Cytokine and Chemokine Levels in COVID-19 Convalescent Plasma

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Short summary: Compared to controls, COVID-19 convalescent plasma (CCP) had higher distributions of IFN-γ, IL-10, IL-15, IL-21 and MCP-1, but lower IL-1RA, IL-8, IL-16 and VEGF-A. Between CCP with low vs. high neutralizing antibody titers, IL-8, IL-15 and IP-10 distributions varied significantly. (40/40 words)

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ABSTRACT

Background

The efficacy of COVID-19 convalescent plasma (CCP) is primarily ascribed as a source of neutralizing anti-SARS-CoV-2 antibodies. However, the composition of other immune components in CCP and their potential roles remain largely unexplored. This study aimed to describe the composition and concentrations of plasma cytokines and chemokines in eligible CCP donors.

Methods

A cross-sectional study was conducted among 20 pre-pandemic healthy blood donors without SARS-CoV-2 infection and 140 eligible CCP donors with confirmed SARS-CoV-2 infection. Electrochemiluminescence detection based multiplexed sandwich immunoassays were used to quantify plasma cytokine and chemokine concentrations (n=35 analytes). A SARS-CoV-2 microneutralization assay was also performed. Differences in the percent detection and distribution of cytokine and chemokine concentrations were examined by categorical groups using Fisher's exact and Wilcoxon rank-sum tests, respectively.

Results

Among CCP donors (n=140), the median time since molecular diagnosis of SARS-CoV-2 was 44 days(interquartile range=38-50) and 9%(n=12) were hospitalized due to COVID-19. Compared to healthy blood donor controls, CCP donors had significantly higher plasma levels of IFN-γ, IL-10, IL-15, IL-21 and MCP-1, but lower levels of IL-1RA, IL-8, IL-16, and VEGF-A($P<0.0014$). Significant differences were also observed in plasma levels of IL-8, IL-15 and IP-10 between CCP donors with low(<40) vs. high(≥160) anti-SARS-CoV-2 neutralizing antibody titers($P<0.0014$). The median levels of IL-6, IL-8, TNF-α, IL-12/IL23p40, MDC were significantly higher among CCP donors who were hospitalized vs. non-hospitalized($P<0.05$).

Conclusion

Heterogeneity in cytokine and chemokine composition of CCP suggests there is a different inflammatory state among the CCP donors as compared to SARS-CoV-2 naïve, healthy blood donors.

Keywords (3–10): SARS-CoV-2, COVID-19, cytokine, chemokine, convalescent plasma, neutralizing antibodies

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BACKGROUND

The ongoing coronavirus disease 2019 (COVID-19) pandemic is a challenging global health crisis. While the incidence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which causes COVID-19, continues to surge, the scientific community is exploring effective prophylactic and therapeutic options. COVID-19 convalescent plasma (CCP) is currently one of the leading therapies and was recently granted hospital Emergency Use Authorization (EUA) by the U.S. Food and Drug Administration [1-4]. Although the efficacy of CCP is primarily thought to be ascribed to neutralizing anti-SARS-CoV-2 antibodies, the composition and potential roles of other immune components in the plasma remain largely unexplored. In this study, the composition and concentrations of plasma cytokines and chemokines were evaluated in potential CCP donors with confirmed SARS-CoV-2 infection, and compared to that in healthy blood donor controls.

METHODS

Study Participants

A cross-sectional study of potential CCP donors (hereinafter referred to as CCP donors) was conducted as previously described [5-7]. From April to May 2020, eligible CCP donors (n=140) from the Baltimore/Washington DC area who had a confirmed molecular diagnosis of SARS-CoV-2 RNA (PCR+), were at least 18 years old and met the eligibility criteria for community blood donation (e.g. no pregnancy within last six weeks, never been diagnosed with HIV, HBV or HCV) were included. During this time period, the FDA did not require a specific titer for CCP donors. Plasma was separated from whole blood within 12 hours of collection and stored at -80˚C until further processing. In addition, a convenience sample of plasma from 20 platelet blood donors (New York Blood Center, NY) collected prior to December 2019 served as healthy controls (pre-COVID-19 pandemic blood donors hereinafter referred to as controls). Self-reported demographic and clinical information was collected and available for the CCP donors. However, except for ABO blood group, no other information was available for the controls.

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Patient Consent Statement

The design of the work has been approved by the Johns Hopkins University School of Medicine Institutional Review Board. All study participants (both CCP donors and controls) provided written informed consent.

Microneutralization Assay

SARS-CoV-2 neutralizing antibody (nAbs) titers against 100 50% tissue culture infectious doses ($TCID_{50}$) per 100 μL were determined using a microneutralization (NT) assay, as previously described [7]. The nAb titer was the highest plasma dilution that prevented cytopathic effect (CPE) in 50% of the wells tested. nAb area under the curve (AUC) values were estimated using the exact number of wells protected from infection at every plasma dilution.

