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Associations between per- and poly-fluoroalkyl substance (PFAS) exposure and immune responses among women in the California Teachers study: A cross-sectional evaluation

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Abstract

Introduction: Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants that have been linked to a number of health outcomes, including those related to immune dysfunction. However, there are limited numbers of epidemiological-based studies that directly examine the association between PFAS exposure and immune responses.

Methods: In this cross-sectional study nested in the California Teachers Study cohort, we measured nine PFAS analytes in serum. Of the 9 analytes, we further evaluated four (PFHxS [perfluorohexane sulfonate], PFNA [perfluorononanoic acid], PFOA [perfluorooctanoic acid], PFOS [perfluorooctanesulfonic acid]) that had detection levels of > 80 %, in relation to 16 systemic inflammatory/immune markers and corresponding immune pathways (Th1 [pro-inflammatory/macrophage activation], B-cell activation, and T-cell activation). Study participants

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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(n = 722) were female, completed a questionnaire regarding various health measures and behaviors, and donated a blood sample between 2013–2016. The association between PFAS analytes and individual immune markers and pathways were evaluated by calculating odds ratios (OR) and 95 % confidence intervals (CI) in a logistic regression model. PFAS analytes were evaluated both as a dichotomous exposure (above or below the respective median) and as a continuous variable (per 1 unit increase [ng/mL]).

Results: The prevalence of detecting any PFAS analyte rose with increasing age, with the highest PFAS prevalence observed among those aged 75 + years and the lowest PFAS prevalence observed among those aged 40–49 years (study participant age range: 40–95 years). Significant associations with BAFF (B-cell activating factor) levels above the median were observed among participants with elevated (defined as above the median) levels of PFHxS (OR=1.53), PFOA (OR=1.43), and PFOS (OR=1.40). Similarly, there were statistically significant associations between elevated levels of PFHxS and TNFR2 (tumor necrosis factor receptor 2) levels (OR=1.78) and IL2R α (interleukin 2 receptor subunit alpha) levels (OR=1.48). We also observed significant inverse associations between elevated PFNA and sCD14 (soluble cluster of differentiation 14) (OR=0.73). No significant associations were observed between elevated PFNA and any immune marker. Evaluation of PFAS exposures as continuous exposures in association with dichotomized cytokines were generally consistent with the dichotomized associations.

Conclusions: PFAS exposure was associated with altered levels of circulating inflammatory/immune markers; the associations were specific to PFAS analyte and immune marker. If validated, our results may suggest potential immune mechanisms underlying associations between the different PFAS analytes and adverse health outcomes.

Keywords

Perfluoroalkyl substances; Polyfluoroalkyl substances; B-cell activating factor (BAFF); Tumor necrosis factor receptor 2 (TNFR2); Interleukin 2 receptor subunit alpha (IL2R α); Human population

1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are ubiquitous chemicals whose use began in the 1940's [1]. They are found in numerous items of daily use, including food packaging, cleaning products, carpeting, and firefighting foam [1]. There are over 12,000 PFAS chemicals, and some of these chemicals are pervasive and persistent environmental contaminants; termed “forever chemicals”, these PFAS do not degrade easily and maintain legacy status, meaning they remain persistent in the environment even after they are no longer used in production. Some PFAS analytes, such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), are thought to persist in the environment for up to 1000 years [2], and some PFAS analytes, including PFOA and PFOS, have a half-life of 3–8 years within the human body [3,4]. PFAS analytes are also known to bioaccumulate in human tissue, act as endocrine disrupting chemicals, and/or promote carcinogenesis [5,6]. PFAS are now ubiquitous in the human population; U.S. data from NHANES in 2011–2012 reported detectable PFAS in 97 % of 1887 human serum samples evaluated [7].

PFAS exposure has been linked to various health outcomes and diseases, including pregnancy related outcomes (e.g., preeclampsia and lower birth weight), higher cholesterol levels, and kidney and testicular cancer [8–10]. More importantly, PFAS exposure have been linked specifically to immune-related endpoints, including altered vaccine responses and immune suppression through decreasing antibody production, hindering immunoglobulin production, and decreasing the amount of circulating immune cells [11–14]. To date, there are limited epidemiology-based studies that have investigated the association between PFAS and cytokine levels circulating in human serum, the latter serving as a surrogate measure for immune function and response [14–20]. Gaps in the current literature include 1) the limited number of PFAS exposures and immune markers evaluated and 2) the conflicting results that have been reported thus far [14]. Because immune dysregulation and chronic inflammation are recognized as key biological mechanisms for disease development and progression [21], we sought to specifically evaluate the link between PFAS and immune dysregulation.

