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Peripheral inflammatory markers during an acute bacterial infection in older patients with and without cognitive dysfunction: A case control study

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ABSTRACT

Dementia is a known risk factor for acute bacterial infections which may also play a significant role in promoting or accelerating cognitive impairment. Pneumonia and urinary tract infections are the main cause of hospitalisation of dementia patients and infections are a major precipitant of delirium. It is well established that peripheral immune signals induce a neuroinflammatory response largely mediated by microglial cells which is amplified with advanced age, neurodegenerative disorders and genetic characteristics. Reversely, the innate immune response to acute bacterial infection is tightly regulated by the brain. It remains unclear whether dysfunctional neural circuits affected by dementia and/or delirium could alter systemic innate immune responses at the periphery. The current study aims to determine if dementia and/or delirium are associated with an altered systemic inflammatory response to an acute bacterial infection. We recruited 46 hospitalised older patients with acute bacterial infections. From these, 29 participants had cognitive dysfunction (6 with delirium, 12 with dementia and 11 with delirium superimposed on dementia) and 17 had normal cognition. We also included a control group of 11 patients with dementia but with no current infection matched for age and educational status. Baseline characteristics were tested between groups using Kruskal-Wallis test and pairwise comparisons were subsequently assessed with Bonferroni correction for multiple comparisons for continuous variables. Chi square test was used to assess differences between groups in categorical data and correlations between peripheral inflammatory parameters were assessed with Spearman's test. The 4 groups with infection and the control group with no infection had similar characteristics except for cognitive function and functionality which was higher for the group of infected cognitively healthy participants. Levels of C-reactive protein were similar between the infected groups and higher than the non-infected dementia group. Infected patients with cognitive dysfunction (delirium and/or dementia) had higher serum levels of IL-6, TNF-alpha and IL-1beta. These participants had reduced expression of miR-145 in circulating exosomes which correlated negatively with miR-155 levels ($r = -0.411$, $p = 0.027$). Expression of CR1 in circulating CD14$^+$ monocytes was higher in infected participants with cognitive dysfunction and, in this group, PICALM correlated both with TNF-alpha and IL-6. In contrast to what was observed in participants with normal cognition, expression of CR1 did not correlate with DAP12 in infected participants with cognitive dysfunction. Taken together, our findings suggest that cognitive dysfunction is associated with an exaggerated proinflammatory response during acute bacterial infection with deregulation of several molecular signalling pathways in circulating exosomes and in monocytes.

1. Background

Dementia, including Alzheimer's Disease (AD), is a known risk factor for common bacterial infections which are associated with high mortality and morbidity (Foley et al., 2015). Pneumonia and urinary tract infections are the main cause of hospitalisation of dementia patients (Bernardes et al., 2018) and infections are a major precipitant of delirium in this population (Lagarto and Cerejeira, 2016). Moreover, acute systemic inflammatory events have been associated with a 2-fold increase in the rate of cognitive decline over a 6-month period (Holmes et al., 2009) and patients with AD who experienced an infection and/or

delirium have accelerated cognitive decline (Fong et al., 2009). A large amount of evidence derived from animal models has established that microglial cells in the Central Nervous System (CNS) react to the presence of peripheral immune signals (e.g. lipopolysaccharide) leading to production of proinflammatory mediators and cell proliferation through complex interactions (Cerejeira et al., 2010). This innate neuroimmune reaction coordinates a central response to combat an acute peripheral infection. Animals with advanced age (Norden and Godbout, 2013) and/or with neurodegenerative disorders show an amplified neuroinflammatory response to acute systemic inflammation (Cunningham et al., 2009). In animal models of chronic neurodegeneration a

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peripheral immune challenge has been shown to induce irreversible cell loss and progression of neurodegenerative disease (Cunningham et al., 2005, 2009). Reversibly, the peripheral innate immune response is influenced by the CNS. The efferent limb of the vagus nerve (Huston and Tracey, 2011) and the activity of peripheral cholinesterases, provide compensatory output to modulate the proinflammatory response to acute inflammatory challenges (Vaknine and Soreq, 2020). Exosomes are a subtype of extracellular vesicles which have been recognized as paracrine messengers released by various cell types of the innate immune system to propagate inflammatory signals during infections and to restore homeostasis (Yates et al., 2022). Notably, micro-RNAs (miRs) delivered by circulating exosomes are able to regulate post-transcriptional gene expression (Correia et al., 2017). Particularly, miR-146 and miR-155 have been both implicated in the activation of a robust host immune response, with anti-inflammatory and pro-inflammatory actions, respectively (Nejad et al., 2018). Down-regulation of miRNA-145 has been associated with an increased inflammatory response to bacterial infection (Fu et al., 2020). Exosomes enriched with miR are also released by activated microglia and are able to cross the brain-blood barrier regulating the immune response at the periphery. Reversely, miRNAs affect microglia phenotype and influence the neuroinflammatory response to peripheral immune challenges (Brites, 2020).

We are far from understanding how exposure to acute infections in the periphery can elicit acute/chronic cognitive dysfunction as in most cases there is no direct involvement of the brain. Also, it is unclear whether the innate immune response to acute infection is altered in patients with delirium and/or dementia. In addition to the classical signalling molecules (cytokines, chemokines) which act through receptor-ligand interactions, the intense trafficking o exosomes between inflammatory cells and different cell types during acute infections exert immunomodulatory effects through cell-to-cell delivery of pathogen and host-derived molecules. The current study aims to determine if dementia and/or delirium are associated with an altered systemic inflammatory response to an acute bacterial infection. Accordingly, we characterised three different components of the inflammatory response: 1) the abundance and content of extracellular vesicles released to the bloodstream; 2) the inflammatory status of circulatory innate immune cells, in particular monocytes, and 3) the circulatory levels of inflammatory cytokines and chemokines.

