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Sex-, Age-, and Metabolic Disorder-Dependent Distributions of Selected Inflammatory Biomarkers among Community-Dwelling Adults

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Background: Inflammatory cytokines are increasingly utilized to detect high-risk individuals for cardiometabolic diseases. However, with large population and assay methodological heterogeneity, no clear reference currently exists.
Methods: Among participants of the Cardiovascular and Metabolic Diseases Etiology Research Center cohort, of community-dwelling adults aged 30 to 64 without overt cardiovascular diseases, we presented distributions of tumor necrosis factor (TNF)-α and -β, interleukin (IL)-1α, -1β, and 6, monocyte chemoattractant protein (MCP)-1 and -3 and high sensitivity C-reactive protein (hsCRP) with and without non-detectable (ND) measurements using multiplex enzyme-linked immunosorbent assay. Then, we compared each markers by sex, age, and prevalence of type 2 diabetes mellitus, hypertension, and dyslipidemia, using the Wilcoxon Rank-Sum Test.
Results: In general, there were inconsistencies in direction and magnitude of differences in distributions by sex, age, and prevalence of cardiometabolic disorders. Overall, the median and the 99th percentiles were higher in men than in women. Older participants had higher TNF-α, high sensitivity IL-6 (hsIL-6), MCP-1, hsCRP, TNF-β, and MCP-3 median, after excluding the NDs. Participants with type 2 diabetes mellitus had higher median for all assayed biomarkers, except for TNF-β, IL-1α, and MCP-3, in which the medians for both groups were 0.00 due to predominant NDs. Compared to normotensive group, participants with hypertension had higher TNF-α, hsIL-6, MCP-1, and hsCRP median. When stratifying by dyslipidemia prevalence, the comparison varied significantly depending on the treatment of NDs.
Conclusion: Our findings provide sex-, age-, and disease-specific reference values to improve risk prediction and diagnostic performance for inflammatory diseases in both population- and clinic-based settings.

Keywords: Biomarkers; Cardiovascular diseases; Inflammation; Metabolic diseases

INTRODUCTION

The utility of inflammatory biomarker assays for detecting individuals at high-risk for cardiovascular and metabolic diseases has become increasingly prevalent in clinical and research settings [1-5]. In adjunct to the conventional chemistry and imaging tests, these biomarkers aid in predicting or diagnosing cardiovascular and metabolic diseases in the context of an appropriate clinical presentation [6-8].

Considering that the major contribution of inflammatory mechanisms to cardiometabolic diseases has been repeatedly emphasized, many efforts are continuously being made to discover novel markers and assay methodologies. In particular, cytokines/chemokines are critical in homeostatic trafficking and positioning of immune cells in response to inflammation [9-11]; they trigger and modify intracellular signaling path-

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ways, thereby orchestrating innate and adaptive immune responses [12].

Of many, interleukin (IL), monocyte chemotactic proteins (MCP) and tumor necrosis factors (TNF) are critical to macrophagic inhibition [13]. They activate growth factors, stimulate procoagulant activity, and suppress antithrombotic pathways in endothelial cells [6]. By measuring their expression in atherosclerotic lesions, previous studies have observed the association between the aforementioned biomarkers and cardiometabolic outcomes, such as type 2 diabetes mellitus (T2DM), myocardial infarction (MI) and more [14-16]. However, particularly with the advent of newer assay methodologies and large population heterogeneity, no clear consensus exists regarding the universal reference range. Moreover, little is known about the distribution of these biomarkers across healthy community population.

To address this knowledge gap, the objective of this study was to determine distributions of selected inflammatory biomarkers among middle-aged Koreans. Then, we compared their descriptive statistics by sex, age, and metabolic disorders, including T2DM, hypertension and dyslipidemia.

METHODS

Approvals and participant consent

As part of the ethical committee process, the Cardiovascular and Metabolic Diseases Etiology Research Center (CMERC) Study has been approved by the Institutional Review Boards of Severance Hospital, Yonsei University Health System, Seoul, Korea (4-2013-0661) and Ajou University Hospital, Suwon, Korea (AJIRB-BMR-SUR-13-272). Written informed consent has been obtained from all participants prior to the baseline survey. Participants were ensured that they can withdraw from the study at any time, regardless of its cause, without any repercussions.

Study population

The CMERC cohort is a multi-centered, prospective, observational study of community-dwelling population residing in Seoul and capital regions in Republic of Korea [17]. The participants are between the age of 30 to 64 years and without history overt cardiovascular diseases (CVDs), recent history of malignant cancer and autoimmune or chronic inflammatory diseases. In the present study, among 8,108 participants who have undergone baseline examination between 2013 and 2018, 4,058 participants (excluding two with missing variables) from Center 1 underwent high sensitivity C-reactive protein (hsCRP) measurements. Among them, a random subset of participants enrolled between 2013 to 2015 underwent additional measurements via multiplex enzyme-linked immunosorbent assay (ELISA) assay, including TNF-α, TNF-β, IL-1α, IL-1β, hsIL-1β, IL-6, hsIL-6, MCP-1, and MCP-3. The varying number of measurements for each biomarker was due to different number of non-detectable (ND) measurements, requiring higher sensitivity analyses.

Data collection

Overnight-fasting blood samples were obtained in the morning. All biomarker assays were performed in accordance to standard protocols at a single laboratory (Seoul Clinical Laboratory R&D Center, Seoul, Korea).

hsCRP

We collected bubble-free serum samples in plastic containers and allowed them to clot for at least 30 minutes before centrifugation for 10 minutes at 1,000×g. Then, we aliquot and stored the samples below –20°C. During the sample preparation, we allowed all regents to warm to room temperature, premixed beat bottle for 30 seconds, vortexed for 1 minute and centrifuged thoroughly prior to use. No samples were frozen nor thawed for multiple times.

The samples were measured by the immunoturbidimetric CRP-N Assay LA CRP-S Nittobo D-Type high sensitive assay (Nittobo, Tokyo, Japan). The samples were loaded and measured using the latex-enhanced nephelometry technique on an automated analyzer ADVIA 1800 Chemistry System (Siemens Medical Sol., Malvern, PA, USA). The assays were based on the principle of particle-enhanced immunological agglutination method with a commercial test kit (N assay LA, CRP-S, Nittobo). A human CRP calibrator N assay LA CRP-S multi-point was employed to delineate the calibration curve.

The lower and upper detection limits of the hsCRP assay were 0.02 and 40 mg/dL, respectively. The functional sensitivity of the hsCRP assay was 0.45 mg/dL. To calculate intra-assay variability, we repeated the pool serum assay 20 times; the absorbance coefficient variability was less than 5%. Measurement was done in duplicates and any duplicates that were not within a three assay standard deviation from one another were re-run.