Here, we measured 9 PFAS analytes and 16 immune markers and cytokines in 722 women cross-sectionally from the California Teachers Study. Specifically, we aimed to understand the association between levels of PFAS chemicals detected in the blood to circulating levels of cytokine and immune markers in blood at the same point in time, thereby providing critical clues on whether exposures to PFAS are linked to inflammation or immune alterations. Additionally, this study of largely post-menopausal women is particularly significant as a study of a potentially susceptibility population where immune response is known to be depressed and the occurrence of autoimmune conditions is higher. Moreover, the older age group of our population further embodies a higher cumulative exposure, particularly for this population which has been exposed to PFAS virtually their entire lifetime. Our investigation of PFAS and altered immune regulation in this population is thus timely and of biological significance in understanding potential downstream health effects [22,23].

The PFAS analytes included in our study (specifically, PFOA, PFOS, PFNA, and PFHxS [perfluorohexane sulfonate]) are considered legacy analytes, thus still prevalent in our environment despite being phased out in the early 2000's by the EPA (Environmental Protection Agency) [24]. As recently as 2022, the Centers for Disease Control and Prevention reported that legacy PFAS analytes continue to be detectable in human serum samples. Specifically, 10 communities identified across multiple states within the United States, including Massachusetts, West Virginia, Delaware, New York, Washington, Texas, Colorado, and Arkansas, had legacy PFAS analytes present in their water [25], and, in these same 10 communities, the populations had higher age-adjusted blood levels of PFHxS, PFOS, PFOA and PFNA than national levels [25]. A 2016–2021 study further reported legacy PFAS contamination in 45 % of United States drinking water samples (of 716 tap water samples) [26]. Given the ubiquity of PFAS, the growing number of health outcomes associated with PFAS exposure and the dearth of understanding of what biological mechanisms by which PFAS leads to these outcomes, our study is timely and fills current gaps in the literature. We hypothesized that individuals with a higher burden of detected PFAS exposure would be associated with detectable differences in immune markers.

2. Methods

2.1. Study population

The California Teachers study (CTS) is a prospective cohort study that began in 1995 and included a population of women that were active or recently retired public school professionals (mainly teachers). The CTS cohort has been previously described [27], and the CTS has been approved by the Institutional Review Board of City of Hope. Initially, there were 133,477 participants enrolled in the CTS; of which, 65,229 participants completed a questionnaire from 2012 to 2015 (Questionnaire 5 or Q5). Further standard exclusion, including those who lived outside of California at baseline, who only consented to breast cancer research, and who provided invalid baseline data, were applied, resulting in 61,984 eligible participants. For this cross-sectional study, 722 participants (of the 61,984) were selected based on their participation in completing a questionnaire from 2012 to 2015 (Questionnaire 5 or Q5) and participating in a biobanking study from 2013 to 2016 [28] and had to be free of cancer at the time of the blood draw. The participants are a subset of the population originally selected as part of a larger study on sleep characteristics and for whom cytokine measurements were previously conducted. As described in Wang et al (2022), this subpopulation's demographic characteristics did not differ from that of the overall study population or from those that participated in the biobanking study [29].

2.2. Population characteristics

In the baseline questionnaire, participants answered questions regarding their age, race/ethnicity (non-Hispanic White or other), socioeconomic status (SES; in quartiles determined by occupation, education, and income), and rural/urban residence (rural, town, city, metropolitan suburban, or metropolitan urban; according to the 1990 census block groups). In the follow-up Q5, participants provided updated information on physical activity (moderate/strenuous: 0–2.37, 2.38–5.88, 5.88 + hrs/week), nonsteroidal anti-inflammatory drug (NSAID) use (none/1/week or > 1/week), statin use (none or > 1/week), and weight (for which updated body mass index was calculated [BMI: 15–24, 25–29, or 30 + kg/m²]). These covariates were selected based on our prior evaluation of host characteristics that demonstrated their association with immune markers [29].

2.3. Cytokine measurements

We previously measured cytokines using two multiplex panels, as described in [29]. Briefly, Human Biomarker A Panel was used to measure human inflammatory cytokines (i.e., interleukin-1 beta [IL-1 β], interleukin-2 [IL-2], interleukin-4 [IL-4], interleukin-6 [IL-6], interleukin-8 [IL-8], interleukin-10 [IL-10], interferon gamma [IFN- γ], and tumor necrosis factor alpha [TNF- α]), and Soluble Receptor Human Panel was used to measure soluble receptors and chemokines (i.e., B-cell activating factor [BAFF], C-X-C motif chemokine ligand 13 [CXCL13], soluble cluster of differentiation 14 [sCD14], soluble cluster of differentiation 27 [sCD27], glycoprotein 130 [GP130], interleukin-2 receptor subunit alpha [IL2R α], interleukin-6 receptor subunit alpha [IL6R α], and tumor necrosis factor receptor 2 [TNFR2]). Limit of detection per immune marker and coefficient of variations for these samples and panels have been previously described [29].