2. Materials and methods

2.1. Recruitment of participants

All individuals aged over 65 years old with unplanned acute admission to Internal Medicine wards between January 2018 and January 2020 with acute bacterial infection were eligible for inclusion in the study. Acute bacterial infection was defined as an infection not directly involving the CNS with C-reactive protein plasma levels superior to 1 mcg/mL and requiring treatment with antibiotics. Participants were excluded if they had less than 48h of hospitalisation, were not able to undergo neuropsychological assessment or had a current episode of trauma, a history of chronic inflammatory diseases, chronic use of anti-inflammatory drugs, infection of the CNS, having suffered a head trauma that resulted in a loss of consciousness or other neurological diseases, drug or alcohol addiction as well as prior chronic exposure to neurotoxic substances. A control group with dementia but with no current infection was recruited from older patients in the outpatient clinic of old age psychiatry (previously diagnosed with dementia) and able to undergo cognitive assessment with MoCA and/or MMSE. A written informed consent was obtained for each participant and/or their legal representatives. This study was submitted and approved by the Ethical Committee of Centro Hospitalar Universitário de Coimbra (Ethics approval Ref. CHUC-065-18).

2.2. Clinical assessment

2.2.1. Participants hospitalised with an acute bacterial infection

a) Diagnosis of dementia and delirium

All participants were assessed within 72 h of admission by a psychiatrist and were first screened with the Richmond Agitation and Sedation Scale (RASS) (Sessler et al., 2002) to assess the level of consciousness. Participants with RASS > -3 were assessed with the Confusion Assessment Method (CAM) (Inouye, 2003) during a formal cognitive test with the validated Portuguese version of Montreal Cognitive Assessment (MoCA) (Freitas et al., 2012). The Portuguese version of Mini Mental State Examination (MMSE) (Freitas et al., 2015) was used for illiterate participants or those with no formal education. Participants with RASS ≤ -3 were reassessed daily until they were able to undergo cognitive assessment. If they remained sedated throughout hospitalisation they were excluded from further analysis.

A diagnosis of prior dementia was obtained following a structured clinical assessment based on DSM-IV-TR criteria (APA, 2000). Information on premorbid function was also gathered from relatives (if available) or formal/informal caregivers based on the Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE-SF) (Jorm, 1994) and review of clinical records. Dementia-related information included family history of dementia and duration of dementia symptoms before diagnosis.

Participants were assessed daily for the development of new episodes of delirium, based on all sources of information available, using RASS and CAM. The classification of participants as having delirium was made if they developed at least one episode of delirium (irrespective of its severity) during hospitalisation. Participants with acute bacterial infections were classified based on their cognitive status during hospitalisation: a) normal cognition (Group 1); b) delirium (Group 2); c) dementia (Group 3); delirium superimposed on dementia (Group 4). Although delirium and dementia are classified as distinct and mutually exclusive conditions in DSM-5, there is a close relation between these two syndromes with significant clinical overlap and common pathophysiological mechanisms, including cholinergic deficiency, inflammation, and reduced cerebral oxidative metabolism ultimately resulting in brain dysfunction (Fong et al., 2015). Therefore, we opted to include an additional classification joining participants with delirium, dementia and delirium superimposed on dementia in a group with "cognitive dysfunction".

2.2.1.1. Demographic and medical data. We gathered from clinical records information about: demographic data (age, gender, education, place of residence); current medication list, smoking habits, alcohol consumption and previous psychiatric or neurologic diseases; severity of chronic comorbidities (Charlson Co-Morbidity scale; Charlson et al., 1987), type of acute medical illness classified according to ICD-10 (World Health Organization, 1993); functionality status measured with Barthel Index (BI; Mahoney and Barthel, 1965); length of hospital stay and mortality at 12 months; number and severity of neuropsychiatric symptoms prior to hospital admission, using the Neuropsychiatric Inventory (NPI; Cummings et al., 1994).

2.2.2. Participants with dementia and no acute bacterial infection

Participants with a prior diagnosis of dementia (based on DSM-IV-TR criteria) were assessed with MoCA and/or MMSE for cognitive function. Absence of a current acute bacterial infection was confirmed with an unremarkable history of acute symptoms during the previous 2 weeks associated with negative findings in physical examination and blood markers of infection (hemogram and C-reactive protein). Clinical and demographic data were collected for each participant.

2.3. Blood samples collection, processing and analysis

Venous blood samples were drawn from each participant with an acute bacterial infection within 2 days following inclusion in the study. A total of 30 mL of blood were collected from each participant and divided between one 10 ml gel-barrier serum separator tube and two 10 mL sterile EDTA-coated tubes.

2.3.1. Levels of IL-6, IL-1β, TNF-α and CCL2 in serum samples

Quantification of IL-6, IL-1β, TNF-α and CCL2s in serum samples was performed by ELISA, employing kits from R&D Systems (D6050, DLB50, DTA00D and DCP00, respectively). Highly purified recombinant proteins were used to generate six-point standard curves (concentration range between 100 pg/ml and 3.13 pg/ml for IL-6; between 125 pg/ml and 3.9 pg/ml for IL-1β; between 1000 pg/ml and 15.6 pg/ml for TNF-α and between 1000 pg/ml and 31.3 pg/ml for CCL2). When specified in the manufacturer's protocol, serum samples were diluted 2-fold with the appropriate Calibrator Diluent. For each ELISA, 200 µl of sample or calibrator were loaded in each well of the provided 96-well plate. The optical density in each well was determined at 450 nm, using a SpectraMax Plus spectrophotometer (Molecular Devices), with wavelength correction set at 570 nm. A standard curve was generated for each protein using a four-parameter logistics (4-PL) curve-fit and, for each sample, the obtained concentrations of each cytokine/chemokine were multiplied by the appropriate dilution factor. Results are presented in pg/ml of serum.

