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# Reduction of household air pollution through clean fuel intervention and recovery of cellular immune balance

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ABSTRACT

*Background:* We conducted a clean fuel intervention trial (Bangladesh Global Environmental and Occupational Health (GEOHealth) (NCT02824237) with liquefied petroleum gas (LPG) for 26 months among rural Bangladeshi women chronically exposed to household air pollution (HAP) from biomass fuel (BMF) use. We aimed to evaluate the effect of HAP reduction following LPG intervention on immune response outcome.

*Methods:* We supplied LPG cook stove and refills in cylinder in 200 households for 26 months. We measured personal exposure to HAP [particulate matter 2.5 ($PM_{2.5}$), black carbon (BC) and carbon monoxide (CO)] in 200 women (main cook) by personal monitors at pre- and post-intervention. Immune function was assessed before and after intervention, in blood collected within 2 weeks of HAP measurements. Primary endpoints included reduction in HAP, lymphocyte proliferation and oxidative stress response, and alterations in T and B cell proportions.

*Findings:* Exclusive LPG use for 26 months resulted in significant reduction in $PM_{2.5}$ (43.5%), BC (13%) and CO (48%) exposure in the women. For one unit decrease in BC, Treg cells and memory B cells increased by 7% and 34% respectively, in the peripheral circulation. One unit decrease in CO was significantly associated with increase in early B cells and plasmablasts by 66% and 5% respectively. For one unit decrease in BC, percent-dividing cells, proliferation and expansion indices increased by 2%, 0.4%, and 1%, respectively.

*Interpretation:* Reduced personal exposure to HAP through clean fuel intervention was related to a return towards cellular immune balance.

## 1. Introduction

Globally about 2.6–3 billion people and 90% of rural households rely on biomass fuel (BMF) for indoor cooking and heating due to limited access to modern technology-based energy sources (WHO 2022). Solid biomass fuel refers to organic matter derived from animal and plant remains (such as wood, coal, charcoal, dried straw, crop residue, animal dung). Burning of BMF is a leading source of household air pollution (HAP), particularly in low-and-middle-income countries. Unfortunately, Bangladesh has been reported as one of the most polluted countries for $PM_{2.5}$ exposure based on a weighted population average (IQAir 2022a).

Household air pollution arising from polluting fuel use poses a serious threat to health and contributes to over 3·8 million premature deaths every year from illness related to HAP(WHO 2022). Exposure to HAP can lead to a wide range of adverse health outcomes including stroke, ischemic heart disease, respiratory diseases, and lung cancer (Ali et al. 2021). Fortunately, air pollution is also a significant preventable and manageable threat to people's health(Academy of Science of South

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et al. 2019). Clean fuel intervention studies have demonstrated marked reduction in air pollutants exposure, however, post-intervention exposures mostly appear to remain above the WHO health-relevant targets (Pope et al. 2017; Shupler et al. 2020). Only recently published HAPIN study reported that LPG intervention can reduce $PM_{2.5}$ exposures to levels at or below WHO targets (Johnson et al. 2022). There is no clear-cut evidence on positive impact of air pollution reduction on lowering respiratory or noncommunicable disease incidences, which probably require long term sustained intervention (Chillrud et al. 2021; Smith et al. 2011). One of the potential mechanisms through which air pollution adversely impacts health is disruption of the functions of different immune cells and dysregulation of the host immune response (Glencross et al. 2020). Adverse effects of BMF generated HAP on immune response in participants have been studied (Dutta et al. 2012; Raqib et al. 2022; Rylance et al. 2015), however there are no published data evaluating immune function outcomes after clean fuel interventions. Dutta et al. (2012) showed that indoor air pollution was associated with suppression of CD4, CD19, and increase in CD8 and NK cells in rural Indian women using BMF (Dutta et al. 2012). In an in vitro study, Rylance et al (2015) showed a dose dependent effect of BC on increase in inflammatory response of macrophages (proinflammatory cytokines) and their functional impairment (oxidative burst capacity) (Rylance et al. 2015). In the Bangladesh GEOHealth study (Raqib et al. 2022), we have earlier shown in a cross-sectional assessment at baseline that chronic HAP exposure through BMF use had a detrimental effect on immune response of 200 Bangladeshi women. We found that $PM_{2.5}$ and BC was associated with decline in memory B cells, while CO was associated with reduction in CD19 cells. Additionally, we also reported that


**Fig. 1.** Map of Bangladesh showing the location of the two study sites, Matlab and Araihazar sub-districts and locations of villages in each site. Six villages in Matlab, and 36 villages in Araihazar were purposively selected for the study purpose. The distance between Matlab and Araihazar is 102 km by road (aerial distance is 40 km). Distance by road between Dhaka to Matlab is 106 km, and Dhaka to Araihazar is 40 km.

HAP exposure was associated with impaired functioning of antigen presenting cells (macrophages and dendritic cells) and elevated plasma IgE level (indicator of allergic response). Another major objective of Bangladesh GEOHealth study was to evaluate whether intervention with clean fuel LPG reduces exposure to air pollutants and thereby reduces measures of immune dysfunctions in the same women. Accordingly, women were provided with improved cookstoves and a free supply of LPG for 26 months in the single group intervention trial (NCT02824237). In this paper, we report on the impact of exclusive clean fuel use for 26 months on reduction of HAP exposure levels measured in personal air samples of the participants and the effects of HAP reduction on cellular and humoral immune function as well as oxidative stress in these women.

## 2. Methods

### 2.1. Study area and population

The study was carried out in two rural sites in Bangladesh, Araihazar and Matlab subdistricts, which are located at a distance of about 40 to 106 km respectively, by road from Dhaka, the capital city (Fig. 1). The distance between the two sites is 102 km by road. The International Centre for Diarrheal Disease Research, Bangladesh (acronym icddr,b), maintains a longitudinal health and demographic surveillance system (HDSS) in rural Matlab covering an average population of about 240,000 in 142 villages (2018–2019). Precise demographic data are available for each individual within the HDSS that are yearly updated in computerized databases (Alam et al. 2017; https://www.icddrb.org/research/platforms/health-and-demographic-surveillance-systems-hdss 2023). Similarly, the University of Chicago Research Bangladesh (URB) in collaboration with Colombia University has been maintaining a cohort of about 35,000 populations in Araihazar in 117 villages under the study "Health Effects of Arsenic Longitudinal Study (HEALS)" which was developed to investigate health outcomes associated with chronic arsenic exposure from groundwater (Ahsan et al. 2006; Huhmann et al. 2019; van Geen et al. 2014). This population database is also regularly updated by URB once in every two years. The villages were purposively identified from both sites, the selection criteria were that study areas should be easily reachable by road, road condition should be reasonably good, LPG vendor shop should be available near the village, so that monthly LPG deliveries for the duration of study was possible and unavailability of piped natural gas supply. In Bangladesh, about 80% of the households use biomass fuel for cooking food and among those who use piped gas (natural gas/liquid petroleum gas/ biogas), most (56%) are located in urban areas (BDHS 2017–2018).

Both Matlab and Araihazar are known to have tubewell water widely contaminated with arsenic. It is well established that arsenic has adverse impact on immune function (Dangleben, Skibola, and Smith 2013; Giles and Mann 2022; Raqib et al. 2023), thus to avoid bias due to arsenic-mediated immune alterations, we incorporated an arsenic-related inclusion criterion. In the study areas, each tubewell is numbered and linked with the unique registered identification number of the head of the household. Water collected from these tubewells were tested for arsenic using atomic absorption spectrophotometry method (Ahsan et al. 2006; Huhmann et al. 2019; Rahman et al. 2006; van Geen et al. 2014). From the HDSS database, information was obtained on tubewell water arsenic levels. At the household level, the criteria were to select households using tubewells with water arsenic levels $< 10$ μg/L. During selection of households in the villages, attention was given to choose households with a gap of at least 200 yards between the adjacent houses to avoid contamination of exposure between households, and only one woman per Bari (a cluster of 3–4 households for the extended family i.e. grandparents, siblings, grandchildren) was included in the study (Shahriar et al. 2021). At baseline, some households were found to own LPG gas stoves with cylinders, however, they were primarily using BMF; LPG was used on rare occasions, e.g. at night for warming baby food.

Inclusion criteria for study participants were as follows- healthy women (age range, 25 to 65 years), being the main cook of the household, non-smokers and living with non-smoker spouses, using biomass fuel (BMF) for cooking purposes for at least or>5 years, apparently healthy, not known to have any immune related illness, not taking any prescription medication that affects immune function, and not known to have any clinical events of lung disease or cardiovascular diseases (CVD) including stroke or coronary heart disease. From 2534 women screened in the two sites, 1054 women were found eligible (Fig. 2). After obtaining informed consents, 100 women each, were randomly selected from 601 eligible women from Matlab and 453 women from Araihazar, respectively, using computer generated randomized numbers.

### 2.2. Data collection

Structured questionnaire-based data was collected at enrolment on the number and types of stoves used, daily duration and frequency of use, types of BMF used, kitchen type, distance of kitchen to living quarters and other sources of exposures. The participants were invited to the field office and a general health check-up was performed, clinical history was obtained, and height and weight were measured. Weight was measured using a digital weighing scale (MEGA Smart body Scale M05) that was standardized daily in the morning using a reference weight. Height was measured using a wooden stadiometer (precision of 0.1 cm). The stadiometer was calibrated before the start of the study. The same information was collected at post-intervention. Blood was collected by trained phlebotomists in the field office before and after the intervention. Exposure to air pollutants ($PM_{2.5}$, BC and CO) and health outcomes were assessed, before and after the intervention (Raqib et al. 2022; Shahriar et al. 2021).

The study was approved by the Ethical Review Committee of icddr,b (PR-15111).