2.4. PFAS measurements

Of the participants with cytokine measurements available, 722 had sufficient serum aliquots required for PFAS measurements. Serum aliquots (1 mL) were sent to Mount Sinai Targeted Analysis Laboratory Hub where 9 PFAS analytes were measured: perfluorooctane sulfonate total (PFOS), perfluorooctanoic acid total (PFOA), perfluorohexane sulfonate (PFHxS), perfluorononanoic acid (PFNA), perfluorohexanoic acid (PFHXA), perfluoroheptanoic acid (PFHPA), perfluoroheptanesulfonic acid (PFHPS), perfluorodecanoic acid (PFDA), and perfluoroundecanoic acid (PFUNDA).

Quantification of PFAS analytes in serum was conducted as described in Kato et al. (2018) with minor modifications [30–32]. Initially, each sample received ^{13}C stable isotope labeled internal standards, then they were processed by solid-phase extraction with an Oasis WAX mixed-mode polymeric reversed-phase 96-well plate (30 mg sorbent per well, 30 μm particle size; Waters Corporation, Milford, MA). The LC-MS/MS (Agilent 1290 Infinity II UHPLC coupled with 6470A triple quadrupole MS, Agilent Technologies, Wilmington, DE) was operated in electrospray negative mode and multiple reaction monitoring (MRM) was performed for ionization and quantification, respectively. Chromatographic separation was performed on an InfinityLab Poroshell 120 EC-C18, 1.9 μm , 100 $\times$ 2.1 mm analytical column with 5 $\times$ 2.1 mm guard cartridge (Agilent Technologies, Wilmington, DE). Instrument-specific PFAS background levels were removed by Eclipse Plus C18, 3.5 μm , 50 $\times$ 4.6 mm delay column (Agilent Technologies, Wilmington, DE). The PFAS assay has been validated through proficiency testing and consistently met acceptance criteria in the G-EQUAS [33] and CTQ-AMAP programs [34]. The laboratory's internal quality control accounted for 20 % and contained reagent- and matrix-based experimental blanks, matrix spikes at three different validation levels (low, medium, and high), NIST standard reference materials (SRM 1957 and SRM 1958), and archived proficiency testing material [30,32], as outlined in Human Health Exposure Analysis Resource (HHEAR) [35]. The limits of quantification (LOQ) for the PFAS analytes in the assay ranged from 0.3 to 0.7 ng/mL. Machine-read values were provided for all measurements and were used for biomarker values below the LOQ. For external quality control and to assess assay performance, 50 blinded QC paired-duplicate samples were run in parallel with the study samples. PFAS analytes that had a percent detection ≥ 80 % (PFHxS, PFNA, PFOA and PFOS) were included in subsequent analyses, concordant with previous human studies on PFAS [36–38].

2.5. Statistical analysis

We created natural log-transformed values of each cytokine/immune marker and each PFAS analyte. For logistic regression models, both exposure (PFAS) and outcome (cytokine/immune markers) were dichotomized as above or below the respective median. Select cytokine/immune markers with detection levels < 50 % (IL-2, IL-4, and IFN- γ) were dichotomized as detectable vs non-detectable.

Logistic regression models were first performed to evaluate the association between participant characteristics and PFAS analytes, specifically among covariates previously identified to be associated with cytokine/immune markers [29]. Because of the pronounced association between age and PFAS analytes, and because no other covariate was associated

with both PFAS and cytokines, only age was included in all subsequent models. We note that even with a conservative approach whereby a fully saturated model was tested including all covariates, the resulting associations did not change by > 10 %, further justifying the inclusion of only age in our final logistic regression models. In sensitivity analyses, we also evaluated PFAS analytes as a continuous variable (per ng/mL) in relation to dichotomized immune markers as the outcome.

Evaluation of PFAS analytes (logistic and continuous) were further conducted with immune pathways as the outcome (dichotomized as either having circulating immune markers reflecting the respective immune pathway or not). Briefly, immune pathways were defined as: 1) Th1: elevated IFN- γ and decreased IL-10 and IL-4) pro-inflammatory/macrophage activation: elevated levels of TNF- α , TNFR2, IL-6, IL-1 β , IL-8, IL6R α , IL-10, and sCD14, 3) B-cell activation: elevated levels of BAFF, IL-10, IL-4, IL-6, sCD27, CXCL13, and 4) T-cell activation: elevated levels of IL-2, IL2R α , IFN- γ , IL-4, IL-6 [29]. For each pathway, the distribution (and median) of the number of elevated immune markers was determined based on the presence of each marker defined above. Each pathway was dichotomized based on the median number of elevated immune markers; those above the median number of markers present were defined as exerting the specific pathway versus not having characteristics of that pathway (e.g., below median).

Statistical analyses were performed using SAS 9.4 and all statistical tests were two-sided (SAS Institute Inc., Cary, NC). Any figures were created using GraphPad Prism 9.5.0 [39] and all analyses were performed via the CTS Researcher Platform [40].

3. Results

3.1. Study population characteristics

Study population characteristics are described in Table 1. Of 722 participants, 59.1 % were greater than 60 years old and 79.2 % identified as non-Hispanic White. The majority (72.1 %) of the study population also reported living in suburban locations. Population characteristics of the analytic subset were comparable to those from the original biobanking study, including body mass index, diabetes, physical activity and NSAID use (Table 1).