2.3.2. Isolation of serum and CD14+ blood monocytes

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood by density gradient centrifugation using Histopaque® (Sigma, USA). Monocyte isolation was performed using magnetic separation, employing CD14 MicroBeads (Miltenyi Biotec, Germany). The purity of the CD14+ monocytes was determined by flow cytometry, using a monoclonal anti-CD14− FITC antibody (Sigma, USA), and was shown to be more than 96%.

2.3.3. Isolation of and characterization of extracellular vesicles (EVs) and exosomes

Extracellular vesicles (EV) present in serum samples for the participants with an infection were isolated by precipitation using the Total Exosome Isolation Reagent from Serum (Invitrogen #4478360). A sample of 10 µl was immediately used for EV characterization and the remaining sample was frozen at −80 °C for miRNA and protein extraction. The mean size and relative % of the different EV populations in each sample, including those with sizes compatible with exosomes (30–120 nm) were determined using dynamic light scattering (DLS), employing a N5 Submicron Particle Size Analyzer (Beckam-Coulter). Exosome abundance (number of CD63 positive exosome particles/ml) in each sample was quantified using the ExoELISA-ULTRA CD63 kit (System Biosciences), according to the manufacturer's instructions.

2.3.4. Total RNA isolation from CD14+ monocytes and exosomes with miRNA and mRNA quantification by qRT-PCR

Total RNA from 2 million CD14+ monocytes was extracted using the Quick-RNA MiniPrep Kit (Zymo Research, #339340), while miRNA extraction from 100 µl of isolated exosomes was performed employing the Total Exosome RNA & Protein Isolation Kit (Invitrogen, #4478545), according to the manufacturer's recommendations. RNA elution was performed in 35 µl of RNAse free water for all samples, followed by RNA quantification using NanoDrop. All RNA samples were immediately transcribed or stored at −80 °C until further processing.

2.3.5. miRNA levels in CD14+ monocytes and exosomes

In order to screen for the levels of miR-155, miR-146a and miR-145 in both exosomes and monocytes, 20 ng of total RNA from each hospitalised participant with an infection were transcribed using the MIRCURY LNA RT Kit (Qiagen), according to the following protocol: 60 min at 42°, followed by heat-inactivation of the reverse transcriptase for 5 min at 95 °C. The resulting cDNA was diluted 40 × with RNAse free water before quantification by qRT-PCR. qRT-PCR was performed in the Real-Time PCR System Step-One plus (Applied Biosystems), using 96-well microtiter plates. For each reaction, 6 µl of the master mix, containing MIRCURY LNA SYBR Green, ROX dye and primers (pre-designed LNA primers by Qiagen: miR-155–5p #YP00204308; miR-145–5p #YP00204483; mir-146a-5p #YP00204688; miR-23a-3p #YP00204772), was combined with 4 µl of cDNA template. All reactions were performed in duplicate, at a final volume of 10 µl/well. The reaction conditions consisted of polymerase activation/denaturation and well-factor determination at 95 °C for 30s, followed by 40 amplification cycles of 5s at 95 °C and 5s at 65 °C (ram-rate of 1.6 °C/s). miR-23a was used as a reference gene.

2.3.6. Differential gene expression analysis

The expression of specific genes was also assessed by qRT-PCR for the groups with infection. In this case, 250 ng of total RNA were transcribed using the NZY First-Strand cDNA Synthesis Kit (Nzytech, Portugal) by applying the following protocol: 10 min at 25 °C, 30 min at 42 °C and 5 min at 85 °C. The obtained cDNA was diluted 20x using RNAse free water. The relative expression of CRA1, CCR2, TNF-α, IL-6, IL10, PICALM and DAP12 mRNA was quantified using the NZY qPCR Green Master Mix with ROX (Nzytech, Portugal). Primers for quantification were pre-designed by Qiagen. For each primer set, both primers were resuspended from a 100 µM concentration to a 10 µM concentration by adding 10 µL of the forward and 10 µL of the reverse primer to 80 µL of RNAse-free water. A master mix was prepared containing SYBR Green master mix and an appropriate volume of primer stock solution to yield a final primer concentration of 1 µM. For each reaction, 6 µl of this master mix was added to 4 µl of template cDNA. All reactions were performed in duplicate, at a final volume of 10 µl/well. The reaction conditions consisted of polymerase activation/denaturation and well-factor determination at 95 °C for 2 min, followed by 40 amplification cycles of 5s at 58 °C (primer annealing) and 30s at 72 °C (amplification). HPRT was used as a reference gene. For both miRNA and mRNA quantification, two negative controls (no reverse transcription – NRT and no target control – NTC) were run for each gene/miRNA and a melting curve protocol was initiated immediately after amplification and consisted of 1 min heating at 55 °C (mRNA) or 65 °C (miRNA), followed by 80 steps of 10s, with a 0.5 °C increase in each step. Threshold values for threshold cycle determination (Ct) were generated using the StepOne TM Software v2.3. The miRNA fold increase or fold decrease, with respect to control samples, was determined using the Pfaffl method, taking into consideration the different amplification efficiencies of the different genes and miRNAs. The amplification efficiency of each target or reference RNA was determined according to the formula: E = 10(−1/S)-1, where S is the slope of the obtained standard curve.