Cytokines and chemokines

The sample collection and preparation methods are the same

as the aforementioned hsCRP assay. We quantitatively determined the steady state level of the circulating inflammatory cytokines/chemokines of interest: TNF-α, TNF-β, IL-1α, IL-1β, IL-6, MCP-1, and MCP-3. Their serum concentrations were measured using a Milliplex MAP Human Cytokine/Chemokine Multiplex Bead-based kit (Millipore, Burlington, MA, USA), with a 38-plex (HMCP3-MAG, HIL1A-MAG, HCYIL1B-MAG, HCYIL6-MAG, HCYMCP1-MAG, HCYTNFA-MAG, HTNFB-MAG) Millipore Human Cytokine Panel Kits. Specifically, 25 μL of serum was incubated with fluorescently labeled capture antibody-coated beads in a 96-well filter bottomed plate on a plate shaker overnight at 4°C. After incubation, the sample bead mix was removed, and the plate was washed two times using a vacuum manifold. The beads were resuspended in sheath fluid for 5 minutes on the plate shaker. Distinctly colored bead sets of 500 5.6-Nm polystyrene microspheres or 80 6.45-μm magnetic microspheres were created, each of which was coated with a distinctive capture antibody. After an analyte from the sample was captured by the bead, a biotinylated detection antibody was introduced and incubated on a plate shaker at room temperature for 30 minutes. The reaction mixture was incubated with streptavidin-phycoerythrin (Streptavidin-PE) conjugate to complete the reaction on the surface of each microsphere.

The Luminex Bio-Plex 100 analyzer (MAGPIX) identified individual microsphere, and the results were quantified based on fluorescent reporter signals using Luminex xPONENT acquisition software, Milliplex Analyst 5.1. We analyzed the median fluorescence intensity (MFI) using a 5-parameter logistic or spline curve-fitting method to calculate cytokine/chemokines concentrations in each sample. The Luminex MAGPIX instrument was calibrated with the MAGPIX Calibration Kit (EMD Millipore Catalog #40-049), and the performance was verified with the MAGPIX Performance Verification Kit (EMD Millipore Catalog #40-050).

All assays were performed by the same operator according to the manufacturers' instructions. For quality assurance, each sample was run twice, and the mean derived for each sample was used as the index value. Additionally, we reconstituted two kit-supplied quality control (#1, 2) with 250 μL of deionized water to run on each plate in duplicate. After inverting the vial several times, we allowed the vial to sit for 5 to 10 minutes, and then transferred the controls to appropriately labeled polypropylene microfuge tubes. We confirmed the samples to fall within the expected range in accordance with the kit-specific protocols provided by Millipore. Less than 0.5% cross-reactivity and interference were observed.

High sensitivity cytokines

The sample collection and preparation methods are the same as the aforementioned cytokine/chemokine assay. Here, the samples underwent a 2-fold dilution, maintaining a 125 μL of sample and 125 μL of Calibrator Diluent RD6-40 ratio, respectively.

We quantitatively determined the steady state level of the circulating inflammatory cytokines of interest: hsIL-1 and hsIL-6. Their serum concentrations were modulated using Milliplex MAP Human High Sensitivity Cytokine/Chemokine Base kit A (Millipore, Billerica, Burlington, USA) on a 96-well filter bottomed plate. Analyte-specific antibodies were precoated onto color-coded magnetic microparticles. Microparticles, standards, and samples were pipetted into wells, and the immobilized antibodies captured the analytes of interest. After washing away unbound substances, corresponding biotinylated antibody cocktail was added to each well. After a thorough wash, Streptavidin-PE conjugate was added to each well. A final wash removed residual unbound conjugate, and the microparticles were resuspended in buffer. The Luminex Bio-Plex 100 analyzer (MAGPIX) identified individual microsphere, and the results were quantified based on fluorescent reporter signals within 90 minutes of the run. The results were analyzed using Luminex xPONENT acquisition software, Milliplex Analyst 5.1.

To calculate a 4-fold dilution for the remaining levels, we referred to the standard concentrations provided by the manufacturer. We averaged the duplicate reading for each standard and sample, and subtracted the average blank MFI using a 5-parameter spline curve-fitting method. Since the samples were diluted, the concentration read from the standard curve were multiplied by the corresponding dilution factor. The Luminex MAGPIX instrument was calibrated with the MAGPIX Calibration Kit (EMD Millipore Catalog #40-049), and its performance was verified with the MAGPIX Performance Verification Kit (EMD Millipore Catalog #40-050).

Similar to the aforementioned cytokine and chemokine assay, we employed the manufacturer-recommended quality control (#11). Likewise, less than 0.5% cross-reactivity and interference were observed.

Data for each kit was analyzed with strict adherence to the manufacturers' guidelines. The proportion of the samples that

had both readings within the accepted recovery range (between 70% and 130%) was determined. The reproducibility of the different multiplex methods was evaluated by describing the limits of agreement between duplicates in range for each combination of method and analyte using the Bland-Altman test.

Type 2 diabetes mellitus

Fasting plasma glucose levels were measured using colorimetry method (ADVIA1800 Auto Analyzer; Siemens Medical Sol.), and glycosylated hemoglobin (HbA1c) measurements were obtained via high-performance liquid chromatography (Variant II Turbo Hemoglobin Testing System; Bio-Rad., Hercules, CA, USA). T2DM was defined based on the Korean Clinical Practice Guidelines for T2DM [18]: participants with fasting glucose level ≥126 mg/dL, HbA1c ≥6.5% or current use of oral glucose-lowering drugs or insulin injection were considered to have T2DM.

Hypertension

Blood pressure was measured using a single-arm automated oscillometric device (HEM-7080; Omron Health, Matsusaka, Japan). Information regarding hypertension treatment was obtained via self-report. Hypertension was defined according to the 2013 Korean Society of Hypertension guidelines for the management of hypertension [19], equivalent to the Eight Joint National Committee guidelines [20]: participants with a mean systolic blood pressure (SBP) ≥140 mm Hg, diastolic blood pressure (DBP) ≥90 mm Hg or currently using antihypertensive medications were considered to have hypertension.

Dyslipidemia

Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were analyzed enzymatically with an ADVIA 1800 Auto Analyzer. Dyslipidemia was defined based on the Korean Dyslipidemia Diagnosis criteria [21], equivalent to Adult Treatment Panel III (ATP III) guidelines [22]. Hypercholesterolemia was defined as TC ≥240 mg/dL; hypertriglyceridemia was defined as TG ≥200 mg/dL; hypoalphalipoproteinemia was defined as HDL-C <40 mg/dL; hyper-LDL-cholesterolemia was defined as LDL-C ≥160 mg/dL. Having any one type of the aforementioned cholesterol abnormality or current intake of lipid-lowering was regarded as prevalent dyslipidemia.