Multiplexed Sandwich Immunoassays

Highly sensitive, multiplexed sandwich immunoassays using MULTI-ARRAY® electrochemiluminescence detection technology (MesoScale Discovery, Gaithersburg, MD, USA) were used for the quantitative evaluation of 35 different human cytokine and chemokine analytes in plasma samples from CCP donors and controls following the manufacturer's instructions. Based on previously published reports [8] and preliminary discussions with COVID-19 researchers at Johns Hopkins University, the analytes evaluated included: colony stimulating factors [GM-CSF, G-CSF (CSF3)]; eosinophil chemotactic proteins [Eotaxin, Eotaxin-3]; interleukins [IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-12/IL23p40, IL-13, IL-15, IL-16, IL-17A, IL-18, IL-21, IL-33 and receptor antagonist IL-1RA]; interferons [IFN-γ, IFN-α2a]; IFN-γ inducible protein-10 [IP-10]; macrophage-inflammatory protein [MIP-1α, MIP-1β]; macrophage-derived chemokine [MDC];

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monocyte chemoattractant protein [MCP-1, MCP-4]; tumor necrosis factors [TNF-α, TNF-β]; thymus- and activation-regulated chemokine [TARC] and vascular endothelial growth factor [VEGF-A]. Analyte concentrations were calculated per manufacturer protocol (MSD DISCOVERY WORKBENCH® analysis software) and were considered "detectable" if both runs of each sample had a signal greater than the analyte- and plate-specific lower limit of detection (LLOD) (i.e., 2.5 standard deviations of the plate-specific blank). Analyte concentrations (pg/mL) from both runs of each analyte were averaged for analysis.

Statistical Analysis

Cytokine and chemokine analytes with <80% detectability in the overall sample were analyzed as binary variables (detectable vs. not detectable) and analytes with ≥80% detectability in the overall sample were analyzed continuously, as previously published [9]. Values for analytes with results below the LLOD were imputed using a single stochastic imputation from truncated log-normal distributions fitted to the detectable values of each analyte (i.e., random values were drawn from the extrapolated tail of the distribution below the LLOD). CCP donors were categorized into groups based on the level of nAb (AUC cutoffs of <40, 40-159, ≥160 as low, middle and high titer, respectively). Differences in analyte detectability were examined between categorical groups using Fisher's exact χ^2 tests. Differences in the distribution of continuous analytes were examined between categorical groups using Wilcoxon rank-sum tests.

To assess whether the distribution of $\log_2$-transformed cytokine and chemokine analytes differed by nAb groups (<40, 40-159, or ≥160 AUC) among CCP donors, least-squares linear regression was performed separately for each analyte (dependent variable). For each analyte, adjusted β coefficients were estimated from a multivariable model that included adjustment for age (continuous), sex, and days from PCR+ diagnosis (continuous). As a sensitivity analysis, a second model was also

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constructed that additionally included adjustment for history of COVID-19 hospitalization status as a measure of severity of illness. Similar analyses were performed using modified Poisson regression for binary analytes. Adjustment for multiple comparisons across analytes was performed by using a family-wise Bonferroni correction to control the family-wise error rate at an α of 0.05 [10]; a two-sided *P value* < 0.0014 (0.05/35 analytes) was considered statistically significant. Analyses were performed in R statistical software (version 4.0.2).

RESULTS

Study Participants Characteristics

The sample of CCP donors (n=140) was 54% male with a mean age of 42 years (Supplementary Table 1). CCP donors were predominantly white (n=108), followed by Asian (n=14), Hispanic (n=5), African American (n=4), and mixed or unknown race (n=9). While most CCP donors had minor symptoms and did not require any hospitalization, 12 (9%) donors had previously been hospitalized due to COVID-19. At the time of blood collection, a median of 44 days (IQR=38-50) had elapsed since participants had been diagnosed with SARS-CoV-2 infection by PCR. There were 60 CCP donors in the low nAb group (43%), 35 in the middle nAb group (25%), and 45 in the high nAb group (32%), for whom epidemiologic characteristics are given in Supplementary Table 1.

Plasma cytokine and chemokine levels in CCP donors

For each of the cytokine and chemokine analytes tested, LLOD range (pg/mL) and total number of detectable samples in CCP donors and controls were calculated. For all analytes, the overall mean coefficient of variation between runs was <15% (Supplementary Tables 2 and 3). The correlation between cytokines and chemokines varied substantially and is shown in (Figure 1).

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Among the analytes with ≥80% overall detectability, the median levels of IL-6, IL-8, TNF-α, IL-12/IL23p40 and MDC were significantly higher among CCP donors who were hospitalized vs. non-hospitalized (Wilcoxon rank sum P <0.05, Supplementary Table 4); the median levels of Eotaxin were significantly lower among those hospitalized vs. non-hospitalized (Wilcoxon rank sum P <0.05, Supplementary Table 4). For analytes with <80% detectability, no significant difference in percent detection was observed among CCP donors by hospitalization status (Supplementary Table 5).

