequencing techniques will be used to provide information on oral bacterial profiles throughout treatment.

In our previous BRACE funded study involving people without dementia we identified a simple means of assessing bacterial overgrowth by analysing the levels of DNA in a single saliva swill. The amount of DNA purified (that was mostly bacterial) was significantly, on average 25 fold higher, in the periodontitis patients compared to controls. We plan to use this simple test

to monitor the effectiveness of the dental intervention to reduce oral bacterial load by intervention and changing oral hygiene habits.

Analysis of human DNA and proteins in saliva

The DNA in the oral cells present in the saliva will also be analysed for single nucleotide polymporphisms (SNPs) to identify those which are specific to those with periodontal and Alzheimer's Disease.

A large database of SNPs, or single nucleotide polymorphisms, which are small differences in DNA sequence in genes, has been produced from data from hundreds of thousands of patients. The database enables users to look up SNPs of interest and determine whether they are associated with specific risk factors. Genes including APOE, TREM2, BIN1, CLU and many others have SNP variants known to either increase risk for dementia or reduce it, sometimes this risk is very small. We intend to assess a series of SNPs in each patient and using the information stored in the database, see if there is a combination which is related to AD or periodontitis. (https://gwas.biosciencedbc.jp/). To assess which SNP is present on human DNA, DNA probes are used which have DNA nucleotide sequence complementary to the SNP site of interest. Using polymerase chain reaction (PCR) techniques it is possible to copy just that chosen DNA site. For instance, APOE3 has the three DNA nucleotides TGC in the first half of the gene whereas apoE4 has CGC at the same position. The presence of SNPs in the chosen DNA site that is copied can be identified in a number of ways including visualization using DNA gel electrophoresis [38].

The human proteins found in the saliva will also be analysed to determine the spectrum of proteins present, specifically pre and post treatment. Saliva from patients will be examined firstly for the range and quantity of human salivary proteins by mass spectrometry. This will also indicate which proteins are increased and those which are diminished. Additionally, measurements will be made of beta-amyloid and phosphorylated tau content; this could be useful towards finding an early biomarker of Alzheimer's which can distinguish it from other neurodegenerative disorders. Likewise, components of the inflammatory system such as: the interleukins, microglial proteins or the complement cascade will be measured, as will other proteins relevant to the identification of a distinct protein profile for Alzheimer's. ELISAs or western blotting, or other similar methods, will be used for identification and quantification.

Psychological assessments

Baseline psychological assessments of the patients living with mild dementia will include standard cognitive tests such as the Addenbrooke's Cognitive Examination III, Alzheimer's disease quality of life assessment (DEMQOL), and Bristol Activities of Daily Living (BADLS). Information on these tests is placed in appendices at the end of this document
Carer/friends views will be canvassed at follow-up visits.

Approximately 6 months after the baseline scores study participants will be required to have further cognitive and functional assessments; this will be repeated once again at 1 and 2 year intervals. These will, as far as possible, be scheduled to coincide with dental treatment/review appointments.

All psychological and dental assessments and interventions will be carried out at Bristol Dental Hospital. During the study participants should be registered with a General Dental Practitioner.

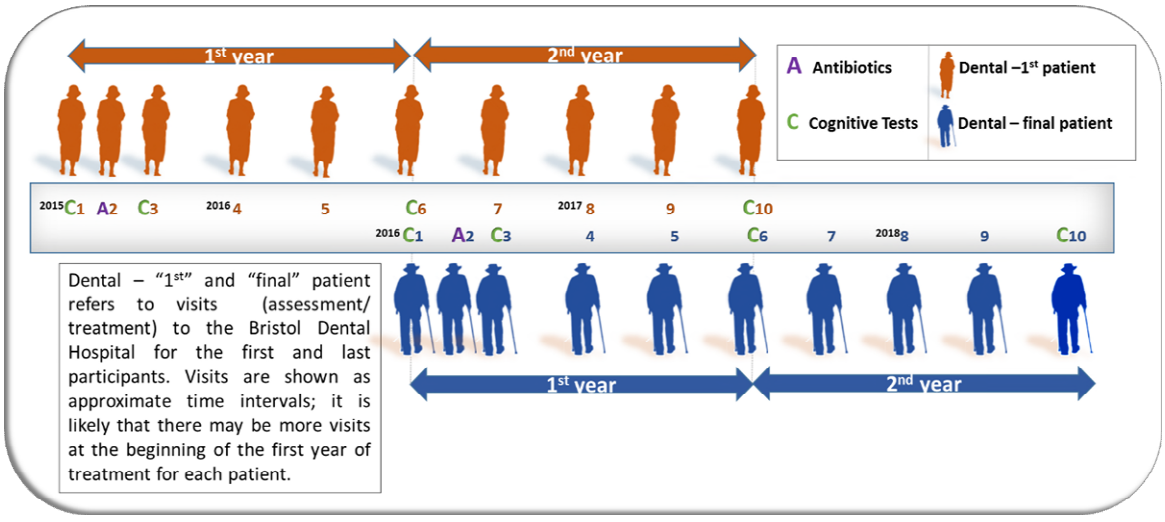
Ongoing oral healthcare at the end of the study

At the end of treatment a letter will be written to the participant's General Dental Practitioner to explain the details of their dental health, the treatment they have received and proposed maintenance regime.