Statistical analysis

We defined the minimum, median, 75th, 90th, 95th, 99th percentile, maximum and mean levels for each biomarker using a non-parametric procedure. We presented distribution statistics of each inflammatory marker with and without NDs below the mechanical detection threshold. We additionally stratified by sex, age, T2DM, hypertension and dyslipidemia prevalence, and compared using Wilcoxon rank-sum test. No detectable outliers were excluded in the analysis to preserve the natural integrity of the cohort characteristics. All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

A total of 4,058 participants underwent hsCRP measurements between 2013 and 2018 (Fig. 1). Among them, 1,285 participants whom enrolled in the CMERC study between 2013 and 2015, also underwent TNF-α, IL-1α, IL-1β, IL-6, and MCP-1 and three measurements. Additionally, we assayed hsIL-6 and hsIL-1β from 819 participants and TNF-β from 466 participants. TNF-α, IL-1α, IL-1β, IL-6, and MCP-3 had varying number of NDs.

At baseline, 35.1% of the participants were male, with mean age of 51.3 years (Table 1). In terms of prevalence of cardiometabolic disorder, 372 participants had T2DM (9.2%), 1,070 participants had hypertension (26.4%) and 1,882 participants had dyslipidemia (46.4%). Lifestyle factors (i.e., current smoking and drinking status), anthropometric measurements (i.e. body mass index) and biomarkers related to CVD risk differed significantly by sex.

Table 2, Supplementary Tables 1-4, and Supplementary Figs 1-15 illustrate the distribution of each inflammatory biomarker, including and excluding NDs. Five biomarkers had varying proportion of NDs: TNF-β (70.0%), IL-1α (72.8%), IL-1β (8.3%), IL-6 (8.2%), and MCP-3 (60.8%). In general, the descriptive statistics with and without NDs were different when comparing their respective median and upper percentiles. The overall skewedness to right primarily resulted from upper outliers and zero coding of the NDs.

Table 3 illustrates the Spearman's rank correlation coefficient. Overall, the markers showed weak to moderate positive association among each other. The exceptions are as follows: MCP-1 and TNF-β (r=–0.13), IL-1α and hsIL-6 (r=–0.04), and MCP-1 and MCP-3 (r=–0.01) showed weak yet negative

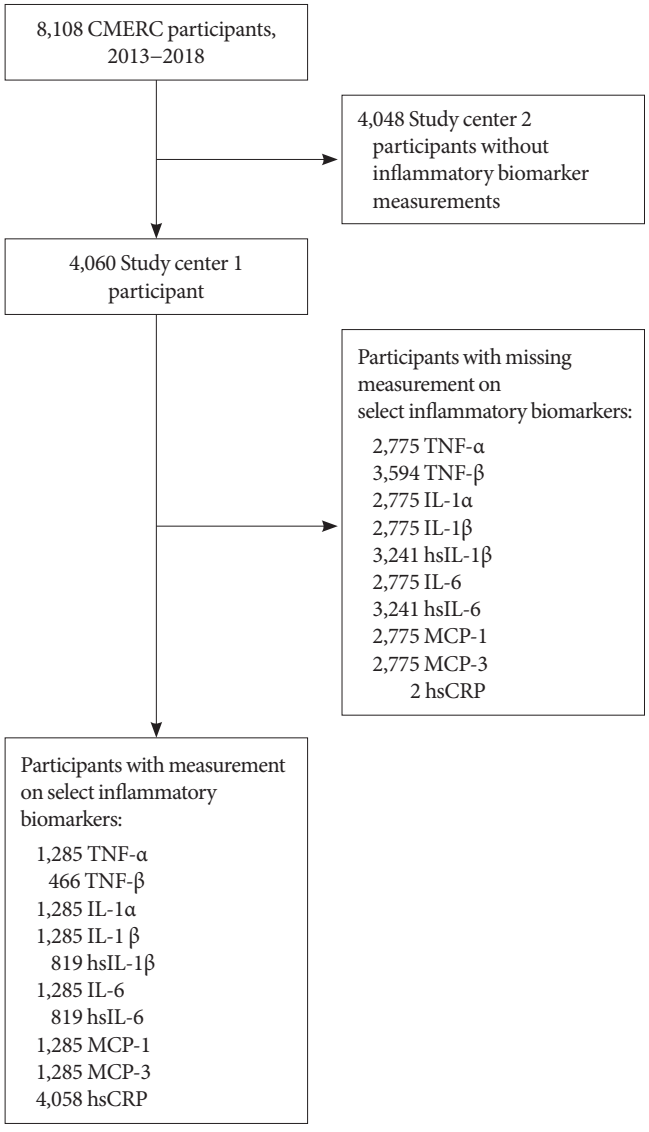

Fig. 1. Flow diagram of the study inclusion criteria. CMERC, Cardiovascular and Metabolic Disease Etiology Research Center; TNF, tumor necrosis factor; IL, interleukin; hsIL, high sensitivity interleukin; MCP, monocyte chemoattractant protein; hsCRP, high sensitivity C-reactive protein.

associations, which persisted even after excluding NDs.

For all biomarkers, the median and the 99th percentiles were higher in male than in female regardless of NDs (Tables 4 and 5). For example, the median and the 99th percentile IL-6 values were 1.38 and 345.97 pg/mL in male and 0.37 and 280.06 pg/mL in female, respectively ($P<0.001$). Such sex-difference extended in high sensitivity analysis, where the median and the 99th percentile hsIL-6 values were 0.83 and 14.06 pg/mL in men and 0.75 and 7.08 pg/mL in women, respectively. Other IL, TNF and MCP subtypes demonstrated similar trend.

When stratifying by the population median age of approximately 55 years, the older group had significantly higher hsIL-6, MCP-1 and hsCRP median (Tables 4 and 5). In contrast, the median value for hsIL-1β was higher in the younger group. Furthermore, the 99th percentile value for hsIL-6 was also markedly higher in the younger group, even after excluding the NDs.

The distributions of the examined markers differed by the presence of each cardiometabolic disorder. Participants with T2DM had higher median for all assayed biomarkers, except for TNF-β, IL-1α, and MCP-3, in which the medians for both groups were 0.00 due to predominant NDs (Table 4). However, when excluding the NDs, the medians for IL-1α and MCP-3 were lower in participants with T2DM than their counterpart (35.24 pg/mL vs. 103.37 pg/mL and 29.85 pg/mL vs. 31.34 pg/mL, respectively), but the differences were statistically insignificant (Table 5). The 99th percentile values for TNF-β, IL-1α, IL-1β, hsIL-1β, hsIL-6, and hsCRP were higher in participants with T2DM, when considering NDs into account.

Compared to the normotensive group, participants with hypertension had higher median TNF-α, hsIL-6, MCP-1, and hsCRP and 99th percentile TNF-β, MCP-3, and hsCRP values. These differences generally persisted even after excluding NDs.