Among the analytes with ≥80% detectability, the distribution of plasma IFN-γ, IL-10, IL-15, IL-21 and MCP-1 were significantly higher in CCP donors as compared to the controls, whereas the distribution of IL-1RA , IL-8, IL-16, and VEGF-A levels were significantly lower in CCP donors than the controls after adjusting for multiple comparison using Bonferroni correction (Wilcoxon rank-sum P<0.0014) (Figure 2). Among CCP donors and the analytes with ≥80% detectability, the distribution of IL-8, IL-15, and IP-10 were significantly higher in the high nAb titer group (≥160 AUC) compared to the low nAb titer group (<40 AUC) (Wilcoxon rank-sum P<0.0014) (Figure 3). In multivariable regression analysis, only the distribution of IL-15 was significantly higher in the high nAb titer group than those in the low nAb titer group (AUC <40) after adjusting for age, sex, days since PCR+ diagnosis as well as for multiple comparisons (P < 0.0014) (Supplementary Table 6). Similar results were obtained in the model that adjusted for prior hospitalization status.

Among analytes with <80% detectability, the percent detection of IL-13, IL-1β, IL-4, and G-CSF was significantly lower among CCP donors than controls after adjusting for multiple comparisons (Fisher's exact P < 0.0014) (Figure 4). There were, however, no statistically significant differences by nAb titer group among CCP donors after adjusting for multiple comparisons (Figure 5; Supplementary Table 7).

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DISCUSSION

In addition to nAbs, convalescent plasma may contain cytokines (pro- and/or anti-inflammatory), clotting factors, natural antibodies, defensins, pentraxins and other undefined proteins [11]. Antibodies in convalescent plasma may also mediate their therapeutic effects through a variety of mechanisms (e.g. direct virus neutralization, antibody mediated complement activation, antibody-dependent cellular cytotoxicity, and/or phagocytosis) [11, 12]. While studies have evaluated the acute phase plasma of COVID-19 patients [13-15], sparse data exist on recovered COVID-19 patients, and the components in convalescent plasma. In addition, little is known regarding whether a particular cytokines or chemokines profile is associated with higher nAb. This study demonstrates that CCP has a different cytokine and chemokine profile than that of the plasma of SARS-CoV-2 naïve controls, and that IL-8, IL-15 and IP-10 were associated with higher nAb among CCP donors.

Distributions of IFN-γ, IL-10, IL-15, IL-21 and MCP-1 were significantly higher in CCP as compared to control plasma. Although no significant difference in median levels of these cytokines and chemokines has been observed in the hospitalized CCP donors (n=12), this may still reflect residual effects of the heightened immune response that were present during acute phase of the disease in severe as well as moderately ill COVID-19 patients as previously reported [16, 17]. From a functional standpoint, some of these cytokines (IL-10, IL-15 and IL-21) may be involved in B cell survival, differentiation into plasma cells and class switching, while others may impart an inhibitory effect on B cells in certain situations (e.g. IL-8, IFN-γ) [18-20]. In addition, it has recently been reported that individuals with inborn errors of type I IFN immunity is associated with life-threatening COVID-19 pneumonia [21]. Of note, some of the analytes tested (e.g. IL-1RA, IL-8, IL-16, and VEGF-A) were significantly lower in CCP donors than those from the control cohort, the biological relevance of which requires further investigation. This is in contrast to a previous report by Chi et. al. where levels of all 48 cytokines and chemokines tested were very similar between a small sample of convalescent (n=4) and control subjects (n=4) [17].

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In a well-controlled immune response, cytokine and chemokine expression is tightly regulated, and loss of this control may have unintended consequences. For instance, IL-10, a key regulator of immune system homeostasis and an anti-inflammatory cytokine, was also associated with sustained chronic infection when expressed aberrantly [22]. Virus-induced aberrant inflammatory responses have been associated with the pathogenesis of many viral diseases including the coronaviruses [8]. Excessive cytokine production, also known as hypercytokinemia, has been linked to pulmonary inflammation and acute lung injury in SARS, MERS-CoV, and in SARS-CoV-2 infected patients [8, 23-26]. Longitudinal immune profiling of COVID-19 has revealed an inflammatory cluster of IL-1α, IL-1β, IL-6, IL-10, IL-12 p70, IL-17A, IFNα, thrombopoietin (TPO), IL-33, IL-16, IL-21, IL-23, IFNλ, eotaxin and eotaxin 3 in patients with severe disease [16]. Transfusion of plasma with elevated levels of some of these cytokines, could contribute to some of the biological effects associated with CCP administration in COVID-19 patients. In this regard, the administration of these cytokine and chemokines may have an immunomodulatory effect in less critical patients via amelioration of the severe inflammatory response (e.g. anti-inflammatory IL-10) and/or antibody production (e.g. IL-21 in plasma cell generation).

There are limitations to this study. The study is cross-sectional and not designed to infer causal associations. The number of available controls was relatively low, and limited demographic and clinical data were available for the control group, which may have resulted in unmeasured confounding. Nevertheless, findings of this study suggest heterogeneity in cytokine and chemokine composition and levels in CCP. Indeed, the biological significance of the variations in several cytokine and chemokine levels observed in this study requires investigation.

