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Systemic infection exacerbates cerebrovascular dysfunction in Alzheimer's disease

Short running title: Systemic infection, cerebrovascular dysfunction and dementia

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Abstract

We studied the effects of systemic infection on brain cytokine level and cerebral vascular function in Alzheimer's disease (AD) and vascular dementia (VaD), in superior temporal cortex (BA22) from AD (n = 75), VaD (n = 22) and age-matched controls (n = 46), stratified according to the presence or absence of terminal systemic infection. Brain cytokine levels were measured using Mesoscale Discovery Multiplex Assays and markers of cerebrovascular function were assessed by ELISA. Multiple brain cytokines were elevated in AD and VaD: interleukin (IL)-15 and IL-17A were maximally elevated in end-stage Alzheimer's disease (Braak tangle stage V-VI) whereas IL-2, IL-5, IL12p40 and IL-16 were highest in intermediate Braak tangle stage III-IV disease. Several cytokines (IL-1β, IL-6, TNF-α, IL-8 and IL-15) were further raised in AD with systemic infection. Cerebral hypoperfusion, indicated by decreased myelin-associated glycoprotein:proteolipid protein-1 (MAG:PLP1) and increased vascular endothelial growth factor-A (VEGF), and blood-brain barrier leakiness, indicated by raised levels of fibrinogen, were exacerbated in AD and VaD, and also in non-dementia controls, with systemic infection. Aβ42 level did not vary with infection or in association with brain cytokine levels. In controls, cortical perfusion declined with increasing interferon-γ (IFN-γ), IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-13 and tumour necrosis factor-α (TNF-α) but these relationships were lost with progression of AD, and with infection (even in BS 0-II brains). Cortical platelet-derived growth factor receptor-β (PDGFRβ), a pericyte marker, was reduced, and endothelin-1 (EDN1) level was increased in AD; these were related to Aβ level and disease progression and only modestly affected by systemic infection. Our findings indicate that systemic infection alters brain cytokine levels and exacerbates cerebral hypoperfusion and BBB leakiness associated with AD and VaD, independently of the level of insoluble Aβ. Our findings highlight systemic infection as an important contributor to dementia, requiring early identification and treatment in the elderly

population.

Keywords: Alzheimer's disease; systemic infection; neuroinflammation; cerebral hypoperfusion; blood-brain barrier.

Introduction

Systemic infection may be associated with delirium and cognitive decline [1, 2], and cognitive impairment is commonly observed in survivors of sepsis [3]. Systemic infection is a risk factor for progression of Alzheimer's disease (AD) [4, 5] and systemic infection and cognitive decline in AD are associated with raised serum IL-1β [6] and TNF-α [7]. Modelling of acute systemic infection in rodents induces microglial activation and elevated pro-inflammatory cytokine production (IL-1β, IL6 and TNF-α), and exacerbates cognitive decline, neurodegeneration, and AD-like (Aβ and tau) pathology in mouse models [8-11]. Post-mortem brain studies indicate that terminal systemic infection, recorded as the primary cause of death, is associated with activation of endothelial cells, perivascular macrophages and microglia [12-14], and we recently reported that the neuroinflammatory response to terminal systemic infection is modified in end-stage AD [15].

Cerebrovascular dysfunction has been highlighted as a major contributor to cognitive decline and disease progression in AD (reviewed [16, 17]). Most AD patients have post-mortem evidence of vascular disease [18], and clinical imaging and cerebrospinal fluid (CSF) biomarker studies have demonstrated blood-brain barrier (BBB) breakdown [19, 20] and reduced cerebral blood flow up to 10-20 years before the onset of clinical symptoms [21]. Disease modelling suggests that vascular dysfunction begins very early in the genesis of AD, around the time of initial Aβ accumulation [22]. CSF changes in markers of pericyte injury and BBB breakdown were reported to predict cognitive decline in patients with mild cognitive impairment (MCI) independently of changes in Aβ and tau [20, 23].

We previously demonstrated that biochemical changes associated with subacute and acute reduction in oxygenation of the cerebral cortex can be detected in post-mortem brain tissue in AD [24, 25]. These comprise a reduction in the level of myelin-associated glycoprotein (MAG) relative to proteolipid protein-1 (PLP1), two myelin proteins with similar long *in-vivo* half-lives (several months) and post-mortem stability but with differential sensitivity to tissue hypoxia [24-28], and an increase in vascular endothelial growth factor-A (VEGF), an hypoxia-inducible factor-1α (HIF-1α) [29]. The extent of reduction in MAG:PLP1 ratio and elevation of VEGF correlate with (i) Aβ42 level [28], (ii) the level of fibrinogen (associated with BBB leakiness), (iii) the decline in platelet-derived growth factor receptor-β (PDGFRβ) (reflecting loss of pericytes within the brain in AD), and (iv) the concentration of endothelin-1 (EDN1) [24], a potent vasoconstrictor peptide that we previously showed to be elevated in AD [30, 31].

Systemic infection has a range of indirect effects on the extracranial vasculature. It increases the risk of coronary artery disease (CAD) [32, 33], renal stenosis, and peripheral atherosclerosis [34, 35]. Infection upregulates proatherogenic mediators including pro-inflammatory cytokines (IL-1β, IL-6), and cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM1) and vascular adhesion molecule-1 (VCAM-1) [36, 37]. A combination of elevated cytokine levels, increased blood viscosity, endothelial activation [38, 39], smooth muscle cell proliferation, vascular remodelling and vasomotor dysfunction contribute to reduced perfusion, increased vascular permeability and increased risk of thrombosis in many tissues (reviewed [40]). Autoimmune mimicry can also contribute to remote vascular damage, e.g. in patients with periodontal disease [41].

In view of the contribution of vascular dysfunction to the development and progression of AD, the accelerated cognitive decline in AD patients with systemic infection, and the known effects of infection and inflammation on extracranial vascular function, we hypothesised that the deleterious influence of systemic infection in dementia, particularly in

AD, is at least partly mediated by exacerbated vascular dysfunction. We have used human post-mortem brain tissue to examine whether terminal systemic infection alters cytokine levels within the brain, and biochemical markers of cerebral oxygenation, BBB function and other measures of vascular integrity and function, at different stages of AD as indicated by Braak tangle stage, in comparison with the effects in non-dementia controls and in cases with vascular dementia (VaD) and mixed vascular and AD pathology. We show that systemic infection causes neuroinflammation and cerebral vascular dysfunction even in non-dementia controls, and exacerbates these processes in AD and VaD.

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Materials and Methods

Study cohort

The use of human brain tissue for this study was approved by the management committee of the South West Dementia Brain Bank (Human Tissue Authority licence number 12273) under the terms of Bristol Research Ethics Committee approval (18/SW/0029). The right cerebral hemisphere had previously been fixed in buffered formalin for three weeks and was used for pathological assessment. The left cerebral hemisphere had been sliced and frozen at −80 ℃. Most brains had been dissected within 72 h of death.

We studied seventy-five AD cases, twenty-two VaD and forty-six age-matched controls. A clinical history, that included post-mortem assessment, and information on the death certificate, was used to subdivide cases according to whether systemic infection was or was not recorded as the primary cause of death in to the following groups: controls who died without systemic infection (Ctrl-, n = 24) or with systemic infection (Ctrl+, n = 22); AD patients, who died without systemic infection (AD-, n = 33) or with systemic infection (AD+,

n = 42), and VaD patients who died without systemic infection (VaD-, n =15) or with systemic infection (VaD+, n = 7).

Established internationally accepted neuropathological criteria were used to identify AD and VaD cases. AD cases had a clinical diagnosis of AD during life and were subjected to detailed neuropathological assessment. We included cases with either intermediate or high AD neuropathological change that according to the NIA-AA guidelines [42] was a sufficient explanation for the dementia. No other significant brain pathologies such as stroke, primary or metastatic brain tumour, or traumatic lesions were present in the AD cases. Cases with VaD/mixed dementia had a clinical history of dementia, only occasional neuritic plaques, histopathological evidence of multiple infarcts/ischaemic lesions and moderate to severe atheroma and/or arteriosclerosis. In most of the cases there was no evidence of other disease likely to contribute to dementia but in addition to the occasional neuritic plaques, three of the cases had moderate tangle pathology. Control brains were from people with no history of dementia, few or absent neuritic plaques, a Braak tangle stage of III or less, and no other neuropathological abnormalities. A summary of the demographic and clinical features of the cohorts are presented in Table 1. For this study, the superior temporal gyrus (BA 22) was the brain area explored.

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Multiplex analysis of brain cytokine and inflammatory markers in post-mortem brain tissue

Brain tissue (100 mg) was homogenised in 500 µl RIPA buffer (Thermo Fisher Scientific, Loughborough, UK) supplemented with protease inhibitor cocktail (Complete mini; cat no. 04693124001) (Roche, Welwyn Garden City, UK) and phosphatase inhibitor cocktail (phosSTOP; cat no. 4906845001) (Roche) using a Precellys automated tissue processor.

Inflammatory proteins were measured on the V-Plex MSD electrochemiluminescence multi-spot assay platform (MesoScale Diagnostics, Rockville USA) using the V-Plex MSD Proinflammatory Human Protein Panel (cat. no. K15049D) and Cytokine Human Protein Panel (cat. no. K15050D), respectively. 25 µl of brain homogenate (1:2 dilution) was used for each assay according to the manufacturer's protocol, as previously described [15]. Each plate was imaged on the Meso QuickplexSQ120 (MesoScale Discovery) according to manufacturer's instructions for 384-well plates. Protein concentration was expressed in pg/ml for each analyte after adjustment for total protein level, which was measured using the Total Protein kit (Sigma Aldrich, Dorset, UK).

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Biochemical assessment of vascular markers

Fresh frozen superior temporal cortex (BA22) (200 mg) was dissected and proteins were extracted in 1 ml of 1% sodium dodecyl sulfate lysis buffer, in a Precellys automated tissue processor (Stretton Scientific, Derbyshire, UK) (Bertin Technologies, France) as previously described [24, 25, 28]. Homogenates were centrifuged at 12,460 g for 15 min at 4°C for and then aliquoted and stored at −80°C until required. Total protein was measured for all samples by use of Total Protein Kit according to manufacturer's guidelines (Sigma Aldrich, Dorset, UK)

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MAG:PLP1 ratio

The level of MAG was measured in homogenates diluted 1 in 10 in PBS, by in-house direct ELISA as previously described [24-28]. A mouse monoclonal anti-MAG1 antibody (cat. no. ab89780) (Abcam, Cambridge, UK) diluted 1:1000 was used in the direct ELISA. PLP1 level was measured in brain tissue homogenates diluted 1 in 10 in PBS using a commercially

available sandwich ELISA (cat no SEA417Hu, USCN, Wuhan, China), as previously [24-28]. The absorbance was measured at 450 nM in a FLUOstar Optima plate reader (BMG Labtech, Aylesbury, UK) after the addition of 2 N sulfuric acid. The concentration of MAG was interpolated from a serial dilution of recombinant human MAG (6.25-400 ng/ml) and adjusted for total protein level within each sample. The concentration of PLP1 was interpolated from a standard curve generated by serial dilution of recombinant human PLP1 (0.156–10 ng/ml) and adjusted for total protein. The ratio of MAG:PLP1 was calculated and is presented for each individual.

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VEGF ELISA

VEGF level was measured using the human VEGF-A ELISA kit (R&D Systems, Oxford, UK), as previously [24, 25, 28]. Brain tissue homogenates were diluted 1:10 in 1% BSA / PBS. Absorbance was measured at 450 nm in a FLUOstar Optima plate reader after the addition of 2N sulfuric acid. VEGF concentration was interpolated from serial dilutions of recombinant human VEGF (2000–31.25 pg/ml) and adjusted for total protein level.

Fibrinogen ELISA

Fibrinogen level was measured in brain tissue homogenates (2 µl + 248 µl PBS) using a commercially available sandwich ELISA (Human Fibrinogen ELISA kit, Cat no EH3057, Wuhan Fine Biological Technology Co, Wuhan City, Hubei Province, China) as previously [28]. The concentration of fibrinogen was interpolated from measurements of serially diluted recombinant human fibrinogen (600–9.375 ng/ml) and adjusted for total protein level.

PDGFRβ ELISA

PDGFRβ level was measured by sandwich ELISA (Cat no DYC385, R&D systems, Oxford, UK) as previously [28]. Absorbance was read at 450 nM following the addition of 2 N sulfuric acid, in a FLUOstar OPTIMA plate reader (BMG labtech, Aylesbury, UK). The absolute concentration of PDGFRβ was interpolated from the standard curve for each case, derived from serial dilution of recombinant PDGFRβ, and adjusted for total protein.

Endothelin-1 ELISA

EDN1 was measured in tissue samples by a commercial sandwich ELISA (cat. no. QET00B, R&D systems, Cambridge, UK) as previously [24-26]. Each sample was individually diluted to achieve a final concentration of 1 mg / ml total protein and 50 µl of sample was added to each well. Relative luminescence was measured using a FLUOstar Optima plate reader (BMG Labtech, Aylesbury, UK). Absolute EDN1 level was interpolated from a standard curve generated by assaying serial dilutions of recombinant human EDN1 (0.343–250 pg/ml).

Aβ42 ELISA

Soluble and insoluble (guanidine-extractable) fractions for Aβ42 measurement were prepared as reported previously [28]. A commercial sandwich ELISA (R&D systems, Cambridge, UK) was used according to manufacturer's instructions to measure Aβ42 in guanidine samples (diluted 1:2500 for AD samples and 1:625 for control and VaD samples). Aβ42 concentration was interpolated from serial dilutions of recombinant human Aβ42 (7.8–500 pg/ml) and corrected for sample dilution. Samples were measured in duplicate and the means calculated.