Notably, PFOA, PFOS, PFNA, and PFHxS levels all increased with age (Fig. 1). Statistically significant stepwise increases in concentration (ng/mL) for all four analytes were observed by increasing decade of age (PFOA p-trend < 0.0001; PFOS p-trend < 0.0001; PFNA p-trend = 0.0257; PFHxS p-trend < 0.0001). The age-association was most pronounced for PFHxS, and as further shown in Supplemental Table 1, among women 50–59 years old we observed a 3.84-fold association with elevated PFHxS levels (compared to women 40–49 years old), which increased to 9.40 and 15.82 for women 60–69 years old and 70 + years old, respectively. Associations were also striking for PFOS and PFOA, and least pronounced for PFNA, though still statistically significant (Supplemental Table 1).

3.2. PFAS analyte and cytokine measurements

Descriptive statistics for the 9 measured PFAS analytes are shown in Supplemental Table 2. Consistent with the current population-based literature, 4 of the 9 PFAS analytes were

detected in ≥ 80 % of study participants: PFHxS, PFNA, PFOA and PFOS [16,37,41]. The medians for PFHxS, PFNA, PFOA, and PFOS were 1.28 ng/mL, 0.78 ng/mL, 2.03 ng/mL, and 4.63 ng/mL, respectively (Supplemental Table 2). The remaining PFAS analytes had medians that were below the limit of quantification. Descriptive statistics for the 16 measured immune markers are shown in Supplemental Table 3.

3.3. Covariate determination for multivariable models

Results of univariate models between population characteristics and PFAS levels are shown in Supplemental Table 1. In addition to associations with older age, statin use (>1 /week) was also associated with a ~ 2 -fold association with higher levels of all 4 PFAS analytes (Supplemental Table 1); other sporadic associations were noted between higher SES and PFOA and between NSAID use and PFHxS. No other covariate previously found associated with our immune markers [29] were associated with any PFAS analyte. However, we note that the associations with statins, SES and NSAID use were no longer significant once age was included in the model; it is thus likely that these associations are confounded by age (e.g., older age groups accounted for the use of statins/NSAIDs and with higher SES). This also further supports the inclusion of age as the singular covariate of interest for our multivariate logistic regression models.

3.4. Cytokine levels and PFAS exposure associations

Dichotomized, age-adjusted associations between PFAS exposure and cytokine levels are shown in Table 2. Elevated levels (defined as above the median) of PFHxS (OR=1.53, 95 % CI=1.12–2.09), PFOA (OR=1.43, 95 % CI=1.06–1.95) and PFOS (OR=1.40, 95 % CI=1.03–1.90) were statistically significantly associated with increased (above the median) levels of BAFF, compared to below the median. PFHxS was also associated with elevated levels of TNFR2 (OR=1.78, 95 % CI=1.29–2.45) and elevated levels of IL2R α (OR=1.48, 95 % CI=1.08–2.03) (Table 2). No significant positive associations were observed between PFNA exposure and any immune marker; however, increased levels of PFNA were associated with decreased levels of sCD14 (Table 2).

Evaluation of PFAS exposures as continuous exposures in association with dichotomized cytokines were generally consistent with the dichotomized associations discussed previously (Supplemental Table 4). Associations with BAFF were also statistically significant between per ng/mL increases in PFHxS (OR=1.34, 95 % CI=1.06–1.68) and PFOS (OR=1.53, 95 % CI=1.20–1.94); associations with TNFR2 and IL2R α were also similarly significant in PFHxS as a continuous variable (Supplemental Table 4). The inverse association between PFNA and sCD14 was not statistically significant when PFNA was evaluated as a continuous variable. No statistically significant associations were observed between any of the 4 PFAS analytes and TNF- α , CXCL13, IFN- γ , IL-2, IL-8, IL-10, IL-1 β , IL6R α , and GP130.

When the four PFAS analytes were evaluated for associations with 4 immune pathways, only elevated levels of PFHxS exposure (defined as above the median) was positively associated with B-cell activation markers (OR=1.37, 95 % CI=1.00–1.87) (Supplemental Table 5). No other statistically significant associations between the 4 PFAS analytes and immune

pathways were observed. Notably, the 1.37 odds ratio is lower than the 1.53 odds ratio observed for the association between PFHxS and BAFF alone, indicating the aggregate median of immune markers in the B-cell activation pathway likely diluted the specific association with BAFF, rather than enhance the overall association with the pathway.