Participants with elevated lipid levels had higher median TNF-α, IL-1β, hsIL-1β, hsIL-6, MCP-1, and hsCRP values than those within normal cholesterol range. However, when examining distributions without NDs, participants without dyslipidemia had higher median TNF-β, IL-1α, IL-6, and MCP-3 levels. In terms of the 99th percentile, participants with dyslipidemia had higher IL-1α, hsIL-1β, hsIL-6, and MCP-1 levels, and such difference remained after excluding NDs.

DISCUSSION

In this middle-aged community-dwelling Korean population, we presented the distributions of varying groups of inflammatory biomarkers, which were distinctive by sex, age and prevalence of cardiometabolic disorders. No explicit recommendations nor universal reference range currently exist for these cytokines/chemokines, owing to insufficient evidence from the general population. Without existing manufacturer-recommended 99th percentile nor referent range, we illustrated their distributions detected in relatively healthy population without any data refinement. These results have important clinical im-

Table 1. General characteristics of the study participants by sex (n=4,058)

Variable	Total (n=4,058)	Sex		P value
		Male (n=1,426)	Female (n=2,632)	
Age, yr	51.3±9.4	50.2±10.2	51.9±8.8	<0.001
BMI, kg/m^2	23.9±3.1	24.9±2.9	23.4±3.0	<0.001
Alcohol intake				<0.001
Non-drinker	905 (22.3)	118 (8.3)	787 (29.9)	
Previous drinker	184 (4.5)	86 (6.0)	98 (3.7)	
Current drinker	2,969 (73.2)	1,222 (85.7)	1,747 (66.4)	
Smoking status				<0.001
Non-smoker	2,778 (68.5)	322 (22.6)	2,456 (93.3)	
Previous smoker	724 (17.8)	626 (43.9)	98 (3.7)	
Current smoker	556 (13.7)	478 (33.5)	78 (3.0)	0.045
History of CVD				
Yes	26 (0.6)	14 (1.0)	12 (0.5)	
No	4,032 (99.4)	1,412 (99.0)	2,620 (99.5)	
Fasting glucose, mg/dL	91.8±19.3	96.1±23.9	89.5±15.8	<0.001
HbA1c, %	5.7±0.7	5.7±0.8	5.6±0.6	<0.001
Diabetes mellitus				<0.001
Yes	372 (9.2)	178 (12.5)	194 (7.4)	
No	3,686 (90.8)	1,248 (87.5)	2,438 (92.6)	
SBP, mm Hg	118.6±14.9	125.0±13.6	115.2±14.4	<0.001
DBP, mm Hg	76.2±9.9	80.6±9.9	73.8±9.1	<0.001
Hypertension				<0.001
Yes	1,070 (26.4)	509 (35.7)	561 (21.3)	
No	2,988 (73.6)	917 (64.3)	2,071 (78.7)	
Total cholesterol, mg/dL	198.4±35.4	195.3±35.3	200.1±35.3	<0.001
HDL-C, mg/dL	57.6±14.7	51.3±12.8	61.0±14.5	<0.001
LDL-C, mg/dL	115.3±32.1	112.8±33.3	116.6±31.4	<0.001
Triglyceride, mg/dL	127.9±87.8	155.9±115.9	112.7±62.8	<0.001
Dyslipidemia				<0.001
Yes	1,882 (46.4)	793 (55.6)	1,089 (41.4)	
No	2,176 (53.6)	633 (44.4)	1,543 (58.6)	

Values are presented as mean±standard deviation or number (%). P value was derived from the independent t-test, the Wilcoxon rank-sum test, or chi-square test.
BMI, body mass index; CVD, cardiovascular disease; HbA1c, glycosylated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

plications that are almost certainly relevant to the application of all modern multiplex assays to both epidemiologic and clinical studies.

hsCRP

Previous literature in this field has noted heterogeneous distributions and reference ranges derived from their own study population. In a Chinese population, the median hsCRP was 0.99 mg/L in the middle-aged group and 1.76 mg/L in the elderly group without gender difference [23]. In a Thai adult population, the hsCRP concentration ranged from 0.2 to 7.9 mg/L without significant sex- nor age-differences [24]. Despite

Table 2. Distribution of inflammatory biomarkers in total participants (n=4,058)

Biomarker	No. of measurement	No. of ND measurement	Mean	SD	Minimum	25th percentile	Median	75th percentile	90th percentile	95th percentile	99th percentile	Maximum
Including ND measurements												
TNF-α, pg/mL	1,285	0	19.59	28.62	0.47	8.11	12.26	19.49	33.23	58.52	150.29	301.65
TNF-β, pg/mL	466	326	7.98	88.97	0.00	0.00	0.00	0.07	1.15	9.48	168.50	1,840.00
IL-1α, pg/mL	1,285	936	79.19	281.69	0.00	0.00	0.00	2.28	193.19	534.02	1,296.00	4,444.00
IL-1β, pg/mL	1,285	106	5.10	18.35	0.00	0.13	0.52	2.01	9.54	24.37	95.95	276.47
hsIL-1β, pg/mL	819	0	0.90	1.00	0.10	0.40	0.70	1.00	1.80	2.57	4.90	15.20
IL-6, pg/mL	1,285	105	23.50	59.64	0.00	0.09	0.61	11.46	79.77	145.27	300.15	505.69
hsIL-6, pg/mL	819	0	1.31	3.32	0.22	0.60	0.76	1.13	1.97	3.03	10.25	69.60
MCP-1, pg/mL	1,285	0	709.40	355.67	80.75	511.57	667.21	843.22	1,056	1,192	1,698	8,129.00
MCP-3, pg/mL	1,285	781	20.50	48.36	0.00	0.00	0.00	17.76	69.45	104.44	243.70	525.80
hsCRP, mg/L	4,058	0	1.44	3.87	0.01	0.33	0.58	1.19	2.66	4.89	16.29	88.49
Excluding ND measurements												
TNF-β, pg/mL	140	-	26.55	161.20	0.01	0.13	0.33	3.16	26.71	106.57	381.59	1,840.00
IL-1α, pg/mL	349	-	291.57	480.27	0.02	15.39	91.43	375.53	853.18	1,151	2,233	4,444.00
IL-1β, pg/mL	1,179	-	5.55	19.09	0.01	0.22	0.62	2.25	10.52	28.00	97.68	276.47
IL-6, pg/L	1,180	-	25.59	61.81	0.01	0.14	0.87	15.11	86.56	150.38	301.61	505.69
MCP-3, pg/mL	504	-	52.26	65.62	0.01	10.19	31.16	70.68	123.65	193.87	273.65	525.80

ND, non-detectable; SD, standard deviation; TNF, tumor necrosis factor; IL, interleukin; hsIL, high sensitivity interleukin; MCP, monocyte chemoattractant protein; hsCRP, high sensitivity C-reactive protein.