Statistical analyses

The distribution of the data and identification of potential outliers were examined for all markers assessed by examination of quantile-quantile plots (not shown). To assess the effect of AD and/or systemic infection on inflammatory brain cytokines and vascular markers, we used both 1-way and 2-way ANOVAs, or their non-parametric equivalents (if the data was deemed to be not normally distributed), as appropriate. Data are presented as mean ± standard error of the mean (SEM). Pearson's or Spearman's test was used as appropriate to assess linear correlation. All statistical analysis was performed with the help of SPSS version 21 (SPSS, Chicago) and GraphPad Prism version 8 (GraphPad Software, La Jolla, CA). *p*-values < 0.05 were considered statistically significant.

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Data availability

All data within the article are linked to the MRC UK-BBN by unique numeric MRC UK-BBN identifier (Supplementary Table 3). Further samples from the cases studied are available on request.

Results

Study cohort

We studied one hundred and forty-three cases in total: seventy-five AD (forty-two with terminal systemic infection and thirty-three without), twenty-two VaD/mixed (seven with and fifteen without terminal systemic infection) and forty-six age-matched controls (twenty-two with and twenty-four without terminal systemic infection). The age-at-onset of dementia, disease duration and Braak tangle stage for each of the six groups is shown in Table 1. Within

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each cohort, the distribution of Braak tangle stages was similar for the infection and non-infection groups. The groups were approximately matched for age-at-death and post-mortem delay. The gender split was approximately equal within the control cohort but skewed towards a higher proportion of females in the disease groups, as expected in the population. There was a higher proportion of *APOE* ϵ4 homozygotes and heterozygotes in the AD cohort but with a similar distribution of these alleles between the infection and non-infection AD groups.

Recorded causes of death in addition to dementia are listed in Table 1. Bronchopneumonia was the leading cause of death in the three groups with terminal systemic infection (33/42 AD cases, 6/7 VaD cases and 14/22 controls); a smaller number of cases were recorded with terminal urinary tract infections (3/42 AD, 1/7 VaD and 2/22 controls) or "other" unclassified infections (2/22 AD, 5/42 AD, 0/7 VaD). Causes of death in the non-infection cohort included systemic (non-stroke) cardiovascular disease (11/33 AD, 3/15 VaD and 19/24 controls) and non-CNS tumours (3/33 AD, 2/15 VaD, 2/24 controls).

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Brain cytokines are raised in AD and VaD, and with systemic infection

We performed 1-way ANOVAs to assess differences in brain cytokine level between control, AD and VaD groups after stratification according to the presence of infection (Fig. 1). In controls, IL-5, GM-CSF, IL-13 and IFN-γ were elevated in brains from people with infection (Con+) compared to those without (Con-) (Fig. 1a,c,d and j). In AD cases, IL-15, IL-1β, IL-6, TNF-α, and IL-8 were higher in those with (AD+) than without infection (AD-) (Fig. 1e-i). In VaD, IL-15 was higher in VaD with (VaD+) than without infection (VaD-) (Fig. 1e). In contrast, IL-13 and IL-1β levels were lower in VaD+ than VaD- (Fig. 1d and f). We performed 2-way ANOVAs to investigate differences in the interactions between dementia

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status and systemic infection (Supplementary Table 1). An interaction effect was observed between AD status and systemic infection indicating that GM-CSF, IL-17A, IFN-γ, and IL-12 were significantly altered by infection in AD (Supplementary Table 1). Interaction between VaD status and systemic infection was seen for IL-13 and IL-1β (as shown by 1-way ANOVA) and in addition, GM-CSF and IL-8. IL-2, IL-4, IL-7, IL-10, IL-12-23p40, IL-12p70, and IL-16 did not differ with dementia or in association with terminal systemic infection (Supplementary Fig. 1).

We assessed brain cytokine levels in relation to tangle progression, a proxy marker of disease stage in AD, in a combined AD and control cohort stratified into Braak stage 0-II (BS0-II), III-IV (BSIII-IV), and V-VI (BSV-VI). IL-15 and IL-17A were significantly elevated in end-stage disease (BS V-VI) compared to BS0-II (IL-15 was also elevated in BSIII-IV brains) (Supplementary Fig. 2a-b). IL-5 rose in mid-stage disease (BSIII-IV) only (Supplementary Fig. 2c), and IL-2, IL-12p40 and IL-16 declined in end-stage disease (BSV-VI) (Supplementary Fig. 2d-f). Several other brain cytokines – IL12-p70, IL-4, IL-7, IL-6 and IL-13 – did not vary significantly with Braak stage, although the levels tended to be highest in BSIII-IV. GM-CSF and IL-1β did not vary with Braak stage and IFN-γ and IL-8 declined with increasing Braak stage (Supplementary Fig. 3).

Cerebral hypoperfusion was exacerbated by systemic infection in controls and dementia

The MAG:PLP1 ratio was highly significantly reduced in AD and VaD compared to age-matched controls (Supplementary Table 2). In a combined AD and control group, MAG:PLP1 was significantly reduced in BSIII-IV and BSV-VI compared to BS0-II (Supplementary Fig. 4a). 1-way ANOVA, to assess differences between control and disease

groups after stratification according to the presence of infection, showed MAG:PLP1 to be reduced in controls with infection (Con+), to a level comparable to that in AD or VaD without infection (AD-, VaD-) (Fig. 2a). MAG:PLP1 was still further reduced in AD brains with (AD+) than without infection (AD-) but did not differ between VaD+ compared to VaD- (Fig. 2a). 2-way ANOVA revealed a highly significant interaction effect between infection and dementia status for MAG:PLP1 in both AD and VaD (Supplementary Table 2).

VEGF, an independent marker of acute cerebral ischaemia [24, 25], was highly significantly elevated in AD and VaD compared to controls (Supplementary Table 2). Analysis of VEGF according to progression of tangle pathology in a combined cohort of AD and controls indicated that VEGF was higher in BSV-VI than BS0-II (Supplementary Fig. 4b). 1-way ANOVA showed that VEGF was significantly elevated in AD+ vs. AD- and VaD+ vs. VaD- (Fig. 2b). 2-way ANOVA assessment of effects of interaction between infection and dementia status on VEGF level indicated that infection did not contribute significantly to the elevated VEGF in AD but did so in VaD (interaction effect p = 0.008) (Supplementary Table 2).

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Blood-brain barrier leakiness was exacerbated by systemic infection in controls and dementia brains

Fibrinogen level within the brain, a marker of BBB leakiness, was significantly higher in both AD and VaD than controls (Supplementary Table 2), and significantly higher in VaD than AD (Supplementary Table 2). Analysis of the effect of Braak tangle stage showed that fibrinogen was significantly higher in BSV-VI than in BS0-II (Supplementary Fig. 4c). When cases were stratified according to systemic infection, a 1-way ANOVA indicated that fibrinogen level was elevated in across all groups (Con+, AD+ and VaD+) in the presence of

systemic infection (Fig. 2c). A significant interaction effect of systemic infection on fibrinogen level was not, however, observed for Con vs. AD and Con vs. VaD, in 2-way ANOVAs, suggesting that the overall impact of systemic infection on BBB leakiness was modest (Supplementary Table 2).

PDGFRβ and EDN1 levels are altered in dementia and only modestly affected by systemic infection

The level of PDGFRβ, a protein expressed mainly by pericytes [28], was highly significantly lower in AD and VaD than controls (Fig. 2d, Supplementary Table 2). In relation to disease stage, PDGFRβ was lower in BSIII-IV ($p < 0.05$) and BS V-VI ($p < 0.01$) than in BS0-II (Supplementary Fig. 4d). When AD and controls were stratified according to systemic infection, 1-WAY ANOVA indicated that PDGFRβ did not differ between groups according to the presence of infection (Fig. 2d); however, a weak but significant effect of systemic infection on PDGFRβ was observed for AD vs. controls (Supplementary Table 2; interaction effect, $p = 0.039$) but not for VaD vs. controls running a 2-way ANOVA.

We have previously shown that cortical EDN1 level is elevated in AD [24]. EDN1 level tended to be higher in AD, and lower in VaD, compared to controls in the superior temporal cortex (Supplementary Table 2). When compared to controls without infection (Con-), EDN1 level was higher in AD groups irrespective of infection status (Fig. 2e).

Aβ42 in AD was unaltered by systemic infection.

Aβ42 level in guanidine-HCL extracts (i.e. in the insoluble pellet fraction) was significantly increased in AD, and to a much lesser extent VaD, compared to age-matched controls (Fig.

3). Aβ42 did not vary according to the presence of systemic infection in any of the groups and did not correlate with brain cytokine levels (data not shown).

Cerebral perfusion was related to brain cytokine levels in early stages of AD

In the absence of infection or substantial AD tangle pathology (i.e. in BS 0-II), cortical perfusion, as indicated by MAG:PLP1, correlated negatively with the levels of several cytokines (IFN-γ, IL-2, IL-12p70, IL-6, IL-10, IL-13, IL-4) but with few exceptions this correlation was lost with infection or progression of tangle pathology (Fig. 4). Notably, TNF-α and IL-10 correlated positively with MAG:PLP1 in BSV-VI only (Fig. 4).

Similarly, VEGF correlated positively with IFN-γ, IL-13 and IL-16 in BS0-II in the absence of systemic infection or substantial tangle pathology but the association was again lost in BSIII-IV and V-VI, and even sooner, in BS0-II, in cases with infection (Fig. 5).

MAG:PLP1 and VEGF showed the expected negative correlation, as previously reported [24, 26] in BS0-II but this relationship was lost in BSIII-IV and BSV-VI and in all brains with systemic infection (Supplementary Fig. 5).

Blood-brain barrier leakiness was related to elevated IL-1β in early and Aβ42 in late disease stage.

As previously reported in the precuneus, fibrinogen level in the superior temporal cortex, was inversely corelated with markers of cerebral hypoperfusion (reduced MAG:PLP1 and elevated VEGF) (Supplementary Figure 6a-b) and positively correlated with Aβ42 in controls and AD cases, but not VaD (Supplementary Figure 6c). Fibrinogen also correlated with reduced pericyte marker, PDGFRβ, level in controls (Supplementary Figure 6d).

Brain fibrinogen correlated positively with IL-1β in the early stages of disease (BS0-II and BSIII-IV) – the relationship was lost in BSV-VI cases and with systemic infection as early as BS0-II (Supplementary Fig. 7a-b). Fibrinogen also correlated weakly with IL-13 in BSIII-IV without infection but not when infection was present (Supplementary Fig. 7c-d). Fibrinogen correlated positively with Aβ42 in Braak tangle stage V-VI only – the relationship between fibrinogen and Aβ42 at each stage of disease was not substantially affected by systemic infection (Fig. 6a-b).

Brain fibrinogen level was also raised in AD in individuals homozygous for *APOE ε4*, as was EDN1 level (Supplementary Figure 8). MAG:PLP1 tended to be lower, and VEGF and PDGFRβ tended to be higher, in individuals heterozygous or homozygous for *APOE ε4* but these differences did not reach statistical significance. With the exception of IL-6 and IL-13, brain cytokine level was not related to possession of *APOE* ε4 in either controls or AD brains (Supplementary Figure 9).

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PDGFRβ and EDN1 level were only modestly affected by systemic infection

PDGFRβ tended to decline with increasing Aβ42 in AD brains in the absence of infection and to increase slightly in the presence infection but none of these trends was significant (Fig. 6c-d). PDGFRβ correlated negatively in BSIII-IV and positively in BSV-VI with several cytokines (IL-10, IL-12, IL-13, IL-2, IL-4 and TNF-α) but only in the absence of infection (Supplementary Fig. 10).

EDN1 correlated with Aβ42 in BSV-VI, only in those cases without infection ($r = 0.560$, $p < 0.01$) (Fig. 6e). Systemic infection had only a modest effect on this relationship (Fig. 6f).

In the absence of systemic infection, EDN1 correlated positively with the level of IL-15, IL-5, IL-1β and IL-17A in BSIII-IV or BSV-VI disease. These relationships were lost in systemic infection (Supplementary Fig. 11). TNF-α was an exception, in that the level did not correlate with EDN1 in the absence of infection; however, in cases with terminal infection, TNF-α showed a weak negative correlation with EDN1 in BS0-II disease and a strong positive correlation in advanced AD (BSV-VI).

Discussion

In this post-mortem study, we show that brain cytokine levels and markers of cerebrovascular dysfunction in the superior temporal gyrus are exacerbated in the presence of terminal systemic infection in AD and VaD, and in healthy age-matched controls. The influence of systemic infection on brain cytokines and vascular function varied with the stage of disease (as indicated by Braak tangle stage (BS)) - brain cytokines were often highest at BS III-IV and markers of cerebral vascular function were often impaired at this early to intermediate stage of disease. Our data indicate that systemic infection, independently of Aβ42 level, contributes to raised brain cytokine level and vascular insufficiency, particularly cerebral hypoperfusion and BBB leakiness in early AD. Markers of cerebral hypoperfusion and BBB breakdown were associated with elevated levels of brain cytokines in early disease (BS0-II) but these relationships were often lost in the presence of systemic infection or disease pathology. In contrast, systemic infection only contributed modestly to disease-related changes in late-stage disease and the expression of the vasoconstrictor, EDN1, and the pericyte marker, PDGFRβ, were associated with Aβ42 at a later stage of disease. These data indicate that the contribution of systemic infection to brain cytokine expression and vascular insufficiency varies according to disease stage: cerebral hypoperfusion and BBB is

exacerbated by infection and is related to elevated brain cytokine expression at an early stage of AD, independently of Aβ, whereas pericyte loss, raised EDN1, and further BBB breakdown, are related to Aβ accumulation in late-stage disease.