4. Discussion

In our evaluation of 722 women from the California Teachers Study whose sera, collected from 2013 to 2016, were evaluated for both PFAS and cytokine/immune markers at a single time point, we found: 1) positive associations between increasing levels of PFHxS and elevated levels of BAFF, TNFR1I, and IL2R α , 2) positive associations between increasing levels of PFOA and PFOS with elevated BAFF, and 3) inverse associations between PFNA and sCD14. Pathway-based analyses did not appear to add additional information beyond the individual cytokine/immune marker associations. These results add important data to the literature by providing robust data linking PFAS analytes with specific immune markers, specifically BAFF, TNFR1I and IL2R α , which, to our knowledge, have not previously been reported. Our data adds to the growing evidence from prior studies that have reported associations between PFAS analytes and increased risk for long-term adverse immune-related health outcomes, such as persistent infections, rheumatoid arthritis, vaccine responses, and ulcerative colitis [11,42,43].

Consistent with the current literature, older age was significantly associated with higher detectable levels for each of the PFAS analytes [37,44]. Our results are consistent with Kato et al.'s (2011) prevalence estimates for PFOA, PFOS, PFNA, and PFHxS at an earlier timepoint (1999–2008) in the United States among both men and women whereby those in the oldest age group (60 + years) had the highest prevalences and levels of PFOA, PFOS and PFNA [44]. Similar results were reported by Stein et al. (2016) in a more recent but smaller subset of younger U.S. men and women. [37]. We also previously reported elevated levels of PFAS in older women compared to younger women in a different subset of 1,257 women in the California Teachers Study [45].

The descriptive statistics for PFOA and PFNA analytes are relatively consistent with other studies that utilized serum and reported median values for each analyte [16,18,41,46]. We reported similar medians (1.28 ng/mL) for PFHxS [18,41,46], except for Barton et al. (2022) who reported a much higher median (16.6 ng/mL) and whose results were based on both men and women [16]. Notably, Zota et al. (2018) reported a similar PFOS median (2.83 ng/mL) to our median (4.63 ng/mL), which could be attributed to study population similarities (all female cohorts) [18]; otherwise, higher PFOS medians were reported in general though these studies also included both men and women (8.2 ng/mL [16] to 45.86 ng/mL [41]). Geographic differences and differences in laboratory methods may also contribute to quantitative differences. It is noteworthy that the median levels of at least two of our PFAS analytes measured in the present study exceeded 2 ng/mL, which is the threshold whereby advanced screenings and health assessments are recommended [47].

Each PFAS analyte displayed different associations with the immune markers assessed in our study. With respect to PFOA exposure, there was a positive association with higher

PFOA and elevated levels of BAFF. Of note, BAFF has been shown to play a role in controlling B cell maturation and acting as a survival factor for B cells [48]. Increased levels of BAFF due to a genetic deletion have been linked to an increased risk of developing autoimmune diseases, such as multiple sclerosis (MS) [49]. Similar to PFOA, higher levels of PFOS were also associated with elevated BAFF. PFOA and PFOS are among the most well documented PFAS analytes, but, to our knowledge, they have not been previously linked to BAFF. The robust and novel associations found here thus warrant further investigation as a potentially important biological pathway for understanding downstream health effects.

Our data yielded an association between increased PFNA exposure with decreased levels of sCD14. CD14 is expressed by monocytes, such as macrophages, and plays a role in innate immunity [50]. CD14 has also been shown to contribute to cancer-related inflammation and alternatively promote an immunosuppressive environment that helps drive tumor development [50]. According to Zhang et al. (2022), PFNA exposure and CD14 have been minimally studied via various study types, and the results are conflicting [14]. In an *in vivo* mouse study, PFNA exposure decreased the number of overall CD14 + cells [51]. In a follow-up *in vivo* mouse study, Rockwell et al. (2017) described that PFNA exposure caused an initial decrease in CD14 + cells followed by an increase in CD14 + cells over time [52]. It is unclear how our results, which found increased PFNA levels associated with decreased levels of sCD14, fit into the larger published data, but further investigations in population studies appear warranted.

Similar to both PFOA and PFOS, elevated levels of PFHxS exposure were robustly associated with higher levels of BAFF. The positive associations of all three PFAS analytes with BAFF are notable and warrant further investigation. PFHxS exposure also yielded a positive association with elevated levels of TNFR2. TNFR2 has been linked to susceptibility to immune conditions, such as multiple sclerosis, due to its role in suppressing the activation of regulatory T cells (Tregs) [53]. As well-documented in current literature, the number of Tregs increases as an individual ages, serving as a mechanism to prevent excessive inflammation or possible autoimmune conditions; however, when the production and activation of Tregs is altered, this protective function is decreased (e.g., such as in the case of PFAS exposure) [23,54]. Aging women represent a particularly susceptible population – as immune response is depressed among post-menopausal women and as autoimmune condition risk is higher – understanding the role that PFAS plays in perturbing the immune response is thus of particular importance for understanding downstream health effects in this susceptible population [22,54]. Lastly, TNFR2 has also been linked to the activation of the NFκB signaling pathway, which subsequently increases the production of TNF-α, a ligand for TNFR2 [55]. PFHxS exposure was positively associated with increased IL2Rα. As a receptor for IL-2, IL2Rα and has been shown to assist with Treg activation and development [56]. PFAS exposure has also been found to increase the levels of Th2 cytokines, including IL2Rα, that can increase the risk for immune conditions, such as rheumatoid arthritis [43,57]. Further investigations to confirm the associations between PFHxS with TNFR2 and IL2Rα in population-based studies are thus warranted to illuminate its potential role in Treg and/or NFκB activation that impacts long-term health outcomes.

