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Intraindividual Long-term Immune Marker Stability in Plasma Samples Collected in Median 9.4 Years Apart in 304 Adult Cancer-free Individuals

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ABSTRACT

Background: Changes in immune marker levels in the blood could be used to improve the early detection of tumor-associated inflammatory processes. To increase predictiveness and utility in cancer detection, intraindividual long-term stability in cancer-free individuals is critical for biomarker candidates as to facilitate the detection of deviation from the norm.

Methods: We assessed intraindividual long-term stability for 19 immune markers (IL10, IL13, TNFα, CXCL13, MCP-3, MIP-1α, MIP-1β, fractalkine, VEGF, FGF-2, TGFα, sIL2Rα, sIL6R, sVEGF-R2, sTNF-R1, sTNF-R2, sCD23, sCD27, and sCD30) in 304 cancer-free individuals. Repeated blood samples were collected up to 20 years apart. Intraindividual reproducibility was assessed by calculating intraclass correlation coefficients (ICC) using a linear mixed model.

Results: ICCs indicated fair to good reproducibility (ICCs $\geq$ 0.40 and < 0.75) for 17 of 19 investigated immune markers, including IL10, IL13, TNFα, CXCL13, MCP-3, MIP-1α, MIP-1β, fractalkine, VEGF, FGF-2, TGFα, sIL2Rα, sIL6R, sTNF-R1, sTNF-R2, sCD27, and sCD30. Reproducibility was strong (ICC $\geq$ 0.75) for sCD23, while reproducibility was poor (ICC < 0.40) for sVEGF-R2. Using a more stringent criterion for reproducibility (ICC $\geq$ 0.55), we observed either acceptable or better reproducibility for IL10, IL13, CXCL13, MCP-3, MIP-1α, MIP-1β, VEGF, FGF-2, sTNF-R1, sCD23, sCD27, and sCD30.

Conclusions: IL10, IL13, CXCL13, MCP-3, MIP-1α, MIP-1β, VEGF, FGF-2, sTNF-R1, sCD23, sCD27, and sCD30 displayed ICCs consistent with intraindividual long-term stability in cancer-free individuals.

Impact: Our data support using these markers in prospective longitudinal studies seeking early cancer detection biomarkers.

Introduction

Worldwide cancer mortality is increasing and in many countries cancer has become the leading cause of death (1). These observations support early cancer detection programs to improve cancer prognosis (2). As immune alteration and inflammation promote tumor development and progression (3), changes in immune or inflammatory markers in the blood could help to improve early detection of cancer (4, 5). In cancer-free individuals, early cancer detection biomarkers should be stable over time (6, 7).

Several previous studies have investigated intraindividual immune marker stability (8–27). These studies were characterized by: small study size ($N < 40$; refs. 8–15); limited generalizability to more diverse populations (e.g., the participants were either female or male or healthy and young; refs. 8–13, 16–24); and/or blood samples collected within a short period (<2 years; refs. 10–14, 22, 23, 25–27; i.e., the samples do not provide accurate information on intraindividual long-term marker stability). However, for the evaluation of early cancer detection biomarker candidates, it is important to provide information about the degree of the long-term intraindividual marker stability in cancer-free individuals.

In this study, we determined the intraindividual long-term immune marker stability by assessing the intraclass correlation coefficients (ICC) of 19 immune markers in repeated blood samples collected 9.4 years (median) apart in a cohort of 304 middle-aged cancer-free women and men. The analyzed immune markers were: cytokines (IL10, IL13, and TNFα); chemokines [C-X-C motif ligand 13 (CXCL13), monocyte chemoattractant protein (MCP)-3, macrophage inflammatory protein (MIP)-1α, MIP-1β, and fractalkine]; proangiogenic or mitogenic growth factors (VEGF, FGF-2, and TGFα); and soluble (s) forms of cell surface receptors (sIL2Rα, sIL6R, sVEGF-R2, sTNF-R1, sTNF-R2, sCD23, sCD27, and sCD30).

Materials and Methods

Study population

Participants were recruited from three nested case–control studies. These earlier studies investigated disease risk in relation to changes in blood immune marker levels or metabolomic patterns (28–30). Participants of the present study ($N = 304$) were cancer-free individuals who had two (or more) blood samples available within the Northern Sweden Health and Disease Study (NSHDS; ref. 31). NSHDS consists of three subcohorts: the Västerbotten Intervention Program (VIP), the Mammary Screening Project (MA), and the Northern Sweden Monica Project. For this study, one first and one repeated blood sample were selected for each participant using both VIP and MA. VIP is a population-based cohort of individuals 40, 50, and 60 years of age

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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who have donated blood samples within health surveys since 1985. MA is a cohort of females attending mammary screening with repeated blood samples available between 1995 and 2006. Linkage of NSHDS to the Swedish Cancer Registry (median follow-up time after the last sample: 4 years) facilitated selection of cancer-free individuals (32). All blood samples were frozen within one hour of collection and stored at −80°C at Umeå University Hospital (Umeå, Sweden). This study was approved by the Ethical Review Board at Umeå University (Umeå, Sweden) and was performed in compliance with the declaration of Helsinki.