### 2.3. Clean fuel intervention with LPG

At baseline (pre-intervention), after HAP exposure levels were measured in personal air samples using personal monitors/devices (described below), each household included in this study was gifted with LPG cylinder (~36 $), 2-burner LPG cook stove (~18 $) and uninterrupted supply of LPG refill (~18 $/ refill) (Omera LP Gas Ltd, Bangladesh) delivered free of cost for the duration of the intervention.

The distribution of stoves and cylinders continued for a month (June 25, 2018 to July 26, 2018) and the intervention was started within 2 weeks (August 2018). Thereafter LPG cylinders were made available within easy access of the participant through regular sale outlets of the LPG supplier company. All participants were provided with a supply card and they collected a new cylinder by showing the card and returning the empty cylinder. Fieldworkers made monthly home visits to the household to check the condition of cookstoves, arrange maintenance and repair of stoves when necessary, collect stove usage data through interview and collect health status data. To encourage participants, various strategies were followed to convince the households to continue using LPG exclusively during the period of intervention. A series of focus group discussion (FGD) was performed in the study community to understand the barriers of using LPG for cooking in households. Based on the information obtained from the pre-intervention FGDs, messages were developed for sharing with participants. Educational sessions were arranged providing sufficient information of the benefits of exclusive use of LPG (e.g. easy handling of stove, reduced cooking time, better health, better time management and availability of time for relaxation, clean kitchen and home environment) as well as the adverse health effect of biomass use (e.g. eye irritation, sneezing, coughing and other health hazards) and associated difficulties (e.g. drudgery related to BMF collection, requirement of extra storage space for BMF) at the time of LPG distribution. Field staff visited the households monthly to monitor free supply of LPG as needed, whether

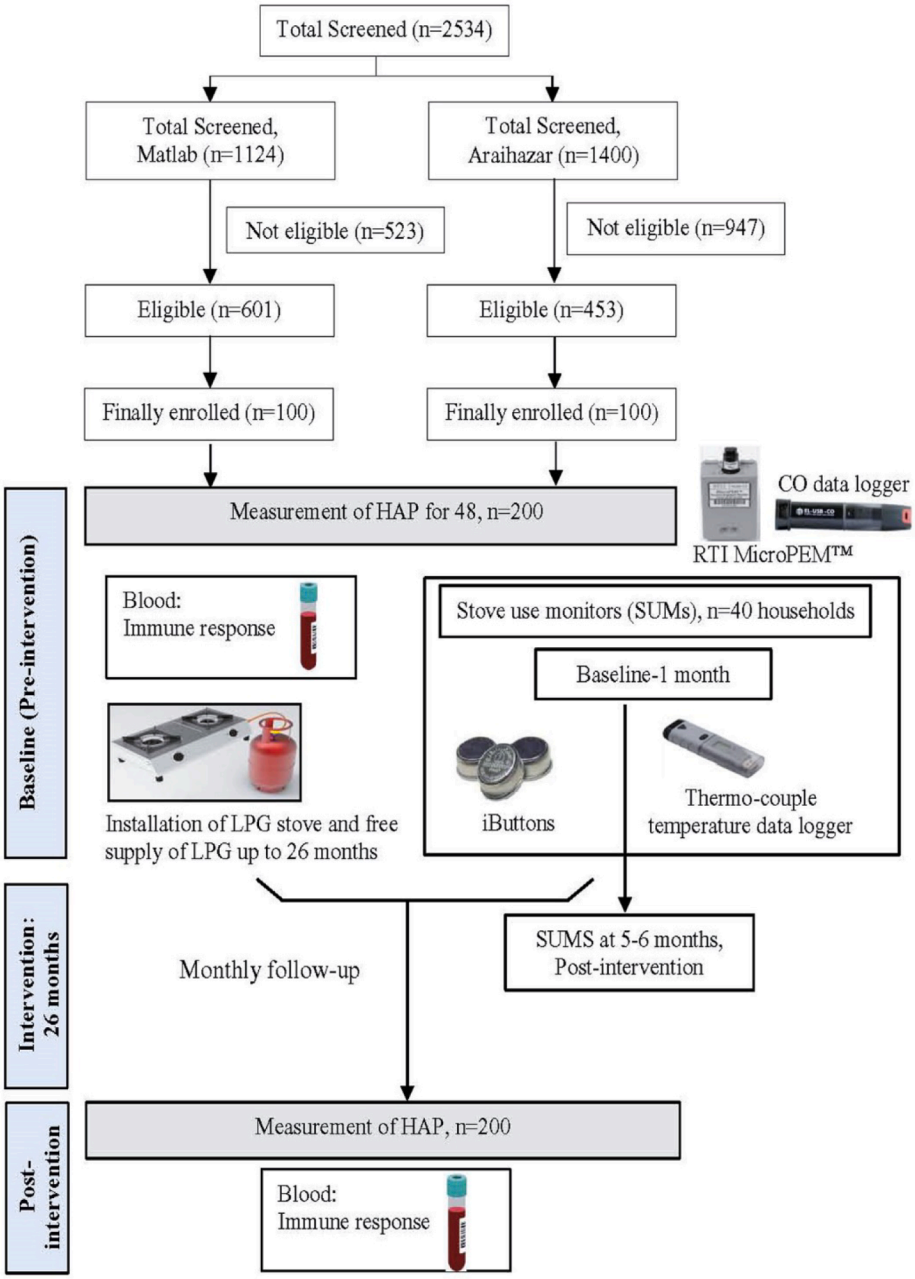


**Fig. 2.** Flow chart depicting the study design, distribution of enrolled study participants in two locations, intervention with liquefied petroleum gas (LPG); timing and assessment procedure of personal exposure to household air pollution (HAP), time of blood sample collection, routine monitoring visits and outcome analysis.

stoves required any repair, the usage pattern of LPG and encouraged the participant for its exclusive use.

In addition to collecting data on stove usage pattern through interview, for unbiased objective assessment of stove use patterns, we employed stove use monitoring system (SUMS). SUMS is a combination of SUMS iButtons (model DS1922L, Maxim, USA) and thermocouple temperature data loggers (model SSN61, Wellzion, China). The sensors/devices were attached to all available cookstoves and the resulting temperature profiles were analyzed to determine the frequency and duration of stove usage events (cooking time) in a subset of randomly selected 40 households, 20 in each site. The devices were placed two weeks prior to the distribution of the LPG cook stoves and were left for additional one month after LPG distribution to assess the stove use patterns at pre-intervention and during the early uptake phase, and the presence of stove stacking. The process was repeated at month 5 until the end of six months intervention to collect information about long term pattern of stove use and adoption beyond the initial phase.

### 2.4. Personal air pollution exposure assessment

The personal exposure monitor (RTI MicroPEM™, version 3.2, RTI International) is a wearable device placed in a small pouch near breathing zone of individuals. Trained field staff explained the function of the personal air sampling devices/monitors and organized wearing of the portable devices by each participant and retrieved it from their homes. The trained field supervisors loaded filters into the device, performed calibration of the air samplers before every assessment, changed air filters and downloaded the data files in the data repository after every use. The participants were instructed to wear the device during their usual daily activities and remove the device from the body during

bathing and sleeping time, keeping it in a nearby safe place (within 1 m). Additionally, during the assessment period the field staffs made follow up visits next day to encourage and ensure continuous wearing of the device. An internal accelerometer placed in MicroPEM sensitive enough to detect breathing movement, also checked the wearing compliance of MicroPEM devices.

The MicroPEM device collects the air into a filter (diameter: 25 mm; pore size: 3·0 μm) (Teflon PTFE Filters, Zefon International) at a constant flow rate of 0·4 L/min. The air filter was weighted before and after completion of 48-h/24-h exposure measurement (Supplementary Method S1), for PM mass gravimetrically. After exposure assessment, field supervisors removed the air filters from the MicroPEM in a temperature and humidity-controlled room and checked for any damage to the filter. Each filter was preserved in an individual case and stored in an air-conditioned room before being transported to the laboratory for measurement. Within two weeks of exposure assessment, the air filters were sent to Atomic Energy Center Dhaka (AEC), Bangladesh for measuring $PM_{2.5}$ and BC. The filters were weighed in a microbalance (METTLER Model MT5) in the designated Laboratory of AEC that maintained room temperature at 22 °C and relative humidity at 50%, and particle concentration was calculated by dividing the PM mass with total volume of air pumped by MicroPEM during the measurement period (time-weighted average). Before every weighing, all filters were kept in constant humidity and temperature for 24 hr. Laboratory blank filters were used for every 33 filters to ensure the quality control of filter weight. After weighing the sample filters and subtracting the pre- from post-assessment weight, the final weight was adjusted by further subtracting the average weight of laboratory blank filters for humidity correction. To eliminate the static charge accumulated on the filters before each weighing, STATICMASTER, a U-shape electrostatic charge eliminator was used. The RTI MicroPEM device included a low-voltage, precision centigrade temperature sensor (P/N TMP-36GT9Z-ND, Analog Devices Inc., Norwood, MA), located in the sample flow stream, downstream of the filter. The sensor allowed the device to record temperature at intervals of 30 s. According to the manufacturer, the temperature range of the device was −40 °C to 100 °C with an accuracy of ± 3 °C at 25 °C. Milà and colleagues have earlier evaluated the performance of the RTI MicroPEM temperature sensor by comparing with a high-precision (accuracy of ± 0.1 °C) thermohygrometer (Testo 635–2) that showed excellent agreement (intraclass correlation coefficient = 1) (Mila et al. 2020).

