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On the Potential Role of Periodontitis in the Pathogenesis of Alzheimer's Disease

Friedrich Leblhuber[1], Julia Huemer[2], Kostja Steiner[1], Dietmar Fuchs[3*]

[1] *Department of Gerontology, Kepler University Clinic, Linz, Austria*
[2] *Freelance Certified Dental Hygienist, Linz, Austria*
[3] *Division of Biological Chemistry, Biocenter, Innsbruck Medical University, Innsbruck, Austria*

Abstract

Neuroinflammation caused by activation of microglial cells plays a key role in the pathogenesis of Alzheimer's disease which can be enhanced by low grade upstream systemic inflammation and *vice versa*. There is growing evidence that age related chronic periodontitis drives systemic inflammation and thus finally Alzheimer's disease, therefore a causal link might exist between certain oral pathogens and Alzheimer's disease.

In an own pilot study, twenty patients with probable Alzheimer's disease were investigated. In seven of these pathogenic periodontal bacteria were found. The presence of *Porphyromonas gingivalis*, the key pathogen and one of the species involved in chronic periodontitis, was found to be associated with lower cognitive test scores (mini mental state examination scores $p < 0.05$; clock drawing test $p = 0.056$). Further, association between lower serum concentrations of immune biomarker neopterin and the presence of *Treponema denticola* ($p < 0.01$). Serum neopterin concentrations correlated highly significantly with kynurenine to tryptophan ratios indicating activity of cytokine interferon-γ. These preliminary findings point to a possible role of an altered salivary microbiome as a causal link between chronic periodontitis and cognitive impairment in Alzheimer's disease.

Keywords:

Резюме

Невроинфламацията, причинена от активиране на микроглиални клетки, играе ключова роля в патогенезата на болестта на Алцхаймер, която може да бъде засилена чрез нискостепенно системно възпаление и обратно. Има все повече доказателства, че свързаният с възрастта хроничен пародонтит води до системно възпаление и по този начин най-накрая болестта на Алцхаймер, следователно може да съществува причинно-следствена връзка между определени орални патогени и болестта на Алцхаймер.

В нашето пилотно проучване са изследвани двадесет пациенти с вероятна болест на Алцхаймер. В седем от тези патогенни пародонтални бактерии са открити. Установено е, че наличието на *Porphyromonas gingivalis*, ключов патоген и един от видовете, участващи в хроничен пародонтит, е свързано с по-ниски резултати от когнитивни тестове (мини оценка на психичното състояние $p < 0{,}05$; тест за рисуване на часовник $p = 0{,}056$). Освен това, връзката между по-ниските серумни концентрации на имунен биомаркер неоптерин и наличието на *Treponema denticola* ($p < 0.01$). Серумните концентрации на неоптерин са в голяма степен свързани с съотношенията на кинурин и триптофан, показващи активността на цитокин интерферон-γ. Тези предварителни резултати сочат за възможна роля на променен слюнчен микробиом като причинно-следствена връзка между хроничен пародонтит и когнитивно увреждане при болестта на Алцхаймер.

* Corresponding author: dietmar.fuchs@i-med.ac.at

Introduction

Dysbiosis of microbiota in different body regions may compromise blood brain barrier (BBB) integrity promoting pathogenesis of Alzheimer's disease (AD) by translocation of pathogenic agents into the brain (Caracciolo *et al*., 2014; Fox *et al*., 2019). Chronic periodontitis is a common infection in humans accompanied by destruction of the tissue supporting the teeth (Page and Eke, 2007). Loss of more than 16 teeth in early to mid-life is significantly associated with the development of dementia (Gatz *et al*., 2006; Lou *et al*., 2015). Conversely, retaining teeth and not brushing them, also exposes individuals to the risk of developing dementia. Taken together, the plausible explanation for missing and unclean teeth is poor oral hygiene (Paganini-Hill *et al*., 2012). Periodontitis is prevalent in individuals with poor oral hygiene and high dental plaque index (Wadhawan *et al*., 2020).

