*Viral and bacterial co-infection in severe pneumonia triggers innate immune responses and specifically enhances IP-10: a translational study*. Jonathan Hoffmann[1], Daniela Machado[1], Olivier Terrier[2], Stephane Pouzol[1], Mélina Messaoudi[1], Wilma Basualdo[3], Emilio E Espínola[4], Rosa M. Guillen[4], Manuel Rosa-Calatrava[2], Valentina Picot[1], Thomas Bénet[5], Hubert Endtz[1], Graciela Russomando[4], Gláucia Paranhos-Baccalà[1*].
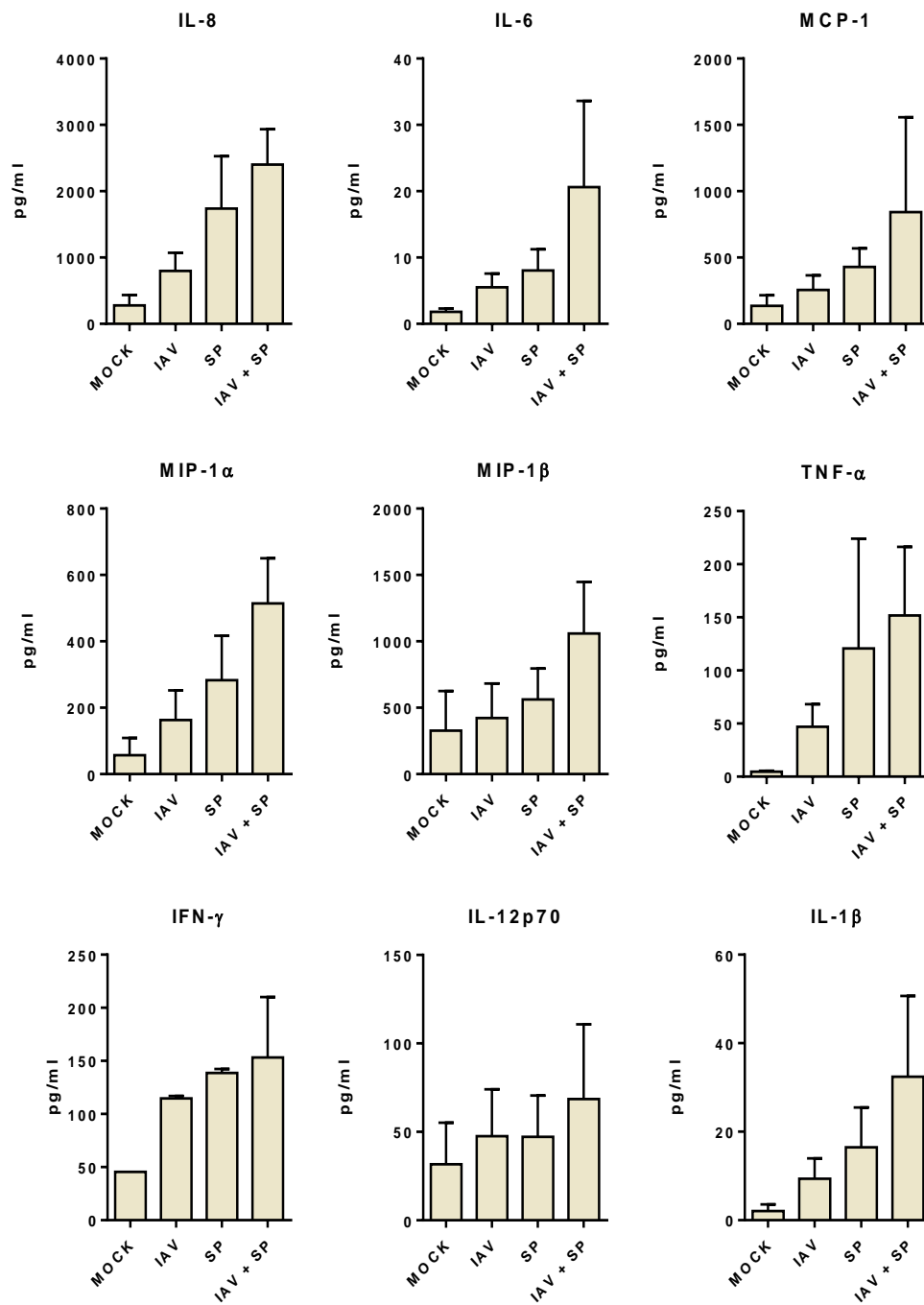
**Figure S1 legend: Cytokine and chemokine responses of MDMs after single or mixed infection with IAV and SP**.

Preceding IAV infection leads to elevated secretion of pro-inflammatory cytokines and chemokines after co-infection with SP. MDMs were infected with or without IAV for 4 hours, then infected with or without SP, incubated for 20 h, and the concentrations of 27 cytokines and chemokines in the cell supernatants were measured using a quantitative multiplex immunoassay. A selection of deregulated expression profiles at 24 hours post-infection is shown: IP-10, IL-8, IL-6, MCP-1, MIP-1α, MIP-1β, TNF-α, IFN-γ and IL-12p70. Values are mean (± SEM) of three independent experiments. Statistical analyses were performed using one-way ANOVA with Tukey's post-hoc test.

**Table S1: Complete mRNA expression dataset of human monocyte-derived macrophages following single or mixed IAV and/or SP infection.**

| Gene | Description | Fold Change (comparing to control group) | | |
|------|-------------|------|------|------|
| | | IAV | SP | IAV + SP |
| CXCL10 | Chemokine (C-X-C motif) ligand 10 | 67,9 | 183,5 | 240,9 |
| MX1 | Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse) | 134,0 | 120,3 | 151,4 |
| IL1B | Interleukin 1, beta | 0,6 | 61,4 | 59,4 |
| IL23A | Interleukin 23, alpha subunit p19 | 3,3 | 68,1 | 56,2 |
| CD80 | CD80 molecule | 2,4 | 49,5 | 54,3 |
| STAT1 | Signal transducer and activator of transcription 1, 91kDa | 31,0 | 44,2 | 52,3 |
| CCL2 | Chemokine (C-C motif) ligand 2 | 2,2 | 26,9 | 34,6 |
| DDX58 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 | 11,2 | 19,3 | 22,2 |
| STAT4 | Signal transducer and activator of transcription 4 | 1,7 | 16,4 | 15,8 |
| IL10 | Interleukin 10 | 1,3 | 11,6 | 10,6 |