Due to availability of limited numbers of MicroPEM device for HAP measurement among 200 participants and time constraints, we did not analyze within device variability of exposure measurements in the same women. Instead, data from the same MicroPEM monitor used to measure personal exposure in different women from different households in the same village/location within a month was used as a proxy for calculating within-device variability. We also determined between device variability or concordance when different RTI monitors were used in the same village/location in different participants within a period of one month. The analyses showed that the performance of RTI device was generally robust and the personal exposure was assessed with good accuracy in both intra- and inter-monitor measurements (Supplementary Method S2).

BC was measured from the Teflon filters by EEL-type Smoke Stain Reflectometer. The concentration of light absorbing BC was determined based on the amount of reflected light absorbed by the filter, by using standards of carbon with known areal density and an assumed mass absorption coefficient (10 $m^2/g$) (Supplementary Method S3) for BC calculation (Biswas et al. 2003). Even though iron has a moderate light absorption coefficient, it can have limited influence on the BC value measured by reflectance. The uncertainty associated with BC measurement was high (4–9%), and therefore, the small influence of variation in iron concentration on BC measurement was not considered. Secondary standards of known BC concentrations were used to calibrate the reflectometer.

A Data Logger (EL-USB-CO, Lascar Electronics Inc, Erie, PA) was also placed in the pouch next to MicroPEM to detect CO by an electrochemical method (detection capacity up to 1000 ppm). The EL-USB-CO devices were pre-calibrated from the factory by the manufacturer. Before each assessment the device was set on zero and the time was synchronized. Data from CO Logger was downloaded and analyzed separately. The details of the measurement of personal air is given elsewhere (Ahmed et al. 2022; Raqib et al. 2022; Shahriar et al. 2021).

The Bangladesh GEOHealth study had two components, one was a cross-sectional assessment of association of HAP with different health parameters; and the other was a clean fuel intervention trial (pre- and post-intervention). In the cross-sectional component, a group of 100 women underwent 24-hr HAP measurement by the same devices. In the intervention group (n = 200) at baseline, we measured 48-hr of HAP exposure assessment, however, at post-intervention, we faced many challenges from the participants for compliance of wearing the heavy device for 48-hr. We compared 24-hr HAP data from 100 women with 48-hr HAP data collected at baseline from intervention group (n = 200) and found no significant difference (Table S1). Finally, 24-h exposure measurement was performed at post-intervention.

### 2.5. Immune response assessment

Blood obtained within 2 weeks of HAP exposure assessment, was collected in sodium heparin vacutainer tubes (Becton Dickinson, New Jersey, United States) at the field office twice, before and after the intervention. Blood samples were transported to Immunobiology, Nutrition and Toxicology Laboratory of icddr,b in Dhaka within 3–4 h. Peripheral blood mononuclear cells (PBMC) and plasma were separated from whole blood by Ficoll-paque (Amersham Pharmacia Biotech Inc; NJ) density gradient centrifugation. Plasma was stored in aliquots in −80 °C for later use. PBMC were washed, counted and divided into two aliquots- one was used for cell phenotyping and the other for lymphocyte proliferation assay.

The proportions of different subpopulations of B and T lymphocytes were measured by flow cytometry (BD Accuri C6 flow cytometer) as described earlier (Raqib et al. 2022). The samples and the fluorescence-minus-one (FMO) control were stained with fluorescently tagged antibodies. Daily FMO-matched controls were used to set the appropriate regions for specified cell populations. Validation of the Accuri C6 was performed once a week using 6- and 8-Peak Validation Beads (BD Biosciences, San Jose, CA). Data were expressed as frequency or proportion of B or T cells. Fig. S1A showed the gating process of subpopulations of B and T lymphocytes.

To evaluate cell-mediated immunity, T cell proliferation response was determined by flow cytometry based CellTrace™ CFSE Cell Proliferation Kit (Invitrogen, ThermoFisher Scientific, Waltham, MA). PBMC ($10 \times 10^6$) was loaded with CFSE cell dye, and following incubation in the dark, cells were washed and stimulated with or without phytohaemagglutinin (10 μg/ml; Sigma-Aldrich, Steinheim, Germany) for 72 h at 37 °C in 5% $CO_2$. The CFSE intensity was determined and data was analyzed using FlowJo™ v10 software. The proliferation response data was expressed as percent (%) dividing cells, proliferation index, expansion index, and replication index. The % dividing cells data determines the proportion of T cells that are mitogen/antigen-specific and respond by proliferating at least once during the entire culture period. The Expansion index determines the fold-expansion of the entire cell population. The Replication index determines the fold-expansion of only the responding/ replicating cells. The Proliferation index is the average number of divisions among the responding cells that have undergone from the beginning of the culture. Fig. S1B. shows the plots defining the proliferation response analysis and CFSE intensity histogram.

IgE concentration in plasma was measured by electrochemiluminescence immunoassay using Elecsys IgE II on immunoassay analyzer Cobas e601(Roche Diagnostics GmbH, Mannheim) which has been standardized against WHO Reference Standard. PreciControl

Universal was used as internal quality control to check accuracy and precision. The inter-assay coefficient of variation was 4·6% and 5·5% for these controls.

### 2.6. Oxidative stress

To assess the oxidative stress levels, 8-isoprostane concentration was measured in plasma using competitive ELISA (Abcam, Cambridge, UK). This kit allows colorimetric detection of 8-isoprostane in the range of 10–5,000 pg/mL; the sensitivity of the assay was 1 pg/mL. Plasma samples required pre-treatment and purification before the ELISA assay could be performed to determine 8-isoprostane level. Briefly, 1 mL of stored plasma was taken, acidified to pH 4 using acetic acid and mixed with equal volume of ethyl acetate to obtain three separate phases: upper organic phase containing lipoproteins, interphase of protein and lower aqueous phase. Protein containing interphase was discarded. The aqueous phase was transferred to separate glass tubes to repeat the ethyl acetate extraction step and the pooled organic phase was collected and evaporated by nitrogen gas. To cleave fatty acid from glycerol backbone, the dried organic residues were dissolved in 2 mL of 20% potassium hydroxide solution, mixed and incubated for 1 h at 50 °C for yielding an aqueous solution. The aqueous solution was further extracted with ethyl acetate, and the organic phase was finally evaporated by nitrogen gas to obtain dried sample sediment which was stored at −80 °C. On the day of performing ELISA, the sample sediment was dissolved in sample dilution buffer. Thereafter, ELISA was performed according to manufacturer's instruction.

### 2.7. Statistical analysis

For exploratory data analysis, we used descriptive statistics that included mean and standard deviation for continuous variables and percentage for categorical variables. We used paired sample *t*-test to compare the difference in HAP and immune markers before and after LPG intervention (Table S2). We also used bivariate regression model and Locally Weighted Scatterplot Smoothing (LOWESS) approach to assess and visualize the relationship between HAP ($PM_{2.5}$, BC and CO) and immune variables (B and T lymphocyte subpopulation, lymphocyte proliferation response, plasma IgE and oxidative stress marker 8-isoprostane). Data that were not normally distributed were log-transformed, which included CO, plasmablast cells and all proliferation responses. To control for multiple comparison, we considered family wise error rate Bonferroni-type approach when the FEW = 0.05, using stepwise procedure.

In this study, we considered intervention (pre - vs post -intervention) as a predictor variable for the HAP regression models. The differences in HAP exposure and immune markers between pre- and post-LPG intervention were evaluated using the linear mixed effect regression model considering the pre- to post-intervention impact as fixed factors (Table 2), and within subject impact as random factor (Table 2). To address the repeated nature of data and clustering of participants within subject, we fitted a subject-specific random intercept and a random intercept model. To rule out potential confounding effects, we included age, body mass index (BMI), and seasonality of HAP measurement (dry (October to March) and wet (April to September)) as covariates. HAP markers were found to be associated, such as, $PM_{2.5}$ and BC (Spearman's rho = 0.432, $p < 0.001$) and $PM_{2.5}$ and CO (Spearman's rho = 0.314, $p < 0.001$). We next checked for multicollinearity (variance inflation factor (VIF)) between all 3 HAP markers and individual immune markers and found no multicollinearity. Thus, we used mixed effect model by including all HAP markers in the same regression model, to evaluate the effects of reduction of HAP (post-intervention – pre-intervention) on changes in immune markers (post-intervention – pre-intervention) following LPG intervention (Tables 3 and 4). The possibility of natural temporal changes occurring in HAP and immune markers were minimized in the regression models by adjusting with other confounding variables including age, personal temperature and season (Tables 3 and 4).

**Table 1**
Demographic characteristic of the study participants.

| | | Pre (n = 200) | Post (n = 200) |
|---|---|---|---|
| Women, age, years | | 38·1 ± 7·46 | 39·95 ± 7·39 |
| Women, BMI, kg/m$^2$ | | 24·22 ± 4·30 | 25·1 ± 4·28 |
| Years of schooling | | | |
| | No schooling | 38 (19%) | |
| | 1–5 years | 73 (36·5%) | |
| | 6–10 years | 82 (41%) | |
| | >11 years | 7 (3·5%) | |
| Occupation | | | |
| | Unskilled | 1 (0·5%) | |
| | Professional | 1 (0·5%) | |
| | Homemaker | 197 (98·5%) | |
| | Others | 1 (0·5%) | |
| Household income, BDT | | | |
| | Monthly average | 10,355 ± 7,113 | 12,837 ± 7,253 |
| | <5000 | 35 (17·5%) | 10(5·0%) |
| | 5000–10,000 | 122 (61·0%) | 93(46·5%) |
| | >10,000–20,000 | 30 (15·0%) | 81(40·5%) |
| | >20,000 | 13 (6·5%) | 16(8·0%) |
| Types of stove | | | |
| | Mud stove with three stones, fixed | 158 (79%) | 0 |
| | Cylinder gas stove | 42 (21%)* | 200(100·0%) |
| ¥Fuel for cooking | | | |
| | Wood | 176 (88%) | 0 |
| | Dried leaf/straw/water hyacinth | 182 (91%) | 0 |
| | Jute straw | 125 (62·5%) | 0 |
| | Wood powder | 97 (48·5%) | 0 |
| | Occasional use of LPG | 42 (21%) | 0 |

Data given as mean ± standard deviation, or number with percentage in brackets. BMI, body mass index; BDT, Bangladeshi Taka; LPG, liquified petroleum gas. *Some households already owned LPG gas stoves at baseline that were used in a limited manner; they primarily used biomass fuel for cooking. ¥Each household used multiple types of cooking fuel, therefore the type of fuel use is not 100%, even after combining some fuel types.