The markedly different inflammatory states in the CCP donors observed in this study may have particular significance to long-term recovery from COVID-19, and also the therapeutic potential of CCP. A recent study of 55 people recovering from COVID-19 in China showed abnormal lung scans

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and lung function three months after discharge from the hospital, and elevated serum levels of D-dimer was associated to people with more lasting lung problems [27]. Even in outpatients with milder COVID-19 illness, delay in complete recovery with prolonged symptoms like cough, fatigue, or shortness of breath has been reported in the US [28].These studies demonstrate that individuals have markedly different inflammatory states long after symptoms have resolved, and these may contribute to the lingering symptoms (e.g. shortness of breath, cough, gastrointestinal problems, headache, or fatigue) reported in the COVID-19 long-haulers. Thus, the levels of CCP cytokines and chemokines provide important insights into overall recovery status of the convalescing patients, as well as its suitability as a therapy for COVID-19 patients with different disease severity. Larger and longer-term CCP studies are also necessary to evaluate their immunomodulatory effects in COVID-19 patients.

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Potential conflict of interest

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All other authors declare no conflicts of interest.

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Figure 1. Correlation heatmap between cytokines and chemokines among COVID-19 convalescent plasma (CCP) donors.

Note: This analysis is restricted to analytes that had ≥80% overall detectability.

Figure 2. Distribution of log2 cytokine and chemokine levels among pre-COVID-19 pandemic blood donors (controls) to COVID-19 convalescent plasma (CCP) donors.

Note: There were 20 controls and 140 CCP donors. P values were determined from Wilcoxon rank sum test. Only p values < 0.05 are presented. Unit of the analytes: log2-transformed pg/mL.

* significant p values after adjusting for multiple comparison using Bonferroni correction (P < 0.0014).

Figure 3. Distribution of log_2 cytokine and chemokine levels by neutralizing antibody (nAb) titer group among COVID-19 convalescent plasma donors.

Note: There were 60 samples in the <40 group, 35 in the 40-159 group, and 45 in the ≥160 group. *P* values were determined by Wilcoxon rank sum test. Only *p* values < 0.05 are presented. Unit of the analytes: log_2-transformed pg/mL.

* significant *p* values after adjusting for multiple comparison using Bonferroni correction (*P* < 0.0014).

Figure 4. Comparisons of percent detection of cytokines and chemokines among pre COVID-19 blood donors (controls) to COVID-19 convalescent plasma (CCP) donors.

Note: This analysis is restricted to analytes that had <80% detectability. There were 20 controls and 140 CCP donors. *P* values were determined by Fisher's exact test. Only *p* values < 0.05 are presented.

* significant *p* values after adjusting for multiple comparison using Bonferroni correction (*P* < 0.0014).

Figure 5. Comparisons of percent detection of cytokines and chemokines by neutralizing antibody (nAb) titer group among COVID-19 convalescent plasma (CCP) donors.

Note: This analysis is restricted to analytes that had <80% detectability. P values were determined by fisher's exact tests. Only *p* values < 0.05 are presented. No *p* value was significant after adjusting for multiple comparison using Bonferroni correction (*P* < 0.0014).