2.4. Statistical analysis

Results are reported as mean and standard deviation (SD) if a normal distribution was found using the Kolmogorov-Smirnov test. Nonparametric data is expressed in median and interquartile range (IQR). Baseline characteristics were tested between groups using Kruskal-Wallis test and pairwise comparisons were subsequently assessed with Bonferroni correction for multiple comparisons for continuous variables. Chi square test was used to assess differences between groups in categorical data. Correlations between peripheral inflammatory parameters were assessed with Spearman's test. The analyses were performed using IBM Corp. IBM SPSS Statistics for Macintosh, Version 22.0. (Armonk, NY: IBM Corp.). Figures were made in GraphPad Prism version 5.03.

3. Results

3.1. Sample characteristics

We recruited a total of 46 hospitalised participants, aged 83.6 (SD = 7.0), who were mostly females (60.9%) with acute respiratory infections (72.4%). From these, 29 participants had cognitive dysfunction (dementia and/or delirium) and 17 had normal cognition (Table 1). We also included a control group of 11 participants with dementia but with no current infection matched for age and educational status who had a higher cognitive performance (assessed by MoCA) than all infected groups with cognitive dysfunction (p < 0.001) and lower than the infected group with normal cognition (p < 0.001). Baseline dementia severity (assessed by IQCODE) was similar between infected groups with dementia and with delirium superimposed on dementia (p = 0.25) as well as between infected and non-infected dementia groups (p = 0.25). Participants with dementia and no infection had lower levels of functionality (assessed by Barthel Index) than participants with normal cognition and those with delirium but higher than infected participants with dementia (p < 0.001). Levels of C-reactive protein were similar between the infected groups and higher than the non-infected dementia group (p < 0.001).

3.2. Cytokine and chemokine levels in serum samples

For each group the levels of cytokines and chemokines in serum were determined in a subset of participants. We observed that participants with acute bacterial infection had higher levels of IL-6, TNF-alpha and IL-1beta than non-infected participants (Group 5). No differences were observed between groups with infection (Table 2). When considering participants with infection and cognitive dysfunction (Groups 2, 3 and 4) higher levels of IL-6 were observed compared with those with normal cognition (Fig. 1).

Serum levels of IL-6 correlated with TNF-alpha (r = 0.514, p = 0.007) and MCP-1/CCL2 (r = 0.607, p = 0.001) in the group of participants with acute bacterial infection and cognitive dysfunction (but not in those with normal cognition).

3.3. Circulating exosomes

In the groups of participants with acute bacterial infection we observed no differences in the mean size of the total extracellular vesicle population, in the percentage of extracellular vesicles with size compatible with exosome (30–120 nm) or in the mean size of the exosome-like population. Dynamic light scattering analysis revealed that more than 80% of the recovered extracellular vesicles presented a size compatible with exosomes.

Table 1

Characteristics of the sample according to the presence of acute bacterial infection and cognitive status.

	Group 1 (n = 17)	Group 2 (N = 6)	Group 3 (N = 12)	Group 4 (N = 11)	Group 5 (n = 11)	Test statistic p value
Age	82.7 (8.8)	82.0 (5.86)	84.2 (5.93)	85.5 (5.50)	79.3 (6.8)	6.25 0.181
Female gender (n, %)	9 (52.9)	4 (66.7)	10 (66.7)	7 (63.6)	6 (54.5)	7.32 0.539
Years of Formal education (%)						
No formal education	3 (17.6)	1 (16.7)	1 (8.3)	2 (18.2)	2 (18.2)	58.42
<5 years	10 (58.8)	5 (83.3)	11 (91.7)	9 (81.8)	7 (63.6)	0.089
≥ 5 years	4 (23.4)	0 (0)	0 (0)	0 (0)	2 (18.2)	
Nursing home care before hospitalisation (n, %)	2 (11.8)	1 (16.7)	5 (41.7)	1 (9.1)	0 (0)	11.22 0.286
Barthel Index before hospitalisation (M%/SD)	82.85 (19.18)	75.0 (33.1)	47.5 (31.4)	33.6 (30.7)	64.55 (29.45)	**15.27** **0.002**
MoCA	18.86 (3.43)	7.8 (4.1)	8.7 (3.4)	4.4 (1.9)	13.9 (4.7)	**39.67** **<.001**
IQCODE (M/SD)	3.00 (0.5)	2.93 (0.41)	3.81 (0.76)	4.00 (.00)	4.00 (.00)	**26.47** **<.001**
Type of infection						
respiratory	11 (64.7)	5 (83.3)	9 (75.0)	6 (54.5)	NA	
urinary	4 (23.5)	0 (0)	1 (8.3)	5 (45.5)	NA	6.45
other	2 (11.8)	1 (16.7)	2 (16.7)	0 (0)	NA	0.849
Pain score	3.5 (2.8)	3.0 (2.0)	2.13 (1.8)	3.12 (2.4)	NA	2.34 0.506
Length of stay (LOS)	7.7 (3.8)	10.8 (4.2)	9.53 (6.3)	11.0 (8.2)	NA	2.66 0.447
Mortality at 18 months (N/%)	1 (6.3)	3 (50)	4 (33.3)	5 (45.5)	1 (9.0)	6.18 0.107
Leucocytes	10.4 (4.5)	10.8 (7.6)	10.18 (4.4)	8.79 (4.19)	7.51 (1.82)	1.92 0.589
Neutrophiles	5.2 (5.3)	6.22 (3.48)	5.68 (4.38)	4.78 (3.44)	4.84 (1.31)	1.84 0.607
Lymphocytes	1.4 (0.86)	1.28 (1.11)	1.12 (0.92)	1.23 (0.92)	1.9 (0.61)	0.514 0.916
Monocytes	0.59 (0.37)	0.44 (0.37)	0.41 (0.29)	0.51 (0.34)	0.55 (0.17)	1.54 0.673
C-reactive protein	10.0 (9.7)	4.12 (2.45)	7.08 (5.64)	6.96 (4.85)	0.22 (0.18)	**23.72** **0.001**