| IRF7 | Interferon regulatory factor 7 | 6,6 | 6,1 | 6,8 |
| CSF2 | Colony stimulating factor 2 (granulocyte-macrophage) | 0,7 | 5,7 | 6,3 |
| IL8 | Interleukin 8 | 0,1 | 7,1 | 6,2 |
| LYZ | Lysozyme | 12,0 | 6,6 | 5,5 |
| IFNB1 | Interferon, beta 1, fibroblast | 2,3 | 5,1 | 5,2 |
| ICAM1 | Intercellular adhesion molecule 1 | 0,5 | 5,0 | 4,7 |
| CD40 | CD40 molecule, TNF receptor superfamily member 5 | 0,9 | 4,4 | 4,7 |
| IL6 | Interleukin 6 (interferon, beta 2) | 0,7 | 3,7 | 4,0 |
| TNF | Tumor necrosis factor | 0,5 | 3,7 | 3,7 |
| IL1R1 | Interleukin 1 receptor, type I | 0,4 | 3,9 | 3,5 |
| NFKB1 | Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 | 1,1 | 3,4 | 3,4 |
| CCL5 | Chemokine (C-C motif) ligand 5 | 0,8 | 3,5 | 3,4 |
| NFKBIA | Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha | 0,4 | 3,4 | 3,2 |
| CCR4 | Chemokine (C-C motif) receptor 4 | 1,1 | 2,5 | 3,1 |
| MYD88 | Myeloid differentiation primary response gene (88) | 2,1 | 2,3 | 2,9 |
| TLR8 | Toll-like receptor 8 | 0,8 | 2,4 | 2,4 |
| TLR9 | Toll-like receptor 9 | 3,9 | 2,4 | 2,1 |
| IL1A | Interleukin 1, alpha | 0,5 | 3,7 | 2,1 |
| FOXP3 | Forkhead box P3 | 1,8 | 2,4 | 2,1 |
| CASP1 | Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase) | 1,5 | 2,0 | 2,0 |
| TYK2 | Tyrosine kinase 2 | 1,3 | 2,0 | 1,9 |
| TICAM1 | Toll-like receptor adaptor molecule 1 | 1,2 | 2,2 | 1,9 |
| NOD2 | Nucleotide-binding oligomerization domain containing 2 | 1,4 | 1,8 | 1,8 |
| IRF3 | Interferon regulatory factor 3 | 1,3 | 1,6 | 1,8 |
| HLA-A | Major histocompatibility complex, class I, A | 1,5 | 1,6 | 1,7 |
| TLR2 | Toll-like receptor 2 | 1,2 | 1,5 | 1,5 |
| STAT6 | Signal transducer and activator of transcription 6, interleukin-4 induced | 1,6 | 1,4 | 1,5 |
| IL5 | Interleukin 5 (colony-stimulating factor, eosinophil) | 0,7 | 1,1 | 1,4 |
| STAT3 | Signal transducer and activator of transcription 3 (acute-phase response factor) | 1,1 | 1,3 | 1,3 |
| NLRP3 | NLR family, pyrin domain containing 3 | 1,1 | 1,2 | 1,3 |
| IFNAR1 | Interferon (alpha, beta and omega) receptor 1 | 0,6 | 1,4 | 1,2 |
| HLA-E | Major histocompatibility complex, class I, E | 1,3 | 1,1 | 1,2 |
| CXCR3 | Chemokine (C-X-C motif) receptor 3 | 0,6 | 1,4 | 1,2 |