For visual examination of scatter plots of the associations of HAP markers with immune cells we combined the HAP data, as well as the immune outcome data, from baseline and post-intervention and applied Lowess smoothing to see an overall trend (Figure. S2). We observed an almost linear association of outcomes with respect to $PM_{2.5}$. However, BC and CO exhibited non-monotonic relationship with immune variables in scatterplots, that either increased or decreased up to a threshold level of HAP and then started to decline or increase showing deviation from a linear pattern. Thus, spline knots were introduced at these thresholds (at 6·0 μg/m$^3$ BC and 1·0 μg/m$^3$ CO), and associations of BC and CO with immune outcome variables were assessed by mixed effect model below and above the knots (adjusted with age, locality and seasonality). The p values < 0.05 were considered statistically significant. Analyses were performed using STATA 15 (StataCorp, LP, College Station, Texas, USA) and figures prepared using GraphPad Prism-8·3·0·538.

## 3. Results

### 3.1. Study participants

Two hundred eligible women were enrolled during August 2017 to April 2018 in the intervention trial. The trial profile (Fig. 2.) describes the total number of women screened from both Matlab and Araihazar and finally enrolled in the study. A summary of demographics, participant characteristics and contextual background data is presented Table 1. At baseline, the women have been cooking for an average of

**Table 2**
Mean difference and percent changes in house hold air pollution (HAP) and immune response from baseline to post-intervention with LPG.

| | Pre (n = 198) | Post (n = 198) | β-coefficient (95% CI) | Percent change | p-value |
|---|---|---|---|---|---|
| $PM_{2.5}$ (μg/m$^3$) | 158·9 ± 73·7 | 85·6 ± 57·3 | −69.1(−83.4, −54.7) | 43.5 (32.4, 56.2) | <0.001 |
| BC (μg/m$^3$) | 7·36 ± 3·07 | 6·27 ± 4·53 | −0.95(−1.77, −0.06) | 12.9 (3.75, 18.7) | 0.036 |
| CO (μg/m$^3$) | 1·02 ± 0·52 | 0·25 ± 0·42 | −0.49(−0.73, −0.25) | 47.6 (22.7, 76.8) | <0.001 |
| CD4 + T cells | 22·5 ± 0·41 | 32·7 ± 0·52 | 9.85(8.42, 11.3) | 43.8 (38.8, 48.7) | <0.001 |
| T regulatory cells | 7·19 ± 0·13 | 3·56 ± 0·18 | −3.49(−3.95, −3.02) | 48.7 (40.8, 56.9) | <0.001 |
| CD8 + T cells | 24·4 ± 0·46 | 17·9 ± 0·42 | −5.38(−6.71, −4.06) | 22.2 (16.1, 28.8) | <0.001 |
| CD19 + B cells | 19·0 ± 0·50 | 6·68 ± 0·29 | −12.5(−13.8, −11.3) | 65.4 (56.2, 75.8) | <0.001 |
| Early/ immature B cells | 4·76 ± 3·36 | 14·2 ± 8·16 | 10.0(8.56, 11.5) | 210(201, 219) | <0.001 |
| Plasmablasts | 1·77 ± 0·21 | 5·33 ± 0·35 | 3.76(2.88, 4.64) | 215(213, 218) | <0.001 |
| Memory B cells | 27·6 ± 9·88 | 39·2 ± 11·4 | 12.7(10.2, 15.2) | 46.0 (38.9, 52.2) | <0.001 |
| % Dividing Cells | 15·9 ± 4·28 | 19·6 ± 3·80 | 4.36(−0.078, 8.82) | 22.3 (0.42, 39.0) | 0.056 |
| Proliferation Index | 2·12 ± 0·69 | 1·72 ± 0·61 | −0.47(−0.61, −0.33) | 22.0 (14.7, 29.9) | <0.001 |
| Expansion Index | 1·93 ± 0·21 | 1·56 ± 0·06 | −0.55(−0.92, −0.18) | 25.6 (7.31, 50.3) | 0.003 |
| Replication Index | 6·66 ± 0·76 | 3·72 ± 0·18 | −4.17(−5.37, −2.96) | 54.9 (34.1, 82.2) | <0.001 |
| IgE, IU/ml | 478·6 ± 3·63 | 338·8 ± 3·47 | −187.0 (−378.7, 4.61) | 64.1 (0.89, 111.5) | 0.056 |
| 8-isoprostane, pg/ml | 1674 ± 837·2 | 1520 ± 678·8 | −289(−430, −148) | 9.67(3.0, 16.4) | <0.001 |

17.42 years (median 20 years). The age range of the enrolled women at post-intervention was 27 to 57 years. Most participants had not studied beyond primary school, were predominantly homemakers and were generally of low socioeconomic status (average monthly income 10,355 BDT that is equivalent to $126·0). The intervention ended in 30th September 2020.

### 3.2. Compliance of LPG use

The stove usage as assessed by stove use monitoring system (SUMS) data at the two time points (from pre-intervention up to one-month post-intervention and again from 5 to 6 months), together with the questionnaire data showed that almost 98% of the selected households used LPG stove exclusively, during the early uptake phase (96·5%) and at 6 months (97·8%). At the end of the study, none of the participants were using BMF for any cooking purposes (Table 1).

### 3.3. Reduction in air pollutant concentrations after clean fuel LPG intervention

The LPG intervention was started in July 2018 and completed in September 2020. After 26 months of exclusive use of LPG, marked decline in personal exposure to HAP was noted in the rural women. $PM_{2.5}$ levels decreased by 43·5% ($p < 0·001$), BC levels decreased by 12·9% ($p = 0·03$) and CO levels by 47·6% ($p < 0·001$) as compared to the pre-intervention levels (Table 2).

Data is presented as geometric mean ± standard deviation (SD) or β-coefficient and percent change with 95% Confidence interval in parentheses, comparing pre- with post-intervention period. Subject-mean adjusted linear mixed-effect model was used to estimate the p-value. Each subject was included as a random effect, that enabled each subject to serve as its own control in this model and accounted for within subject correlations between repeated measurements. The regression model was adjusted by age, body mass index, and seasonality.

### 3.4. Changes in immune outcomes after LPG intervention

Compared to pre-intervention, frequencies of $CD4^+$T cells increased by 43·8% ($p < 0·001$), while frequencies of $T_{reg}$ and $CD8^+$ T cells decreased by 48·7% and 22·2% respectively ($p < 0·001$) after 26 months of LPG intervention (Table 2). Frequencies of total $CD19^+$B cells (pan B cell marker) decreased by 65·4% ($p < 0·001$) after the intervention compared to baseline. Within $CD19^+$B cell compartment, frequencies of B cell subpopulation increased significantly after the intervention; early B cells increased by 210% ($p < 0·001$), plasmablasts by 215% ($p < 0·001$) and memory B cells by 46% ($p < 0·001$).

There was a marked reduction in almost all aspects of proliferation response of T lymphocytes at the end of intervention compared to baseline; proliferation, expansion and replication indices decreased by 22%, 25·6% and 55% respectively (Table 2). Concentration of IgE and 8-isoprostane levels also decreased by 64% and 10% respectively after the clean fuel intervention compared to baseline (Table 2).

### 3.5. Effect of HAP reduction following LPG intervention on immune response outcome

We found that reduction in BC and CO exposure following exclusive LPG intervention was significantly associated with changes in lymphocyte frequencies (Table 3). For one unit decrease in BC, $T_{reg}$ cells increased by 6% and memory B cells by 17% in the peripheral circulation. One unit decrease in CO was associated with increase in early B cells by 188% and plasmablasts by 11%. There were significant increases in the frequency of $CD4^+$T cells, early B cells, plasmablasts and memory B cells, and significant reduction in $CD8^+$T cells, Treg cells and $CD19^+$ B cells at post-intervention compared to pre-intervention time. At any time, participants from Araihazar had an average of 2.12 units higher CD4 + T cells compared to that of Matlab participants. A one-year increase in age was associated with 16% increase in $CD4^+$T cells, and 20% and 1% decreases in CD8 + T cells and plasmablasts, respectively. Any change in seasons was associated with 150% increase in early B cells.

We also carried out single-pollutant models to estimate the individual effect of single HAP pollutants on immune biomarkers, following LPG intervention. The results indicated that the estimates of the associations of single-pollutants (Tables S3, S4 and S5) were in the same direction and of similar magnitude to that of the 3 combined pollutant models (Table 3).

