How does human cytomegalovirus regulate autoimmune responses in multiple sclerosis? Role of osteopontin and CD46

Background

- Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system. Relapsing-remitting MS (RRMS) is the most common form
- Cytomegalovirus (CMV) is a human herpes virus, which may have a negative association with RRMS (1).
- CD46 is a membrane-bound glycoprotein, which is involved in the entry and spread of human CMV(2). It also normally mediates the differentiation of inflammatory T helper 1 cells to anti-inflammatory Treg cells, a pathway that is dysregulated in MS (3).
- Osteopontin (OPN) is a proinflammatory cytokine that is raised in MS relapses (4).
- This project aims to find a mechanism explaining the reduction in MS risk due to CMV infection

References

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(3) Killick J, Hay J, Morandi E, et al. Vitamin D/CD46 Crosstalk in Human T Cells in Multiple Sclerosis. Front Immunol. 2020;11:598727. Published 2020 Nov 24. doi:10.3389/fimmu.2020.598727

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Methods

- A retrospective case-control study of RRMS serum samples and controls from the Italian Biobank in North-western Italy
- Enzyme-linked immunosorbent assay testing in duplicate for CD46 and OPN.
- Statistical analyses done with:
  - CD46: student t-test with Bonferroni adjusted level of significance (p = 0.00455).
  - OPN: Mann-Whitney U test with level of significance p= 0.05 (no adjustment as small sample size meant likelihood of type 2 error, which is increased by Bonferroni was high).

Results

OPN

- Serum levels of OPN were not significantly higher in CMV IgG negative (median=16570 [IQR: 11448-23555]) individuals than CMV IgG positive (median=16440 [IQR: 13525-19185]) individuals, U=79.0, p=0.478.
- Serum levels of OPN were not significantly higher in RRMS patients (median=16280 [IQR: 12895-21188]) than controls (median=18340 [IQR: 12068-23070]), U=47.0, p=0.420.
- Serum levels of OPN were significantly lower in individuals over 40 (median=14400 [IQR: 9834-17995]), than in individuals under 40 years (median=20545 [IQR: 13825-27258]), U=41, p=0.044, shown in **Figure 2.**

CD46

- The serum levels of CD46 were higher (p=0.150) in controls ($\bar{x}$=15661 pg/ml [SD:3984]) than in RRMS patients ($\bar{x}$=14749 pg/ml [SD:4443]), but not to a significant level.
- Serum levels of CD46 in CMV negative individuals ($\bar{x}$=14364 [SD:4449]) were lower (p=0.058) than CMV positive individuals ($\bar{x}$=15751 [SD:4023]), but not to a statistically significant level.
- Serum levels of CD46 were not significantly higher (p=0.043) in individuals over 40 years of age ($\bar{x}$=16081 [SD:3552]) than in individuals under 40 years of age ($\bar{x}$=14351 [SD:4655]), shown in **Figure 1.**

Figure 1 – Seroprevalence of OPN presented as median + IQR values (n=25).

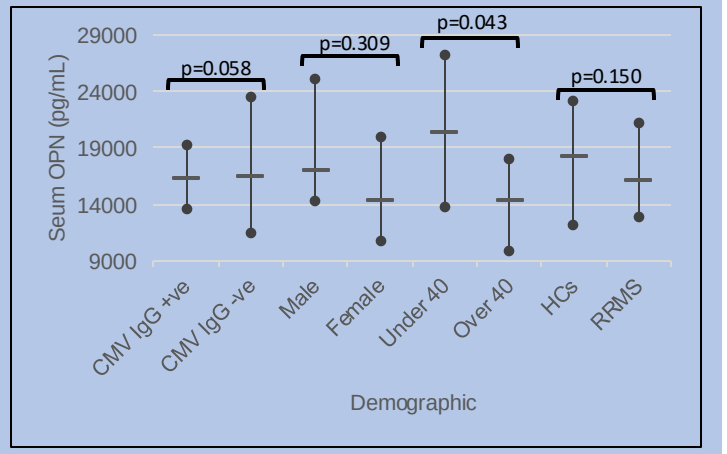
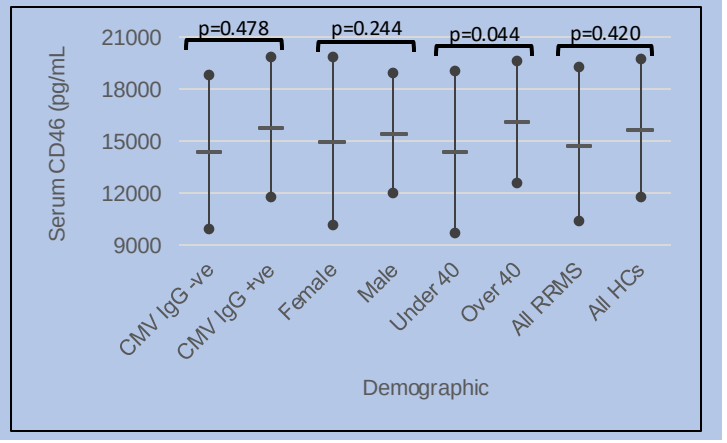


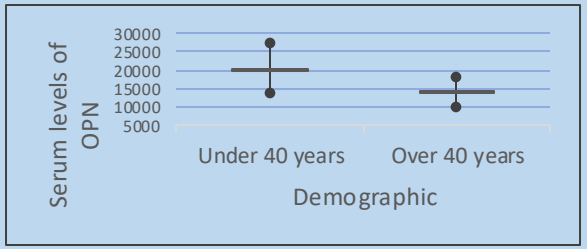
Figure 2 – Seroprevalence of CD46 presented as mean ± SD (n=95).



Significant Results

- Serum levels of OPN were significantly lower in individuals over 40, than in individuals under 40 years, shown in **Figure 3.**

Figure 3 – Direct comparison of serum levels of OPN presented as median ± IQR values between samples ≥40 years of age and <40 years of age (p=0.044).



Conclusions

- Serum levels of CD46 and OPN are not significantly altered by CMV IgG status or RRMS diagnosis.
- Results do not support a role for CD46 or OPN in the reduced risk of MS due to CMV infection.
- Higher OPN levels in individuals under 40 could be due to raised inflammatory cytokines at the average RRMS age of onset, which is 33.37 (5).
- OPN is not a biomarker specific to MS and so this relationship could also be explained by skew due to the effects of other conditions, eg CKD.

Recommendations for Future Research

- Future research into the role of CMV infection could be focused on proteins with stronger evidence than CD46 for involvement in CMV infection, such as the Nrp2 receptor, done without multiple analyses, sampling at point of CMV infection.
- This further research could transfer to better care for MS patients by giving understanding of the mechanism of CMV infection controlling RRMS risk to develop novel therapies for MS treatment.