Immune marker measurement

Repeated immune marker levels were assessed in all participants in one laboratory effort using multiplex bead-based immunoassays (Luminex): the Milliplex HCYTOMAG-60K kit (Millipore) was used to analyze IL10, IL13, TNFα, MCP-3, MIP-1α, MIP-1β, fractalkine, VEGF, FGF-2, and, TGFα; the Milliplex HSCRMAG-32K kit (Millipore) was used to analyze sIL2Rα, sIL6R, sVEGF-R2, and sTNF-R2; and the LXSAHM kit (R&D Systems) was used to analyze CXCL13 and sTNF-R1. ELISAs (eBioscience) were used to analyze blood levels of sCD23, sCD27, and sCD30. In most study participants ($N = 286$), the first and repeated sample were placed in random order on the same analysis plate to minimize plate-mediated effects. Laboratory personnel were blinded concerning the chronological order of the samples. The same experienced lab technician performed all analyses according to the manufacturers' protocols. All markers were measured in duplicate with exception of the quantification of sCD30 (due to limited sample volume). For statistical analyses, duplicate immune marker measures were averaged.

Statistical analyses

Immune marker concentration data were visually inspected and evaluated for normality. Because of right-skewing, all immune markers were natural log transformed to improve normality. To reduce the influence of extreme values, immune marker concentrations were winsorized to the 1st and 99th percentile (28, 29). Immune marker concentrations below the limit of quantification (LoQ; 2.4% of all performed measurements) were treated as missing values.

To assess the temporal reproducibility of repeated immune marker measures, we calculated intraclass correlation coefficients (ICC) and 95% confidence intervals (CI) for each immune marker [i.e., between-individual variability in relation to the total variability (between-individual variability + within-individual variability)]. ICCs and 95% CIs were estimated by fitting a linear mixed model using the lme4 package (33) in the R software. The linear mixed model included analysis plate and subject (nested within analysis plate) as random intercepts to account for repeated immune marker measures. The model was also adjusted for sex and age (continuous in years at the time of blood draw) by including both variables as fixed effects. The 95% CIs were calculated for all estimated ICCs using the bootstrapping approach. Rosner interpretation of ICCs was applied to evaluate intraindividual reproducibility of repeated immune marker measurements: ICCs < 0.4 indicate poor reproducibility; ICCs between 0.40 and 0.75 indicate fair to good reproducibility; and ICCs ≥ 0.75 indicate strong reproducibility (34). In addition, we used a more stringent criterion for evaluating immune marker reproducibility with ICCs ≥ 0.55 indicating acceptable reproducibility (19).

Intraindividual marker stability was evaluated in subpopulations. To investigate the influence of the time interval between blood sample collections on the marker stability, we stratified individuals by the time between the first and repeated blood draw (>3–10 years and >10–20 years). Next, we stratified the dataset by sex to investigate intraindividual marker stability in female ($N = 212$) and male ($N = 92$) study participants separately. In addition, we investigated differences in intraindividual marker stability with regard to body mass index (BMI) and smoking status.

Sensitivity analyses were performed to assess the robustness of the results. To exclude plate-mediated effects on estimated ICCs, we restricted analyses to a dataset with all participants having their first and repeated blood sample placed on the same analysis plate ($N = 286$). Other processes such as the participant's fasting status or diurnal variation of immune marker concentrations could influence the estimated intraindividual marker stability. To minimize the influence of such processes, we restricted analyses to a dataset with all participants donating their first and repeated blood sample to VIP ($N = 135$) because within VIP the majority of blood samples are drawn in the morning after overnight fasting.

All statistical analyses were performed using the R environment for biostatistical computing (The R Foundation for Biostatistical Computing).

Results

Intraindividual long-term stability of 19 immune markers including cytokines, chemokines, growth factors, and soluble cell surface receptors was investigated in repeated blood samples. Blood samples were collected from 304 individuals in median 9 years apart between November 1986 and May 2010. Participants of this study ($N = 304$) were selected from the general population with a median cancer-free follow-up time of 4 years (**Table 1**). At the first and repeated blood sample draw, the median age was 50 and 60 years, respectively. About one-third ($N = 224$) of all samples ($N = 608$) were retrieved from MA (mammography cohort), explaining the higher proportion of females (70%) in this study (**Table 1**).