The reduction in $PM_{2.5}$ exposure following LPG intervention compared to baseline, did not show any association with changes in any of the lymphocyte proliferation indices. Only BC exposure had an impact on the proliferation responses- 1 μg/m$^3$ unit increase in BC was significantly associated with decrease in percent-dividing cells, proliferation and expansion indices by 3%, 0.4%, and 1%, respectively (Table 4). Compared to pre-intervention, there were marked reductions in proliferation index by 0.13 units ($p < 0·001$), expansion index by 0.06 units ($p = 0·003$), and replication index by 0.28 units ($p < 0·001$) at post-intervention. Only percent-dividing cells showed increase by 0.16

**Table 3**
The effect of reduction of household air pollution ($PM_{2.5}$, BC and CO) following LPG intervention on changes in proportions of peripheral lymphocytes.

| | $CD4^+$ T cells β (95% CI) | T regulatory cells β (95% CI) | $CD8^+$ T cells β (95% CI) | $CD19^+$ B cells β (95% CI) | Early B cells β (95% CI) | Plasmablasts[§] β (95% CI) | Memory B cells β (95% CI) |
|---|---|---|---|---|---|---|---|
| $PM_{2.5}$ | −0·007(−0·02, 0·03) | −0·001(−0·01, 0·002) | 0·001(−0·003, 0·003) | 0·003(−0·01, 0·01) | 0·003(−0·01, 0·01) | −0·001(−0·001, 0·001) | −0·01(−0·03, 0·01) |
| BC | 0·09(−0·074, 0·25) | −0·06(−0·11, −0·01)* | 0·04(−0·08, 0·16) | 0·02(−0·13, 0·17) | 0·001(−0·17, 0·17) | −0·004(−0·01, 0·01) | −0·17(−0·41, −0·01)* |
| [§]CO | 0·66(−0·80, 2·13) | 0·16(−0·28, 0·60) | 0·08(−1·21, 01·04) | −0·69(−2·0, 0·62) | −1·88(−3·34, −0·43)* | −0·11(−0·20, −0·02)** | −0·748(−3·17, 1·68) |
| Intervention | 9·26(7·71, 10·8)** | −3·94(−4·42, −3·46)** | −5·96(−7·13, −4·79)** | −12·6(−14·1, −11·0)** | 8·67(6·95, 10·4)** | 0·46(0·35, 0·57)** | 10·6(7·82, 13·4)** |
| Locality | 2·12(0·49, 3·75)* | 0·28(−0·14, 0·70) | 1·25(−0·35, 2·84) | 1·25(−0·35, 2·84) | 0·28(−0·95, 1·49) | −0·03(−0·11, 0·05) | 0·44(−2·25, 3·12) |
| Seasons | −0·83(−2·08, 0·41) | 0·04(−0·44, 0·35) | 0·80(−0·12, 2·84) | −0·28(−1·49, 0·94) | 1·50(0·15, 2·85)* | 0·05(−0·03, 0·14) | 0·25(−1·94, 2·46) |
| Age | 0·16(0·05, 0·27)** | 0·01(−0·02, 0·04) | −0·20−(−0·31, −0·09)** | 0·03(−0·06, 0·11) | −0·05(−0·15, 0·04) | −0·01(−0·01, −0·001)* | −0·11(−0·33, 0·22) |
| [ϒ]Temperature | 0.04(−0.13, 0.20) | 0.05(−0.01, 0.10) | 0.07(−0.05, 0.19) | 0.02(−0.14, 0.17) | 0.15(−0.02, 0.32) | 0.01(−0.002, 0.02) | −0.05(−0.33, 1.68) |

Note. Estimates were generated by using subject specific mixed effect models controlling for locality, seasonality age and [ϒ]personal temperature. Change in HAP markers = post-intervention – pre-intervention; change in immune responses = post-intervention – pre-intervention. Intervention (post- compared to pre-intervention); season (wet compared to dry season); locality (Matlab compared to Araihazar).
[§]Natural log transformation· * *p*-value ≤ 0·05, ** *p*-value ≤ 0·01· $PM_{2.5}$, particulate matter 2·5; BC, Black carbon; CO, Carbon monoxide.

**Table 4**
The effect of reduction of household air pollution ($PM_{2.5}$, BC and CO) following LPG intervention on changes in lymphocyte proliferation response.

| | % Dividing Cells β (95% CI) | Proliferation Index β (95% CI) | Expansion Index β (95% CI) | Replication Index β (95% CI) |
|---|---|---|---|---|
| $PM_{2.5}$ | 0·001 (−0·001, 0·001) | −0·001 (−0·003, 0·001) | 0·001 (−0·001, 0·0004) | −0·001 (−0·003, 0·004) |
| BC | −0·03 (−0·05, −0·01)* | −0·004(−0·01, −0·0002)* | −0·01 (−0·01, −0·002)** | −0·01(−0·01, 0·0004) |
| [§]CO | 0·04 (−0·08, 0·16) | −0·02(−0·07, 0·01) | −0·002 (−0·05, 0·05) | −0·04(−0·10, 0·02) |
| Intervention | 0·16(0·04, 0·29)* | −0·13(−0·17, −0·09)** | −0·06 (−0·12, −0·01)** | −0·28(−0·35, −0·22)** |
| Locality | −0·03 (−0·12, 0·06) | −0·03(−0·05, 0·01) | −0·03 (−0·07, 0·01) | −0·04(−0·08, 0·01) |
| Seasons | −0·03 (−0·13, 0·07) | −0·01(−0·04, 0·03) | 0·01(−0·03, 0·05) | −0·01(−0·06, 0·04) |
| Age | −0·01 (−0·02, 0·002) | 0·002(0·001, 0·004)* | 0·002 (−0·001, 0·01) | 0·003 (−0·004, 0·01) |
| [ϒ]Temperature | −0.01 (−0.02, 0.002) | 0.003(−0.002, 0.01) | −0.002 (−0.01, 0.003) | 0.002 (−0.004, 0.01) |

Note. All outcome and one exposure ([§]CO) data were log transformed (natural). Estimates were generated by using subject specific mixed effect models controlling for locality, seasonality age and [ϒ]personal temperature. Change in HAP exposures = post-intervention – preintervention and change in immune responses = postintervention – preintervention. Intervention (post- compared to pre-intervention); season (wet compared to dry season); locality (Matlab compared to Araihazar). * *p*-value ≤ 0·05, ** *p*-value ≤ 0·01.

units after the intervention (p = 0·026) (Table 4). A one-year increase in age was associated with a small increase (0.002 unit) in proliferation index.

However, the reduction in HAP markers following LPG intervention compared to baseline, did not show any association with changes in IgE, and 8-isoprostone levels. IgE level was significantly reduced by 0.17 IU/ml after the intervention, compared to pre-intervention (p = 0.038). Women from Araihazar exhibited 0·15 IU/ml higher IgE levels (95% CI = 0·02, 0·22; p = 0·030) and 492.1 pg/ml lower 8-isoprostone levels (95% CI = -650, −327·4; p < 0·001) compared to the Matlab women (Table S6).

### 3.6. Association of overall HAP exposure with immune outcomes

Mixed effect analysis between the combined HAP data and combined immune outcome data (Table S7) were performed. With increasing concentrations of $PM_{2.5}$, $CD8^+$T, $T_{reg}$, and $CD19^+$B cells showed a positive association, whereas, $CD4^+$T cells, early B cells, plasmablasts and memory B cells showed a decreasing trend reflecting a monotonic relationship. When the model was further adjusted with BC, only the associations with $T_{reg}$ cells remained significant, indicating that the modifying effect was mainly due to BC (Table S7).

We found that below the knot (<6·0 μg/m3), 1 μg/$m^3$ increase in BC was associated with increase in 87% of $CD19^+$B cells but decrease in 64% of early B cells, 40% of plasmablasts and 109% of memory B cells. However, above the knot (>6·0 μg/$m^3$), every 1 μg/$m^3$ increase in BC was associated with 13% decrease in $T_{reg}$ cells (Fig. 3A). The results indicated that the threshold level of BC was crucial, while it was stimulatory for some immune cells below the knot, for other immune cells it exhibited an inhibitory role.

When associations were examined between CO and immune cells, CO below the knot (1·0 μg/$m^3$) was found to show stimulatory effects on $T_{reg}$ cells, $CD8^+$T cells and $CD19^+$ B cells by 3.59, 5.87 and 10.8 units respectively. However, the associations were negative with other cells reflecting immuosuppressive effect, where one unit increase in CO was associated with reduction in 10·1, 9·49, 3·35 and 10·2 units of $CD4^+$ T cells, early B cells, plasmablasts, and memory B cells, respectively (Fig. 3B). Here again, the threshold level of CO was important; the associations of CO above the knot, changed directions with $CD4^+$ T cells, Treg cells, and $CD19^+$ B cells.

BC and CO but not PM2.5 appeared to have some effects on lymphocyte proliferation responses, BC was negatively associated with % dividing cells above the knot exhibiting an immunosuppressive role (Fig. 3C). At lower concentration (below the knot) CO level played immunostimulatory role for lymphocyte proliferation response. One unit increase in CO was associated with 0.43 units increase in proliferation and 3.37 units increase in replication indices. Again, negative associations were found above the knot (Fig. 3D). For 1 μg/$m^3$ increase in CO above the knot, there were 0.18 unit decrease in proliferation and 1.44 unit decrease in replication indices. One unit increase in CO below the knot (1·0 μg/$m^3$) was associated with 0.20 unit increase in IgE, indicator of allergic response (p = 0.048) and 254.3 unit increase in 8-

