Journal of Pharmacy and Pharmacology 5 (2017) 648-653
doi: 10.17265/2328-2150/2017.09.006

On the Role of Intestinal Microbiota in Patients with Cognitive Decline

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Abstract: Objective: The association between gut microbiota composition and biomarkers of immune activation and inflammation was assessed in the elderly. Patients: Serum inflammation markers of fifty-five outpatients (29 females, 26 males, aged 78 ± 8.5 years) were analyzed. Stool specimens and thus data on gut microbiota were available from a subgroup of 23 individuals (9 females and 14 males). Results: Global cerebral atrophy was found in all magnet resonance tomography scans. Mean mini-mental-score examination in Alzheimer's disease patients was 18.8 ± 7.1, in patients with mild cognitive impairment 27.8 ± 1.5. Serum neopterin concentrations correlated with concentrations of fecal S100A12 ($p < 0.001$) and α_1-antitrypsin ($p < 0.05$). *Faecalibacterium prausnitzii* correlated with MMSE ($p < 0.05$), with *Akkermansia muciniphila* ($p < 0.01$) and with serum neopterin ($p < 0.05$). Fecal zonulin correlated inversely with *Clostridium cluster* I ($p < 0.02$). Conclusions: Our results underline earlier *in vitro* and animal studies that cognitive decline associates with age-related changes in the intestinal microbiota and neuroinflammation. However, only correlational evidence can be reported, and a causative relationship still has to be demonstrated.

Key words: Microbiota, brain-gut axis, prevention, cognitive decline.

1. Introduction

AD (Alzheimer's disease) is the most common form of dementia [1] with age as the most prominent out of numerous risk factors [2].

The prevalence of dementia increases exponentially with age, and it doubles every five years from about 1.5% in individuals aged 65-69 to 40% in nonagenarians [3]. Worldwide there are approximately 4.6 million new dementia cases every year [4].

Gastrointestinal health is of increasing interest in preventive medicine. Recent studies indicate that the human microbiota may regulate parts of the neurotransmission in the central nervous system in health and disease [5]. Gastrointestinal barrier function and gut microbiome seem to be the key to gut health.

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of bacteria in the colon and thus improve host health, especially important in the elderly, as there is an age-related decrease in the diversity of gut microbiota.

Probiotics are living organisms, which when administered in adequate amounts confer health benefits on the host: probiotics suppress pathogens, stimulate epithelial cell proliferation, differentiate and fortify the intestinal barrier and mediate immune modulation. However, there is still confusion in the minds of the authorities over whether a probiotic is a drug, a food, or a dietary supplement [6].

Gut microbes can produce hormones and neurotransmitters like SCFAs (short chain fatty acids), GABA, serotonin, tryptophan and other biogenic

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amins like adrenalin and noradrenalin that are identical to those produced by the host. So they are together with prebiotics named psychobiotics, which act on the gut-brain axis. These effects are mediated via the vagus nerve, spinal cord and the hypothalamic-pituitary-adrenal axis.

Dysbiosis (perturbation in microbiome composition) results in dysbalance of neurotransmitters and consecutively increases the risk of depression, anxiety, altered pain perception and other symptoms like cognitive decline and dementia [7-9].

Administration of prebiotics and probiotics may alter the gut microbiome and it appears likely that they can improve the disturbed resorption of neurotransmitters important for the neuropsychiatric status in the elderly [10].

2. Patients and Methods

Fifty-five consecutive outpatients from the Department of Gerontology of the Neuromed Campus at the Kepler University Clinic Upper Austria with symptoms of cognitive decline (29 females, 26 males, aged 78 ± 8.5 years) were included in our study. Fourty-five patients fulfilled the diagnostic ICD-10 criteria of AD (F 00.1), ten the criteria of mild cognitive impairment (MCI, F06.7). Diagnosis was proofed by routine laboratory tests (electrolytes, blood cell count, hemoglobin, hematocrit, platelets, serum enzymes, urine analysis, blood glucose, HbA1c, bilirubin, bicarbonate, creatinine, blood urea nitrogen, C-reactive protein, total serum protein, folic acid, vitamin B_{12}, thyroid parameters) and MRT (magnetic resonance tomography) scans. MMSE (mini mental state examination) and CDT (clock drawing test) scores were examined for evaluation of the cognitive status of the patients investigated. Of a subgroup of 23 patients (9 females and 14 males), 2 g fecal samples, stored immediately at -20 °C were collected from all patients for later analysis of bacterial DNA (*Clostridium cluster I, Faecalibacterium prausnitzii, Akkermansia muciniphila*) and measurement of S100A12, calprotectin, α_1-antitrypsin and zonulin by Biovis Diagnostik MVZ, Limburg an der Lahn-Offheim, Germany.