Recent studies suggest an association between periodontitis and dementia with possible common pathophysiological mechanisms (Pazos *et al*., 2018; Fox *et al*., 2019). AD is the most common form of dementia with a complex multifactorial etiology: diverse pathogens including periodontal bacteria were associated with AD (Pritchard *et al*., 2017). Neuroinflammation - an inflammatory process in the brain, modulated by microglial cells (Zhou *et al*., 2018) - is an early event which can be enhanced by periodontitis and consecutive systemic inflammation and *vice versa* (Caracciolo *et al*., 2014; Leblhuber *et al*., 2017; Wadhawan *et al*. 2020). Increased serum concentrations of neopterin and of tryptophan breakdown were found to be sensitive biomarkers of immune activation caused by stimulation of indoleamine 2,3-dioxygenase-1 (IDO) and of GTP-cyclohydrolase 1 by interferon-γ (IFN- γ) (Murr *et al*., 2002; Schröcksnadel *et al*., 2006). Similar findings were seen due to the age-related immune response even in healthy individuals (Capuron *et al*., 2011), but more often and to a greater extent in AD (Sawada *et al*., 1987; Hamon *et al*., 1988; Giil *et al*., 2017). The decline of tryptophan concentrations and consequently the increase of the neurotoxic tryptophan catabolite quinolinic acid were observed in parallel with cognitive decline in AD patients (Hamon *et al*., 1988; Giil *et al*., 2017; Leblhuber *et al*., 2017).

Periodontal disease was found to be associated with higher brain amyloid load even in the older-aged healthy (Kamer *et al*., 2015). Older-aged individuals frequently are suffering from periodontitis with prevalence increasing with age (Wu and Nakanishi, 2014). In earlier studies, a close and causal link between chronic periodontitis, aggravating systemic inflammation and cognitive impairment was hypothesized (Shoemark and Allen, 2015; Sochocka *et al*., 2017). This hypothesis is underlined by more recent preclinical data (Ilievsky *et al*., 2017). *Porphyromonas gingivalis* (*P. gingivalis*) is the dominant strain of the foremost pathologic complex ("red complex") including also *Tannerella forsythia* and *Treponema denticola* causing polymicrobial synergy, thus gaining greater virulence (Harding *et al*., 2017). Serum IgG levels to these common periodonto-pathogenic bacterial strains are described to be associated with risk for developing incident AD (Noble *et al*., 2014).

Poor dental status, masticatory dysfunction and periodontal disease has repeatedly been linked to reduced cognitive function and AD in a series of reviews (Watanabe *et al*., 2015; Tremlett *et al*., 2017; Wadhawan *et al*., 2020). In the Nun study, participants with the fewest teeth had the highest risk of prevalence and incidence of dementia (Gatz *et al*., 2006; Stein *et al*., 2007). In an earlier *post-mortem* study, pathogenic periodontal disease bacteria *T. denticola*, *T. forsythia* and *P. gingivalis* were identified in brain tissue indicating a link between chronic periodontal disease and AD (Poole *et al*., 2013).

Additionally, dysbiosis of intestinal microbiota in the elderly and in patients with dementia earlier has been described to cause leaky gut (Leblhuber *et al*., 2015; Buford *et al*., 2018), which also results in silent systemic inflammation and via microbiota-gut-brain-axis in neuroinflammation (Caracciolo *et al*., 2014; Marashwari and Eslick, 2015; Minter *et al*., 2016; Leblhuber *et al*., 2017; Leblhuber *et al*., 2018).

Periodontitis in the pathogenesis of Alzheimer's disease, preliminary results of an ongoing study

In a recent pilot study, after written informed consent the diagnosis of AD was established in twenty patients by cerebral magnetic resonance tomography (MRT) and routine laboratory tests as described earlier (Widner *et al*., 1997; Leblhuber *et al*., 2018). Cognitive testing was performed by mini mental state examination (MMSE) and clock drawing test (CDT).