Timelines

- **Four or more** dental interventions are planned in each '*first patient treatment year*' at the BDH.
- **We anticipate that treatment will commence** from January 2016; the last patient will have **begun** dental treatment by the end of December 2017. The deadline for the last patient to finish dental treatment will be the end of December 2019.
- **A second cognitive test will be taken after initial active treatment and approximately 6 months after the initial cognitive assessment.** This will enable us to see if there is a positive change in cognitive behaviour arising from the initial dental care even if this is not sustained for a year or two.
- **The 3rd and 4th cognitive test** will be at the end of treatment year 1 and at the end of treatment year 2.
- **We anticipate that the deadline for the last patient** to have a final '*2nd year*' measurement of cognition is the end of December 2018.

Figure 1 below shows the theoretical first (orange) and last (blue) patient's timeline.
It is possible that the study could finish early if most patients were seen in the 2016/2017 period or if the majority of the participants dropped out/withdrew their consent to take part in the study.



Analysis of Data

Periodontal, psychological, bacteria load and carer/friends' verbal feedback will be analysed using descriptive statistics (graphs and charts), and where relevant simple statistical analysis

to reach a conclusion as to whether it is feasible to reduce oral bacteria in those living with mild dementia.

Adverse Events

A serious adverse event (SAE) is defined as an event which suggests a definite hazard or handicap to the subjects. Serious events are any events resulting in death, life threatening situations, permanent disability, hospitalization or prolonged hospitalisation or congenital abnormality.

When an investigator is notified of a SAE the investigator will promptly (within 24 hours) notify UH Bristol R&D office, regardless of causality. UH Bristol R&D will then make an assessment of whether this needs to be reported to the NHS REC. This is in accordance with UH Bristol research related adverse event reporting policy. UH Bristol will regularly inform the University about SAEs. Expedited reporting takes place where necessary, to agree corrective / preventative actions. Additionally the research ethics committee will be notified of the serious or unexpected event at the time it is reported. Any adverse events (serious or non-serious) will be recorded and those continuing at the end of the study will be followed up to resolution unless documented as 'not clinically significant' or the participant is lost to follow-up by the investigator.

Research Governance

Sponsorship

The Sponsor is the University of Bristol. Funding is from Bristol Research into Alzheimer's and Care of the Elderly (BRACE).

Insurance

The University of Bristol has arranged Public Liability insurance to cover the legal liability of the University as Research Sponsor in the eventuality of harm to a research participant arising from management of the research by the University.

The University of Bristol holds Professional Negligence insurance to cover the legal liability of the University, for harm to participants arising from the design of the research, where the research protocol was designed by the University

Ethics and R&D approval

We are applying for Ethics approval using the online Integrated Research Application System (IRAS). This includes site-specific application (SSA) forms which enable NHS R&D engagement and which will be applied for after a favourable opinion has been granted by the Ethics Committee.

References

[1] Shoemark DK and Allen SJ (2015). The Microbiome and Disease: Reviewing the Links between the Oral Microbiome, Aging, and Alzheimer's Disease. Journal of Alzheimer's Disease 43 725–738

[2] Cerajewska TL, Davies M and West NX. (2015) Periodontitis: a potential risk factor for Alzheimer's disease

[3] Alzheimer's Society (2013). What is Alzheimer's disease? London: Alzheimer's Society,