Group 1: Acute bacterial infection + normal cognition.
Group 2: Acute bacterial infection + Delirium.
Group 3: Acute bacterial infection + Dementia.
Group 4: Acute bacterial infection + Delirium Superimposed on Dementia.
Group 5: No infection + Dementia.
Categorical variables are presented as number and percentages: Continuous variables are expressed as mean and standard deviation. Chi-square statistics were calculated for categorical variables, and Kruskal-Wallis tests and pairwise comparisons with Bonferroni Correction were performed for continuous variables.

Table 2
Serum levels of cytokines and chemokines.

	Group 1 (n = 17)	Group 2 (N = 6)	Group 3 (N = 12)	Group 4 (N = 11)	Group 5 (N = 11)	Test statistic[a] p value
IL-6	6.52 (3.54)	24.49 (15.2)	14.89 (13.4)	18.99 (13.9)	3.50 (1.07)	**19.97** **0.002**
TNF-alpha	1.60 (0.41)	2.03 (0.48)	1.97 (0.83)	1.91 (0.42)	1.52 (0.23)	**12.15** **0.016**
IL-1beta	1.13 (0.15)	1.22 (0.79)	1.27 (0.45)	1.34 (0.36)	0.92 (0.54)	**13.23** **0.020**
MCP-1/CCL2	139.10 (91.35)	363.1 (287.5)	188.06 (79.9)	206.6 (103.1)	155.27 (32.84)	8.08 0.062

Group 1: Acute bacterial infection + normal cognition.
Group 2: Acute bacterial infection + Delirium.
Group 3: Acute bacterial infection + Dementia.
Group 4: Acute bacterial infection + Delirium Superimposed on Dementia.
Group 5: No infection + Dementia.
Values are expressed as mean and standard deviation.
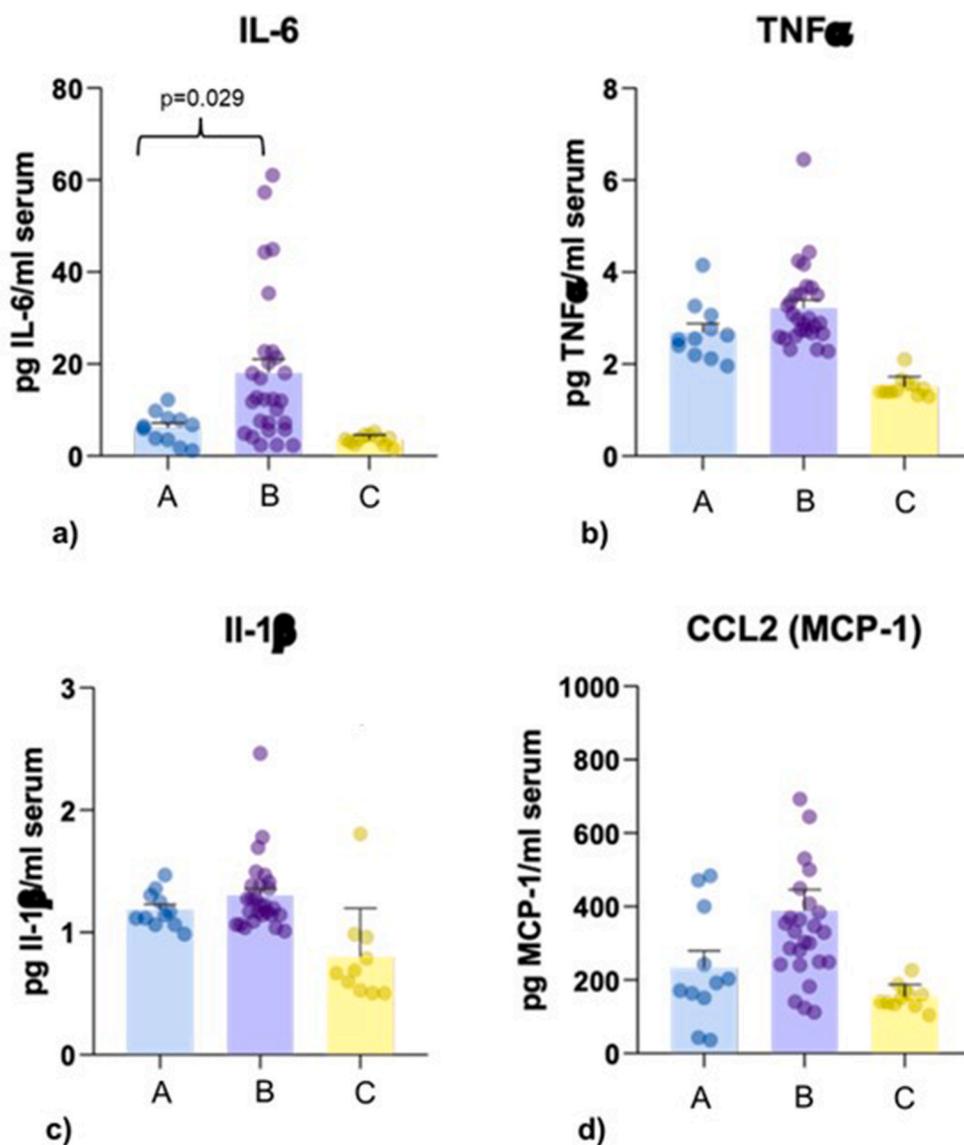 [a] Kruskal-Wallis test.