| NOD1 | Nucleotide-binding oligomerization domain containing 1 | 1,4 | 1,0 | 1,1 |
| CD14 | CD14 molecule | 0,9 | 1,2 | 1,1 |
| IL18 | Interleukin 18 (interferon-gamma-inducing factor) | 0,9 | 1,2 | 1,1 |
| APCS | Amyloid P component, serum | 0,7 | 1,1 | 1,0 |
| CCR6 | Chemokine (C-C motif) receptor 6 | 0,7 | 1,1 | 1,0 |
| CCR8 | Chemokine (C-C motif) receptor 8 | 0,7 | 1,1 | 1,0 |
| CD40LG | CD40 ligand | 0,7 | 1,1 | 1,0 |
| CD8A | CD8a molecule | 0,7 | 1,1 | 1,0 |
| CRP | C-reactive protein, pentraxin-related | 0,7 | 1,1 | 1,0 |
| FASLG | Fas ligand (TNF superfamily, member 6) | 0,7 | 1,1 | 1,0 |
| GATA3 | GATA binding protein 3 | 0,7 | 1,1 | 1,0 |
| IFNA1 | Interferon, alpha 1 | 0,7 | 1,1 | 1,0 |
| IFNG | Interferon, gamma | 0,7 | 1,1 | 1,0 |
| IL13 | Interleukin 13 | 0,7 | 1,1 | 1,0 |
| IL17A | Interleukin 17A | 0,7 | 1,1 | 1,0 |
| IL2 | Interleukin 2 | 0,7 | 1,1 | 1,0 |
| IL4 | Interleukin 4 | 0,7 | 1,1 | 1,0 |
| MBL2 | Mannose-binding lectin (protein C) 2, soluble | 0,7 | 1,1 | 1,0 |
| MPO | Myeloperoxidase | 1,7 | 1,1 | 1,0 |
| RORC | RAR-related orphan receptor C | 0,7 | 1,1 | 1,0 |
| TBX21 | T-box 21 | 0,7 | 1,1 | 1,0 |
| TLR3 | Toll-like receptor 3 | 1,1 | 0,8 | 1,0 |
| CD4 | CD4 molecule | 1,6 | 1,1 | 1,0 |
| TLR5 | Toll-like receptor 5 | 1,3 | 1,1 | 1,0 |
| CCR5 | Chemokine (C-C motif) receptor 5 | 4,4 | 1,1 | 1,0 |
| CD86 | CD86 molecule | 1,2 | 0,9 | 1,0 |
| LY96 | Lymphocyte antigen 96 | 0,9 | 1,0 | 1,0 |
| C3 | Complement component 3 | 0,7 | 1,0 | 0,9 |
| IRAK1 | Interleukin-1 receptor-associated kinase 1 | 1,2 | 0,9 | 0,9 |
| MAPK1 | Mitogen-activated protein kinase 1 | 0,9 | 1,1 | 0,9 |
| TRAF6 | TNF receptor-associated factor 6 | 1,0 | 1,0 | 0,9 |
| JAK2 | Janus kinase 2 | 0,8 | 0,7 | 0,7 |
| TLR1 | Toll-like receptor 1 | 0,6 | 0,6 | 0,7 |
| MAPK8 | Mitogen-activated protein kinase 8 | 0,7 | 0,8 | 0,7 |
| IFNGR1 | Interferon gamma receptor 1 | 0,7 | 0,6 | 0,5 |
| RAG1 | Recombination activating gene 1 | 0,7 | 0,4 | 0,5 |
| SLC11A1 | Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1 | 1,5 | 0,4 | 0,4 |
| TLR6 | Toll-like receptor 6 | 0,5 | 0,4 | 0,4 |
| TLR4 | Toll-like receptor 4 | 0,8 | 0,4 | 0,4 |
| ITGAM | Integrin, alpha M (complement component 3 receptor 3 subunit) | 0,5 | 0,3 | 0,2 |