Table 3. Spearman's correlation among inflammatory biomarkers (n=4,058)

Biomarker	TNF-α	TNF-β	IL-1α	IL-1β	hsIL-1β	IL-6	hsIL-6	MCP-1	MCP-3	hsCRP
TNF-α	1.00	0.03	0.30[c]	0.26[c]	0.21[c]	0.46[c]	0.14[c]	0.32[c]	0.28[c]	0.19[c]
TNF-β	0.43[c]	1.00	0.25[c]	0.26[c]	NA	0.27[c]	NA	−0.13[b]	0.35[c]	0.03
IL-1α	0.27[c]	0.11	1.00	0.44[c]	0.01	0.48[c]	−0.04	0.03	0.29[c]	0.01
IL-1β	0.30[c]	0.42[c]	0.31[c]	1.00	0.23[c]	0.42[c]	0.10[b]	0.12[c]	0.43[c]	0.04
hsIL-1β	0.21[c]	NA	−0.06	0.23[c]	1.00	0.10[b]	0.45[c]	0.17[c]	0.06	0.11[b]
IL-6	0.47[c]	0.39[c]	0.38[c]	0.42[c]	0.04	1.00	0.15[c]	0.12[c]	0.39[c]	0.15[c]
hsIL-6	0.14[c]	NA	−0.01	0.10[b]	0.45[c]	0.10[b]	1.00	0.21[c]	0.01	0.41[c]
MCP-1	0.32[c]	0.16	0.12[a]	0.15[c]	0.17[c]	0.11[b]	0.21[c]	1.00	−0.01	0.13[c]
MCP-3	0.35[c]	0.48[c]	0.30[c]	0.59[c]	0.04	0.33[c]	−0.01	0.10[a]	1.00	0.05
hsCRP	0.19[c]	0.16	−0.01	0.03	0.11[b]	0.14[c]	0.41[c]	0.13[c]	0.06	1.00

The correlation coefficient was obtained from Spearman's rank correlation. The upper right coefficients are obtained from biomarkers including non-detectable (ND) measurements. The lower left coefficients are obtained from biomarkers excluding ND measurements.
TNF, tumor necrosis factor; IL, interleukin; hsIL, high sensitivity interleukin; MCP, monocyte chemoattractant protein; hsCRP, high sensitivity C-reactive protein; NA, not available.
[a]P<0.05, [b]P<0.01, [c]P<0.001.

the different numerical range, our hsCRP measurements uniformly demonstrated significant right-skewed distribution, with older and participants with any of the cardiometabolic disorders embodying higher median. hsCRP is known to increase with wide range of both acute and chronic infections, tissue necrosis, neoplasia, insulin resistance, obesity, smoking,

Table 4. Distribution of inflammatory biomarkers by sex, age, and prevalence of type 2 diabetes mellitus, hypertension and dyslipidemia, including NDs (n=4,058)

Biomarker	Sex			Age, yr			T2DM			Hypertension			Dyslipidemia		
	Male	Female	*P* value	55>	55≤	*P* value	No	Yes	*P* value	No	Yes	*P* value	No	Yes	*P* value
TNF-α, pg/mL															
Number	458	827	-	797	488	-	1,180	105	-	996	289	-	729	556	-
Median	14.51	11.17	<0.001	12.24	12.32	0.889	12.15	14.04	0.027	12.23	12.52	0.428	12.12	12.57	0.101
75th percentile	23.42	17.03	<0.001	20.61	18.67	<0.001	19.36	20.59	0.766	19.66	19.15	0.050	18.72	20.31	0.969
90th percentile	39.99	28.85	0.011	35.84	29.44	<0.001	33.15	33.51	0.798	33.82	31.50	0.042	33.56	32.83	0.387
95th percentile	76.12	54.16	0.107	67.35	48.44	0.002	58.49	64.58	0.989	59.36	53.92	0.240	66.46	50.83	0.041
99th percentile	157.74	139.54	0.405	158.96	135.10	0.205	157.14	101.24	0.252	157.74	150.29	0.788	209.14	112.73	0.025
TNF-β, pg/mL															
Number	127	339	-	246	220	-	430	36	-	353	113	-	274	192	-
Median	0.00	0.00	0.352	0.00	0.00	<0.001	0.00	0.00	0.149	0.00	0.00	0.566	0.00	0.00	0.115
75th percentile	0.00	0.07	0.015	0.20	0.00	<0.001	0.07	0.00	0.119	0.07	0.01	0.086	0.13	0.03	0.002
90th percentile	1.31	1.09	0.440	1.31	0.50	0.276	1.12	4.40	0.876	0.98	3.90	0.386	2.03	0.58	0.088
95th percentile	9.48	10.19	0.539	11.33	7.49	0.513	9.18	10.19	0.571	9.18	11.33	0.755	14.44	3.90	0.035
99th percentile	178.94	92.26	0.244	125.54	168.50	0.601	125.54	1,840.00	0.401	92.26	381.59	0.088	178.94	92.26	0.237
IL-1α, pg/mL															
Number	458	827	-	797	488	-	1,180	105	-	996	289	-	729	556	-
Median	0.00	0.00	0.048	0.00	0.00	0.117	0.00	0.00	0.130	0.00	0.00	0.436	0.00	0.00	0.021
75th percentile	8.33	0.04	0.030	3.77	0.00	<0.001	2.67	0.00	0.130	2.75	0.03	0.013	6.58	0.00	0.003
90th percentile	249.77	186.25	0.492	196.83	159.73	0.003	206.70	46.69	0.015	197.47	123.82	0.155	280.33	82.64	<0.001
95th percentile	647.87	496.05	0.107	544.72	470.97	0.009	555.84	117.21	0.032	543.18	530.10	0.373	644.42	371.13	0.011
99th percentile	1,380.00	1,191.00	0.157	1,270.00	1.380.00	0.876	1,296.00	458.64	0.854	1,311.00	1,135.00	0.368	1,269.00	1,380.00	0.589
IL-1β, pg/mL															
Number	458	827	-	797	488	-	1,180	105	-	996	289	-	729	556	-
Median	0.69	0.45	<0.001	0.66	0.40	<0.001	0.52	0.68	0.478	0.55	0.48	0.133	0.52	0.53	0.814
75th percentile	2.63	1.67	0.001	2.47	1.26	<0.001	2.03	1.57	0.122	2.03	1.83	0.016	2.12	1.85	0.034
90th percentile	10.96	9.09	0.382	10.64	6.42	<0.001	10.00	5.31	0.034	9.38	11.97	0.683	9.09	10.34	0.975
95th percentile	32.99	24.13	0.572	32.99	15.86	<0.001	24.43	9.27	0.678	24.49	24.17	0.373	24.49	24.37	0.591
99th percentile	143.32	81.31	0.405	101.67	71.66	0.068	86.97	125.57	0.437	97.68	71.66	0.368	97.68	81.03	0.567
hsIL-1β, pg/mL															
Number	331	488	-	551	268	-	750	69	-	643	176	-	455	364	-
Median	0.70	0.60	<0.001	0.70	0.60	0.310	0.64	0.72	0.153	0.66	0.64	0.696	0.62	0.67	0.221
75th percentile	1.20	0.90	<0.001	1.00	1.00	<0.001	1.03	1.08	0.764	1.03	1.05	0.190	0.98	1.07	0.573
90th percentile	2.00	1.50	<0.001	1.80	1.80	0.003	1.72	2.49	0.208	1.74	1.90	0.591	1.60	1.94	0.265
95th percentile	3.30	1.98	0.002	2.24	2.61	0.322	2.32	2.72	0.292	2.33	2.56	0.636	2.10	2.56	0.547
99th percentile	6.50	3.90	0.007	4.70	5.32	0.816	4.89	6.47	0.840	5.32	3.77	0.072	4.89	6.28	0.908