Fig. 1. Levels of IL-6 (a), TNF-alpha (b), IL-1 beta (c) and CCLR/MCP-1 (d) in serum samples
A- Acute bacterial infection + normal cognition
B- Acute bacterial infection + cognitive dysfunction (delirium and/or dementia)
C- Dementia (no infection).

3.4. miR expression in circulating exosomes

In hospitalised participants with acute bacterial infection we found a decreased exosome expression of miR-145 in those with delirium or dementia (p = 0.034) (Table 3) and a correlation between expression of miR-145 and miR-155 (r = −0.411, p = 0.027).

Table 3

Expression of miRNA in circulating exosomes of participants with acute bacterial infection.

	Group 1 N = 17	Group 2 N = 6	Group 3 N = 12	Group 4 N = 10	Test statistic[a] p value
miR-145	1.65 (1.6)	0.84 (1.27)[b]	0.65 (0.7)[b]	0.93 (0.94)	**8.66** **0.034**
miR-146a	0.99 (2.01)	1.64 (0.74)	1.49 (1.53)	0.59 (2.2)	2.44 0.486
miR-155	0.69 (9.59)	0.21 (5.7)	0.57 (3.14)	0.59 (2.2)	2.19 0.532

Group 1: Acute bacterial infection + normal cognition.
Group 2: Acute bacterial infection + Delirium.
Group 3: Acute bacterial infection + Dementia.
Group 4: Acute bacterial infection + Delirium Superimposed on Dementia.
The results are presented as miRNA fold increase or decrease, with respect to control samples (determined using the Pfaff method). Values are presented with median (interquartile range).
[a] Kruskal-Wallis test.
[b] Represents significant differences (sig<.05) relatively to Group 1.

3.5. Expression of mRNA in monocytes CD14$^+$ in participants with acute bacterial infection

When considering the 4 groups with infection no differences were observed in mRNA expression between groups (Table 4). Participants with infection and cognitive dysfunction (delirium and/or dementia) showed increased expression of CR1 mRNA in CD14$^+$ monocytes (m = 2.04, IQR = 3.35) compared with participants with infection and normal cognition (m = 1.09, IQR = 1.75) (p = 0.049). No differences between the two groups were found for the remaining mRNAs (Fig. 2).

Expression of TNF-a and IL-6 genes correlated in participants with normal cognition and with cognitive dysfunction. In participants with cognitive dysfunction the expression of PICALM correlated with TNF- and IL-6 (Table 5, upper right) whereas in participants with normal cognition there was a correlation between CR1 and DAP12 expression (Table 5, lower left).

3.6. Expression of miR in CD14$^+$ monocytes in participants with acute bacterial infection

No differences between groups were found regarding the expression of miR-145, miR-146a and miR-155 in CD14$^+$ monocytes.

Table 4

Expression of mRNA in monocytes CD14$^+$ in participants with acute bacterial infection.

	Group 1 N = 12	Group 2 N = 5	Group 3 N = 10	Group 4 N = 10	Test statistic[a] p value
TNF-alpha	0.72 (4.81)	1.24 (6.66)	1.57 (1.69)	1.24 (2.66)	1.45 0.69
IL-6	0.53 (2.98)	0.44 (5.87)	0.32 (3.48)	0.26 (2.59)	0.556 0.91
CCR2	0.72 (4.54)	2.01 (1.52)	0.37 (3.34)	0.56 (4.61)	3.67 0.30
CR1	1.09 (1.75)	3.41 (5.05)	2.25 (4.15)	1.31 (1.55)	6.07 0.09
IL-10	0.48 (2.44)	1.12 (1.37)	0.36 (0.90)	0.97 (6.88)	3.66 0.30
DAP-12	1.03 (1.816)	1.77 (3.59)	1.30 (1.37)	1.63 (1.49)	1.30 0.73
PICALM	0.68 (0.89)	1.61 (1.58)	0.95 (1.28)	0.66 (0.89)	3.14 0.37

Group 1: Acute bacterial infection + normal cognition.
Group 2: Acute bacterial infection + Delirium.
Group 3: Acute bacterial infection + Dementia.
Group 4: Acute bacterial infection + Delirium Superimposed on Dementia.
Values are presented with median (interquartile range).
[a] Kruskal-Wallis test.

4. Discussion

It is well established that during infection or injury the CNS and the peripheral immune system maintain a dynamic cross-talk to tightly coordinate the innate immune response. Growing evidence suggests that acute systemic inflammation translates into acute neuropsychiatric symptoms and acceleration of ongoing neurodegeneration. Whether dysfunctional neural circuits associated with cognitive impairment could alter systemic innate immune responses remains unclear. In this study we evaluated the systemic inflammatory response to an acute bacterial infection in participants with and without cognitive dysfunction (delirium and/or dementia).