Systemic infection has long been recognized as a cause of cognitive impairment and delirium. AD patients with raised serum levels of pro-inflammatory cytokines IL-1β and TNF-α are indeed at increased risk of subsequent cognitive decline [6, 7]. The level of IL-1β within the brain is elevated by peripheral administration of endotoxins, simulating sepsis, suggesting that systemic infection may exacerbate already present brain inflammatory responses in AD [8-11]. In our previous post-mortem study, we found evidence of down-regulation of pro-inflammatory cytokines in brain tissue when infection occurred in end-stage AD [15]. Here, we assessed the impact of systemic infection on brain cytokine expression in superior temporal cortex from brains representing the full spectrum of AD progression, from BS0-II to BSV-VI, as well as from patients with neuropathologically confirmed VaD. Levels of some cytokines (IL-15 and IL-17A) were highest in brains from AD patients with BSV-VI disease. IL-15 is a pleiotropic cytokine that is highly expressed in activated astrocytes and contributes to disease pathology in brain ischaemia [43] and multiple sclerosis [44]. IL-15 level is raised in the CSF in relation to cognitive impairment and disease progression in AD [45]. IL-17, released from activated microglia, is associated with neurodegeneration in vitro [46], and with disease pathology and cognitive decline in a mouse model of Aβ accumulation [47]. It may also have a role in the recruitment of peripheral neutrophils in AD [48]. IL-17 has been found to drive tau hyperphosphorylation [49], and it was notable that IL-17 level was highest in BSV-VI brains.

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For many cytokines, (IL-5, IL-2, IL-12p40 and 1L-16), however, the level was highest in BSIII-IV disease, suggesting perhaps that the deleterious effects of systemic infection on the brain are likely to be maximal at an early to intermediate stage of AD. This is

consistent with clinical observations [45], brain imaging of microglia [50, 51] and post-mortem observation of activated microglia in controls with Aβ pathology, potentially reflecting early-mid stage disease [52] indicating that neuroinflammation occurs at an early presymptomatic stage in AD and contributes to cognitive decline and disease progression. Brain cytokine levels were unrelated to insoluble Aβ42 level, and Aβ42 levels were unchanged by infection, possibly suggesting that the impact of systemic infection of brain cytokines occurred independently of Aβ pathology.

Cerebrovascular dysfunction, associated with reduced cerebral blood flow [21] and cerebrovascular damage, including BBB leakiness, is apparent not only in VaD but also from an early stage in the development of AD [16, 17]. Recent high-resolution imaging studies have revealed leakiness of the BBB in the hippocampus in pre-symptomatic AD [19]. Later studies by the same group indicated that BBB breakdown precedes changes in the levels of Aβ and Tau in the CSF in the earliest stages of AD [20]. These vascular abnormalities are accelerated with possession of *APOE* ε4 [23], in keeping with earlier post-mortem studies indicating that pericyte loss and BBB breakdown are more pronounced in individuals with *APOE* ε4 [28, 53]. Elevated levels of endothelin-1 (EDN1) in AD may contribute to cerebral hypoperfusion via contraction of smooth muscle cells on penetrating arteries and arterioles [24] and pericyte dysfunction, an essential component of the neurovascular unit, contributes to blood flow dysregulation and as mentioned, BBB breakdown. Pericyte injury upon exposure to Aβ peptides or hypoxia in vitro [54], resulting in shedding and elevated CSF level of soluble PDGFRβ (sPDGFRβ) in AD [55] is related to BBB damage [19, 20].

Our recent post-mortem studies indicate that biochemical markers of pathological hypoperfusion and reduced oxygenation of the cerebral cortex in AD and VaD are associated with elevated levels of Aβ42, EDN1, and fibrinogen, and reduced PDGFRβ [24, 25, 28]. The level of fibrinogen, a marker of BBB leakiness, correlated with that of Aβ42 and was

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inversely related to the concentration of PDGFRβ and to the MAG:PLP1 ratio [28]. Raised CSF markers of cerebrovascular function, including YKL-40, ICAM-1, VCAM-1 and VEGF receptor 1 (Flt1), are elevated in presymptomatic AD in association with cognitive decline and markers of cortical thinning [45] and correlated with CSF Tau, as was also the case for CSF levels of soluble PDGFRβ (a marker of pericyte injury) [55]. Here, we show that MAG:PLP1 and PDGFRβ were significantly reduced, at an early stage i.e BSIII-IV, in AD indicating vascular dysfunction from an early to intermediate stage of disease. We found that terminal systemic infection exacerbated cortical perfusion and BBB function not only in AD but also in healthy controls. Cerebral hypoperfusion and BBB, associated with systemic infection, was likely independent of Aβ42 (which was unaltered in late-stage AD in the presence of infection). Indeed, in cases with no or minimal AD pathology (BS0-II), MAG:PLP1 declined and VEGF and fibrinogen increased by magnitudes similar to those in end-stage AD in control donors with terminal infection. In contrast, systemic infection appeared to have a more modest effect on EDN1 and PDGFRβ level. We previously showed that EDN1, fibrinogen and PDGFRβ are related to Aβ42 level [24]. Here we show that PDGFRβ and EDN1 (and to some extent fibrinogen) levels are related to Aβ42 in Braak tangle stage III-IV and V-VI disease but not BS0-II, perhaps reflecting a threshold effect of classical AD pathological processes on the regulation of these vasoactive molecules. Together, these data indicate a complex relationship between cerebrovascular dysfunction in AD, likely to involve multiple mediators, which is both dependent and independent of Aβ and Tau depending on stage of disease.

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It is likely that cerebrovascular dysfunction associated with systemic infection is related to the systemic effects of circulating cytokines as well as localised brain-expressed pro-inflammatory cytokines. In this study, we found that in the absence of significant brain pathology or systemic infection, the expression of several brain cytokines was higher in

brains that were less well perfused, i.e. with lower MAG:PLP1. The relationships were lost in the presence of systemic infection and disease pathology, suggesting that these pathological processes overwhelm the normal, relatively subtle, inflammatory responses to reductions in perfusion. Cytokines play a multifactorial role in vascular injury, mediating both vasoconstriction and dilatation (reviewed in [56]). Systemic inflammation is associated with cerebral hypoperfusion, via EDN1-mediated vasoconstriction [57]. Reduced regional blood flow in the brain in rats exposed to LPS, to model septicaemia that resulted in microglial activation and neuronal loss, was associated with enhanced transcription of several cytokines and chemokines including TNF-α, IL-1β, TGF-β and MCP-1 within the brain [58].

Experimental chronic cerebral hypoperfusion caused an increase in pro-inflammatory cytokines [59], and IL-1β infusion exacerbated cerebral hypoperfusion [60]. Several cytokines modulate signalling pathways that regulate vascular tone, some increasing the production of vasodilators (NO, PGI_2), and other pro-inflammatory cytokines, such as TNF-α, upregulating the expression of the potent vasoconstrictors, Ang-II and EDN1 [61], which are also elevated in AD [24, 25, 30]. Our findings indicate that this complex interrelationship between ischaemia, cytokine production and cerebral perfusion is altered by systemic infection and influenced by the severity of AD pathology, with evidence that aberrant patterns of response of several cytokines in hypoperfused tissue varied according to Braak stage. The patterns of response of some cytokines, such as IFN-γ and IL-12p70, were most abnormal for infection in BS0-II disease, whereas for others, such as IL-10 and TNF-α, the relationships between cytokines and markers of perfusion were most abnormal for infection in BSV-VI disease.

Systemic infection and neuroinflammation alter BBB permeability (reviewed in [62]). BBB leakiness is often associated with raised levels of pro-inflammatory cytokines, such as IL-1β, TNF-α, IL-6 and IL-2 [63-67] and pro-inflammatory cytokines directly influence BBB permeability in rodent endothelial cell cultures [68] and isolated cerebral microvessels from

sheep [69]. For instance, elevated endothelial expression of IL-6, in response to TNF-α, reduces the expression of tight junction proteins including cadherin, occludin and claudin-5 in human brain endothelial cells [70, 71] and TNF-α induces pericytes to produce MMP-9, which increases BBB leakiness [72]. Brain fibrinogen was related to IL-1β (and IL-13 to a smaller extent) in early and intermediate stages of disease (BS0-II and III-IV) but the relationship was lost in end stage disease, or with infection. A recent study revealed that IL-1β released by activated microglia disrupts astrocytic regulation of BBB permeability, by suppressing astrocytic expression of sonic hedgehog protein [73]. Other cytokines, including IL-17A, were shown to reduce or redistribute tight junction proteins in a human cerebral microvascular cell line (hCMEC/D3) [74]. Opening of the BBB allows peripheral cytokines to enter the brain [58], further compromising cerebral vascular function. In late, stage disease, BSV-VI, fibrinogen level was associated with insoluble Aβ42 level and was only modestly affected by systemic infection. The effects of systemic infection [75] on intravascular fibrinogen, and stalling of blood flow in brain capillaries may also have contributed to the elevated brain fibrinogen level. However, the rise in intracerebral fibrinogen was of the order of 100% in our cases with terminal infection, whereas even chronic systemic inflammation (e.g. in rheumatoid arthritis) causes a rise in intravascular fibrinogen of about 50%, [76] and (ii) correction for variations in haemoglobin concentration (a proxy indicator of blood content) made only a small (up to a few percent) difference to the raw measurements of fibrinogen (not shown) in our study, so it is unlikely that changes in intravascular fibrinogen level made more than a modest contribution to the increase in intracerebral fibrinogen in the cases with systemic infection.

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In conclusion, we have found that systemic infection is associated with elevated levels of multiple cytokines within the brain and exacerbates hypoperfusion and BBB leakage at an early/intermediate disease stage possibly independently of Aβ42. PDGFRβ, a marker of

pericytes, EDN1 levels, and fibrinogen level, were associated with Aβ42 level at a more advanced stage of disease and appeared to be only modestly affected by systemic infection. The retrospective, observational, post-mortem nature of this study imposes limitations on the interpretation of our findings, particularly insofar as the evidence is circumstantial and does not inform directly on causality or underlying mechanisms. The extent to which our findings are relevant to the progression of disease in a chronic condition with an extended prodromal phase remains to be determined. However, we know that cerebrovascular dysfunction is a strong predictor of cognitive decline and demonstrable in the early stages of dementia, perhaps independent of Aβ and Tau, and our observations are in keeping with studies in animal models of Aβ accumulation which indicate that both systemic infection and cerebral hypoperfusion exacerbate disease progression and pathology. Preservation of proteins is always a concern in post-mortem studies, but to assess vascular function we have used biochemical markers that we have previously shown that to be stable for up to 72 hours under simulated post-mortem conditions [27, 31].

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In conclusion, our data are in keeping with a range of previous experimental and observational studies of the relationship between systemic inflammation and cytokine levels within the brain; the effects of cytokines on microvascular perfusion and permeability; the association of both hypoperfusion and BBB breakdown with cognitive impairment; and the deleterious impact of systemic infection on the progression of dementia in AD. In AD, vascular dysfunction is strongly associated with the level of insoluble Aβ42. Our findings suggest that systemic infection exacerbates AD mostly through additive, cytokine-mediated vascular dysfunction that is independent of the level of insoluble Aβ42 in the early stages of disease.

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Competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Dr Daniel Asby, Professor Seth Love and Dr Scott Miners. The first draft of the manuscript was written by Dr Scott Miners and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Figure legends

Figure 1. Influence of systemic infection on brain cytokine levels in Alzheimer's disease and vascular dementia. Scatterplots showing cytokine levels in the superior temporal cortex (BA22) in post-mortem brain tissue in Alzheimer's disease (AD) and vascular dementia (VaD) in the absence or presence of terminal systemic infection. Cytokine levels were measured using an MSD multiplex panel. Each point represents the mean of duplicate measurements for an individual. Horizontal bars indicate the cohort mean ± SEM. * significant compared to age-matched controls; # significant in association with terminal systemic infection in the same diagnosis group. */# $p < 0.05$, **/## $p < 0.01$, ***/### $p < 0.001$

Figure 2. Systemic infection and cerebrovascular dysfunction in Alzheimer's disease and vascular dementia. Scatterplots showing levels of several markers of cerebrovascular function/dysfunction in the superior temporal cortex (BA22) in post-mortem brain tissue in Alzheimer's disease (AD) and vascular dementia (VaD) in the absence or presence of terminal systemic infection. Each point represents the mean of duplicate measurements for an individual. Horizontal bars indicate the cohort mean ± SEM. * significant compared to age-matched controls. # significant in association with terminal systemic infection in the same diagnosis group. */# $p < 0.05$, **/## $p < 0.01$, ***/### $p < 0.001$

Figure 3. Amyloid-β42 (Aβ42) level not influenced by terminal systemic infection. Scatterplots showing Aβ42 level in guanidine-HCl extracts (insoluble Aβ42) in superior temporal cortex (BA22) in AD, VaD and age-matched controls, stratified for the absence or presence of terminal systemic infection. Each point represents the mean of duplicate

measurements for an individual. Horizontal bars indicate the cohort mean ± SEM. * significant compared to age-matched control. *** $p < 0.001$

Figure 4. Relationship between biochemical markers of brain perfusion and brain cytokines: influence of Braak stage and infection. In brains with minimal tangle pathology (BS0-II) but not more advance disease, MAG:PLP1 correlated negatively with a large number of brain cytokines. With infection, these correlations were lost, even in BS0-II disease. The best-fit linear regression lines and 95% confidence intervals are shown.

Figure 5. Relationship between VEGF and brain cytokines: influence of Braak stage and infection. In brains with minimal tangle pathology (BS-0-II) but not more advanced disease, VEGF correlated positively with several brain cytokines. This relationship was lost even in BS0-II in the presence of systemic infection. The best-fit linear regression lines and 95% confidence intervals are shown.

Figure 6. Relationships of PDGFRβ, fibrinogen and endothelin-1 with Aβ42: influence of Braak stage and infection. (A-B) Fibrinogen correlated positively with Aβ42 in BSV-VI without and BS0-II with systemic infection. (C-D) PDGFRβ correlated negatively with Aβ42 approaching significance ($r = -.0568$; $p = 0.0541$) in BS III-IV in the absence, and positively in BS V-VI in the presence, of terminal systemic infection in superior temporal cortex. (E-F) EDN1 correlated positively with MAG:PLP1 in BSV-VI in BSV-VI only without infection. The best-fit linear regression lines and 95% confidence intervals are shown.