When assessing PFAS exposure in relation to our constructed immune pathways, there were no notable associations other than those already driven by the specific cytokines. For example, the PFHxS association with the B-cell activation pathway is likely attributed to the specific association between PFHxS and BAFF association which was in fact more pronounced than that of the overall pathway, likely diluted by the null associations from the other markers in the pathway. Overall, the pathway associations did not appear to indicate a cumulative effect of all markers in a given pathway, but rather appeared largely driven by one specific marker.

Our cross-sectional study strengths include the use of a relatively large study cohort, the number of immune markers assessed, and involved standardized specimen collection and minimized variability due to the processing and storage by a single laboratory. Due to the nature of a cross-sectional study, our study does not permit us to measure the long-term implications of PFAS exposure on immune dysregulation. Study limitations also include the evaluation among females only, thereby limiting the generalizability of the study overall; however, we note the strength of evaluating these effects among aging women. Studies have demonstrated that PFAS exposure have been associated with altered female reproductive function, including later age of menarche, irregular and longer menstrual cycles, earlier age of menopause, and altered levels of hormones [58,59]. These effects are posited to result in downstream health outcomes; to date, higher levels of PFOS have been positively associated with ER+, PR+ and ER+/PR+ breast cancer tumors, and PFOA have been positively associated with ER-, PR-, ET+/PR- and ER-/PR- breast cancer tumors [60]. Investigating the role of PFAS exposure within an aging female population is thus a particularly important aspect of understanding the effects of the environment on immunity and related health outcomes, particularly in an immunologically susceptible and highly exposed population. Moreover, based on studies to date, a recent review emphasized that future epidemiology studies should include the evaluation of immunotoxicity among PFAS-associated disease endpoints [11]. Despite the overall consistency of our study results to the current literature, we acknowledge that cannot exclude the possibility that the associations present in our results were due to chance, particularly given the number of PFAS analytes and immune markers evaluated.

5. Conclusions

In summary, our study results assessed the associations between various PFAS analyte levels and circulating inflammatory markers at a single timepoint, providing important insight regarding the biological underpinnings that PFAS exposures may have in downstream development of disease endpoints. Specifically, our data suggests that PFAS exposure may be associated with the elevation of markers in the TNF family or superfamily, and its effects on immune function dysregulation may be associated with Treg production and activation. Importantly, each PFAS analyte displayed different associations with measured cytokines, thus warranting future studies to investigate the mechanistic differences between the PFAS analytes to identify the individual effects of these exposures on immune dysregulation and the array of disease endpoints that may result.

6. Data statement

All data associated with this publication are available for research use. The California Teachers Study welcomes all inquiries (<https://www.calteachersstudy.org/for-researchers>). Additionally, the PFAS analyte dataset presented in this study can be found in the online Human Health Exposure Analysis Resource data repository (<https://hhearprogram.org/data-services>).

Data associated with this publication are also publicly available through the **Human Health Exposure Analysis Resource (HHEAR) Data Center** (<https://hheardatacenter.mssm.edu/>).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

All data associated with this publication are available for research use. The California Teachers Study welcomes all inquiries. Please visit <https://www.calteachersstudy.org/for-researchers>.

Abbreviations:

PFAS	Per- and polyfluoroalkyl substances
PFOA	Perfluorooctanoic acid

PFOS	Perfluorooctanesulfonic acid
PFNA	Perfluorononanoic acid
PFHxS	Perfluorohexane sulfonate
BAFF	B-cell activating factor
TNFR_{II}	Tumor necrosis factor receptor 2
IL2Rα	Interleukin-2 receptor subunit alpha
sCD14	Soluble cluster of differentiation 14
EPA	Environmental Protection Agency
SNURs	Significant New Rules
CTS	California Teachers study
Q5	Questionnaire 5
SES	Socioeconomic status
NSAID	Nonsteroidal anti-inflammatory drug
BMI	Body mass index
PFHXA	Perfluorohexanoic acid
PFHPA	Perfluoroheptanoic acid
PFHPS	Perfluoroheptanesulfonic acid
PFDA	Perfluorodecanoic acid
PFUNDA	Perfluoroundecanoic acid
TNF-α	Tumor necrosis factor alpha
IL-1β	Interleukin-1 beta
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleukin-8
IL-10	Interleukin-10
IFN-γ	Interferon gamma
CXCL13	C-X-C motif chemokine ligand 13
sCD27	Soluble cluster of differentiation 27