(*Continued to the next page*)

Table 4. Continued

Biomarker	Sex			Age, yr			T2DM			Hypertension			Dyslipidemia		
	Male	Female	*P* value	55>	55≤	*P* value	No	Yes	*P* value	No	Yes	*P* value	No	Yes	*P* value
IL-6, pg/mL															
Number	458	827	-	797	488	-	1,180	105	-	996	289	-	729	556	-
Median	1.38	0.37	<0.001	0.72	0.49	0.051	0.61	0.62	0.913	0.61	0.61	0.561	0.74	0.52	0.233
75th percentile	14.37	10.04	0.187	15.79	7.78	<0.001	12.29	7.26	0.056	12.99	8.77	0.004	16.23	8.44	0.002
90th percentile	89.78	69.66	0.070	86.17	59.02	<0.001	83.60	57.19	0.236	80.44	79.77	0.309	87.49	66.73	0.052
95th percentile	168.45	137.77	0.276	149.24	137.79	0.035	147.98	92.42	0.396	147.28	137.34	0.373	148.87	137.34	0.197
99th percentile	345.97	286.06	0.157	336.88	244.24	0.017	300.15	226.26	0.854	329.69	298.67	0.368	300.15	292.51	0.567
hsIL-6, pg/mL															
Number	331	488	-	551	268	-	750	69	-	643	176	-	455	364	-
Median	0.83	0.75	<0.001	0.75	0.81	<0.001	0.75	0.98	<0.001	0.75	0.94	<0.001	0.69	0.85	<0.001
75th percentile	1.30	1.01	<0.001	1.05	1.31	0.024	1.11	1.43	0.103	1.04	1.45	0.010	0.98	1.30	0.003
90th percentile	2.11	1.77	<0.001	1.82	2.11	0.418	1.94	4.10	0.567	1.88	2.24	0.727	1.85	2.13	0.266
95th percentile	3.42	2.34	0.005	3.03	2.91	0.032	2.82	8.32	0.078	2.82	3.70	0.672	2.54	3.42	0.210
99th percentile	14.06	7.08	0.007	14.06	8.13	0.004	8.13	69.60	0.012	13.62	10.25	0.778	7.08	14.06	0.059
MCP-1, pg/mL															
Number	458	827	-	797	488	-	1,180	105	-	996	289	-	729	556	-
Median	753.01	626.03	<0.001	649.71	704.10	0.002	664.72	707.78	0.061	657.93	706.85	0.007	644.85	704.38	<0.001
75th percentile	927.38	801.87	<0.001	836.75	856.04	0.003	836.88	911.94	0.088	834.37	866.72	0.802	815.68	900.94	0.088
90th percentile	1,132.00	985.86	<0.001	1,053.00	1,059.00	0.027	1,045.00	1,132.00	0.500	1,040.00	1,067.00	0.797	1,008.00	1,083.00	0.143
95th percentile	1,256.00	1,132.00	0.004	1,200.00	1,176.00	0.035	1,182.00	1,313.00	0.188	1,182.00	1,244.00	0.598	1,162.00	1,221.00	0.334
99th percentile	1,843.00	1,530.00	0.157	1,735.00	1,642.00	0.205	1,727.00	1,548.00	0.252	1,735.00	1,679.00	0.368	1,679.00	1,727.00	0.987
MCP-3, pg/mL															
Number	458	827	-	797	488	-	1,180	105	-	996	289	-	729	556	-
Median	0.00	0.00	0.089	0.00	0.00	0.050	0.00	0.00	0.135	0.00	0.00	0.544	0.00	0.00	0.036
75th percentile	16.02	19.33	0.671	19.60	14.93	<0.001	18.12	14.45	0.354	18.18	17.04	0.083	20.66	14.54	0.029
90th percentile	73.58	69.26	0.617	70.75	67.06	0.010	70.68	42.37	0.381	66.60	79.65	0.841	70.60	66.87	0.387
95th percentile	115.10	101.10	0.408	116.92	98.13	0.009	104.37	113.04	0.986	101.99	116.92	0.807	118.22	100.31	0.197
99th percentile	256.00	224.82	0.405	245.16	212.02	0.205	243.25	243.70	0.437	228.80	249.45	0.322	252.64	173.63	0.092
hsCRP, mg/L															
Number	1,426	2,632	-	2,097	1,961	-	3,686	372	-	2,988	1,070	-	2,176	1,882	-
Median	0.65	0.54	<0.001	0.55	0.61	<0.001	0.56	0.87	<0.001	0.53	0.75	<0.001	0.50	0.69	<0.001
75th percentile	1.39	1.10	<0.001	1.16	1.23	0.241	1.13	2.03	<0.001	1.07	1.57	<0.001	1.02	1.41	<0.001
90th percentile	3.26	2.45	<0.001	2.58	2.68	0.154	2.51	3.96	<0.001	2.40	3.47	0.007	2.41	2.93	0.022
95th percentile	6.28	4.01	<0.001	4.68	5.35	0.154	4.56	7.75	<0.001	4.21	6.25	0.007	4.23	5.43	0.022
99th percentile	21.53	11.41	0.01	15.62	17.37	0.709	14.30	22.42	<0.001	16.29	16.34	0.946	16.57	16.29	0.996

The *P* value was derived from the two-sided Wilcoxon rank-sum test.
ND, non-detectable; T2DM, type 2 diabetes mellitus; TNF, tumor necrosis factor; IL, interleukin; hsIL, high sensitivity interleukin; MCP, monocyte chemoattractant protein; hsCRP, high sensitivity C-reactive protein.