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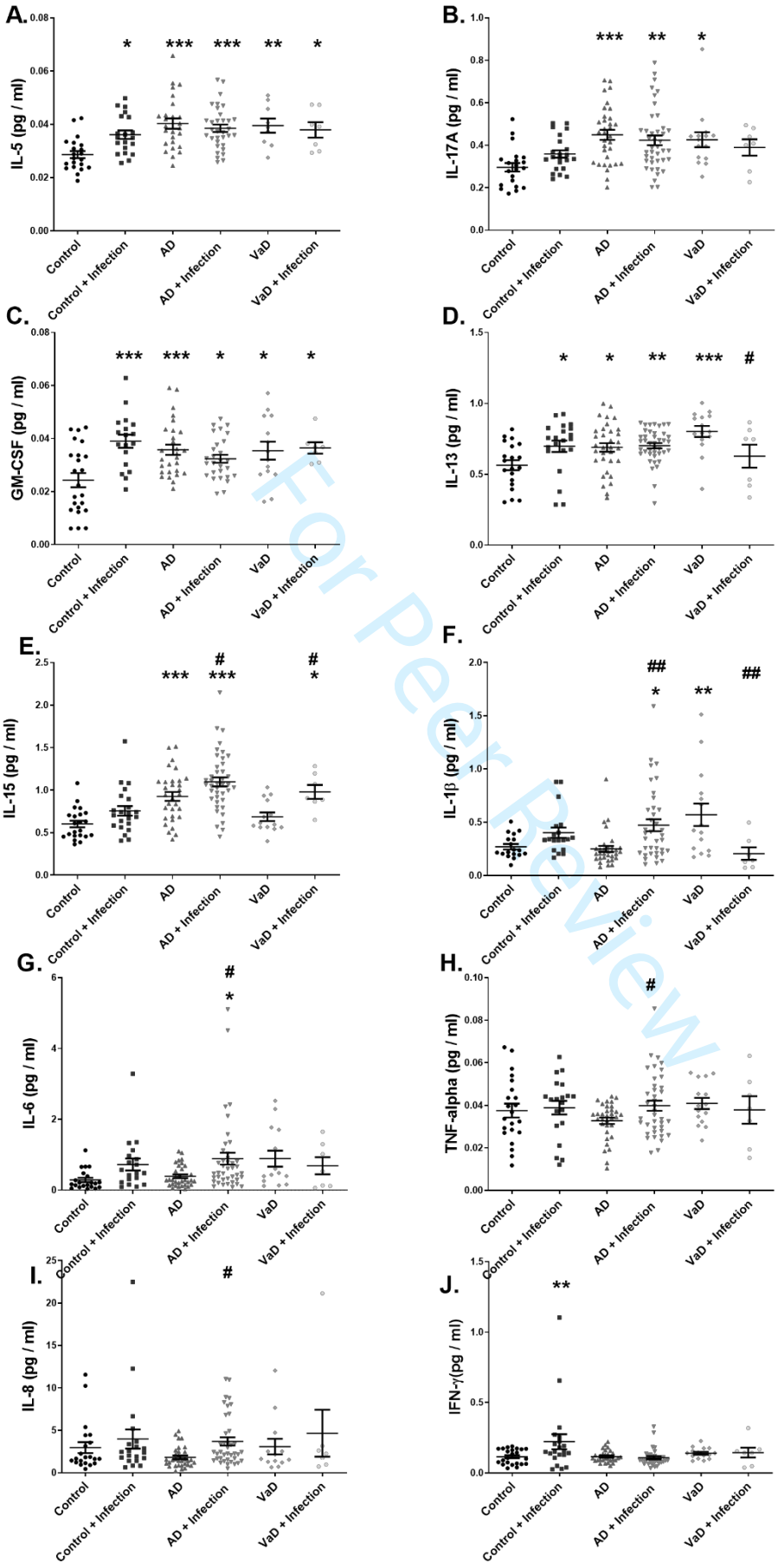


Figure 1. Influence of systemic infection on brain cytokine levels in Alzheimer's disease and vascular dementia. Scatterplots showing cytokine levels in the superior temporal cortex (BA22) in post-mortem brain tissue in Alzheimer's disease (AD) and vascular dementia (VaD) in the absence or presence of terminal systemic infection. Cytokine levels were measured using an MSD multiplex panel. Each point represents the mean of duplicate measurements for an individual. Horizontal bars indicate the cohort mean ± SEM. * significant compared to age-matched controls; # significant in association with terminal systemic infection in the same diagnosis group. */# $p < 0.05$, **/## $p < 0.01$, ***/### $p < 0.001$

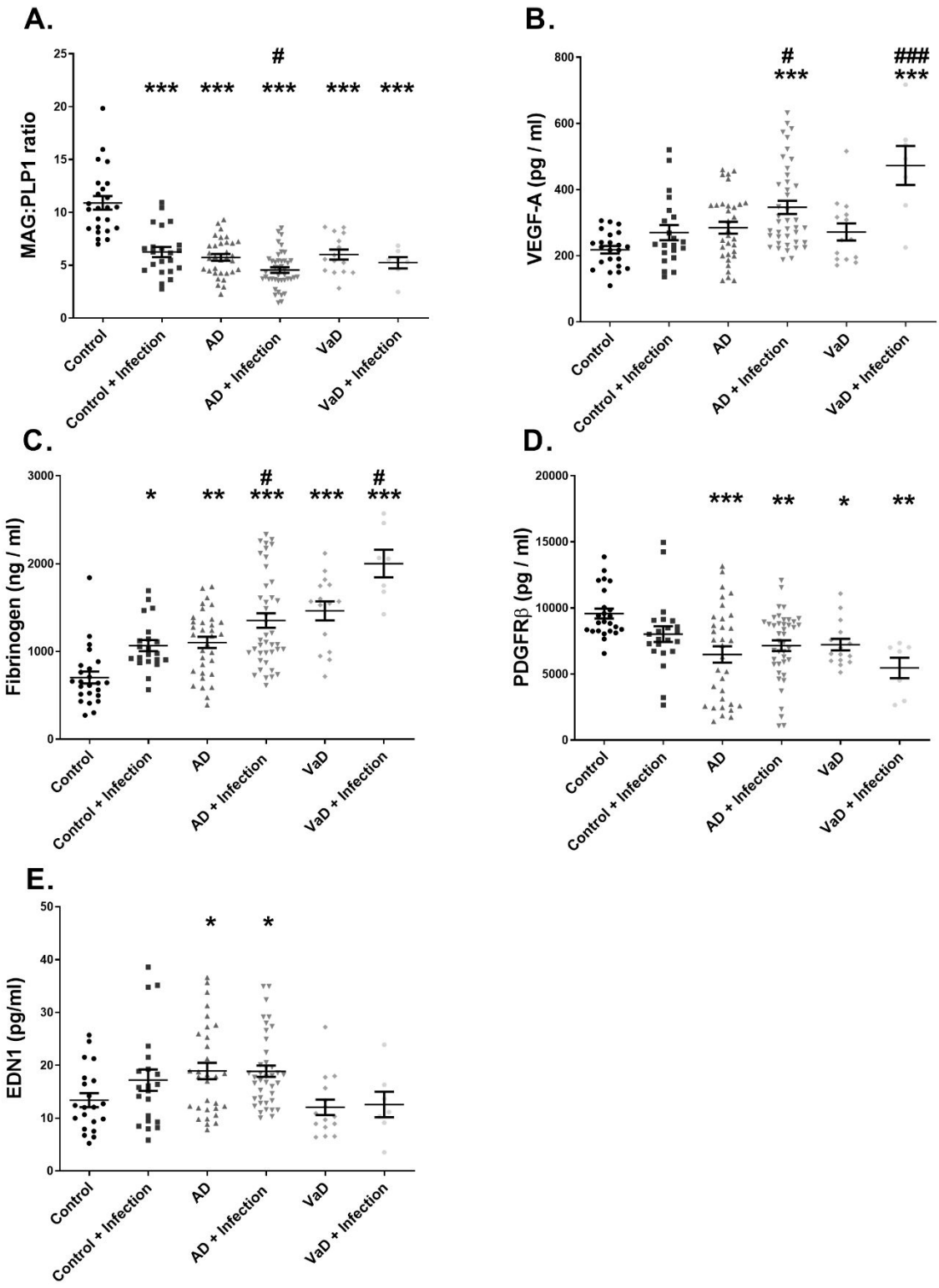


Figure 2. Systemic infection and cerebrovascular dysfunction in Alzheimer's disease and vascular dementia. Scatterplots showing levels of several markers of cerebrovascular function/dysfunction in the superior temporal cortex (BA22) in post-mortem brain tissue in

Alzheimer's disease (AD) and vascular dementia (VaD) in the absence or presence of terminal systemic infection. Each point represents the mean of duplicate measurements for an individual. Horizontal bars indicate the cohort mean ± SEM. * significant compared to age-matched controls. # significant in association with terminal systemic infection in the same diagnosis group. */# $p < 0.05$, **/## $p < 0.01$, ***/### $p < 0.001$

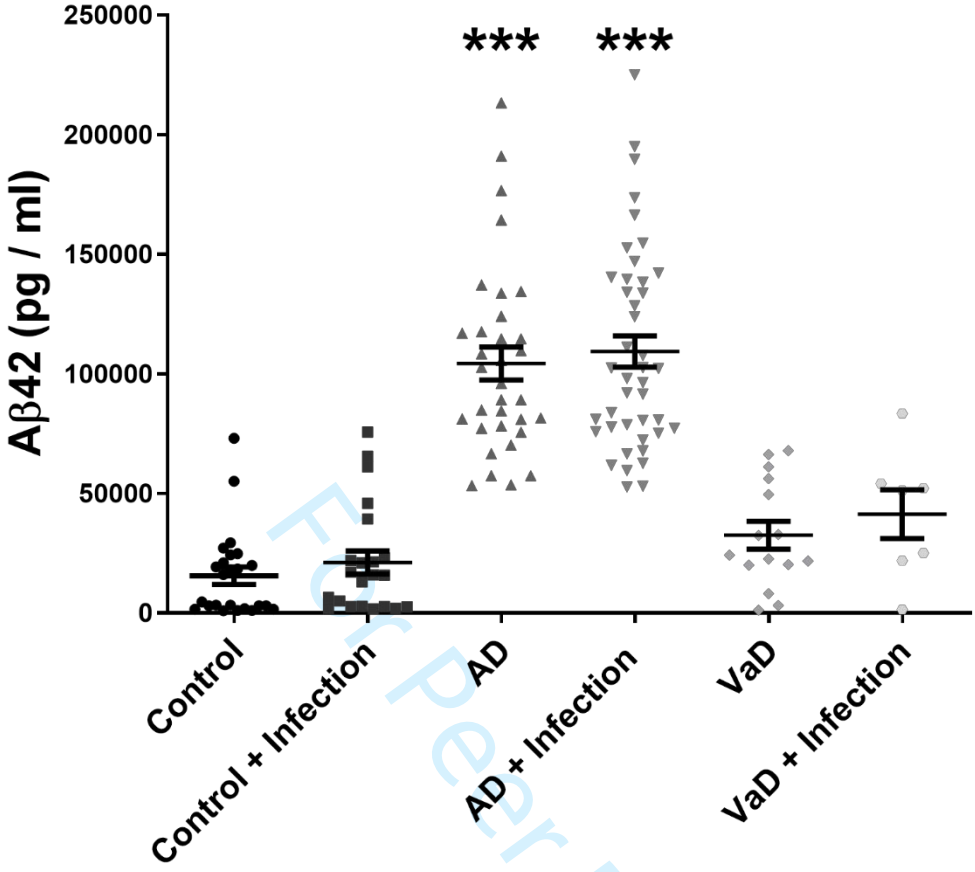


Figure 3. Amyloid-β42 (Aβ42) level not influenced by terminal systemic infection. Scatterplots showing Aβ42 level in guanidine-HCl extracts (insoluble Aβ42) in superior temporal cortex (BA22) in AD, VaD and age-matched controls, stratified for the absence or presence of terminal systemic infection. Each point represents the mean of duplicate measurements for an individual. Horizontal bars indicate the cohort mean ± SEM. * significant compared to age-matched control. *** $p < 0.001$