[4] Braak H, Braak E (1998) Evolution of neuronal changes in the course of Alzheimer's disease. *J Neural Transm Suppl* **53**, 127-140.
[5] Wilcock GK, Esiri MM (1982) Plaques, tangles and dementia. A quantitative study. *J Neurol Sci* **56**, 343-356.]
[6] Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, and Beyreuther K (1985). Amyloid plaque core protein in Alzheimer's disease and Down's syndrome. *Proceedings of the National Academy of Sciences of the USA* **82**, 4245–9.
[7] Akiyama H, Barger S, Barnum S *et al*. (2000). Inflammation and Alzheimer's disease. Neurobiol Aging; **21**: 383-421.
[8] Weiskopf D, Weinberger B, Grubeck-Loebenstein B. (2009). The aging of the immune system. *Transplant International*; **22**: 1041-1050.
[9] Eikelenboom P. Hoozemans J. J, Veerhuis R et al. (2012). Whether, when and how chronic inflammation increases the risk of developing late onset Alzheimer's disease. *Alz Res Ther*; **4**: 15
[10] Poole S, Singharao S. K, Crean, St.J. (2014). Emerging evidence for an association between periodontitis and the development of Alzheimer's disease. *Faculty Dent J*; **5**;39-42.]
[11] Page R. C. (1996). The pathobiology of periodontal diseases may affect systemic diseases: inversion of a paradigm. *Ann Periodontol*; **1**: 821-878.
[12] Offenbacher S. (1996). Periodontal diseases: pathogenesis. *Ann Periodontol*; **1**; 821-878.
[13] Herbert R.P, Harris J, Chong K.P et al. (2012). Cytokines and olfactory bulb microglia in response to bacterial challenge in the compromised primary olfactory pathway. *J Neuroinflammation*; **29**:109]
[14] Lesne S. E. (2013). Breaking the code of Amyloid-beta Oligomers. Int J Cell Biol; 950783
[15] Spires-Jones T.L, Hyman B.T. (2014). The intersection of amyloid-beta and tau at synapses in Alzheimer's disease. Neuron; 82: 756-771.
[16] Pussinen PJ, Tuomisto K, Jousilahti P, Havulinna AS, Sundvall J, Salomaa V (2007) Endotoxemia, immune response to periodontal pathogens, and systemic inflammation associate with incident cardiovascular disease events. *Arterioscler Thromb Vasc Biol* **27**, 1433-1439.
[17] Zaremba M, Gorska R, Suwalski P, Kowalski J (2007). Evaluation of the incidence of periodontitis-associated bacteria in the atherosclerotic plaque of coronary blood vessels. *J Periodontol* **78**, 322-327.
[18] Morre SA, Stooker W, Lagrand WK, van den Brule AJ, Niessen HW (2000). Microorganisms in the aetiology of atherosclerosis. *J Clin Pathol* **53**, 647-654.
[19] Piconi S, Trabattoni D, Luraghi C, Perilli E, Borelli M, Pacei M, Rizzardini G, Lattuada A, Bray DH, Catalano M, Sparaco A, ClericiM (2009). Treatment of periodontal disease results in improvements in endothelial dysfunction and reduction of the carotid intima-media thickness. *FASEB J* **23**, 1196-1204.
[20] Stein PS, Desrosiers M, Donegan SJ, Yepes JF, Kryscio RJ (2007). Tooth loss, dementia and neuropathology in the Nun study. *J Am Dent Assoc* **138**, 1314-1322; quiz 1381-1312
[21] Gatz M, Mortimer JA, Fratiglioni L, Johansson B, Berg S, Reynolds CA, Pedersen NL (2006). Potentially modifiable risk factors for dementia in identical twins. *Alzheimers Dement* **2**, 110-117.
[22] Paganini-Hill A, White SC, Atchison KA (2012). Dentition, dental health habits, and dementia: The LeisureWorld Cohort Study. *J Am Geriatr Soc* **60**, 1556-1563.
[23] http://www.hscic.gov.uk/catalogue/PUB01086/adul-dent-heal-surv-summ-them-exec-2009-rep2.pdf (2009)
[24] Tarkowski E, Blennow K, Wallin A, Tarkowski A (1999). Intracerebral production of tumor necrosis factor-alpha, a local neuroprotective agent, in Alzheimer disease and vascular dementia. *J Clin Immunol* **19**, 223-230.
[25] Loeb MB, Molloy DW, Smieja M, Standish T, Goldsmith CH, Mahony J, Smith S, Borrie M, Decoteau E, Davidson W, McDougall A, Gnarpe J, O'Donnell M, Chernesky M (2004).
A randomized, controlled trial of doxycycline and rifampin for patients with Alzheimer's disease. *J Am Geriatr Soc* **52**, 381-387.
[26] ClinicalTrials.gov Identifier:NCT01463384 Minocycline

[27] Soscia SJ, Kirby JE,Washicosky KJ, Tucker SM, Ingelsson M, Hyman B, Burton MA, Goldstein LE, Duong S, Tanzi RE, Moir RD (2010). The Alzheimer's disease-associated amyloid beta-protein is an antimicrobial peptide. *Plos One* **5**, e9505.

[28] Riviere GR, Riviere KH, Smith KS (2002). Molecular and immunological evidence of oral Treponema in the human brain and their association with Alzheimer's disease. *Oral Microbiol Immunol* **17**, 113-118.

[29] Miklossy J, Kis A, Radenovic A, Miller L, Forro L, Martins R, Reiss K, Darbinian N, Darekar P, Mihaly L, Khalili K (2006). Beta-amyloid deposition and Alzheimer's type changes induced by Borrelia spirochetes. *Neurobiol Aging* **27**, 228-236.