Table 5. Distribution of inflammatory biomarkers by sex, age, and prevalence of type 2 diabetes mellitus, hypertension and dyslipidemia, excluding NDs (n=1,180)

Biomarker	Sex			Age, yr			T2DM			Hypertension			Dyslipidemia		
	Male	Female	*P* value	55>	55≤	*P* value	No	Yes	*P* value	No	Yes	*P* value	No	Yes	*P* value
TNF-β, pg/mL															
Number	33	107	-	95	45	-	134	6	-	110	30	-	89	51	-
Median	0.56	0.30	0.279	0.31	0.42	0.445	0.30	6.95	0.032	0.30	1.12	0.088	0.42	0.30	0.233
75th percentile	6.38	2.31	0.644	1.31	6.38	0.977	2.31	10.19	0.642	1.52	8.59	0.146	4.94	1.31	0.075
90th percentile	120.88	21.03	0.964	14.44	92.26	0.682	22.26	1,840.00	0.793	19.33	182.73	0.427	49.03	9.49	0.181
95th percentile	178.94	62.92	0.223	62.92	168.50	0.641	92.26	1,840.00	0.639	52.78	381.59	0.065	125.54	68.91	0.089
99th percentile	1,840	244.58	0.660	1,840	381.59	0.963	244.58	1,840.00	0.046	168.50	1,840.00	0.018	381.59	1,840	0.919
IL-1α, pg/mL															
Number	140	209	-	229	120	-	326	23	-	275	74	-	213	136	-
Median	74.54	106.12	0.887	105.07	81.66	0.888	103.37	35.24	0.094	101.66	61.48	0.515	134.07	53.98	0.013
75th percentile	413.38	371.13	0.110	373.14	383.13	0.013	410.01	117.21	0.024	375.53	389.99	0.304	442.14	271.13	0.003
90th percentile	940.64	852.97	0.337	902.85	843.77	0.045	902.85	242.31	0.194	902.85	804.14	0.214	967.91	760.22	0.034
95th percentile	1,282.50	1,116	0.408	1,135.00	1,281.50	0.422	1,151.00	458.64	0.595	1,239.00	1,053.00	0.141	1,151	1,311	0.523
99th percentile	2,233	2,054	0.534	2,233	1,769	0.351	2,054	2,233	0.273	2,509	1,552	0.231	1,769	2,233	0.885
IL-1β, pg/mL															
Number	433	746	-	797	488	-	1,082	97	-	918	261	-	671	508	-
Median	0.77	0.53	<0.001	0.75	0.48	<0.001	0.60	0.70	0.501	0.67	0.55	0.242	0.61	0.63	0.975
75th percentile	2.82	1.91	0.001	2.80	1.54	<0.001	2.28	1.67	0.123	2.28	2.09	0.022	2.41	2.04	0.077
90th percentile	12.88	10.00	0.201	12.27	8.72	<0.001	10.96	6.72	0.060	10.00	13.43	0.981	9.56	10.96	0.892
95th percentile	33.86	25.88	0.370	34.35	18.40	<0.001	27.54	48.41	0.853	27.54	28.00	0.643	27.54	28.00	0.720
99th percentile	143.32	86.97	0.280	101.67	71.66	0.106	95.95	156.03	0.368	97.68	71.66	0.445	101.67	81.03	0.364
IL-6, pg/mL															
Number	435	745	-	739	441	-	1,084	96	-	922	258	-	674	506	-
Median	1.90	0.62	<0.001	1.01	0.77	0.157	0.87	0.90	0.835	0.86	1.03	0.754	1.16	0.75	0.395
75th percentile	19.46	13.17	0.291	20.41	10.08	<0.001	17.48	8.77	0.035	17.78	10.89	0.003	19.46	10.22	0.002
90th percentile	94.72	79.71	0.201	94.72	76.49	0.001	87.49	59.03	0.119	87.16	85.52	0.132	94.04	79.46	0.045
95th percentile	170.20	145.27	0.241	154.29	148.68	0.026	151.04	118.07	0.522	149.71	152.64	0.447	168.19	137.79	0.377
99th percentile	345.97	296.78	0.092	336.88	244.24	0.028	301.61	326.23	0.920	329.69	298.67	0.445	329.69	292.51	0.743
MCP-3, pg/mL															
Number	160	344	-	332	172	-	471	33	-	399	105	-	305	199	-
Median	39.31	26.70	0.045	30.47	32.61	0.400	31.34	29.85	0.577	29.00	37.61	0.072	31.34	30.66	0.957
75th percentile	80.09	67.04	0.606	68.92	73.71	0.007	70.75	69.45	0.266	66.60	81.40	0.715	68.58	73.26	0.421
90th percentile	127.49	113.04	0.982	127.57	110.97	0.007	118.22	131.70	0.742	117.95	131.70	0.860	139.47	112.60	0.015
95th percentile	194.87	183.39	0.955	198.58	173.63	0.029	193.87	243.70	0.794	193.87	203.51	0.703	203.51	138.24	0.005
99th percentile	480.20	252.64	0.106	257.04	432.06	0.935	301.97	256	0.436	301.97	256	0.590	346	245.16	0.023

The *P* value was derived from the two-sided Wilcoxon rank-sum test.
ND, non-detectable; T2DM, type 2 diabetes mellitus; TNF, tumor necrosis factor; IL, interleukin; MCP, monocyte chemoattractant protein.

and stress [25]. Considering that the prevalence of such proinflammatory conditions are more prevalent with older age, our results align with other population-based studies, where hsCRP was associated with positively graded risk for frailty [26], incident T2DM [2,27], elevated lipid profile [23], and non-alcoholic fatty liver disease [7].

Yet, descriptive assays from population- or clinic-settings of other multiplex assayed inflammatory biomarkers are currently scarce. Several studies have done genetic, experimental and analytic examinations between cytokines/chemokines and diseases with inflammation as a primary basis for pathogenesis. They may provide biological plausibility for some of our current findings.

IL-6

IL-6 is produced in various tissues, including activated leukocytes, adipocytes, and endothelial cells [28]. Our participants of older age or with T2DM, hypertension or dyslipidemia showed higher median IL-6 levels. Similarly, a cross-sectional data from the Newcastle 85+ Study identified positive association between basal IL-6 and frailty risk, enacting as a mediator in inflammatory processes whilst physiological changes accompanied with aging, such as decreased lean body mass, osteopenia, low-grade anemia, decreased serum albumin and cholesterol and increased prevalence of lymphoproliferative disorders [26]. In terms of high IL-6 levels among participants with cardiometabolic disorder, parallel results were also observed in a study, which implicated the pro-coagulant effect of IL-6 from its production in arterial endothelial and smooth muscle cells [29]. Findings from a population-based study also confirmed that elevated levels of IL-6 are associated with increased risk of future MI, reinforcing its role in cytokine-mediated inflammation during the early stages of atherogenesis [3].