| TLR7 | Toll-like receptor 7 | 0,7 | 0,2 | 0,2 |

**Table S2: Complete microRNA expression dataset of human monocyte-derived macrophages following single or mixed IAV and/or SP infection.**

| Human microRNA | Fold Change (comparing to control group) | | |
| --- | --- | --- | --- |
| | IAV | SP | IAV + SP |
| hsa-miR-200a-3p | 6,9 | 3,7 | **7,3** |
| hsa-miR-298 | 3,3 | 1,1 | **4,8** |
| hsa-miR-130a-3p | 5,4 | 1,9 | **4,8** |
| hsa-miR-20b-5p | 1,9 | 4,9 | **4,7** |
| hsa-miR-196a-5p | 2,0 | 2,3 | **4,3** |
| hsa-miR-147a | 2,3 | 2,0 | **4,1** |
| hsa-miR-138-5p | 2,6 | 2,7 | **3,9** |
| hsa-miR-135a-5p | 1,4 | 2,0 | **3,8** |
| hsa-let-7c-5p | 1,2 | 2,9 | **3,7** |
| hsa-miR-194-5p | 2,8 | 1,2 | **3,7** |
| hsa-miR-451a | 1,9 | 3,1 | **3,6** |
| hsa-miR-205-5p | 4,1 | 0,5 | **3,1** |
| hsa-miR-183-5p | 3,0 | 1,8 | **2,8** |
| hsa-miR-19b-3p | 1,6 | 2,1 | **2,6** |
| hsa-miR-195-5p | 1,4 | 1,9 | **2,6** |
| hsa-miR-17-5p | 1,4 | 2,2 | **2,5** |
| hsa-let-7e-5p | 1,2 | 1,8 | **2,5** |
| hsa-miR-16-5p | 1,7 | 1,5 | **2,4** |
| hsa-miR-20a-5p | 1,3 | 2,3 | **2,3** |
| hsa-miR-31-5p | 2,1 | 2,3 | **2,3** |
| hsa-miR-182-5p | 1,9 | 1,7 | **2,2** |
| hsa-miR-155-5p | 3,0 | 1,3 | **2,2** |
| hsa-miR-142-3p | 1,4 | 2,3 | **2,1** |
| hsa-miR-29c-3p | 1,3 | 2,1 | **2,1** |
| hsa-miR-18a-5p | 1,1 | 1,9 | **2,1** |
| hsa-let-7a-5p | 1,1 | 1,4 | **2,1** |
| hsa-let-7d-5p | 1,0 | 1,8 | **2,0** |
| hsa-miR-19a-3p | 1,4 | 1,6 | **2,0** |
| hsa-miR-30b-5p | 1,3 | 1,6 | **2,0** |
| hsa-miR-34a-5p | 1,2 | 2,3 | **1,9** |
| hsa-miR-363-3p | 0,9 | 1,7 | 1,9 |
| hsa-miR-148a-3p | 1,4 | 1,0 | 1,9 |
| hsa-miR-186-5p | 1,1 | 1,4 | 1,9 |
| hsa-miR-149-5p | 1,7 | 0,9 | 1,8 |
| hsa-miR-135b-5p | 3,6 | 2,1 | 1,8 |
| hsa-miR-181a-5p | 0,9 | 2,0 | 1,8 |
| hsa-let-7g-5p | 1,6 | 1,4 | 1,8 |
| hsa-miR-126-3p | 1,2 | 1,3 | 1,7 |
| hsa-miR-21-5p | 2,0 | 1,2 | 1,7 |
| hsa-miR-210-3p | 0,9 | 1,1 | 1,7 |
| hsa-miR-29b-3p | 1,0 | 2,1 | 1,7 |
| hsa-miR-98-5p | 1,6 | 1,3 | 1,7 |
| hsa-miR-26b-5p | 1,5 | 1,3 | 1,7 |
| hsa-miR-27b-3p | 1,4 | 1,3 | 1,6 |
| hsa-miR-27a-3p | 1,0 | 1,4 | 1,6 |
| hsa-miR-379-5p | 1,7 | 0,7 | 1,6 |
| hsa-miR-15a-5p | 0,9 | 1,9 | 1,5 |
| hsa-miR-128-3p | 1,2 | 1,6 | 1,5 |