[30] Miklossy J (2011). Alzheimer's disease – a neurospirochetosis. Analysis of the evidence following Koch's and Hill's criteria. *J Neuroinflammation* **8**, 90.

[31] Poole S, Singhrao SK, Kesavalu L, Curtis MA, Crean S (2013). Determining the presence of periodontopathic virulence factors in short-term postmortem Alzheimer's disease brain tissue. *J Alzheimers Dis* **36**, 665-677.

[32] Kamer AR, Craig RG, Pirraglia E, Dasanayake AP, Norman RG, Boylan RJ, Nehorayoff A, Glodzik L, Brys M, de Leon MJ (2009). TNF-alpha and antibodies to periodontal bacteria discriminate between Alzheimer's disease patients and normal subjects. *J Neuroimmunol* **216**, 92-97.

[33] Sparks Stein P, Steffen MJ, Smith C, Jicha G, Ebersole JL, Abner E, Dawson D, 3rd (2012). Serum antibodies to periodontal pathogens are a risk factor for Alzheimer's disease. *Alzheimers Dement* **8**, 196-203.

[34] Branton WG, Ellestad KK, Maingat F, Wheatley BM, Rud E,Warren RL, Holt RA, Surette MG, Power C (2013). Brain microbial populations in HIV/AIDS: Alpha-proteobacteria predominate independent of host immune status. *PLoS One* **8**, e54673.

[35] Han YW, Shen T, Chung P, Buhimschi IA, Buhimschi CS (2009). Uncultivated bacteria as etiologic agents of intraamniotic inflammation leading to preterm birth. *J Clin* When these barriers are breached by sub-acute levels of "immunequiet" oronasal bacteria [39-40]

[36] Jenkinson HF, Demuth DR (1997). Structure, function and immunogenicity of streptococcal antigen I/II polypeptides. *Mol Microbiol* **23**, 183-190.

[37] Novak N, Gros E, Bieber T, Allam JP (2010). Human skin and oral mucosal dendritic cells as 'good guys' and 'bad guys' in allergic immune responses. *Clin Exp Immunol* **161**, 28-33.

[38] Zhong L, Xie YZ, Cao TT, Wang Z, Wang T, Li X, Shen RC, Xu H, Bu G, Chen XF (2016). A rapid and cost-effective method for genotyping apolipoprotein E gene polymorphism. *Molecular Neurodegeneration* **11:2**,

APPENDIX I

Cognitive tests

The Addenbrooke's Cognitive Examination-III (ACE-III) is a brief cognitive test that assesses five cognitive domains: attention, memory, verbal fluency, language and visuospatial abilities. The ACE-III replaces the previous Addenbrooke's Cognitive Examination-Revised and was developed at Neuroscience Research Australia (NeuRA;www.neura.edu.au). The total score is 100 with higher scores indicating better cognitive functioning. Administration of the ACE-III takes, on average, 15 minutes and scoring takes about 5 minutes.

It may be important to use slightly different versions of cognitive tests if they are to be repeated over time in order to avoid learning.

The following are provided as pdfs for the committee:

ACE-III (UK Version A).pdf
ACE-III (UK Version B).pdf
ACE-III (UK Version C).pdf
ACE-III (UK) – Scoring.pdf

APPENDIX II

Dementia Quality of Life (DEMQOL)

Purpose: The DEMQOL is a 29-item measure designed to assess quality of life of a person with mild to moderate dementia from both the patient and the caregiver.
Admin time: 10-20 Min
User Friendly: High
Administered by: Interviewer with patient and/or informant.
Most Appropriate: Primary, Community and Residential Care. QoL for persons with dementia regardless of age.

It generally takes caregivers about 10 minutes to complete the questions concerning their friend/relative. For patients, the interview takes about 10 to 20 minutes to administer, depending on whether a break is needed. Detailed instructions for the interviewer are available.

The following are provided as pdfs for the committee:

- **DEMQOL.pdf**
- **DEMQOL_PROXY- carer.pdf**
- **Interviewer_manual_DEMQOL_and_DEMQOL-Proxy.pdf**

This is accompanied by a paper which explains the assessment:

Moyle 2011 comparison of the DEMQOL and the QOL-AD.pdf

APPENDIX III

Assessment of Activities of Daily Living in Dementia:

The Bristol Activities of Daily Living Scale (BADLS) is a 20-item questionnaire designed to measure the ability of someone with dementia to carry out daily activities such as dressing, preparing food and using transport.

The following is provided as a pdf for the committee:

BADLS questionnaire.pdf

This is accompanied by a paper which explains the assessment:
Bristol Assessment of Activities of Daily Living - Bucks 1996.pdf