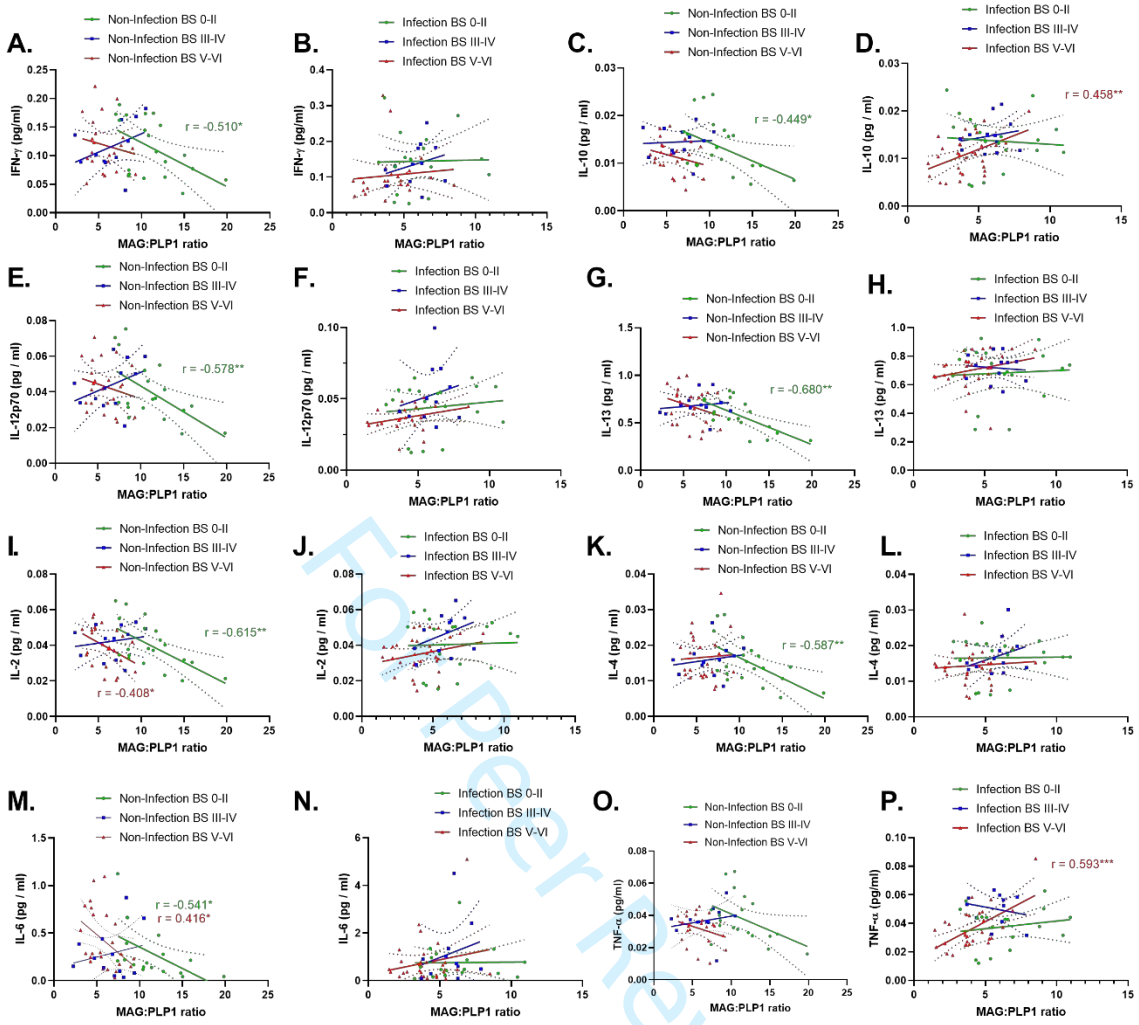


Figure 4. Relationship between biochemical markers of brain perfusion and brain cytokines: influence of Braak stage and infection. In brains with minimal tangle pathology (BS0-II) but not more advance disease, MAG:PLP1 correlated negatively with a large number of brain cytokines. With infection, these correlations were lost, even in BS0-II disease. The best-fit linear regression lines and 95% confidence intervals are shown.

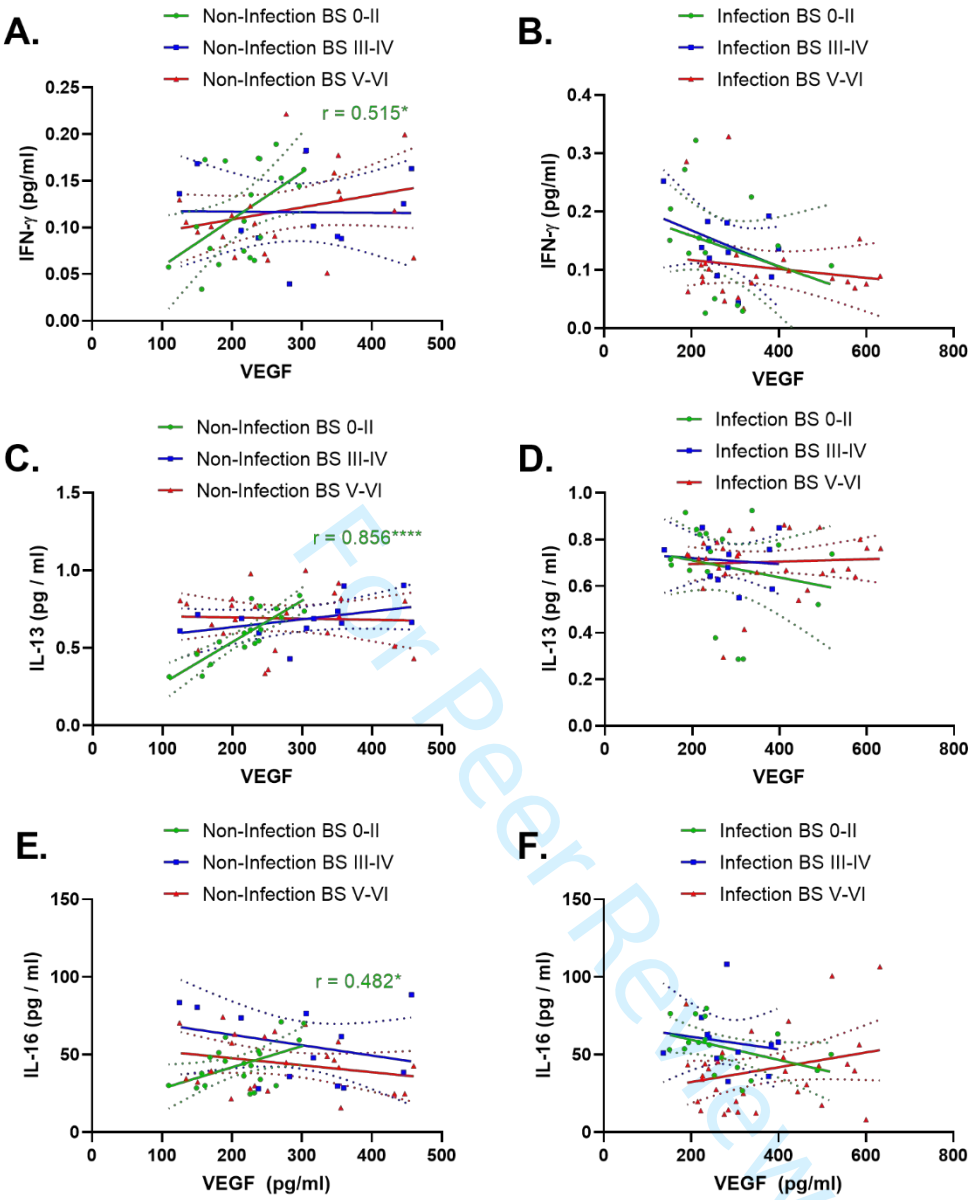


Figure 5. Relationship between VEGF and brain cytokines: influence of Braak stage and infection. In brains with minimal tangle pathology (BS-0-II) but not more advanced disease, VEGF correlated positively with several brain cytokines. This relationship was lost even in BS0-II in the presence of systemic infection. The best-fit linear regression lines and 95% confidence intervals are shown.

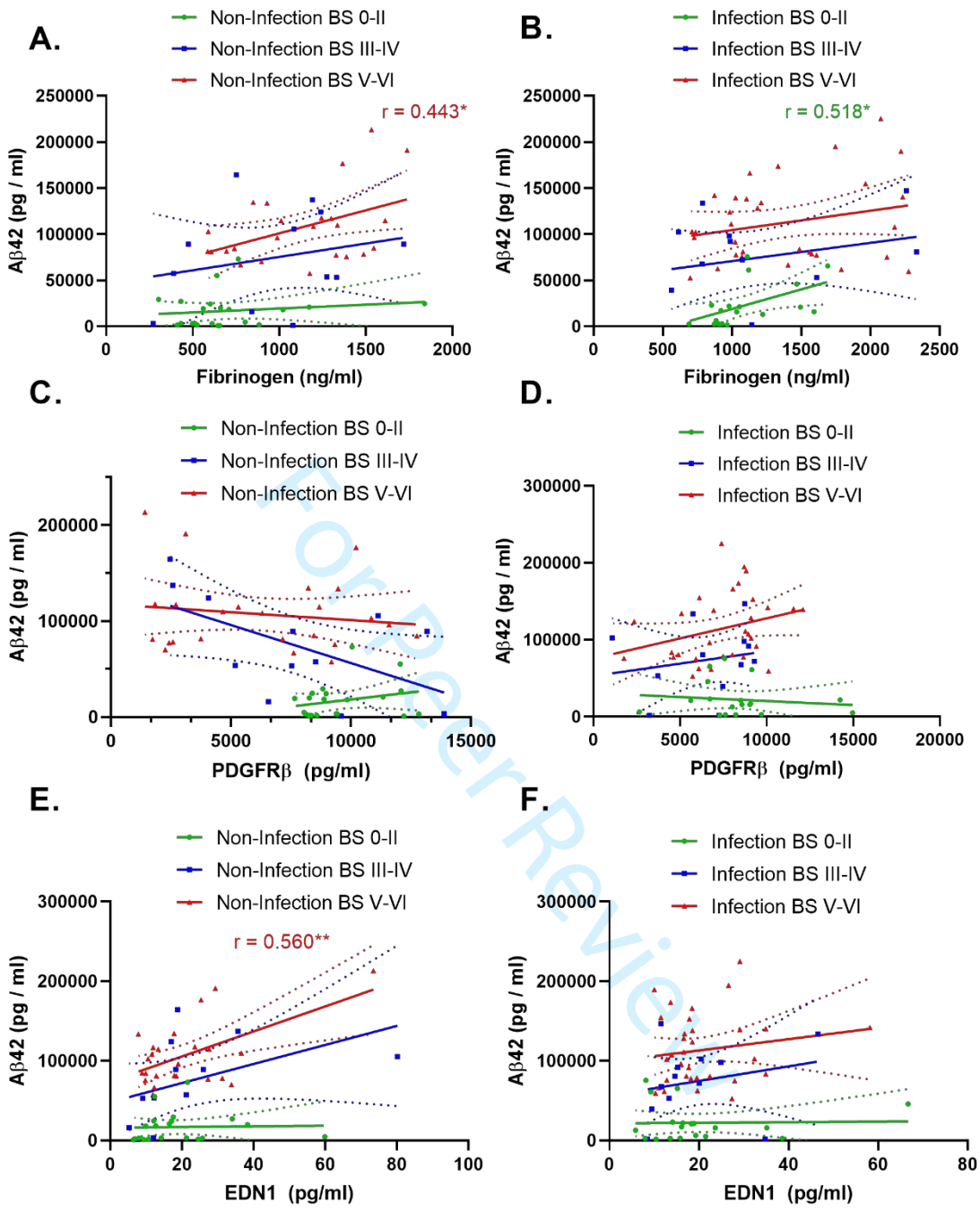


Figure 6. Relationships of PDGFRβ, fibrinogen and endothelin-1 with Aβ42: influence of Braak stage and infection. (A-B) Fibrinogen correlated positively with Aβ42 in BSV-VI without and BS0-II with systemic infection. (C-D) PDGFRβ correlated negatively with Aβ42 approaching significance (r = -.0568; p = 0.0541) in BS III-IV in the absence, and positively in BS V-VI in the presence, of terminal systemic infection in superior temporal cortex. (E-F)

EDN1 correlated positively with MAG:PLP1 in BSV-VI in BSV-VI only without infection. The best-fit linear regression lines and 95% confidence intervals are shown.