In the current study infected participants with cognitive dysfunction had higher serum levels of IL-6 compared to cognitively normal controls indicating a stronger M1 inflammatory response mediated by CD14$^+$ monocytes. Our findings are in line with previous evidence showing elevated levels of plasma C-reactive protein and higher ratio of proinflammatory to anti-inflammatory cytokines in participants with acute cognitive dysfunction (Macdonald et al., 2007; Burkhart et al., 2010; Cerejeira et al., 2012). We also found that the expression of miR-145 was downregulated in circulating exosomes obtained from participants with cognitive dysfunction. Serum levels of IL-6 correlated with TNF-alpha (r = 0.514, p = 0.007) and MCP-1/CCL2 (r = 0.607, p = 0.001) suggesting a more robust interplay between these mediators during the early phase of the innate response to infection. Among the circulating miRs, miR-155 is a promoter of inflammatory responses following toll-like receptor activation, while miR-146a is a mediator of immune suppression (Alexander et al., 2015). Previous studies reported that the expression of miR-145 reduces cell viability and the inflammatory reaction in macrophages infected with Mycobacterium tuberculosis (Fu et al., 2020) while, in animal models, inhibition of miR-145 induced macrophage infiltration in the liver and mononuclear cells proliferation in bone marrow (He et al., 2020). In participants who developed postoperative delirium miR-146a was up-regulated in the cerebro-spinal fluid and down-regulated in the serum (Dong et al., 2017). The anti-inflammatory role of miR-145 is likely to be mediated by several protein targets including the Toll-interleukin-1 receptor domain-containing adaptor protein and KLF5, thereby inhibiting TLR4 and NF-kB signalling pathways respectively (Nejad et al., 2018; He et al., 2020). Therefore, a stronger proinflammatory response to bacterial infection observed in the group of patients with cognitive dysfunction might be a consequence of a downregulation of miR-145 in circulating exosomes which negatively correlated with miR-155 levels (r = −0.411, p = 0.027).

Monocytes/macrophages are the first barrier against infection and are a key player in the recognition of bacteria, recruitment of immune cells to the local infected tissue, elimination of pathogens, and conversion to suppressive cells thus restoration of tissue homeostasis (Zhang and Wang, 2014). We observed that expression of CR1 in circulating CD14$^+$ monocytes was higher in infected participants with cognitive dysfunction and, in these participants, PICALM correlated both with TNF-alpha and IL-6. Complement C3b/C4b receptor 1 (CR1) regulates complement activation and acts as a promoter of phagocytosis in macrophages while the phosphatidylinositol binding clathrin assembly (PICALM) protein plays a key role in endocytosis, thereby influencing the expression of receptors and synaptic transmission. Recent evidence shows that CR-mediated phagocytosis increases the levels of TNF-a, IL-1b, IL-6, and MMP-9, compared to FcgR-mediated phagocytosis upon calpain-mediated activation of NFkB (Acharya et al., 2020). In contrast to what was observed in participants with normal cognition, expression of CR1 did not correlate with DAP12 in infected participants with cognitive dysfunction. Activation of TREM-2/DAP12 signalling exerts an anti-inflammatory function in immune cells with the reduction of pro-inflammatory cytokines. The current study suggests that during acute infection activation of TREM-2/DAP12 signalling is less robust in circulating monocytes of participants with cognitive dysfunction.
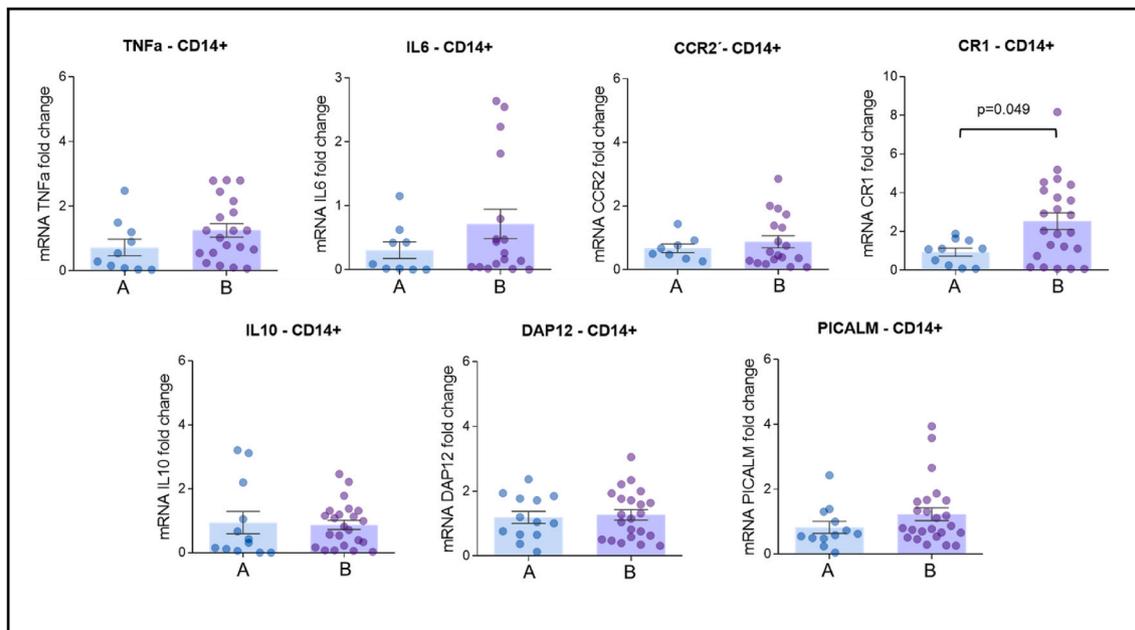
Fig. 2. Expression of mRNA in monocytes CD14$^+$ in participants with acute bacterial infection
A- Acute bacterial infection + normal cognition
B- Acute bacterial infection + cognitive dysfunction (delirium and/or dementia)
The results are presented as mRNA fold increase or decrease, with respect to control samples (determined using the Pfaff method). Values are presented with median (interquartile range).