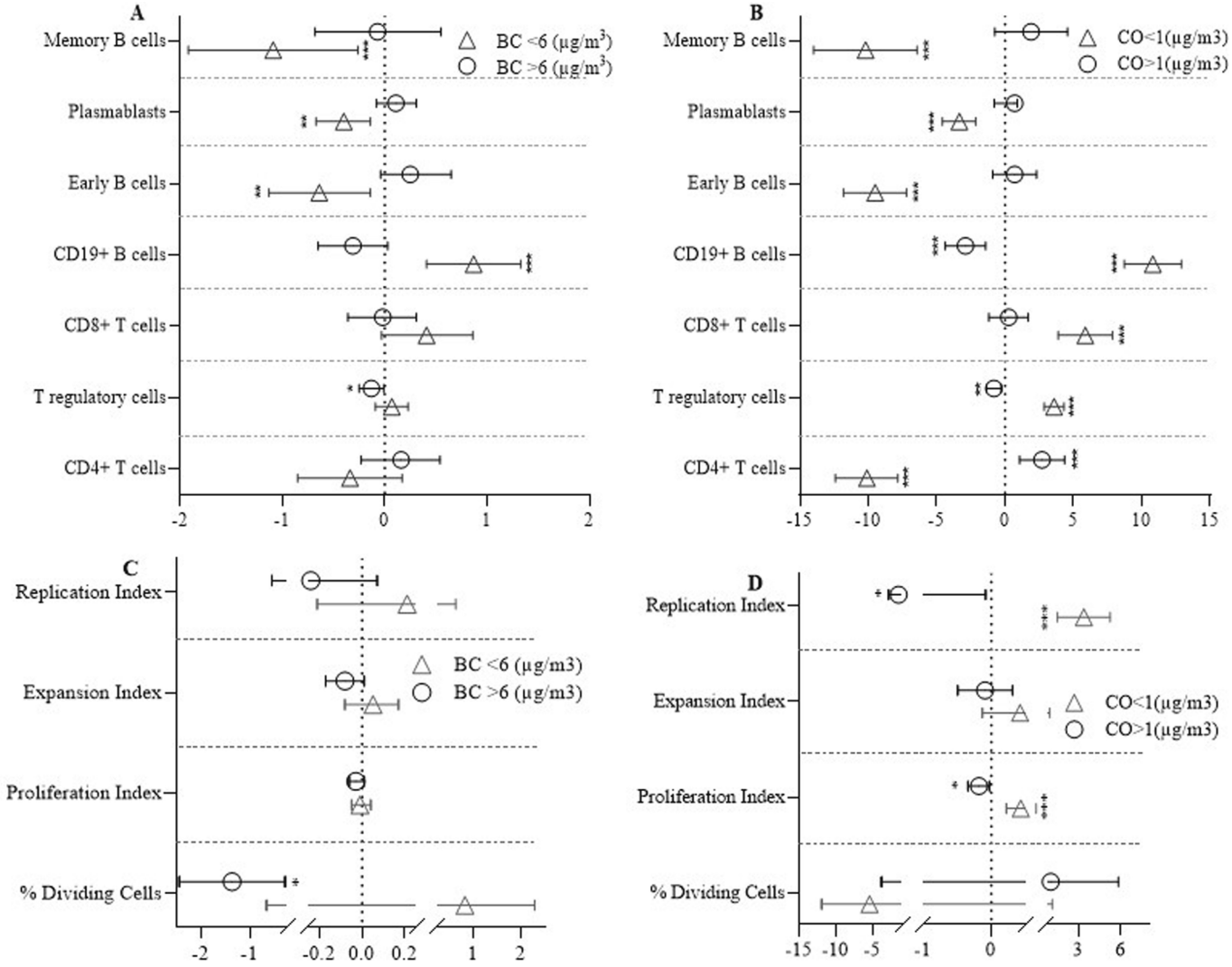


**Fig. 3.** Association between exposure to household air pollutants BC (A), and CO (B) and proportions of lymphocytes (C); and lymphocyte proliferation response (D). Spline regression model was applied to determine association between household air pollution and outcome variables at values below and above spline knots introduced at BC (6·0 µg/m$^3$) and CO (1·0 µg/m$^3$). The mixed effect model was adjusted by time, locality, seasonality, and age. ***indicates $p < 0{\cdot}001$, **indicates $p < 0{\cdot}01$ and *indicates $p < 0{\cdot}05$.

isoprostane levels, indicator of oxidative stress ($p = 0{\cdot}033$) (Table S8). Above the knot, the associations no longer remained significant.

## 4. Discussion

In a cohort of 200 rural Bangladeshi women chronically exposed to HAP through BMF use, we previously carried out a cross-sectional assessment of association of HAP with immune markers at pre-intervention and demonstrated that HAP exposure was associated with altered immune status (Raqib et al. 2022). Here, we report that exclusive use of clean fuel intervention for 26 months resulted in significant reduction in HAP exposure particularly $PM_{2·5}$, BC, and CO, in these women, which was associated with reduced lymphocyte proliferation and recovery towards T and B cell immune balance. Even though IgE and 8-isoprostane levels decreased from baseline to post-LPG intervention, we did not find any association between reduction in HAP levels and changes in these biomarker levels. This is the first intervention trial to report the impact of clean fuel intervention on immune function in women in relation to reduction in household air pollution. The findings suggested that HAP reduction through LPG intervention modulated immune function towards normalization of the T and B cell pools.

Earlier epidemiological studies used fixed-site concentrations in the kitchen or room that acted as a proxy for personal exposure; this may lead to misclassification of personal exposures (Pope et al. 2017; Shupler et al. 2020). Recent clean fuel intervention trials have used personal air monitors to obtain more precise data on personal HAP exposure demonstrating considerable reduction in HAP exposure among the participants (32–69% reduction in $PM_{2·5}$ exposure, 33–89% reduction in BC and 47–82% reduction in CO) (Chillrud et al. 2021; Johnson et al. 2022; Sharma and Jain 2020; Shupler et al. 2020). According to a recent report from the HAPIN study, LPG intervention could reduce $PM_{2·5}$ exposures to levels at or below WHO targets (Johnson M 2022). However, few studies have also reported null effect (Kumar et al. 2021). With the use of personal monitors, our study also showed that exclusive use of LPG for 26 months reduced 44%, 13%, and 48% of $PM_{2·5}$, BC and CO, respectively. Despite the clean fuel intervention, it was evident that $PM_{2·5}$ levels were still well above (Table 2) the WHO Global Air Quality Guidelines (AQG) limit of 5 µg/m$^3$ in a year as long-term exposure limit, and 15 µg/m$^3$ in 24 h as short-term exposure limit (IQAiR 2022b). For CO, the average indoor concentration set by the AQG is 7 mg/m$^3$ in 24 h (IQAiR 2022b). Among the BMF using rural Bangladeshi participants, average personal CO exposure was much lower ($1{\cdot}02 \pm 0{\cdot}52$ µg/m$^3$). For BC, there are no exposure limit set by AQG. Epidemiological studies have described mean BC concentrations in low ranges from 0·7-to-12·6

μg/m$^3$ reaching up to as high as 83 μg/m$^3$ and 399 μg/m$^3$ in a number of studies from Asia and Africa (Curto et al. 2019; Norris et al. 2016; Sharma and Jain 2020; Zhou et al. 2020). In our study, the percent decrease in BC levels after the intervention was the lowest among the three HAP markers which remained at relatively elevated levels (6·27 ± 4·53 μg/m$^3$) after the intervention, similar to the levels seen in countries where BMF use is common (Curto et al. 2019; Norris et al. 2016). The fairly high levels of $PM_{2·5}$ and BC in rural Bangladesh after the exclusive use of clean fuel, probably resulted from contamination from the neighboring household kitchens and the ambient atmosphere (Pope et al. 2017; Shupler et al. 2020).

Air pollution is recognized to be associated with increased risk of diseases, particularly non-communicable and respiratory diseases (Schraufnagel et al. 2019). However, only a handful of studies have demonstrated direct influence of HAP reduction on different disease and mortality outcomes (Kumar et al. 2021; Pratiti et al. 2020). Risk of disease prevalence is closely linked with poor immune function. The immune system consists of multiple types of immune cells that act jointly to mount immune responses against any insult. Earlier studies by Dutta et al found that indoor air pollution was associated with suppression of CD4, CD19, and increase in CD8 and NK cells among women using BMF (Dutta et al. 2012). Another study described a dose dependent effect of BC on increase in inflammatory response and impairment of phagocytic response of macrophages in *in vitro* experiments (Rylance et al, 2015). In the GEOHealth study at baseline (pre-intervention), we showed that chronic HAP exposure was associated with altered frequencies of immune cells, impaired functioning of antigen presenting cells and elevated plasma IgE level reflecting a status of immune imbalance in these chronically exposed women. In the present report, we demonstrated that exclusive clean fuel use for a period of 26 months was able to bring changes in the frequency of different lymphocyte subpopulations likely to restore the immune equilibrium in these women. Our findings further suggest that in spite of substantial reductions in BC and CO levels after the intervention, the residual levels still adversely impacted lymphocyte numbers and function. In an attempt to find out threshold concentrations of BC and CO, above or below which the pollutants may behave differently, the pre- and post-intervention HAP data were combined. For some cell types, BC acted as a stimulant below the knot (<6·0 μg/m$^3$), while above the knot a suppressive effect was noted. Carbon monoxide exposure also behaved differently, by inducing proliferation and replication responses below the knot (>1·0 μg/m$^3$), and at concentrations above the knot, expressing inhibitory effects on these responses. *In vitro* and animal studies have reported that BC (Moller et al. 2002; Pierdominici et al. 2014) and CO (Song et al. 2004; Yan et al. 2020) adversely impact cell proliferation response by impairing cellular synthesis, cellular stress, cell-cycle activity and cytoskeletal toxicity. Lymphocyte activation and proliferation is a crucial immune function that is indicative of cellular fitness and is involved in many pathophysiological processes; their dysregulated function may have diverse health consequences. $T_{reg}$ cells, a specialized subpopulation of $CD4^+$T cells, act to suppress exaggerated immune response, and maintain a noninflammatory balanced environment and self-tolerance. $T_{reg}$ cells suppress excessive B cell responses, and B cell-mediated antibody production. Both BC and CO below the thresholds, exerted inhibitory effects on B cell subpopulations while stimulating $T_{reg}$ cells. However, at higher concentrations, suppressive influence on $T_{reg}$ cells suggested further dysregulation of humoral immunity via their action on B cell lineage cells.