GP130)	Glycoprotein 130
IL6Rα	Interleukin-6 receptor subunit alpha
MRM	Multiple reaction monitoring
HHEAR	Human Health Exposure Analysis Resource
LOQ	Limits of quantification
ORs	Odd ratios
Min	Minimum value
Max	Maximum value
MS	Multiple sclerosis
Tregs	Regulatory T cells

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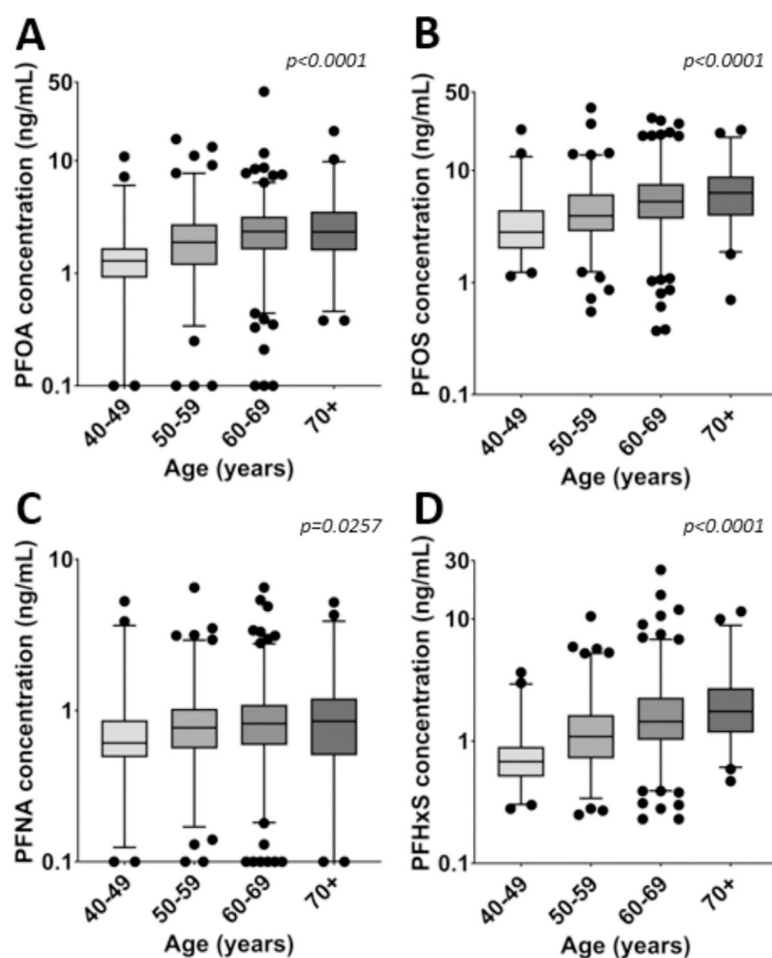


Fig. 1. Median PFAS analyte concentrations per 10-year age groups (40–49, 50–59, 60–69, 70 + years) of 722 women in the California Teachers Study who had serum immune marker and PFAS measurements from the same serum (blood) collection sample (2013–2016): (A) PFOA: Perfluorooctanoic acid (total), (B) PFOS: Perfluorooctane sulfonate (total), (C) PFNA: Perfluorononanoic acid, (D) PFHxS: Perfluorohexane sulfonate. The upper limit represents the 97.5 percentile; the lower limit indicates the 2.5 percentile. The p-value denotes the linear p-trend for median PFAS levels with each decade of age.

Table 1

Comparison of study participant characteristics who had serum immune marker and PFAS measurements (n = 722), participated in the larger biobanking study (n = 13,888), and completed questionnaire 5 after standard exclusions (n = 61,984) in the California Teachers Study cohort.

Characteristic	Cohort with completed Q5 questionnaire (n = 61,984*)		Cohort with completed Q5 questionnaire and blood sample collected (n = 13,888*)		Cohort with cytokine and PFAS measurements (n = 722*)	
	N	%	N	%	N	%
Age (in years)						
40–49	4393	7.1 %	1333	9.6 %	93	12.9 %
50–59	10,244	16.5 %	3452	24.9 %	203	28.1 %
60–69	22,272	35.9 %	6586	47.4 %	326	45.2 %
70+	25,044	40.4 %	2517	18.1 %	100	13.9 %
Race						
Non-Hispanic White	54,294	87.6 %	12,391	89.2 %	572	79.2 %
Other	7690	12.4 %	1497	10.8 %	150	20.8 %
Socioeconomic status (SES)						
Quartile 1	2376	3.9 %	457	3.3 %	23	3.2 %
Quartile 2	9867	16.2 %	2155	15.7 %	105	14.7 %
Quartile 3	19,987	32.8 %	4607	33.5 %	245	34.3 %
Quartile 4	28,755	47.2 %	6533	47.5 %	342	47.8 %
Body mass index (BMI)						
15–24	29,196	49.7 %	6744	50.1 %	347	49.2 %
25–29	17,967	30.6 %	4056	30.1 %	209	29.7 %
30+	11,580	19.7 %	2672	19.8 %	148	21.1 %
Physical Activity (hr/week)						
0–2.37	20,947	34.3 %	3879	28.2 %	239	33.1 %
2.38–5.88	20,136	33.0 %	4771	34.6 %	238	33.0 %
5.88+	20,037	32.8 %	5132	37.2 %	244	33.9 %
NSAID use						
None or 1/week	24,616	42.3 %	5784	43.3 %	314	44.8 %
>1/week	33,630	57.7 %	7581	56.7 %	388	55.2 %
Diabetes						
No	56,246	91.6 %	12,891	93.4 %	657	91.4 %
Yes	5128	8.4 %	907	6.6 %	62	8.6 %
Statin use						
None	42,336	69.9 %	10,188	74.4 %	529	73.5 %
>1/week	18,223	30.1 %	3513	25.6 %	190	26.5 %
Rural/Urban residence						
Rural	8813	14.4 %	1782	13.0 %	49	6.9 %
Town	2032	3.3 %	423	3.1 %	12	1.7 %
City	10,905	17.9 %	2627	19.1 %	52	7.3 %