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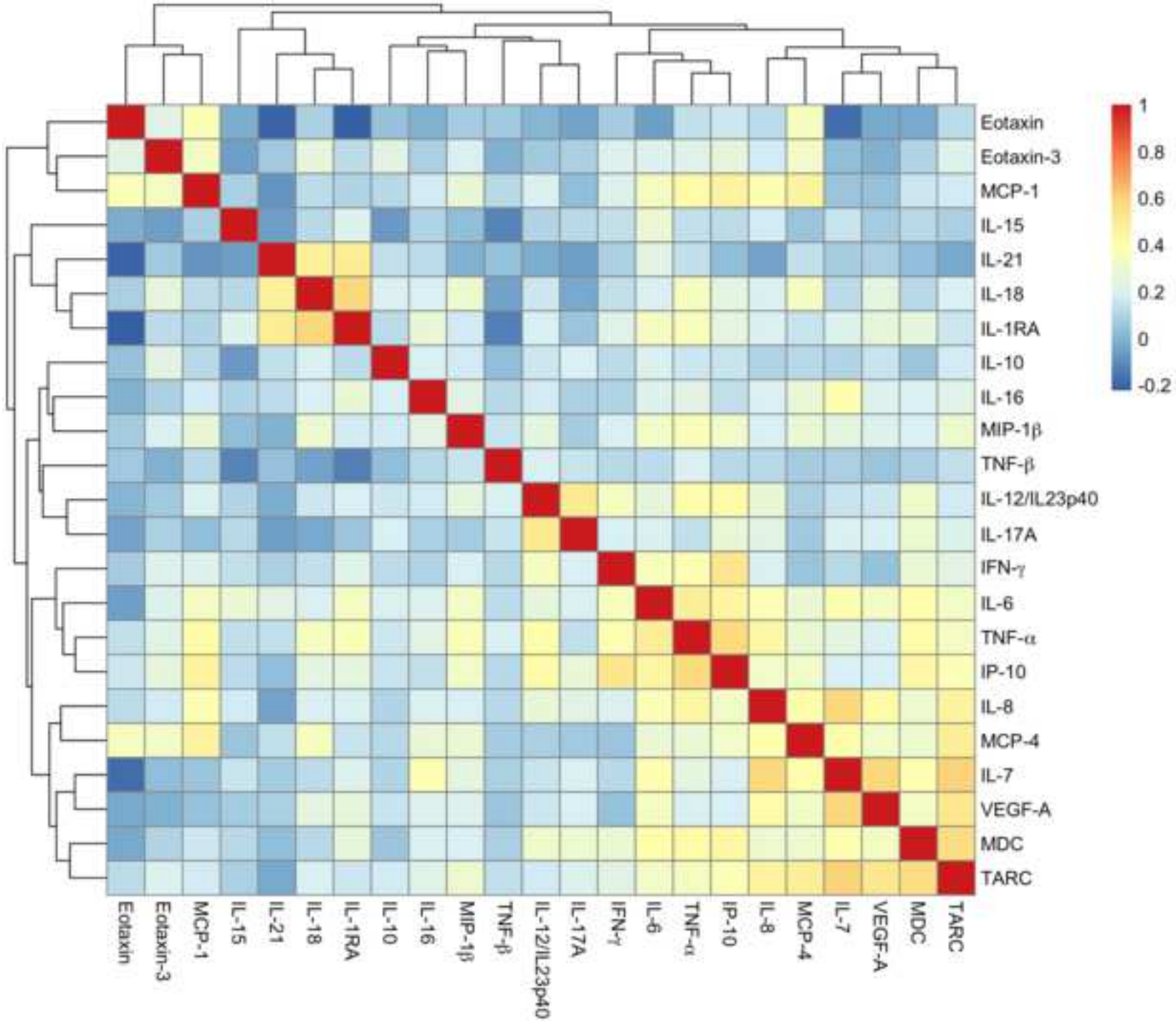
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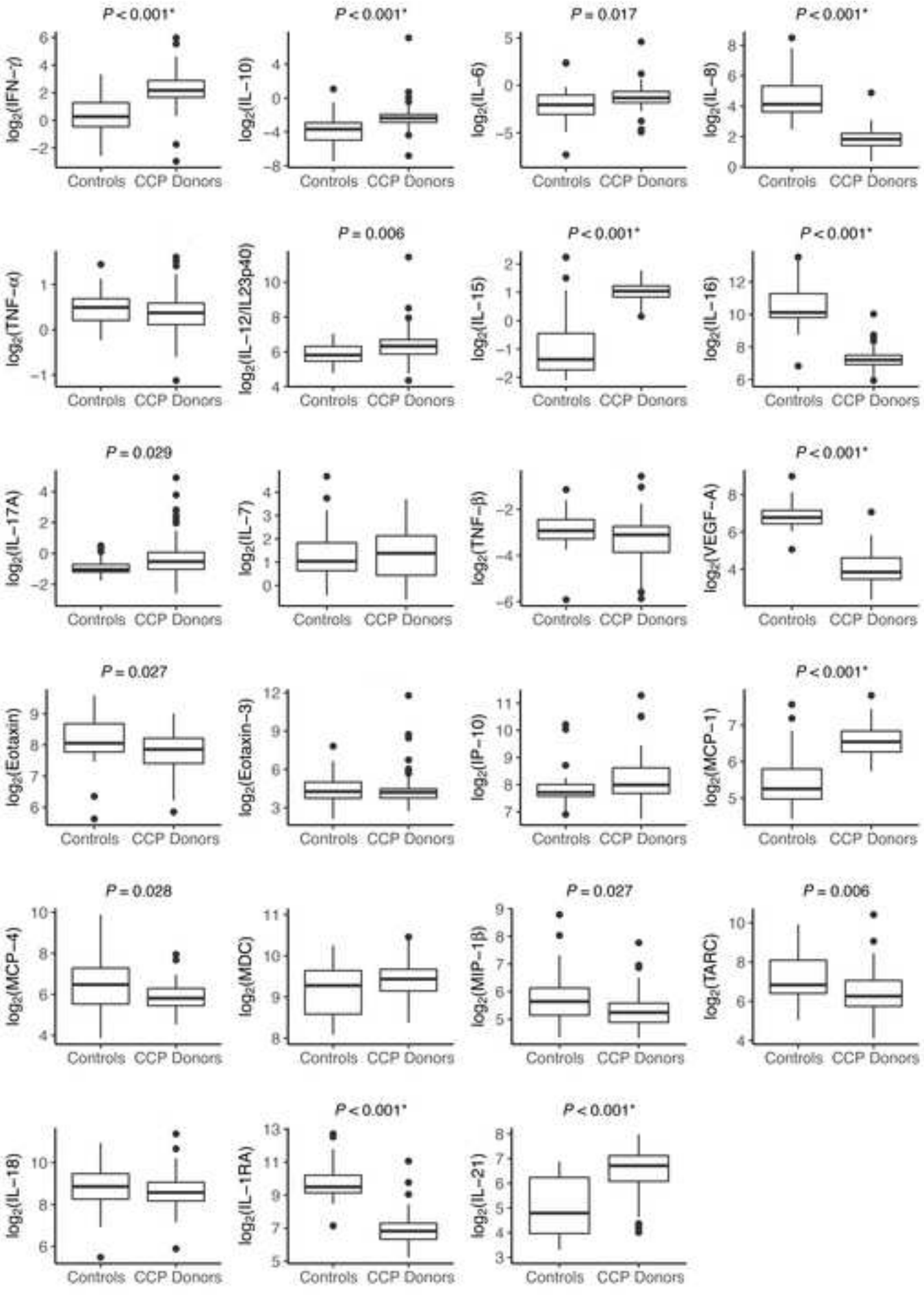
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P = 0.017
log2(IL-6)
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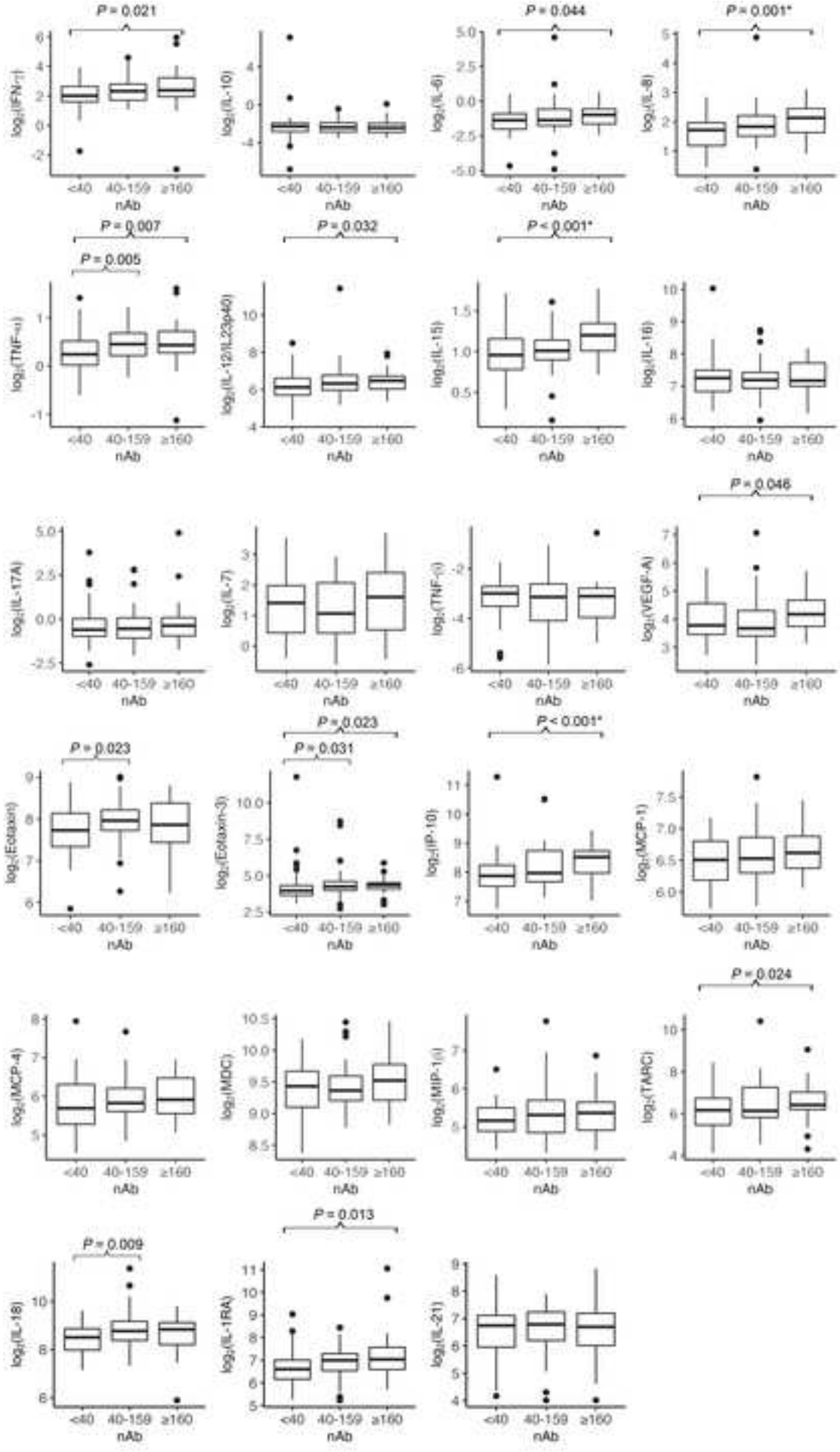
log2(IL-8)
Controls CCP Donors
P = 0.006
log2(TNF-α)