Serum concentrations of neopterin as well as tryptophan and kynurenine were also analyzed, calculating the kynurenine to tryptophan ratio (Kyn/Trp) as an index of tryptophan breakdown (Widner *et al*., 1997; Schröcksnadel *et al*., 2006). Additionally, alveolar fluid was tested by RNA-based analy-

sis (PerioPOC®, Genspeed Biotech, Henry Schein Dental, Vienna, Austria) for the presence of the following periodontal pathogens: *T. denticola*, *T. forsythia*, *P. gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* described by Polonyi *et al*. (2013).

Twenty consecutive patients (aged 78.1 ±2.2y, 9 females) with symptoms of progressive cognitive decline were recruited, none of them was smoking. The procedure was well-tolerated by all patients. In MRT scans all patients showed global cerebral atrophy without circumscribed lesions. The MMSE in our patients was mean ± SD: 20.8 ± 1.70 and the CDT scores were 6.0 ± 0.77.

In seven of the patients investigated with clinical signs of periodontitis stage III and IV (Tonetti *et al*., 2018) pathological periodontitis strains were found. *P. intermedia* and *A. actinomycetemcomitans* could be detected in none of our cases studied. A significant association between the salivary presence of *P. gingivalis* and lower MMSE ($p < 0.05$; Fig. 1) was observed.

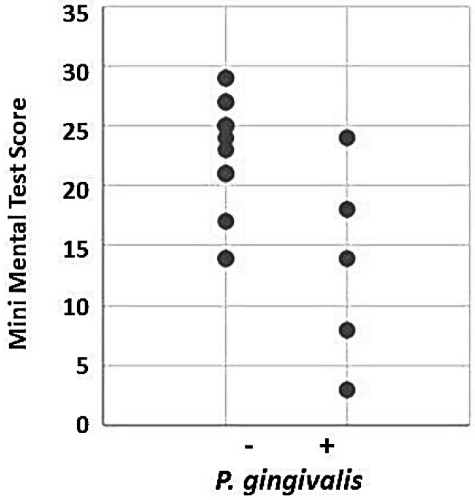

Fig. 1. Patients with AD saliva positive for *P. gingivalis* present with statistically lower scores in the mini mental state examination test (MMSE), $p < 0.05$ (adapted from: Leblhuber F, *et al*., *Wien Klin Wochenschr* 2020, in press, doi.org/10.1007/s00508-020-01638-5).

There was also a tendency to lower scores in the CDT ($p = 0.056$) when this particular pathogen was present. No other pathogen was associated with an unequal distribution of cognitive results in this group of patients. Further, the presence of *T. denticola* was associated with lower serum neopterin concentrations ($p < 0.01$; Fig. 2) and also kynurenine levels showed a tendency to lower levels, the difference however not reaching the level of statistical significance ($p = 0.064$).

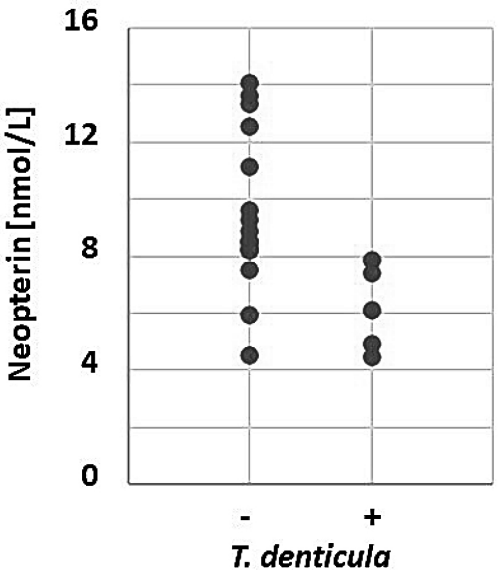

Fig. 2. Lower serum neopterin concentrations in AD patients saliva positive vs. negative for *T. denticola*, $p < 0.01$ (adapted from: Leblhuber F, *et al*., *Wien Klin Wochenschr* 2020, in press, doi.org/10.1007/s00508-020-01638-5).