Early studies on effects of HAP on immune function in humans largely described pro-inflammatory responses including oxidative stress, impaired function of innate cells, augmented IgE responses and altered frequencies of immune cells (Lee et al. 2015; Raqib et al. 2022; Stiller-Winkler et al. 1996). Systematic reviews have indicated that air pollution may be correlated with IgE-mediated allergic diseases though the existing findings are inconclusive (Wang et al. 2022). We found a positive association between CO and plasma IgE at baseline (Raqib et al. 2022). However, the decrease in air pollutants and plasma IgE levels post-LPG intervention no longer showed any association. Decrements in IgE levels were probably not directly related to reduced HAP, and may have occurred through different mechanisms (Wang et al. 2022). Various studies have reported positive associations between air pollutants and 8-isoprostane levels, predominantly measured in exhaled breath condensate (Hashemzadeh et al. 2019; Rosa et al. 2014). We did not find any association between any of the HAP markers and plasma 8-isoprostane. Methodological differences in measurement or source of specimen (exhaled breath vs plasma) may partially explain the lack of association.

Our study had some limitations. There was no control group without LPG intervention to compare immune responses between the groups. However, each participant served as her own control comparing pre-intervention with post-intervention HAP exposure status. There is a possibility of natural temporal changes in HAP and immune biomarkers; to minimize the causal effects of time duration, we incorporated all HAP exposures in the same model along with other confounding variables (e.g. age, seasonality) (Tables 3 and 4). Due to the absence of a GPS tracking device installed in the MicroPEM, we could not objectively monitor the movement of the participants within or outside the household. However, participants were requested to write in a notepad about their movement from their household to other nearby places such as to neighboring households or to a nearby pond, during the period when they were wearing the MicroPEM. We mostly relied on self-reported fuel usage pattern through monthly interview by field workers. However, a sub-sample of households was monitored by SUMS at baseline and twice after the intervention, which showed exclusive use of clean fuel. We did not measure ambient air pollution levels in parallel to HAP measurement in our selected communities, therefore the relative effect of localized ambient air pollution on personal HAP exposure could not be examined. Nevertheless, we assume that the residual HAP found after the exclusive use of clean fuel, were contributions from emissions due to use of BMF from neighboring households or from trash burning in the courtyard.

Strengths of this study includes HAP measurement using personal monitoring device which were calibrated before every assessment meticulously; wearing compliance of the monitors was ensured through assessment of accelerometer data at both baseline and end line at almost 100% level; intra- and inter-device measurements showed good accuracy; and exclusive use of clean fuel for all cooking without stove stacking for the entire duration of intervention, with close monitoring. Another major strength was assessment of immunophenotyping and lymphocyte proliferation response in a large number of participants (200 × 2) at pre- and post-LPG intervention. Furthermore, we have found indication of crucial threshold concentrations of BC and CO at personal exposure level, that may lead to a loss in immune regulation and inflammatory processes. Future studies can focus on functional capacities of immune cells and adaptive immune responses to assess relationship with susceptibility to infectious diseases, and investigate whether the indicated threshold concentrations of HAP in exposed Bangladeshi populations are applicable to other geographical settings in relation to immunological outcomes.

## 5. Conclusions

The findings of this study suggest that exclusive use of clean fuel LPG for 26 months was able to reduce personal HAP exposure level considerably, which in turn modulated distribution of B cell subpopulation in the peripheral circulation. The study further showed that sustained reductions in HAP through clean fuel usage also require controlled ambient air pollution generated from anthropogenic activities in parallel, and require more focused attention from the policy makers in monitoring and implementing actions to sustain uptake and improve health. Air pollution is a cross-cutting aspect of many United Nations' Sustainable Development Goals. Clean fuel intervention studies

demonstrating beneficial health impacts in terms of immunological or clinical outcomes will be useful to implement effective interventions and policies based on scientific evidence.

## 6. Data sharing

icddr,b data is shared in compliance with its Data Policies and through an instrument called Data Licensing Application and Agreement (DLAA). However, anything written in the agreement/contract between icddr,b and the sponsor regarding data generated from a funded research project and agreed upon by both parties overrides the requirements of icddr,b data policies. icddr,b Data Policies protect data rights of the Principal Investigator of a study and also privacy of study participants.

## CRediT authorship contribution statement

**Rubhana Raqib:** Conceptualization, Funding acquisition, Methodology, Resources, Writing – original draft, Writing – review and editing. **Evana Akhtar:** Investigation, Validation, Visualization, Writing – review and editing. **Md. Ahsanul Haq:** Data curation, Formal analysis, Software, Writing – original draft. **Shyfuddin Ahmed:** Methodology, Validation, Project administration, Writing – review and editing. **Farjana Haque:** Investigation. **Muhammad Ashique Hyder Chowdhury:** Project administration, Methodology, Writing – review and editing. **Mohammad Hasan Shahriar:** Project administration, Methodology, Writing – review and editing. **Bilkis Ara Begum:** Validation, Investigation. **Mahbub Eunus:** Project administration, Investigation, Supervision. **Golam Sarwar:** Data Curation, Software. **Faruque Parvez:** Writing – review and editing. **Yushuf Sharker:** Formal analysis, Writing – review and editing. **Habibul Ahsan:** Conceptualization, Funding acquisition, Methodology, Resources. **Mohammed Yunus:** Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Writing – review and editing.

## 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.108137.

## References

Academy of Science of South, Africa, Sciences Brazilian Academy of, Leopoldina German National Academy of Sciences, U. S. National Academy of Medicine, and U. S. National Academy of Sciences, 2019. 'Air Pollution and Health - A Science-Policy Initiative', *Ann Glob Health*, 85, 140.

Ahmed, S., Chowdhury, M.A.H., Kader, S.B., Shahriar, M.H., Begum, B.A., Eunus, M., Sarwar, G., Islam, T., Alam, D.S., Parvez, F., Raqib, R., Ahsan, H., Yunus, M., 2022. Personal exposure to household air pollution and lung function in rural Bangladesh: A population-based cross-sectional study. Int. J. Environ. Health Res. 1–13.

Ahsan, H., Chen, Y., Parvez, F., Argos, M., Hussain, A.I., Momotaj, H., Levy, D., van Geen, A., Howe, G., Graziano, J., 2006. Health Effects of Arsenic Longitudinal Study (HEALS): description of a multidisciplinary epidemiologic investigation. J. Eposure Sci. Environ. Epidemiol. 16, 191–205.

Alam, N., Ali, T., Razzaque, A., Rahman, M., Zahirul Haq, M., Saha, S.K., Ahmed, A., Sarder, A.M., Moinuddin Haider, M., Yunus, M., Nahar, Q., Kim Streatfield, P., 2017. 'Health and Demographic Surveillance System (HDSS) in Matlab. Bangladesh', *Int J Epidemiol* 46, 809–816.

Ali, M.U., Yu, Y., Yousaf, B., Munir, M.A.M., Ullah, S., Zheng, C., Kuang, X., Wong, M.H., 2021. Health impacts of indoor air pollution from household solid fuel on children and women. J. Hazard. Mater. 416, 126127.

BDHS, 2017-2018. "National Institute of Population Research and Training (NIPORT), and ICF. 2020. Bangladesh Demographic and Health Survey 2017-18. Dhaka, Bangladesh, and Rockville, Maryland, USA." In.

Biswas, S.K., Tarafdar, S.A., Islam, A., Khaliquzzaman, M., Tervahattu, H., Kupiainen, K., 2003. 'Impact of unleaded gasoline introduction on the concentration of lead in the air of Dhaka. Bangladesh', *J Air Waste Manag Assoc* 53, 1355–1362.

Chillrud, S.N., Ae-Ngibise, K.A., Gould, C.F., Owusu-Agyei, S., Mujtaba, M., Manu, G., Burkart, K., Kinney, P.L., Quinn, A., Jack, D.W., Asante, K.P., 2021. The effect of clean cooking interventions on mother and child personal exposure to air pollution: results from the Ghana Randomized Air Pollution and Health Study (GRAPHS). J. Eposure Sci. Environ. Epidemiol. 31, 683–698.

Curto, A., Donaire-Gonzalez, D., Manaca, M.N., Gonzalez, R., Sacoor, C., Rivas, I., Gascon, M., Wellenius, G.A., Querol, X., Sunyer, J., Macete, E., Menendez, C., Tonne, C., 2019. Predictors of personal exposure to black carbon among women in southern semi-rural Mozambique. Environ. Int. 131, 104962.

Dangleben, N.L., Skibola, C.F., Smith, M.T., 2013. Arsenic immunotoxicity: a review. Environ. Health 12, 73.

Dutta, A., Bhattacharya, P., Lahiri, T., Ray, M.R., 2012. Immune cells and cardiovascular health in premenopausal women of rural India chronically exposed to biomass smoke during daily household cooking. Sci. Total Environ. 438, 293–298.

Giles, B.H., Mann, K.K., 2022. Arsenic as an immunotoxicant. Toxicol. Appl. Pharmacol. 454, 116248.

Glencross, D.A., Ho, T.R., Camina, N., Hawrylowicz, C.M., Pfeffer, P.E., 2020. Air pollution and its effects on the immune system. Free Radic. Biol. Med. 151, 56–68.

Hashemzadeh, B., Idani, E., Goudarzi, G., Ankali, K.A., Sakhvidi, M.J.Z., Akbar Babaei, A., Hashemzadeh, H., Vosoughi, M., Mohammadi, M.J., Neisi, A., 2019. Effects of PM2.5 and NO2 on the 8-isoprostane and lung function indices of FVC and FEV1 in students of Ahvaz city, Iran. Saudi J Biol Sci 26, 473–480.

https://www.icddrb.org/research/platforms/health-and-demographic-surveillance-systems-hdss, 2023. "Health and Demographic Surveillance Systems (HDSS)-Matlab Bangladesh.".