log2(IL-12/IL23p40)
P < 0.001*
log2(IL-15)
P < 0.001*
log2(IL-16)
P = 0.029
log2(IL-17A)
log2(IL-7)
log2(TNF-β)
P < 0.001*
log2(VEGF-A)
P = 0.027
log2(Eotaxin)
log2(Eotaxin-3)
log2(IP-10)
P < 0.001*
log2(MCP-1)
P = 0.028
log2(MCP-4)
log2(MDC)
P = 0.027
log2(MIP-1β)
P = 0.006
log2(TARC)
log2(IL-18)
P < 0.001*
log2(IL-1RA)
P < 0.001*
log2(IL-21)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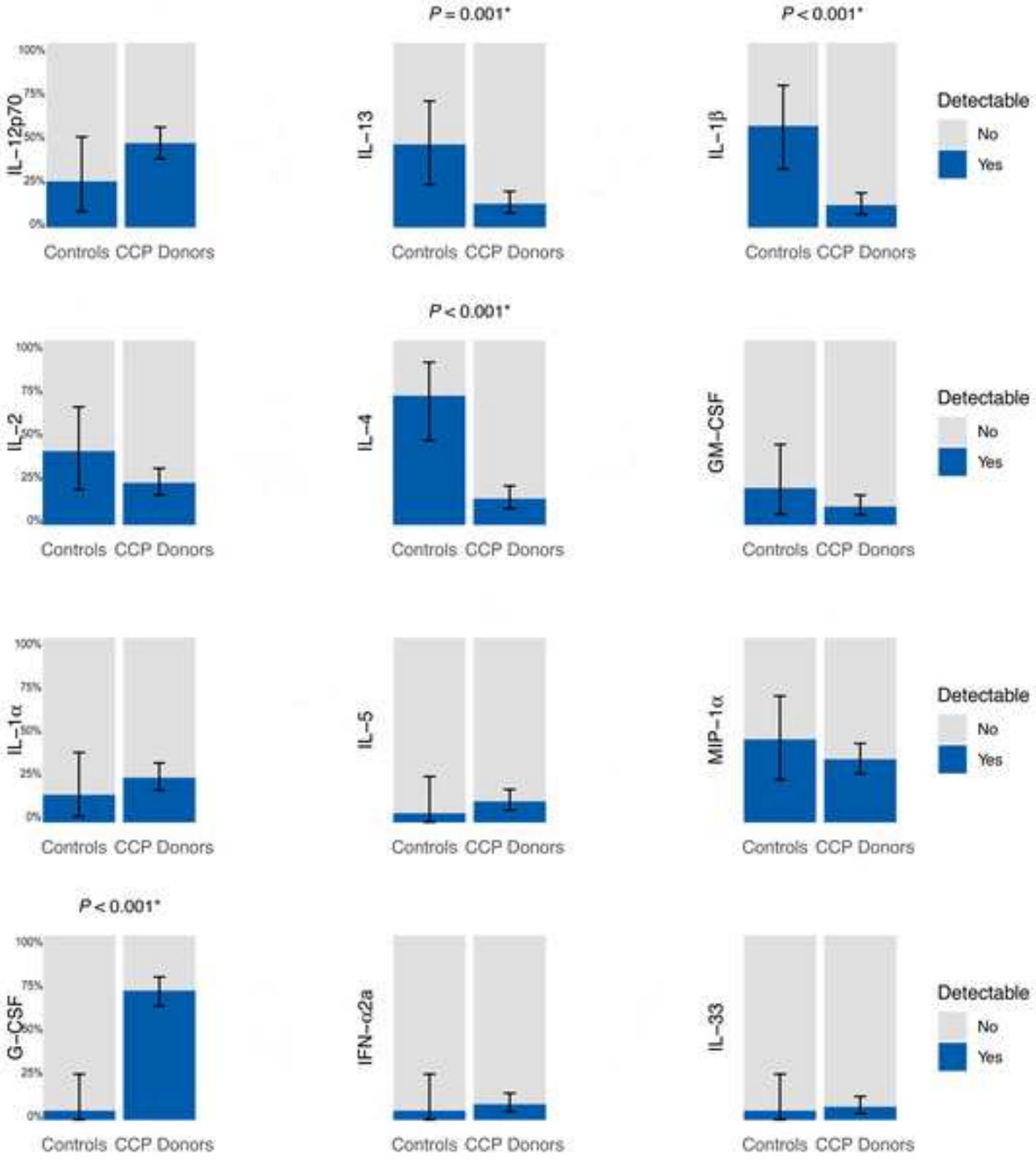