Vice versa, the presence of *T. forsythia* resulted in higher serum kynurenine concentrations compared to patients with a negative saliva microbiological test result ($p < 0.05$). Still in the whole data set, a significant positive correlation existed between serum concentrations of neopterin and Kyn/Trp ($r_s = 0.674$, $p < 0.001$; Fig. 3) confirming earlier results (Widner *et al*., 2000; Leblhuber *et al*., 2017) and indicating induction of Th1-type cytokine IFN-γ. No significant effect was observed for any other measurement; the results of this pilot study were published recently (Leblhuber *et al*., 2020).

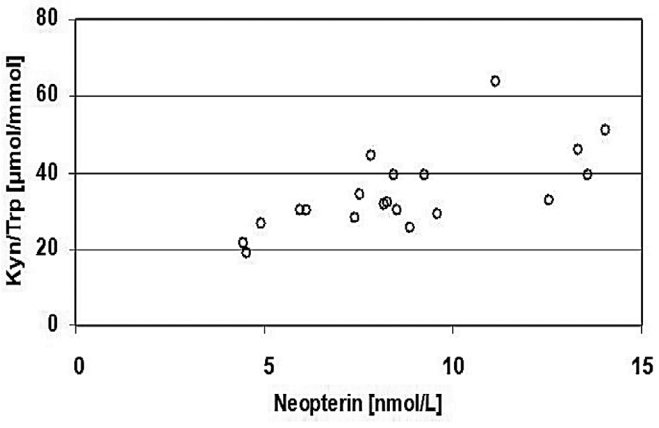

Fig. 3. Correlation between serum neopterin concentrations and serum kynurenine to tryptophan ratios in AD patients ($r_s = 0.674$, $p < 0.001$ (adapted from Leblhuber F, *et al*., *Wien Klin Wochenschr* 2020, in press, doi.org/10.1007/s00508-020-01638-5).

Discussion

Chronic low-grade immune activation and inflammation alter monoamine metabolism that is involved in the development of neuropsychiatric symptoms characteristic for age and dementia (Widner *et al*., 2000; Neurauter *et al*., 2008; Capuron *et al*., 2011; Leblhuber *et al*., 2017). Systemic peripheral inflammation may induce neuroinflamation together with an increase of permeability of the BBB (Fox *et al*., 2019; Panza *et al*., 2019). Altered gut microbiota (dysbiosis) results in systemic inflammation and microglia dysfunction implicated in AD pathogenesis (Caracciolo *et al*., 2014; Mosher and Wyss Coray, 2014; Erny *et al*., 2015).

Furthermore, elevated antibodies to periodontopathogenic strains were found in subjects years before cognitive impairment became manifest (Spars Stein *et al*., 2012). Dysbiosis of oral microbiota leads to microbial translocation to the brain either through the blood stream, via damaged oral mucosal barriers or directly via the trigeminal nerve (Fox *et al*., 2019). Evidence exists that periodontopathogenic strains as *T. denticola* and *P. gingivalis* finally translocate to brain structures afflicted in AD via saliva, tooth pulp chambers, trigeminal ganglia and pons (Shoemark and Allen, 2015; Dominy *et al*., 2019). Recently it was claimed that infiltration of the brain by *HSV type 1* or *Chlamydia pneumonia* crossing directly the weakened BBB may elicit neuroinflammation from the periphery in a similar way (Panza *et al*., 2019).

Our recent exploratory pilot study (Leblhuber *et al*., 2020) described presence of the periodontal pathogenic bacteria *T. denticola*, *T. forsythia*, and *P. gingivalis* in saliva of a subgroup of demented patients associated with chronic oral inflammation as mentioned earlier (Singhrao *et al*., 2014; Pritchard *et al*., 2017). *P. gingivalis*, the most virulent and intriguing pathogen of the bacteria associated with periodontitis (Harding *et al*., 2017; Wadhawan *et al*., 2020) with remote body inflammatory pathologies, was found to be associated with lower MMSE ($p < 0.05$; Fig. 1) and also albeit only weakly with lower CDT scores ($p < 0.06$) in these patients. Data indicates a link between this periodontal pathogenic strain and neuroinflammation and the dementing process, respectively.