Characteristic	Cohort with completed Q5 questionnaire (n = 61,984*)		Cohort with completed Q5 questionnaire and blood sample collected (n = 13,888*)		Cohort with cytokine and PFAS measurements (n = 722*)	
	N	%	N	%	N	%
Suburban	33,386	54.7 %	7586	55.1 %	516	72.1 %
Urban	5874	9.6 %	1342	9.8 %	86	12.0 %

* Sample sizes for some of the covariates are not 722 due to unknown measures.

Table 2

Associations (odds ratio [OR] and 95% confidence intervals [CI]) between exposures to 4 PFAS analytes (defined as above or below the median) and 16 immune markers (defined as above or below respective median) among 722 women in the California Teachers Study cohort.

	BAFF			TNFR2			TNF-α			sCD14 ^a			sCD27			CXCL13			IFN-γ			IL-2		
	OR	95 % CI		OR	95 % CI		OR	95 % CI		OR	95 % CI		OR	95 % CI		OR	95 % CI		OR	95 % CI		OR	95 % CI	
	1.00	Reference		1.00	Reference		1.00	Reference		1.00	Reference		1.00	Reference		1.00	Reference		1.00	Reference		1.00	Reference	
PFHxS	1.53	(1.12–2.09)		1.78	(1.29–2.45)		1.14	(0.83–1.56)		0.79	(0.58–1.09)		1.27	(0.93–1.74)		1.10	(0.80–1.51)		0.94	(0.44–2.02)		1.12	(0.69–1.83)	
PFNA	0.98	(0.73–1.32)		0.86	(0.63–1.16)		0.82	(0.61–1.10)		0.73	(0.54–0.99)		0.77	(0.57–1.04)		1.24	(0.92–1.67)		1.66	(0.81–3.42)		0.81	(0.51–1.28)	
PFOA	1.43	(1.06–1.95)		1.10	(0.81–1.51)		0.99	(0.73–1.35)		1.04	(0.77–1.41)		0.99	(0.73–1.34)		1.14	(0.84–1.55)		0.62	(0.29–1.31)		0.98	(0.61–1.56)	
PFOS	1.40	(1.03–1.90)		1.07	(0.78–1.46)		1.12	(0.83–1.52)		0.94	(0.69–1.27)		0.92	(0.67–1.24)		1.05	(0.77–1.42)		1.03	(0.50–2.13)		0.89	(0.56–1.43)	
IL-6																								
IL-8																								
IL-10																								
IL-1β																								
IL-2Ra																								
IL-6Ra																								
GP130																								
PFHxS	1.54	(0.79–3.02)		1.06	(0.77–1.44)		0.84	(0.61–1.16)		1.06	(0.77–1.45)		0.94	(0.69–1.28)		1.48	(1.08–2.03)		1.02	(0.74–1.39)		1.11	(0.81–1.52)	
PFNA	0.59	(0.31–1.12)		0.89	(0.66–1.19)		1.32	(0.97–1.78)		1.06	(0.79–1.43)		1.04	(0.77–1.39)		0.80	(0.60–1.08)		0.88	(0.66–1.19)		0.88	(0.65–1.18)	
PFOA	1.04	(0.55–1.97)		0.85	(0.63–1.15)		1.15	(0.84–1.56)		1.04	(0.76–1.40)		0.92	(0.68–1.25)		1.07	(0.79–1.46)		0.97	(0.72–1.32)		0.91	(0.67–1.24)	
PFOS	0.75	(0.40–1.42)		0.99	(0.73–1.34)		1.07	(0.78–1.46)		0.90	(0.66–1.22)		1.08	(0.79–1.46)		1.02	(0.75–1.38)		1.01	(0.74–1.37)		0.97	(0.71–1.32)	

^a CD14; N=721, 1 participant was missing a cytokine measurement. All models adjusted for age.