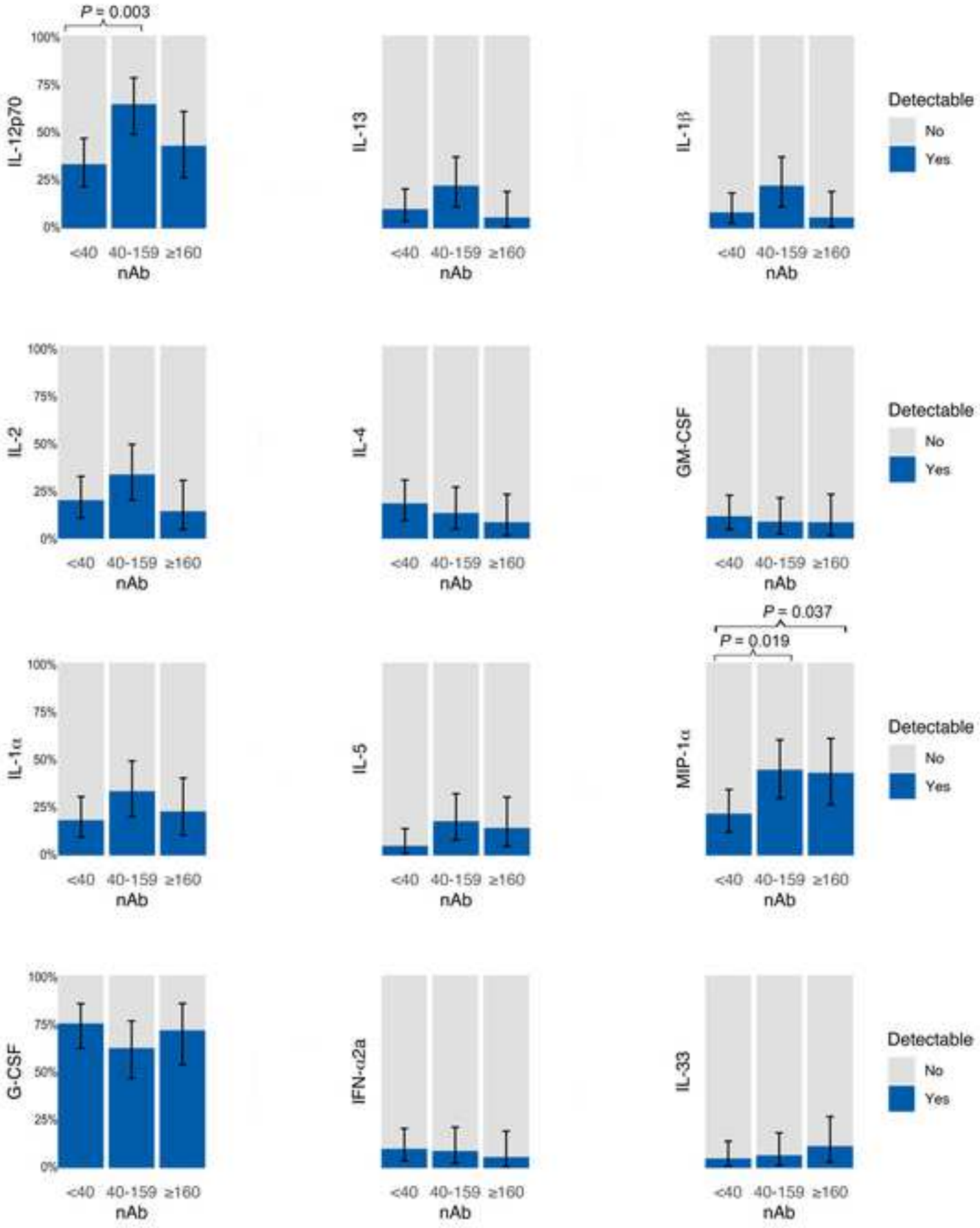
P = 0.044
log2(IL-6)
P = 0.001*
log2(IL-8)
log2(IL-10)
P = 0.007
P = 0.032
P < 0.001*
P = 0.005
log2(TNF-α)
log2(IL-12/IL23p40)
log2(IL-15)
log2(IL-16)
<40
40-159
≥160
nAb
P = 0.046
log2(IL-17A)
log2(IL-7)
log2(TNF-β)
log2(VEGF-A)
P = 0.023
P = 0.031
P < 0.001*
log2(Eotaxin)
log2(Eotaxin-3)
log2(IP-10)
log2(MCP-1)
P = 0.024
log2(MCP-4)
log2(MDC)
log2(MIP-1α)
log2(TARC)
P = 0.009
P = 0.013
log2(IL-18)
log2(IL-1RA)
log2(IL-21)


IL−12p70
IL−13
P = 0.001*
IL−1β
P < 0.001*
Detectable
No
Yes
IL−2
P < 0.001*
IL−4
GM−CSF
IL−1α
IL−5
MIP−1α
G−CSF
P < 0.001*
IFN−α2a
IL−33
100%
75%
50%
25%
0%
Controls
CCP Donors


P = 0.003
IL-12p70
IL-13
IL-1β
IL-2
IL-4
GM-CSF
P = 0.037
P = 0.019
IL-1α
IL-5
MIP-1α
G-CSF
IFN-α2a
IL-33
100%
75%
50%
25%
0%
<40
40-159
≥160
nAb
Detectable
No
Yes