In an animal study, experimental chronic periodontitis was induced by repeated oral application of *P. gingivalis* (IIievsky *et al*., 2018). Gingipain, a protease secreted by this bacterium, could be detected immunohistochemically in the hippocampi of experimental mice confirming its translocation to the brain. Also, signs of neuroinflammation, neurodegeneration and the formation of extracellular Aß42 consistent with neuropathological findings in AD could be shown after repeated oral application of *P. gingivalis* in young adult wild type mice (IIievsky *et al*., 2018). In AD, *P. gingivalis* possibly affects the BBB permeability and influences local IFN-γ response by preventing entry of immune cells into the brain. The scarcity of adaptive immune cells in AD neuropathology implies *P. gingivalis* infection of the brain likely causing impaired clearance of insoluble amyloid and inducing immune-suppression (Olsen *et al*., 2016; Harding *et al*., 2017).

The presence of salivary *T. denticola* was associated with lower serum concentrations of neopterin ($p < 0.01$; Leblhuber *et al*., 2020) and in the whole data set a positive association between neopterin and Kyn/Trp levels was apparent. These results imply that some of the periodontal pathogens may interact with adaptive (Th1-type) immunity probably by triggering regulatory T-cells which finally suppress and downregulate the inflammation process as hypothesized recently (Tremlett *et al*., 2017; Leblhuber *et al*., 2018): The correlation between neopterin and Kyn/Trp concentrations points to the regulatory principle of Th1-type immune activation, and the lower neopterin levels imply lowered production of IFN-γ. Likewise, *P. gingivalis* was reported to suppress adaptive immunity in AD patients (Olsen *et al*., 2016). Probably an imbalance of immune functions and an imbalance of adaptive immunosuppression could be caused by these synergistic pathogenic bacterial species (Soreq and Wolf, 2011; Wadhawan *et al*., 2020).

Interestingly, six of our patients positive for periodontal pathogens were ApoE4 allele carriers, two of them homozygous. In an earlier study, individuals with both a low number of teeth and the ApoE4 allele were performing worse in cognitive tests and showed more rapid cognitive decline over time (Sparks Stein *et al*., 2012). The data set in our recent study (Leblhuber *et al*., 2020) was too small for detailed statistical analysis, and future studies with an appropriate number of ApoE4 allele carriers will be necessary to address this issue.

To conclude in the absence of longitudinal data, our preliminary findings can only provide correlational evidence that chronic periodontal bacterial infections may be additive in the pathogenesis of cognitive decline and AD. Future longitudinal studies including periodontal disease in larger numbers of demented patients as well as age related

non-demented individuals (Kamer *et al*., 2015) respecting the ApoE status are necessary to elucidate the specific role of the oral microbiome in neuroinflammation and neurodegeneration.

The presence of specific oral pathogens relating to immunobiochemical changes in demented patients could probably reflect the fact that these patients are no longer able to perform sufficient dental hygiene. Additional longitudinal studies also should investigate the effects of certain bacterial strain consumption to improve or prevent periodontal disease in the aging population (Tobita *et al*., 2018). Small molecule inhibitors to reduce the bacterial load of certain bacteria like *P. gingivalis* will be developed in the near future (Dominy *et al*., 2019; Panza *et al*., 2019). Thus, encompassing investigation of the symbiotic intestinal, oral as well as nasal microbiome might identify new pathways for AD treatment (Fox *et al*., 2019).

Our recent study (Leblhuber *et al*., 2020) is certainly limited by the thus far too small number of patients. Nevertheless, these preliminary results underline the aspect that the salivary microbiome could play a relevant role in the pathophysiology of AD. It might also emphasize a potential bottom-up contribution in AD (Panza *et al*., 2019; Wadhawan *et al*., 2020) which is usually regarded to be top-down only. Thus, it appears attractive as saliva can be easily assessed without any burden to the patients allowing to monitor aspects in the pathogenesis and progression of AD.

Ethics Aproval and Consent to Paricipate

The study was performed in accordance with the criteria of the Declaration of Helsinki and approved by the local ethics committee <dated 2017/05/11 by the Ethikkommittee des Landes Oberösterreich, Studie Nr. I-24-16>.

Human and Animal Rights

Human specimens were collected following an approved study protocol. The work does not report experiments involving animals.

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