Additionally, the following laboratory parameters were measured in serum specimens obtained in parallel: neopterin by ELISA (BRAHMS, Hennigsdorf, Germany), and tryptophan and kynurenine by HPLC via fluorescence detection (tryptophan) and monitoring UV absorption (kynurenine). The kynurenine to tryptophan ratio (Kyn/Trp) was calculated as an index of tryptophan breakdown [11]. Tyrosine and phenylalanine were measured by HPLC and fluorescence detection, and the phenylalanine to tyrosine ratio (Phe/Tyr) was calculated as an index of PAH (phenylalanine hydroxylase) activity [12].

Data were analyzed using the Statistical Package for the Social Sciences (version 19, SPSS, Chicago, IL, USA). To take into account that not all collected data followed a normal distribution, non-parametric Friedman and Wilcoxon signed-rank test were applied. To test for associations between variables, Spearman rank correlation analysis was performed, and *p* values below 0.05 were considered to indicate significance. The study was approved by the local ethics committee.

3. Results

Global cerebral atrophy was found in all MRT scans. Mean MMSE in 45 AD patients was 18.8 ± 6.3, mean CDT 3.5 ± 2.6, in 10 MCI patients MMSE was 27.8 ±1.5, CDT 8.6 ± 0.9. Fecal specimens were available from a subgroup of 23 patients. Concentrations of fecal calprotectin were mean ± SEM 85 ± 114 mg/L, concentrations of fecal S100A12 were 3.3 ± 5.2 µg/L, of fecal α_1-antitrypsin 67.3 ± 54.5 mg/g and of fecal zonulin 75.1 ± 52.3 ng/ml. Average concentration of CRP was 1.6 ± 2.3 mg/dl, of neopterin 12.4 ± 9.6 nmol/L, of tryptophan 49.3 ± 13.9 µmol/L, of kynurenine 1.7 ± 0.8 µmol/L, of Kyn/Trp 39.4 ± 16.2 µmol/mmol, of tyrosine 119 ±

40.1 µmol/L, of phenylalanine 95.9 ± 100 µmol/L, and of Phe/Tyr was 0.85 ± 0.94 µmol/µmol (see Table 1).

Correlations existed between serum concentrations of neopterin and tryptophan (r_S = -0.517, $p < 0.01$), kynurenine (r_S = 0.429, $p < 0.05$) and Kyn/Trp (r_S = 0.762, $p < 0.001$), and results correspond well with earlier observations [13] and represent signs of an activated immune system. Also, fecal S100A12 (r_S = 0.816, $p < 0.001$; Fig. 1) and fecal α_1-antitrypsin (r_S = 0.396, $p < 0.05$) correlated with serum neopterin, see Table 2. There were no significant correlations between fecal calprotectin and CRP or neopterin concentrations in the blood. There was also no significant association between fecal calprotectin levels and cognitive performance of patients (CDT: r_S = 0.027; MMSE: r_S = 0.064; both n.s.).

Table 1 Concentrations of neurotransmitter precursors in serum and of serum and fecal inflammation markers investigated in 45 patients with cognitive decline, of 23 patients stool specimens were available (Kyn/Trp = kynurenine to tryptophan ratio, Phe/Tyr = phenylalanine to tyrosine ratio).

Serum measurements (n = 45)	
CRP	1.6 ± 2.3 mg/dl
Neopterin	12.4 ± 9.6 nmol/L
Tryptophan	49.3 ± 13.9 µmol/L
Kynurenine	1.7 ± 0.8 µmol/L
Kyn/Trp	39.4 ± 16.2 µmol/mmol
Tyrosine	119 ± 40.1 µmol/L
Phenylalanine	95.9 ± 100 µmol/L
Phe/Tyr	0.85 ± 0.94 µmol/µmol
Stool measurements (n = 23)	
Calprotectin	85.0 ± 114 mg/L
S100A12	3.3 ± 5.2 µg/L
α_1-Antitrypsin	67.3 ± 54.5 mg/g
Zonulin	75.1 ± 52.3 ng/ml

Table 2 Spearman rank correlations r_s and p values between neopterin and tryptophan degradation in serum (upper; n = 45) and between serum neopterin, fecal S100A12 and fecal α_1-antitrypsin concentrations (lower; n = 23), (Kyn/Trp = kynurenine to tryptophan ratio).

