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XIX Convegno Triennale sugli Aspetti Clinici e Sociali dell'Emofilia ABSTRACT N. CO-39

2.02 Laboratory issues

MYELOID CELL FUNCTION AND CYTOKINE PROFILES IN PEDIATRIC HEMOPHILIA A: INSIGHTS FROM FACTOR VIII AND EMICIZUMAB PROPHYLAXIS

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Background and Aims. Haemophilia A (HA) is an X-linked bleeding disorder caused by factor VIII (FVIII) deficiency, treated with FVIII infusions or, more recently, Emicizumab subcutaneously. Although Emicizumab is safe and effective, FVIII is still required for severe bleeding, trauma, or surgery, and few studies have compared these prophylactic options in paediatric patients. This study explores the immunological and haematological profiles of paediatric HA patients receiving FVIII or Emicizumab, using haemophilia B patients and healthy controls.

Materials and Methods. The immunological and cytokine profiles of pediatric patients with hemophilia A (stratified by treatment), hemophilia B, and healthy controls were characterized by flow cytometry and multiplex plasma assays. In parallel, macrophages were generated in vitro from patient monocytes to assess their immune response following FVIII stimulation. Cytokine levels and gene expression profiles of these cultured cells were finally quantified by ELISA and RT-qPCR assays, respectively.

Results. Clinical parameters and immune cell populations showed no major differences aside from age-related variations. However, HA patients displayed higher HLA-DR expression on CD14+ cells than healthy controls, and Emicizumab-treated patients showed increased HLA-DR expression on CD11c+ cells compared with FVIII-treated patients. Plasma cytokines including IL-12p40, TNF-α, CCL-22, IL-18, and CCL-4 were elevated in HA, suggesting a dysregulated myeloid compartment in HA. Patient-derived macrophages exhibited a stronger pro-inflammatory (M1-like) polarization after in vitro FVIII stimulation, with increased TNF-α and reduced TGF-β gene expression. Stratification by prophylaxis showed that macrophages from FVIII-treated patients maintained the M1 phenotype, whereas those from Emicizumab-treated patients showed no clear shift and tended toward an immune-regulatory profile (figure 1).

Conclusions. These findings highlight distinct myeloid and cytokine signatures associated with different prophylaxis, emphasizing the need for optimized therapeutic strategies.

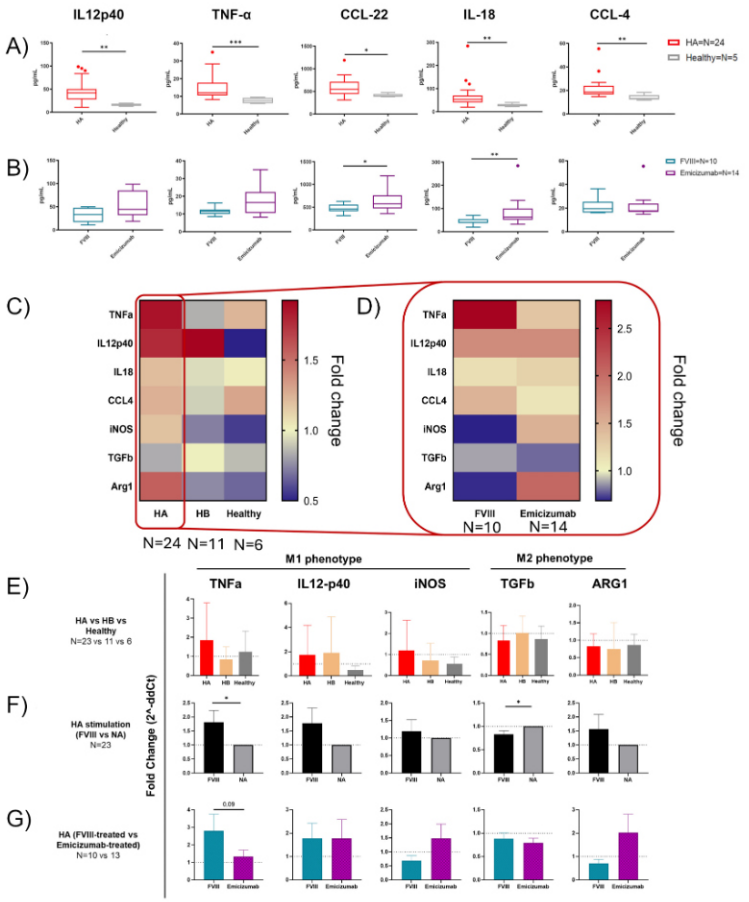


Figure 1. (A-B) Broad spectrum of monocyte/macrophages-related cytokines in haemophilic patient plasma. **(A)** Graphs showing comparison of IL12p40, TNF-a, CCL-22, IL-18 and CCL-4 quantification in plasma of HA patients and healthy donors. **(B)** Stratification of HA patients according to their undergoing treatment, either rFVIII or Emicizumab. All data are represented as a Tukey plot where the out-of-range samples are represented as a dot. Mann–Whitney test has been used for comparing the two groups (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001). **C-G)** Gene expression analysis of patient-derived macrophages stimulated with FVIII in vitro, to understand if they shift toward an M1 or M2 phenotype. **(C-D)** Heatmaps showing gene expression profiles of HA (N = 23), HB (N = 11) and healthy (N = 6) macrophages **(C)**, and subsequent stratification of HA patients according to their undergoing treatment, either rFVIII or Emicizumab **(D)**. Values for each gene are calculated as fold-change of FVIII-treated over non-treated samples and they are represented as double-gradient heatmap as group's mean value. Comparison of TNF-a, IL12p40, iNOS, TGFb and ARG1 mRNAs expression between HA, HB and healthy macrophages **(E)**, HA macrophages stimulated with FVIII and their untreated controls **(F)**, and stratification of HA patients according to their undergoing treatment, either rFVIII or Emicizumab **(G)**. Data are represented as Tukey plots (out-of-range samples are represented as a dot) and Mann–Whitney test has been used for comparing the two groups) **(A-B)**. Histogram plots with bars representing the means of the individual groups ± SD. One-way ANOVA or two-way ANOVA have been used for comparing the three groups, while unpaired t-test was used when two groups were compared **(C-G)** (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).