IL-1α and IL-β

IL-1 is crucial for host-defense responses to infection and injury by triggering inflammation in a pathway initiated through myeloid differentiation primary response 88 (MYD88) activation and culminating in NF-KB-induced transcription of inflammatory genes [30,31]. Whereas IL-1β is secreted and active only upon cleavage of its precursor by caspase-1 on the inflammasome [11], IL-1α can be found constitutively inside cells under normal homeostasis, and is active in its precursor as well as calpain-processed mature form [32,33]. These different order of IL-1 subtype expression and activation may explain the low correlation and varying detection rate despite their similar cellular niche. Likewise, whereas no differences were detected by age group for median IL-1α values, IL-1β, and hsIL-1β levels were higher in the younger group. Such results contrast a previous study that tested age-related augmentation of the systemic and myocardial inflammatory responses, in which the older mice displayed greater myocardial mononuclear cell accumulation and IL-1β production than younger mice due to depressed cardiac function with older age [34]. This result translates to higher vulnerability to endotoxemic cardiac depression with aging [34]. We suspect that such inconsistency may arise from different study population structure and size, as similar studies have also observed varying magnitude of association between IL-6 and age by participant sex, age, and race [34,35]. Yet, our results align in terms of the implication of IL-1 in inflammatory diseases. The genetic assay showed the distribution of oncogenic mutations and single nucleotype polymorphisms (SNPs) in the predicted and experimental structures of protein complexes in the IL-1 pathway, supporting its contributions to cancer development and other inflammatory diseases [36]. Although our study excluded participants with recent history of malignant cancer, the hsIL-1β concentrations remained consistently high among those with metabolic disorders, implying that moderate differences in IL-1 subtypes may be present even in non-critical inflammatory diseases.

TNF-α and TNF-β

TNFs play crucial role in innate and adaptive immunity by inducing proatherogenic changes in lipid metabolism, thereby implicated in lymphoid follicle development, production of proinflammatory cytokines and facilitation of fibroblasts and synviocytes proliferation [37]. Despite TNF-β and TNF-α share many common biological activities, TNF-β is more likely to induce the secretion of IL-6, IL-8, and matrix metallopeptidase 3 (MMP-3) than TNF-α even at low levels, thereby supporting differential productions and detections of TNF subtypes in inflammatory and autoimmune diseases [38,39]. Moreover, TNFs are highly implicated in impaired glucose metabolism and elevated insulin resistance via inhibition of insulin-stimulated tyrosine kinase activity of the insulin receptor by downregulating adiponectin and upregulating leptin production [40,41]. These results align with our current findings, in which the both subtypes of TNF were higher among participants with T2DM.

MCP-1 and MCP-3

MCPs selectively recruit monocytes, neutrophils, and lymphocytes, thereby are found during progression of immune disorders, pulmonary diseases, cancer, and vascular diseases [42,43]. Recent experiments demonstrated a reduction in atherosclerotic lesion formation in MCP-1 deficient mice [44,45]. Conversely, macrophage-specific overexpression of MCP-1 resulted in the acceleration of vascular lesion size and infiltration of macrophages in atherosclerosis-prone mice [45]; such findings are in align with higher MCP-1 levels detected among participants with T2DM, hypertension and dyslipidemia in our study population. Another study indicated high feasibility of MCP-1 as a surrogate measure of biological age, as its circulating levels were higher in frail participants [46], in parallel with higher percentiles embodied by our older participants.

Compared with previous findings, inconsistencies may be explained by the following reasons. First, the upper percentiles were largely derived from small counts of very high values; considering that we did not exclude any upper outliers, those extreme values may distort the true distribution differences between participants with and without metabolic disorder(s). Secondly, the assay methodological discrepancies challenge the direct comparison across different studies. Different kits and software extrapolate antigen concentration with different sample preparation and trade-off of sensitivity, specificity and referent. Lastly, the inflammatory biomarkers are highly dependent on the conditions of extraction. Depending on the study design, number of measurements done at specific time point(s) in disease progression may lead to high heterogeneity. These differences may explain the unexpected negative correlation observed between TNF-β and MCP-1, likely due to varying sample size, number of detectable measurements and its range, compared with previous studies.

In short, every family and subtypes of cytokine/chemokines play differential role in disease-specific inflammatory processes. Their concentrations vary by location and timing of secretion, expression and proliferation, depending on the stages of disease progression, individual- and population heterogeneity and genetic susceptibility. These variations challenge the current multiplex assay to provide a consensus on single reference range for risk prediction and diagnosis.

Strengths and limitations

To the extent of our knowledge, this is the first study to assay and to present a wide array of inflammatory markers in community-dwelling Korean population. Our findings may serve as an addendum in reinforcing biological mechanisms of inflammation and a basis for more comprehensive depiction of inflammatory states for both clinic and research purposes. In addition, despite a considerable proportion of NDs, we were still able to illustrate a wide range of inflammatory states from considerably large-sized population, thereby able to portray realistic distributions found in population settings. Future studies warrant larger population- and clinic-based sample with repeated measurements to obtain more robust reference values for diagnostic purposes.

Several limitations warrant cautious interpretations of our findings. The primary concern involves the study nature and the design of the study. Considering the relatively good health of the participants, a large proportion of NDs were observed. Although some of these were overcome by high sensitivity assay, we were unable to distinguish differences within the ND range. Moreover, due to the cross-sectional nature of the study design, we relied on a single measurement, thereby unable to determine whether the assayed biomarkers reflect acute or persistent inflammatory state. Another limitation arises from the detection methodology used. Although the samples were measured via identical kit and technologies, day-to-day variation in sample collection were inevitable. However, we have minimized potential batch effect by assaying the samples at a single time point. Lastly, our study did not limit eligibility nor collect information regarding history of acute infectious diseases.

In sum, the distributions of inflammatory biomarkers varied by its classes and inclusion/exclusion of NDs. Importantly, the median and the 99th percentile for most biomarkers were different when stratifying participants by sex, age and with and without cardiometabolic disorders. These findings lend weight to provide sex- and age-specific reference values to improve risk predictive and diagnostic performance for inflammatory diseases. A more considered approach would be to tailor the references to individual baseline characteristics and comorbidities. Given that independent clinical feasibility of these biomarkers is not yet warranted, they should yet be referred in conjunction with routine examinations, individual's clinical presentation, and medical history.

SUPPLEMENTARY MATERIALS

Supplementary materials related to this article can be found

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CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: S.M.J.C., H.C.K.
Acquisition, analysis, or interpretation of data: S.M.J.C., H.L., J.S.S., H.C.K.
Drafting the work or revising: S.M.J.C., H.L.
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None

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