| hsa-miR-15b-5p | 1,3 | 1,5 | 1,5 |
| hsa-miR-142-5p | 1,0 | 2,8 | 1,5 |
| hsa-miR-146a-5p | 1,5 | 1,4 | 1,5 |
| hsa-miR-335-5p | 2,4 | 0,5 | 1,5 |
| hsa-miR-103a-3p | 1,2 | 1,5 | 1,4 |
| hsa-miR-30e-5p | 1,0 | 1,3 | 1,4 |
| hsa-miR-134-5p | 0,8 | 1,1 | 1,4 |
| hsa-miR-152-3p | 1,2 | 1,0 | 1,4 |
| hsa-miR-409-3p | 2,0 | 0,5 | 1,4 |
| hsa-miR-223-3p | 1,6 | 1,3 | 1,3 |
| hsa-miR-9-5p | 1,5 | 0,8 | 1,3 |
| hsa-miR-214-3p | 0,4 | 1,7 | 1,3 |
| hsa-miR-99b-5p | 1,3 | 1,0 | 1,2 |
| hsa-miR-493-3p | 1,0 | 1,5 | 1,2 |
| hsa-miR-145-5p | 1,0 | 1,7 | 1,2 |
| hsa-miR-574-3p | 1,1 | 1,1 | 1,2 |
| hsa-miR-185-5p | 1,3 | 1,1 | 1,2 |
| hsa-miR-18b-5p | 1,2 | 1,0 | 1,1 |
| hsa-miR-143-3p | 1,0 | 1,4 | 1,1 |
| hsa-miR-23b-3p | 1,3 | 1,1 | 1,1 |
| hsa-miR-30c-5p | 1,2 | 0,9 | 1,1 |
| hsa-miR-187-3p | 0,9 | 0,9 | 1,1 |
| hsa-miR-191-5p | 1,0 | 1,1 | 1,0 |
| hsa-miR-132-3p | 1,1 | 0,9 | 1,0 |
| hsa-miR-383-5p | 1,7 | 0,5 | 0,9 |
| hsa-miR-26a-5p | 1,2 | 1,1 | 0,9 |
| hsa-miR-125a-5p | 1,2 | 0,9 | 0,9 |
| hsa-miR-299-3p | 1,7 | 0,5 | 0,9 |
| hsa-miR-105-5p | 1,7 | 1,0 | 0,8 |
| hsa-miR-302a-3p | 0,8 | 1,5 | 0,8 |
| hsa-miR-125b-5p | 0,8 | 1,2 | 0,7 |
| hsa-miR-325 | 1,3 | 0,6 | 0,6 |
| hsa-miR-150-5p | 1,7 | 0,5 | 0,5 |
| hsa-miR-184 | 1,7 | 0,5 | 0,5 |
| hsa-miR-203a-3p | 1,7 | 0,5 | 0,5 |
| hsa-miR-206 | 1,7 | 0,5 | 0,5 |

**Table S3: Diagnostic accuracy values of the main significant blood-based biomarkers allowing differentiation between non-severe of severe pneumonia cases.**

| Serum-based biomarker | AUC | SE | [95% CI] | *P* | Cut-off value | Sen. (%) | Spe. (%) | LR |
|---|---|---|---|---|---|---|---|---|
| **PCT** | 0.70 | 0.06 | [0.58 - 0.82] | **<0.01** | >0.68 | 70.0 | 70.4 | 2.37 |
| **IP-10** | 0.69 | 0.06 | [0.57 - 0.82] | **<0.01** | > 4,240 | 63.3 | 63.6 | 1.74 |
| **IL-6** | 0.70 | 0.07 | [0.58 - 0.83] | **<0.01** | > 250.7 | 66.6 | 65.9 | 1.96 |
| **CRP** | 0.67 | 0.07 | [0.54 - 0.79] | *.02* | > 72.00 | 70.0 | 61.3 | 1.81 |
| **IL-8** | 0.65 | 0.07 | [0.51 - 0.78] | *.03* | > 88.70 | 63.3 | 63.6 | 1.74 |

AUC: area under curve; SE: standard error; CI: confidence interval; Sen.: Sensitivity; Spe.: Specificity; LR: likelihood ratio. Significant *P*-values (*P*< 0.05) are in bold.