Table 5
Correlations between gene expression in CD14$^+$ monocytes in participants with acute bacterial infection.

	TNFa	IL-6	IL-10	DAP12	CCR2	CR1	PICALM
TNF-a	–	**0.642**	0.009	0.048	−0.249	0.247	**0.617**
		0.001	0.965	0.821	0.230	0.234	**0.001**
IL-6	**0.580**	–	0.029	−0.121	−0.285	0.089	**0.760**
	0.048		0.894	0.573	0.178	0.680	**<0.001**
IL-10	−0.032	0.270	–	0.019	0.059	0.359	−0.026
	0.922	0.396		0.927	0.779	0.078	0.901
DAP12	0.392	0.147	0.366	–	−0.056	−0.014	0.047
	0.207	0.648	0.243		0.790	0.948	0.824
CCR2	0.018	0.130	0.411	0.172	–	−0.058	−0.133
	0.957	0.688	0.184	0.593		0.784	0.526
CR1	0.385	−0.077	0.148	**0.691**	−0.298	–	0.279
	0.216	0.812	0.647	**0.013**	0.346		0.176
PICALM	0.196	0.350	0.407	0.207	0.256	0.438	–
	0.542	0.265	0.187	0.519	0.422	0.155	

Lower left: correlations in Group A (acute bacterial infection + normal cognition).
Upper right: correlations in Group B (acute bacterial infection + delirium and/or dementia).
Each cell presents the Spearman correlation coefficient and the significance (p-value).

The strength of this study was the ability to assess participants during the exposure to an acute bacterial infection with a close monitoring of their mental state and measurement of several cellular and molecular markers of the inflammatory response at the periphery. This study also has important limitations. Firstly, the recruitment of participants was restricted to those able to be cognitively assessed and having a stable medical condition and this resulted in a small sample. The reduced number of participants with cognitive dysfunction affected the robustness of comparison across the different groups (i.e. delirium, dementia and delirium superimposed on dementia) and the characterisation of dementia subtype. Secondly, while the inflammatory response is a dynamic process, the biomarkers of inflammation were measured only at one time-point demanding a cautious interpretation of the results. Another limitation of our study is that some circulating miRs measured from peripheral blood have several origins, including the CNS, being difficult to disentangle the specific contribution of the peripheral immune system and brain-to-immune pathways.

Despite these limitations, our findings suggest that cognitive dysfunction is associated with an exaggerated proinflammatory response to acute bacterial infection with deregulation of several molecular signalling pathways in circulating exosomes and in monocytes. Our study was underpowered to clarify if these changes in the inflammatory response were specific to delirium or dementia. Nevertheless, delirium and dementia have significant clinical and pathophysiological overlap and are ultimately characterised by brain dysfunction. Therefore, it is plausible that both conditions are associated with an altered CNS response to peripheral infection, including the activation of descending pathways to influence the immune response at the periphery and restore homeostasis. In the context of ageing and chronic neurodegeneration adaptive changes to acute insults are characterised by exaggerated production of pro-inflammatory cytokines by primed microglia coupled with dysfunction of brain-to-immune pathways

(Cerejeira et al., 2014). Dysregulation of bacterial and viral response genes has been reported in participants with AD and vascular dementia (Guo et al., 2019) and a series of polymorphisms in inflammation-related genes have been shown to be associated with AD including CR1 and PICALM (Sims et al., 2020). Moreover, in AD participants, circulating CD14$^+$ monocytes showed decreased chemotaxis in response to MCP-1 and A-beta phagocytosis, suggesting a functional impairment of circulating immune cells in these participants (Guedes et al., 2015). Importantly, miR-155 induces an acute pro-inflammatory M1 state in primary microglia under LPS stimulation whereas miR-145 mediates anti-inflammatory pathways and M2-like activation phenotype of microglia (Freilich et al., 2013). Thus, underexpression of miR-145 in participants with cognitive dysfunction might be associated with a shift of macrophage and microglial polarisation to an M1 phenotype characterised by an excessive production of proinflammatory cytokines. Altered levels of CR1 and PICALM genes in autopsy brain tissue have been associated with cognitive decline and AD (Baig et al., 2010; Chibnik et al., 2011). Within the CNS TREM-2/DAP12 are expressed in microglia inhibiting the production of pro-inflammatory cytokines following a LPS challenge (Zhong et al., 2017). Importantly, mutations in TREM2 (Guerreiro et al., 2013; Jonsson et al., 2013) and DAP12 (Pottier et al., 2013) have been associated with increased risk of AD.

It is clinically relevant to predict the risk of delirium and cognitive decline in subjects with dementia exposed to common bacterial infections. In future studies, analysis of genetic polymorphisms in inflammation-related genes which have been shown to be associated with AD can provide new insights into the origins of the variability in the immune response during a peripheral bacterial infection. Ultimately, if the degree of neuroinflammation during acute systemic infection irreversibly impacts neuronal and cognitive function at long term, this finding would hold substantial clinical implications for prevention and management of dementia. Importantly, miRs are stable in body fluids (e. g. blood plasma, serum, saliva) offering promising perspectives to be used as peripheral biomarkers.

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Declaration of competing interest

None.

Data availability

Data will be made available on request.

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