Neopterin *vs.*		
Tryptophan	r_s = 0.517	$p < 0.01$
Kynurenine	r_s = 0.429	$p < 0.05$
Kyn/Trp	r_s = 0.726	$p < 0.001$
Neopterin *vs.*		
S100A12	r_s = 0.816	$p < 0.001$
α_1-antitrypsin	r_s = 0.396	$p < 0.05$

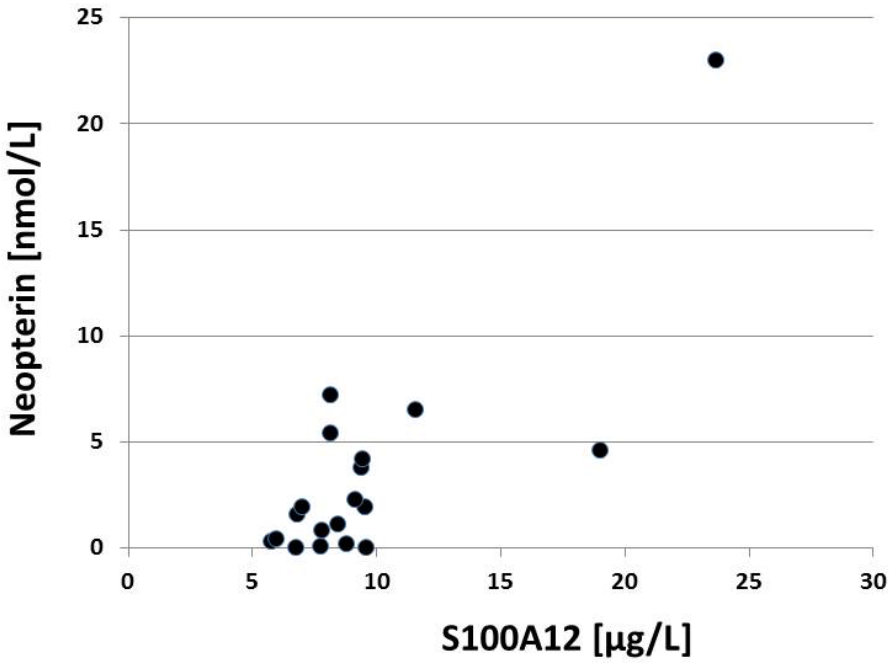

Fig. 1 Fecal S100A12 and serum neopterin concentrations in patients suffering from cognitive decline (rs = 0.816, $p < 0.001$).

Measurement of *Clostridium cluster I* strain revealed 7.4 ± 2.1 (RNA copy/g feces, log10), of *Faecalibacterium prausnitzii* 7.4 ± 1.7 (RNA copy/g feces, log10) and of *Akkermansia muciniphila* 8.0 ± 1.6 (RNA copy/g feces, log10).

Clostridium Cluster I correlated inversely with zonulin (r_S = -0.523, $p < 0.01$) and with the Kyn/Trp ratio (r_S = -0.530, $p < 0.01$). *Faecalibacterium prausnitzii* correlated with MMSE (r_S = 0.418, $p < 0.05$), with *Akkermansia muciniphila* (r_S= 0.593; $p < 0.01$) and with neopterin (r_S= 0.358, $p < 0.05$). No other correlations were seen between the rest of the parameters investigated.

4. Discussion

Alzheimer's disease is the most common form of dementia, however there is no cure available so far. In the recent years there is increasing interest in the role of the human microbiota in different clinical diseases with partly common pathologies including neurodegeneration and dementia [14]. The gut microbiota was studied in AD mouse models, but has not been studied in AD patients so far [15]. We investigated immune and inflammation markers in serum and stool specimens of 55 patients with cognitive deficits, 45 AD patients and 10 patients with MCI, as the extended preclinical phase of AD.