Huhmann, B. L., C. F. Harvey, A. Navas-Acien, J. Graziano, F. Parvez, Y. Chen, M. Argos, A. Ahmed, Akmr Hasan, H. Ahsan, and A. van Geen. 2019. 'Changes in arsenic exposure in Araihazar, Bangladesh from 2001 through 2015 following a blanket well testing and education campaign', *Environ Int*, 125: 82-89.

IQAir. 2022a. "Air quality and pollution city ranking." In.

2022b. "New WHO Global Air Quality Guidelines aim to save millions of lives from air pollution." In.

Johnson, M., A. Pillarisetti, R. Piedrahita, K. Balakrishnan, J. L. Peel, K. Steenland, L. J. Underhill, G. Rosa, M. A. Kirby, A. Diaz-Artiga, J. McCracken, M. L. Clark, L. Waller, H. H. Chang, J. Wang, E. Dusabimana, F. Ndagijimana, S. Sambandam, K. Mukhopadhyay, K. A. Kearns, D. Campbell, J. Kremer, J. P. Rosenthal, W. Checkley, T. Clasen, L. Naeher, and Investigators the Household Air Pollution Intervention Network Trial. 2022. 'Exposure Contrasts of Pregnant Women during the Household Air Pollution Intervention Network Randomized Controlled Trial', *Environ Health Perspect*, 130: 97005.

Kumar, N., Phillip, E., Cooper, H., Davis, M., Langevin, J., Clifford, M., Stanistreet, D., 2021. Do improved biomass cookstove interventions improve indoor air quality and blood pressure? A systematic review and meta-analysis. Environ. Pollut. 290, 117997.

Lee, A., Kinney, P., Chillrud, S., Jack, D., 2015. A Systematic Review of Innate Immunomodulatory Effects of Household Air Pollution Secondary to the Burning of Biomass Fuels. Ann. Glob. Health 81, 368–374.

Mila, C., Curto, A., Dimitrova, A., Sreekanth, V., Kinra, S., Marshall, J.D., Tonne, C., 2020. Identifying predictors of personal exposure to air temperature in peri-urban India. Sci. Total Environ. 707, 136114.

Moller, W., Hofer, T., Ziesenis, A., Karg, E., Heyder, J., 2002. Ultrafine particles cause cytoskeletal dysfunctions in macrophages. Toxicol. Appl. Pharmacol. 182, 197–207.

Norris, C., Goldberg, M.S., Marshall, J.D., Valois, M.F., Pradeep, T., Narayanswamy, M., Jain, G., Sethuraman, K., Baumgartner, J., 2016. A panel study of the acute effects of personal exposure to household air pollution on ambulatory blood pressure in rural Indian women. Environ. Res. 147, 331–342.

Pierdominici, M., Maselli, A., Cecchetti, S., Tinari, A., Mastrofrancesco, A., Alfe, M., Gargiulo, V., Beatrice, C., Di Blasio, G., Carpinelli, G., Ortona, E., Giovannetti, A., Fiorito, S., 2014. Diesel exhaust particle exposure in vitro impacts T lymphocyte phenotype and function. Part. Fibre Toxicol. 11, 74.

Pope, D., Bruce, N., Dherani, M., Jagoe, K., Rehfuess, E., 2017. Real-life effectiveness of 'improved' stoves and clean fuels in reducing PM2.5 and CO: Systematic review and meta-analysis. Environ. Int. 101, 7–18.

Pratiti, R., Vadala, D., Kalynych, Z., Sud, P., 2020. Health effects of household air pollution related to biomass cook stoves in resource limited countries and its mitigation by improved cookstoves. Environ. Res. 186, 109574.
Rahman, M., Vahter, M., Wahed, M.A., Sohel, N., Yunus, M., Streatfield, P.K., El Arifeen, S., Bhuiya, A., Zaman, K., Chowdhury, A.M., Ekstrom, E.C., Persson, L.A., 2006. 'Prevalence of arsenic exposure and skin lesions. A population based survey in Matlab, Bangladesh', *J Epidemiol Community Health* 60, 242–248.
Raqib, R., Akhtar, E., Sultana, T., Ahmed, S., Chowdhury, M.A.H., Shahriar, M.H., Kader, S.B., Eunus, M., Haq, M.A., Sarwar, G., Islam, T., Alam, D.S., Parvez, F., Begum, B.A., Ahsan, H., Yunus, M., 2022. Association of household air pollution with cellular and humoral immune responses among women in rural Bangladesh. Environ. Pollut. 299, 118892.
Raqib, R., Akhtar, E., Haq, M.A., Sarker, P., 2023. 'Effects of prenatal exposure to arsenic on T cell development in children', *Current Opinion on.* Toxicology 34.
Rosa, M.J., Yan, B., Chillrud, S.N., Acosta, L.M., Divjan, A., Jacobson, J.S., Miller, R.L., Goldstein, I.F., Perzanowski, M.S., 2014. Domestic airborne black carbon levels and 8-isoprostane in exhaled breath condensate among children in New York City. Environ. Res. 135, 105–110.
Rylance, J., Fullerton, D.G., Scriven, J., Aljurayyan, A.N., Mzinza, D., Barrett, S., Wright, A.K., Wootton, D.G., Glennie, S.J., Baple, K., Knott, A., Mortimer, K., Russell, D.G., Heyderman, R.S., Gordon, S.B., 2015. Household air pollution causes dose-dependent inflammation and altered phagocytosis in human macrophages. Am. J. Respir. Cell Mol. Biol. 52, 584–593.
Schraufnagel, D.E., Balmes, J.R., De Matteis, S., Hoffman, B., Kim, W.J., Perez-Padilla, R., Rice, M., Sood, A., Vanker, A., Wuebbles, D.J., 2019. Health Benefits of Air Pollution Reduction. Ann. Am. Thorac. Soc. 16, 1478–1487.
Shahriar, M.H., Chowdhury, M.A.H., Ahmed, S., Eunus, M., Kader, S.B., Begum, B.A., Islam, T., Sarwar, G., Al Shams, R., Raqib, R., Alam, D.S., Parvez, F., Ahsan, H., Yunus, M., 2021. Exposure to household air pollutants and endothelial dysfunction in rural Bangladesh: A cross-sectional study. Environ Epidemiol 5, e132.
Sharma, D., Jain, S., 2020. Reduction in black carbon concentration and its exposure in rural settings of Northern India: An intervention analysis. Chemosphere 247, 125838.
Shupler, M., P. Hystad, A. Birch, D. Miller-Lionberg, M. Jeronimo, R. E. Arku, Y. L. Chu, M. Mushtaha, L. Heenan, S. Rangarajan, P. Seron, F. Lanas, F. Cazor, P. Lopez-Jaramillo, P. A. Camacho, M. Perez, K. Yeates, N. West, T. Ncube, B. Ncube, J. Chifamba, R. Yusuf, A. Khan, B. Hu, X. Liu, L. Wei, L. A. Tse, D. Mohan, P. Kumar, R. Gupta, I. Mohan, K. G. Jayachitra, P. K. Mony, K. Rammohan, S. Nair, P. V. M. Lakshmi, V. Sagar, R. Khawaja, R. Iqbal, K. Kazmi, S. Yusuf, M. Brauer, and Pure-Air study. 2020. 'Household and personal air pollution exposure measurements from 120 communities in eight countries: results from the PURE-AIR study', *Lancet Planet Health*, 4: e451-e62.
Smith, K.R., McCracken, J.P., Weber, M.W., Hubbard, A., Jenny, A., Thompson, L.M., Balmes, J., Diaz, A., Arana, B., Bruce, N., 2011. Effect of reduction in household air pollution on childhood pneumonia in Guatemala (RESPIRE): a randomised controlled trial. Lancet 378, 1717–1726.
Song, R., Mahidhara, R.S., Zhou, Z., Hoffman, R.A., Seol, D.W., Flavell, R.A., Billiar, T.R., Otterbein, L.E., Choi, A.M., 2004. Carbon monoxide inhibits T lymphocyte proliferation via caspase-dependent pathway. J. Immunol. 172, 1220–1226.
Stiller-Winkler, R., Idel, H., Leng, G., Spix, C., Dolgner, R., 1996. Influence of air pollution on humoral immune response. J. Clin. Epidemiol. 49, 527–534.
van Geen, A., Ahmed, E.B., Pitcher, L., Mey, J.L., Ahsan, H., Graziano, J.H., Ahmed, K.M., 2014. Comparison of two blanket surveys of arsenic in tubewells conducted 12 years apart in a 25 km(2) area of Bangladesh. Sci. Total Environ. 488–489, 484–492.
Wang, H., Li, X.B., Chu, X.J., Cao, N.W., Wu, H., Huang, R.G., Li, B.Z., Ye, D.Q., 2022. Ambient air pollutants increase the risk of immunoglobulin E-mediated allergic diseases: a systematic review and meta-analysis. Environ. Sci. Pollut. Res. Int. 29, 49534–49552.
WHO. 2022. "Household air pollution and health." In.
Yan, Y., Wang, L., Chen, S., Zhao, G., Fu, C., Xu, B., Tan, X., Xiang, Y., Chen, G., 2020. Carbon Monoxide Inhibits T Cell Proliferation by Suppressing Reactive Oxygen Species Signaling. Antioxid. Redox Signal. 32, 429–446.
Zhou, H., Lin, J., Shen, Y., Deng, F., Gao, Y., Liu, Y., Dong, H., Zhang, Y., Sun, Q., Fang, J., Tang, S., Wang, Y., Du, Y., Cui, L., Ruan, S., Kong, F., Liu, Z., Li, T., 2020. Personal black carbon exposure and its determinants among elderly adults in urban China. Environ. Int. 138, 105607.