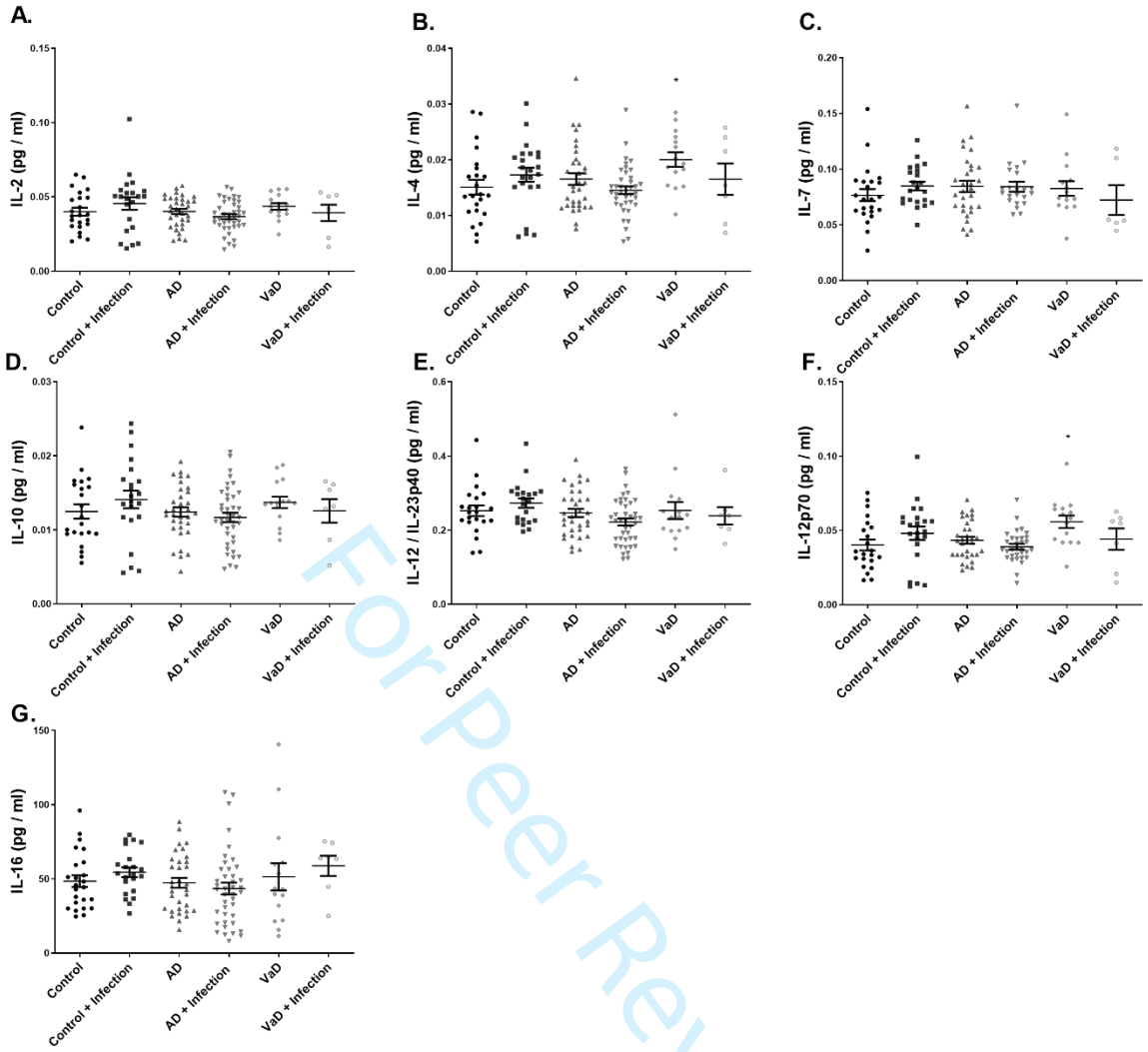
Supplementary Tables and Figures

Con Vs AD	Diagnosis Con Vs AD	Systemic Infection	Interaction effect		Con Vs VaD	Diagnosis Con Vs VaD	Systemic Infection	Interaction effect
IL-5	$p < 0.01$	NS	NS		**IL-5**	$p < 0.05$	NS	NS
IL-17A	$p < 0.0001$	NS	$p < 0.05$		**IL-17A**	$p < 0.01$	NS	NS (0.070)
GM-CSF	NS	0.022	$p < 0.0001$		**GM-CSF**	NS	0.022	0.032
IL-13	$p < 0.05$	$p < 0.05$	NS		**IL-13**	NS (0.098)	NS	$p < 0.01$
IL-15	$p <0.00001$	$p < 0.01$	NS		**IL-15**	$p < 0.05$	$p < 0.01$	NS
IL-1B	NS	$p < 0.01$	NS		**IL-1B**	NS	NS	$p < 0.001$
IL-6	NS	$p < 0.01$	NS		**IL-6**	NS	NS	NS (0.066)
TNF-a	NS	NS	NS		**TNF-a**	NS	NS	NS
IL-8	NS (0.077)	$p < 0.05$	NS		**IL-8**	NS (0.064)	NS	0.025
IFN-g	0.014	$p < 0.05$	$p < 0.01$		**IFN-g**	NS	NS	NS
IL-2	NS	NS	NS		**IL-2**	NS	NS	NS
IL-4	NS	NS	0.043		**IL-4**	NS	NS	NS
IL-7	NS	NS	NS		**IL-7**	NS	NS	NS
IL-10	NS (0.051)	NS	NS		**IL-10**	NS	NS	NS
IL-12/p40	$p < 0.05$	NS	NS (0.055)		**IL-12/p40**	NS	NS	NS
IL-12/p70	NS	NS	$p < 0.05$		**IL-12/p70**	NS	NS	NS
IL-16	NS	NS	NS		**IL-16**	NS	NS	NS

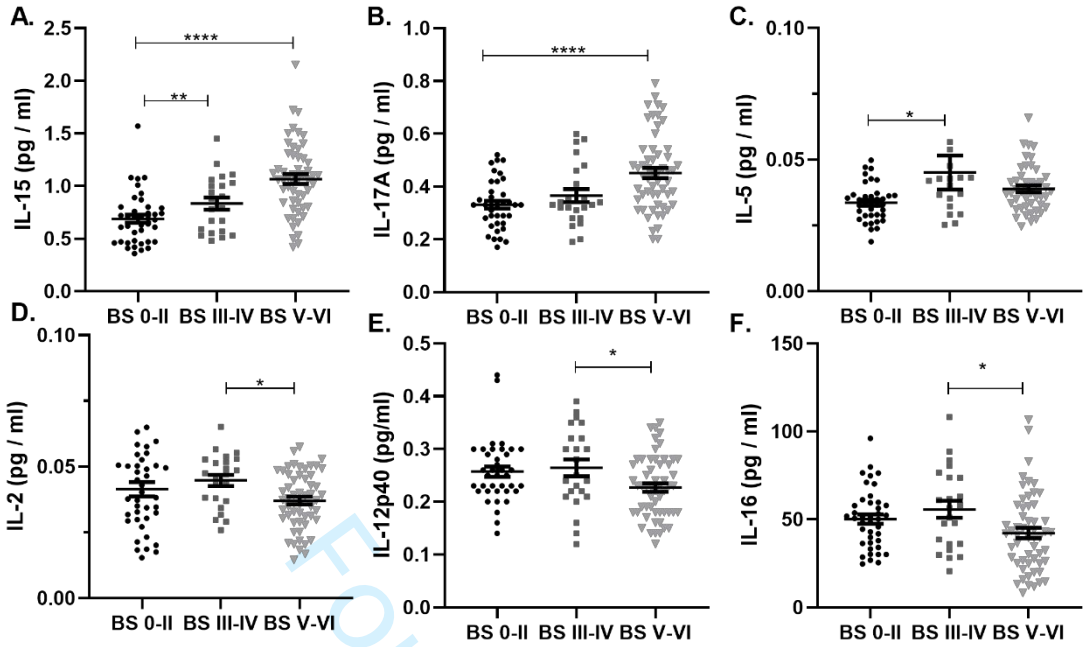
Supplementary Table 1. The contribution of systemic infection on brain cytokine levels in Alzheimer's disease (AD) and Vascular dementia (VaD). 2-WAY ANOVAs were performed to identify disease-specific differences and identify whether systemic infection significantly altered brain cytokine levels in Con Vs AD and Con Vs VaD, indicated by the interaction effect. p-values < 0.05 were considered statistically significant (p-values approaching $p < 0.05$ are shown in parenthesis).

Con Vs AD	Diagnosis Con Vs AD	Systemic infection	Interaction effect		Con Vs VaD	Diagnosis Con Vs VaD	Systemic Infection	Interaction effect
MAG:PLP1	$p < 0.00001$	$p < 0.00001$	$p < 0.00001$		**MAG:PLP1**	$p < 0.0001$	$p < 0.001$	$p < 0.01$
VEGF-A	$P < 0.001$	$P < 0.01$	NS		**VEGF-A**	$p < 0.0001$	$p < 0.0001$	$p < 0.01$
Fibrinogen	$p < 0.001$	$p < 0.001$	NS		**Fibrinogen**	$p < 0.00001$	$p < 0.00001$	NS
PDGFRβ	$p < 0.001$	NS	$p < 0.05$		**PDGFRβ**	$p < 0.001$	$p < 0.01$	NS
EDN1	NS	NS	NS		**EDN1**	$P < 0.05$	NS	NS

Supplementary Table 2. The contribution of systemic infection on vascular dysfunction in the temporal cortex (BA22) in Alzheimer's disease (AD) and Vascular dementia (VaD). 2-WAY ANOVAs were performed to identify differences in vascular marker between disease groups and whether systemic infection contributes to disease-related changes in cerebrovascular markers in Control (Con) Vs AD and Control (Con) Vs VaD. p values < 0.05 were considered statistically significant (p-values approaching $p < 0.05$ are shown in parenthesis).

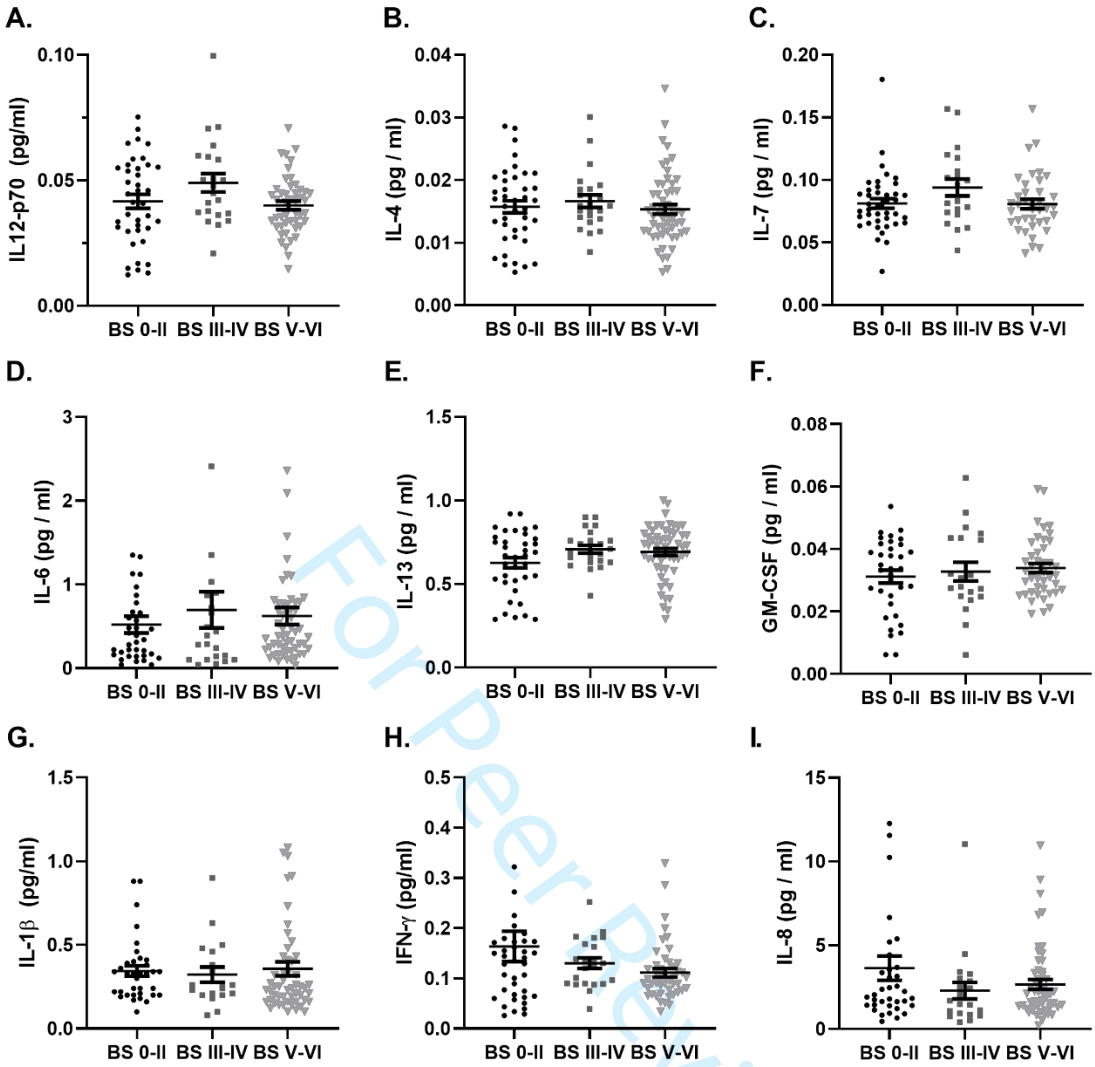


Supplementary Figure 1. Influence of systemic infection on brain cytokine levels in Alzheimer's disease and vascular dementia. Scatterplots showing cytokine levels in the superior temporal cortex in post-mortem brain tissue in Alzheimer's disease (AD) and vascular dementia (VaD) in the absence or presence of terminal systemic infection. Cytokine levels were measured using an MSD multiplex panel. Each point represents the mean of duplicate measurements for an individual. Horizontal bars indicate the cohort mean ± SEM.

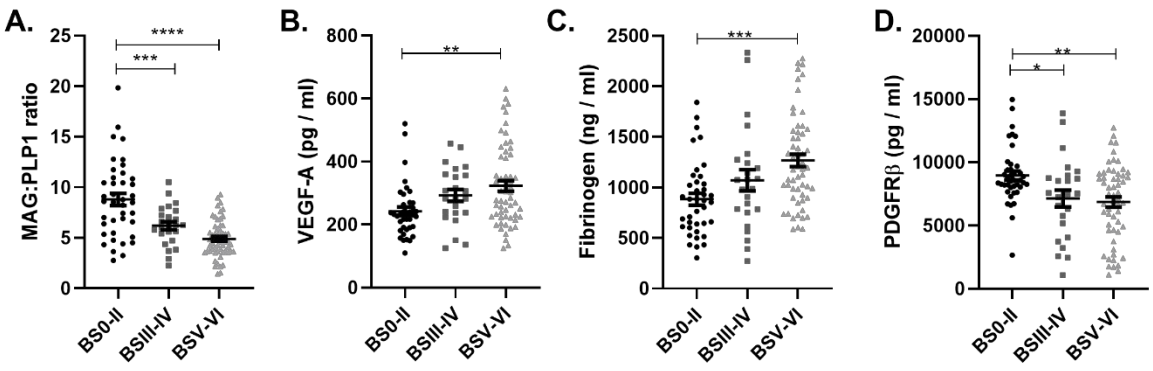


Supplementary Figure 2. Brain cytokine levels in relation to Braak tangle stage.