Similar to earlier studies [10-12] signs of immune activation could be detected: serum neopterin was found elevated as well as the Kyn/Trp ratio, serum levels of the neurotransmitter precursors tryptophan, phenylalanine and tyrosine were decreased as found in an earlier study with indirect correlation to stool calprotectin [10]. Calprotectin—a complex of S100A8 and S100A9—is an inflammatory marker for gut mucosal inflammation [16]. Earlier studies found that calprotectin may trigger the aggregation of ß-amyloid in animal studies [17]. Interestingly, in the present series a rather close correlation was found between stool S100A12 and serum neopterin ($p < 0.001$; Fig. 1), indicating coincident low grade systemic and gut inflammation [18]. These findings again underline the role of neuroinflammation as a possible pathogenic cofactor in cognitive deterioration and dementia. In an earlier study [19], the potential role of pro-inflammatory S100A9 and S100A12 proteins in the pathogenesis of AD was described. In addition, circulating CRP known to affect cognition negatively, was also found elevated in this series—1.6 ± 2.3 mg/L without clinical signs of acute infection—as a sign of low grade inflammation ("inflammaging") [20]. Age related changes in the gut microbiota may thus be part of inflammaging.

Concerning the measurements of stool bacterial strains in our patients with cognitive decline: *Clostridium Cluster I* significantly correlated with *Faecalibacterium prausnitzii* ($p > 0.01$). In a recent paper [21] the stool abundance of selected bacterial stool taxa including *Faecalibacterium prausnitzii* and the blood levels of pro- and anti-inflammatory cytokines in cognitively impaired patients and in a group of controls was measured. Amyloid positive patients showed higher levels of pro-inflammatory cytokines compared with both controls and with amyloid negative patients. A possible causal relation between gut microbiota related inflammation and amyloidosis was suspected in this study [21]. In our series *Faecalibacterium prausnitzii* correlated with MMSE ($p < 0.05$), with *Akkermansia muciniphila* ($p < 0.01$) and with serum neopterin ($p < 0.05$).

Altered gut microbiota might be associated with cerebral accumulation of amyloid-β in AD. It has been shown that gut microbiota can release ß-amyloid and lipopolysaccharides, which might play a role in the production of pro-inflammatory cytokines related to the pathogenesis of AD [22]. Gold M et al. described the anti-inflammatory action of α_1-antiptrypsin on microglial mediated neuroinflammation *in vitro* [23]. A strong correlation was found between anti-inflammatory α_1-antitrypsin and pro-inflammatory S100A12 ($p < 0.001$) in the fecal specimens of our cognitively impaired patients. α_1-antitrypsin was also

correlated to zonulin ($p < 0.01$), a protein modulating tight junction permeability between cells of the digestive tract [24]. These findings also indicate changes in the microbiota-gut-brain-axis correlated to neuroinflammation. Since neuroinflammation is an early event in the pathogenesis of dementia [25], these markers may be important in the very beginning of this devastating process. Emry et al. [26] underlined the importance of microglia in brain function and its dysfunction as primary disease-causing condition in neuropsychiatric and neurodegenerative disorders. They state that microbiota vitally regulate microglia function, again indicating the importance of the gut-brain-axis.

The potential relevance of the association between neopterin production and tryptophan breakdown resulting from immune system activation has been concluded from earlier studies when especially the serum tryptophan levels were found to significantly associate with cognitive scores in AD patients [13]. The findings of this study further strengthen the potential relationship between gut bacteria composition and signs of immune activation in the pathogenesis of AD. The increased immune activation and inflammation could indeed relate to the composition of gut microbiota as is indicated by the close relationship between fecal S1900SA and serum neopterin concentrations.

The results of our measurements seem to be in concordance with earlier in vitro studies and findings in animal models that there is correlation between age related changes of the microbiota and neuroinflammation in cognitive decline. The study is limited by the fact that only correlational evidence could be shown, a causative relationship still has to be demonstrated. Measurements of these inflammation markers in larger populations including cognitively normal controls and during follow-up are warranted. Analysis of microbiota in correlation to the Apo E status could give additional information in terms of progressive cognitive decline and dementia [27, 28].

5. Conclusions

Bacterial composition in the gut is found to correlate to immunological alterations in patients with MCI and AD that are relevant for tryptophan metabolism and other biochemical processes linked with memory and cognition. The therapeutic role of probiotics and prebiotics in preventing dementia [10, 29, 30] and life style-associated cognitive impairment [31] has to be elucidated in future studies. This could be of special relevance in the care taking of patients with cognitive impairment or dementia.

Declaration of Interest

The authors have no relevant interests to declare.

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