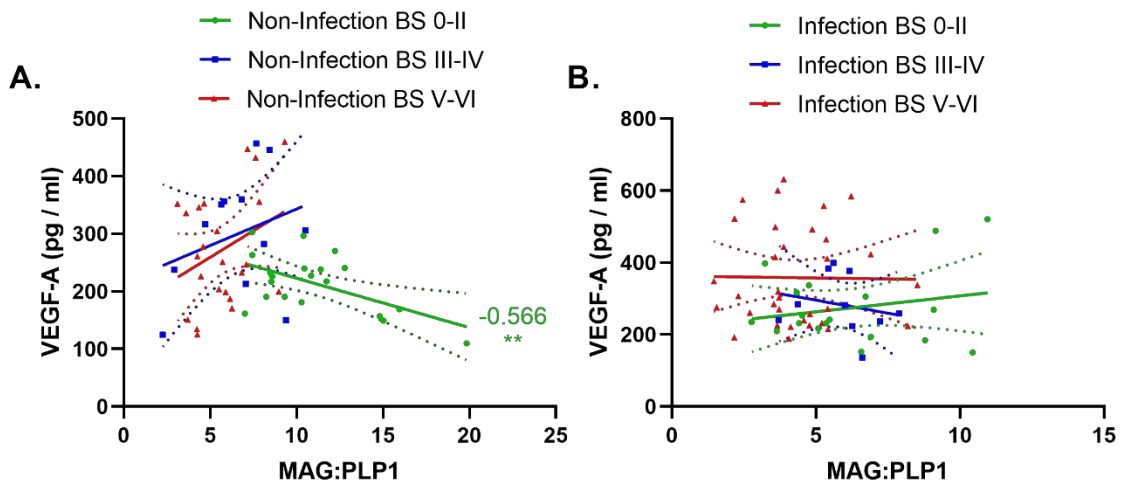
Scatterplots showing cytokine levels in the superior temporal cortex (BA22) in post-mortem brain tissue in a combined AD and control cohort stratified according to Braak tangle stage (BS): BS 0-II, BS III-IV and BS V-VI. Cytokine levels were measured using an MSD multiplex panel. Each point represents the mean of duplicate measurements for an individual. Horizontal bars indicate the cohort mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$



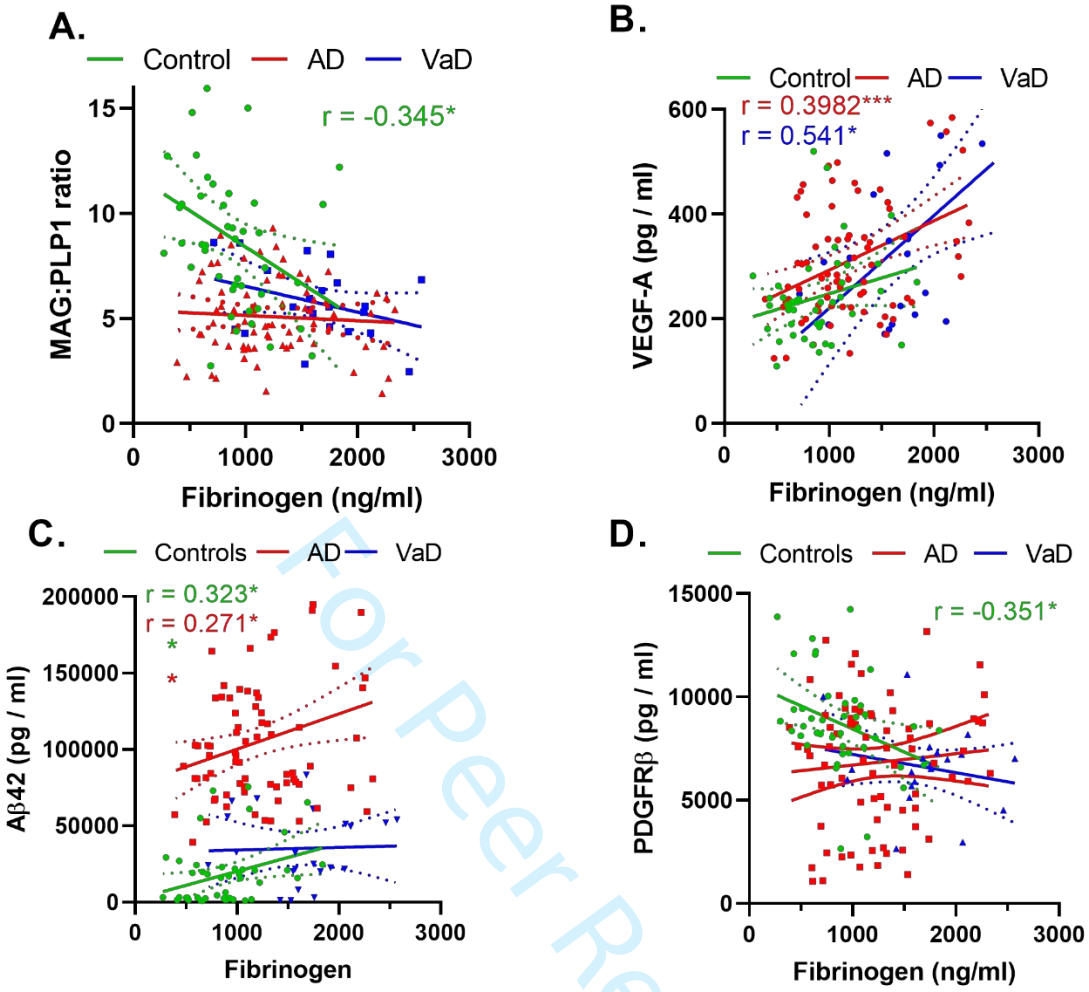
Supplementary Figure 3. Influence of Braak tangle stage pathology on brain cytokine levels. Scatterplots showing cytokine levels in the superior temporal cortex in post-mortem brain tissue in a combined Alzheimer's disease and age-matched control cohort. Each point represents the mean of a duplicate measurement for an individual. Horizontal bars indicate the cohort mean ± SEM



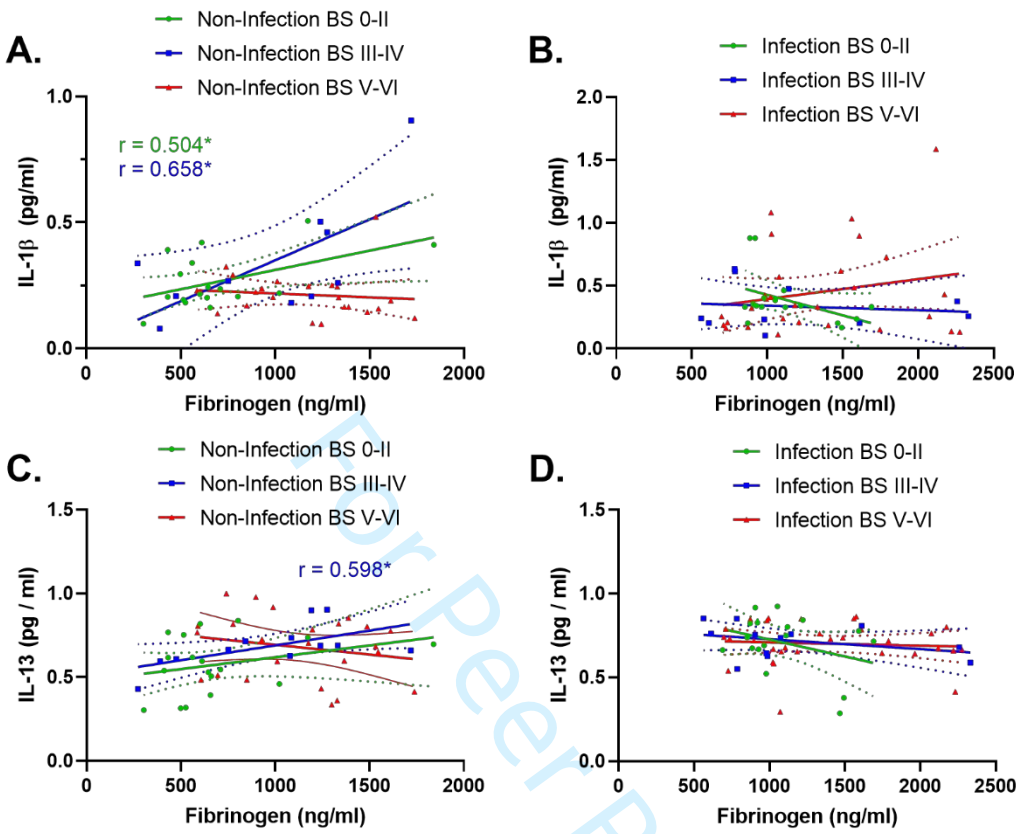
Supplementary Figure 4. Vascular markers in relation to Braak tangle. Scatterplots showing (A) MAG:PLP1 ration (B) VEGF (C) Fibrinogen and (D) PDGFRβ in AD and control cases divided into Braak tangle stage (BS). Each point represents the mean of a duplicate measurement for an individual. Horizontal bars indicate the cohort mean ± SEM. * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ **** $p < 0.0001$



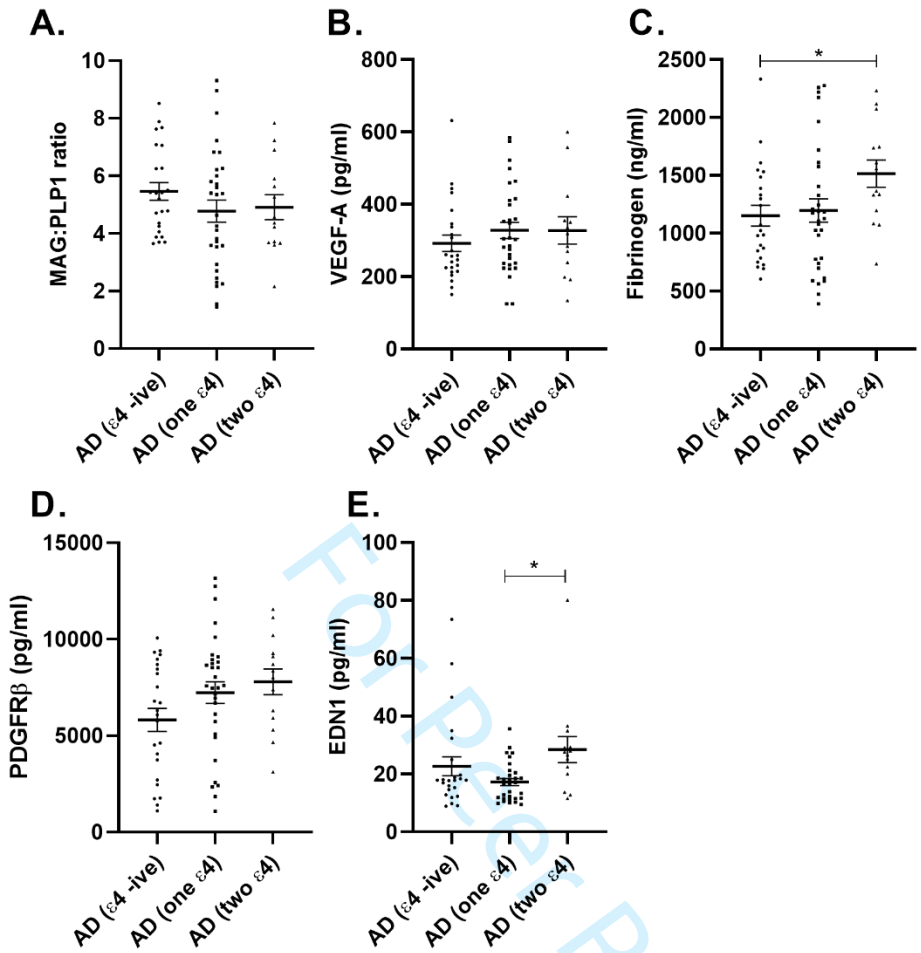
Supplementary Figure 5. Relationship between MAG:PLP and VEGF: influence of infection. In brains with minimal tangle pathology (BS 0-II) but not more advanced disease, VEGF correlated negatively with MAG:PLP1 in superior temporal cortex. With infection, this correlation was lost even in BS 0-II disease. The best-fit linear regression lines and 95% confidence intervals are shown.



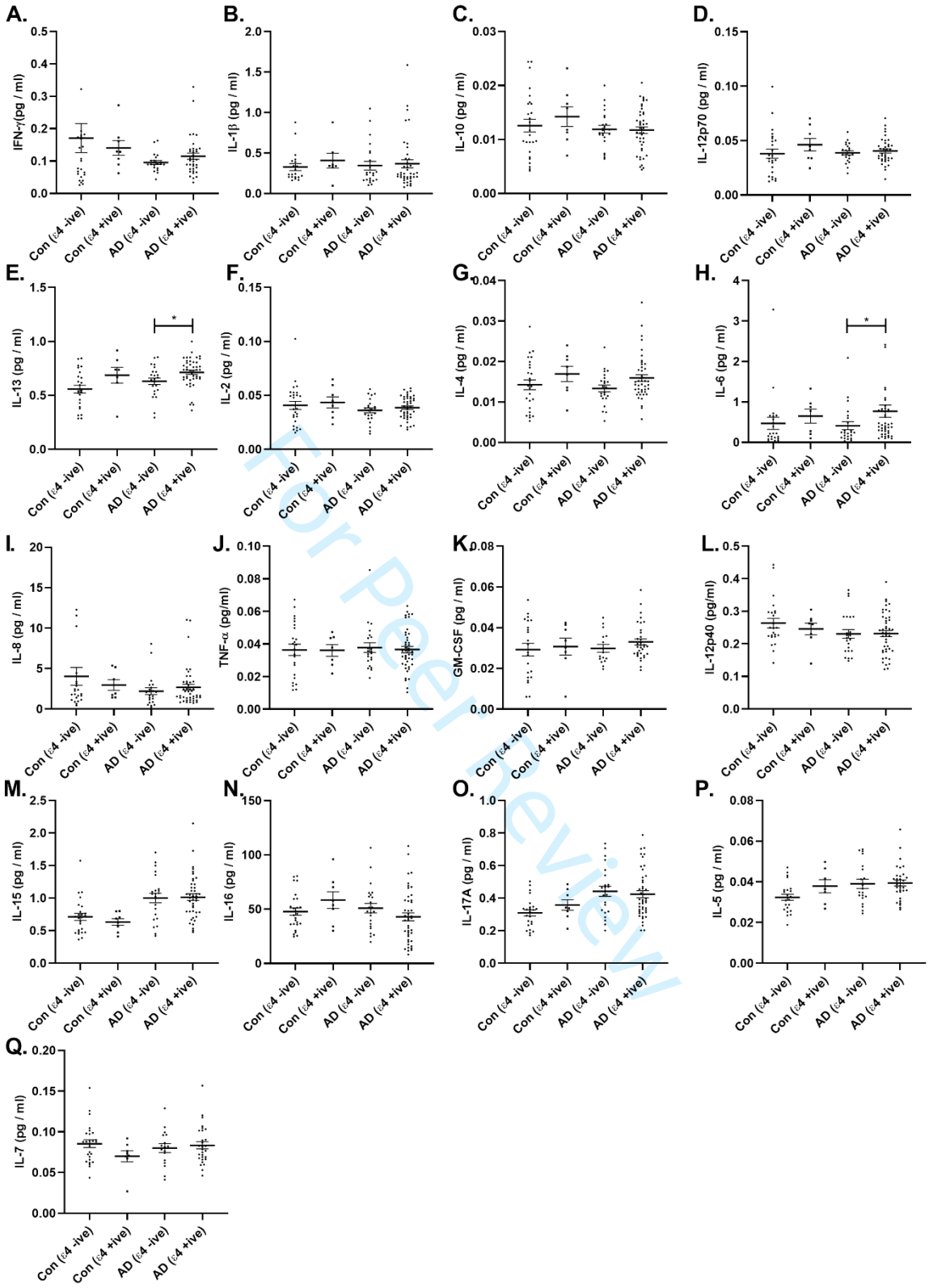
Supplementary Figure 6: Relationships between brain fibrinogen level and markers of brain perfusion (MAG:PLP1), pericyte content (PDGFRβ) and Aβ42 in dementia. (A-B) Brain fibrinogen correlated with MAG:PLP1, a marker of reduced oxygenation and positively with VEGF, a marker of acute ischaemia, in AD and VaD. (C) Fibrinogen was increased in relation to Aβ42 in AD only. (D) Fibrinogen was related to lower PDGFRβ level in controls. The best-fit linear regression lines and 95% confidence intervals are shown.



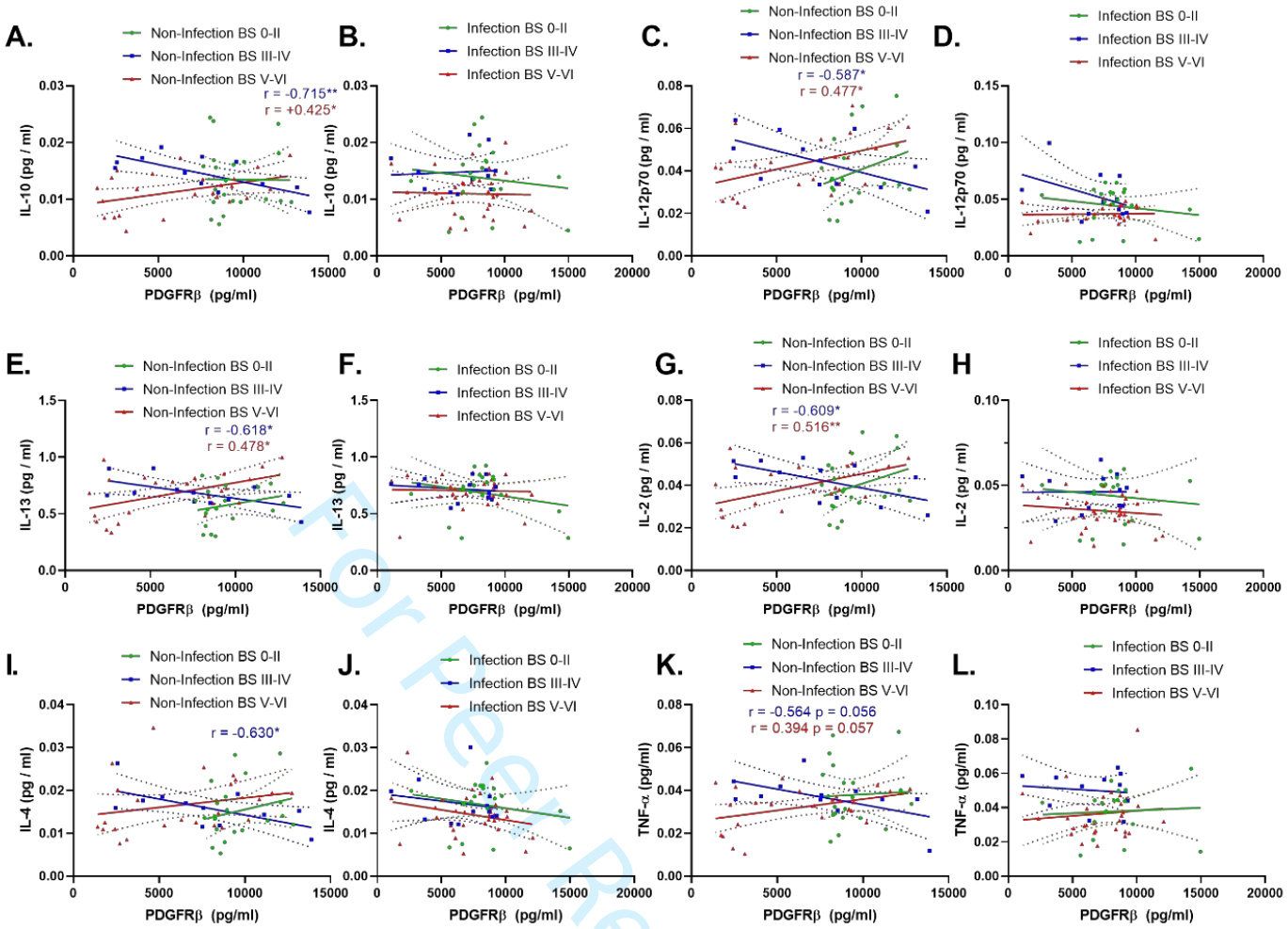
Supplementary Figure 7. Relationship between fibrinogen and brain cytokines: influence of Braak stage and infection. Most brain cytokines did not correlate with fibrinogen level, however, fibrinogen correlated with IL-1β in BS III-IV and V-VI in cases without systemic infection but not with infection. Fibrinogen correlated positively with IL-13 in BS III-IV without but not with infection. The best-fit linear regression lines and 95% confidence intervals are shown.



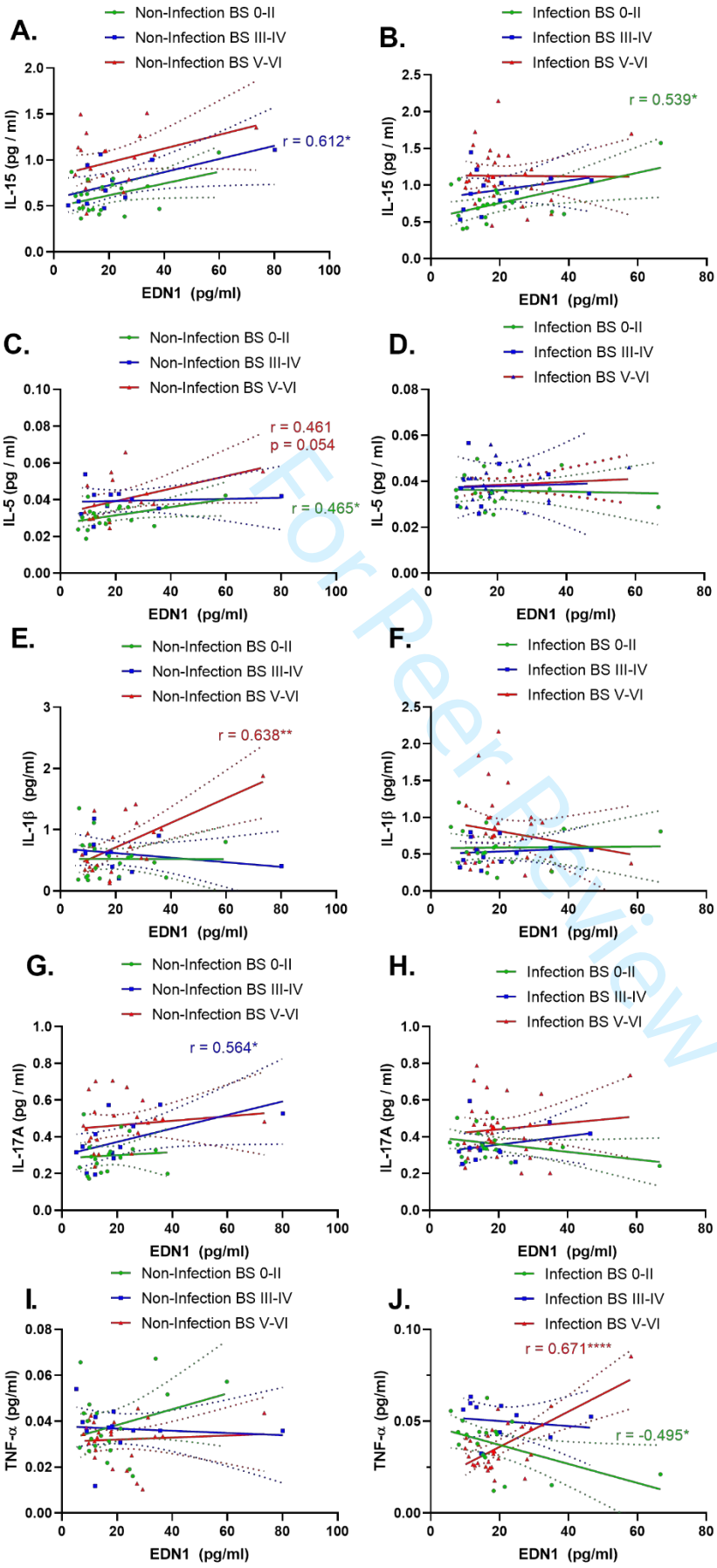
Supplementary Figure 8. Vascular markers in relation to *APOE* genotype. Scatterplots showing (A) MAG:PLP1 ration (B) VEGF (C) Fibrinogen (D) PDGFRβ and EDN1 (E) in AD cases (ε4 -ve indicates absence of ε4 and possession of either APOE ε2 or 3; one ε4 = heterozygosity; two ε4 = homozygosity). Each point represents the mean of a duplicate measurement for an individual. Horizontal bars indicate the cohort mean ± SEM. * $p < 0.05$



Supplementary Figure 9. Brain cytokines in relation to *APOE* genotype. Scatterplots showing individual brain cytokine level (A-Q) in relation to presence (ε4 +ve) and absence (ε4 -ve) of *APOE* ε4 allele in the control and Alzheimer's disease cohort. Each point represents the mean of a duplicate measurement for an individual. Horizontal bars indicate the cohort mean ± SEM. * $p < 0.05$



Supplementary Figure 10. Relationship between PDGFRβ and brain cytokines: influence of Braak stage and infection. In brains with intermediate tau tangle pathology (BS III-IV), PDGFRβ correlated negatively with several brain cytokines, but positively in BS V-VI, in cases without infection. This relationship as lost in cases with systemic infection. The best-fit linear regression lines and 95% confidence intervals are shown.



Supplementary Figure 11. Relationships between endothelin-1 and brain cytokines: influence of Braak stage and infection. EDN1 correlated with several brain cytokines in cases with disease pathology (BS III-IV or BSV-VI) without systemic infection but not in cases with infection. The notable exception was TNF-α, which was strongly positively correlated with EDN1 in BS V-VI in the presence of infection. The best-fit linear regression lines and 95% confidence intervals are shown.

Control (-)	Control (+)	AD (-)	AD (+)	VaD (-)	VaD (+)
BBN_8671	BBN_8725	BBN_8848	BBN_8825	BBN_8861	BBN_8832
BBN_8684	BBN_8732	BBN_8852	BBN_8842	BBN_9313	BBN_4223
BBN_8691	BBN_8735	BBN_8915	BBN_8846	BBN_4208	BBN_9369
BBN_8706	BBN_8751	BBN_8968	BBN_8850	BBN_9387	BBN_14403
BBN_8708	BBN_8770	BBN_8990	BBN_8870	BBN_19611	BBN_19628
BBN_8709	BBN_8888	BBN_9118	BBN_8871	BBN_19622	BBN006.28583
BBN_8728	BBN_9028	BBN_9122	BBN_8892	BBN_24309	BBN006.29152
BBN_8739	BBN_9329	BBN_9189	BBN_8899	BBN_24310	
BBN_8835	BBN_9331	BBN_9198	BBN_8910	BBN_24901	
BBN_8898	BBN_9359	BBN_9200	BBN_8912	BBN_24904	
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BBN_9292	BBN_9408	BBN_9309	BBN_9037	BBN006.31492	
BBN_9340	BBN_9422	BBN_9336	BBN_9076	BBN006.32513	
BBN_9344	BBN_19608	BBN_4200	BBN_9095	BBN006.33638	
BBN_4205	BBN_24337	BBN_4215	BBN_9109		
BBN_9354	BBN_24325	BBN_4216	BBN_9112		
BBN_9365	BBN_26009	BBN_9361	BBN_9123		
BBN_9407	BBN006.26096	BBN_4231	BBN_9150		
BBN_19613	BBN006.28893	BBN_4232	BBN_9156		
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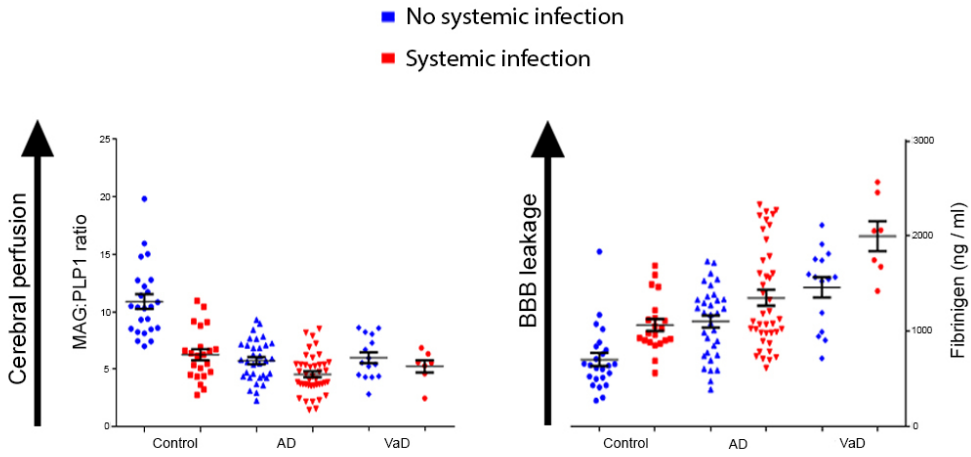
			BBN_9433		
			BBN_19614		

Supplementary Table 3. List of MRC UK-BBN identifier numbers for cases used in this study. Controls (Con-/Con+), Alzheimer's disease (AD+/AD-) and Vascular dementia (VaD+/VaD-) with (+) and without systemic infection (-)



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