The majority (97.6%) of all measurements ($N = 12{,}768$) were above the LoQ (**Table 2**). Intraassay coefficients of variation (CV) of duplicate immune marker measures ranged between 7.6% for sCD23 and 19.1% for TGFα. Inter-assay CVs based on one quality control sample were relatively high for all markers and ranged between 15.0% for sCD30 and 47.4% for sCD27 (**Table 2**). Median immune marker

Table 1. Descriptive statistics of included blood samples and cancer-free participants.

Characteristic	N	Median	Range
Age (years)			
First blood sample	304	50.1	30.0–68.8
Repeated blood sample	304	60.0	39.7–73.7
Cancer-free years from repeated blood sample	304	4.2	0.0–17.0
Year of blood sample collection			
First blood sample	304	1994	1986–2002
Repeated blood sample	304	2002	1995–2010
Years between blood sample collection	304	9.4	0.1–20
Gender			
Female	212		
Male	92		
Body mass index (kg/m^2)			
First blood sample	290	25.1	17.1–40.3
Repeated blood sample	269	25.6	15.8–44.6

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Table 2. Proportion of measurements above the LoQ, and intraassay and interassay CVs.

Marker	LoQ (%)	Intraassay CV (%)	Interassay CV (%)
IL10[a]	95.9	15.4	42.9
IL13[a]	93.6	16.1	36.9
TNFα[a]	96.1	11.9	36.4
CXCL13[a]	99.5	10.7	41.0
MCP-3[a]	96.7	14.4	36.4
MIP-1α[a]	97.9	18.7	44.4
MIP-1β[a]	95.4	13.8	27.9
Fractalkine[a]	99.8	14.9	24.9
VEGF[a]	99.2	17.9	19.3
FGF-2[a]	99.2	15.4	24.1
TGFα[a]	84.4	19.1	48.2
sIL2Rα[a]	98.4	11.8	29.4
sIL6R[a]	100.0	8.9	18.6
sTNF-R1[a]	99.3	8.7	15.3
sTNF-R2[a]	100.0	9.6	17.2
sVEGF-R2[a]	100.0	9.3	33.7
sCD23[b]	100.0	7.6	18.7
sCD27[b]	99.5	16.6	47.4
sCD30[b]	96.9	[c]	15.0

[a]Assessed by Luminex multiplex kit.
[b]Assessed by singleplex ELISA.
[c]sCD30 not assessed in duplicate.

concentrations and median percentage changes of immune markers are presented in **Table 3**.

In all immune markers, we evaluated reproducibility (or intraindividual marker stability) by calculating ICCs between measures of the first and repeated blood draw. On the basis of calculated ICCs, we observed fair to good reproducibility (ICC between 0.40 and 0.75) for 17 of 19 investigated immune markers including IL10, IL13, TNFα, CXCL13, MCP-3, MIP-1α, MIP-1β, fractalkine, VEGF, FGF-2, TGFα, sIL2Rα, sIL6R, sTNF-R1, sTNF-R2, sCD27, and sCD30 (**Fig. 1**). Reproducibility was strong (ICC ≥ 0.75) for sCD23, while reproducibility was poor (ICC < 0.40) for sVEGF-R2 (**Fig. 1**). Using a more stringent criteria for reproducibility (ICC ≥ 0.55), we observed acceptable reproducibility for IL10, IL13, CXCL13, MCP-3, MIP-1α, MIP-1β, VEGF, FGF-2, sTNF-R1, sCD23, sCD27, and sCD30 (**Fig. 1**).

We evaluated the influence of the time interval between the first and repeated blood draw on the estimated marker stability. Participants were stratified according to two different intervals between the first and repeated blood draw (>3–10 years and >10–20 years). In these analyses, ICCs were of approximately similar size for IL10, IL13, CXCL13, MCP-3, sIL6R, sTNF-R2, sCD23, and sCD30 in both time intervals (**Table 4**). ICCs were somewhat lower in individuals within the longest time interval (>10–20 years) between the first and repeated blood draw for most of the investigated markers including TNFα, MIP-1α, MIP-1β, fractalkine, VEGF, FGF-2, TGFα, sIL2Rα, sTNF-R1, and sCD27 (**Table 4**).

Analyses stratified by sex displayed similar ICCs in most immune markers. ICCs were lower in men for MIP-1α, MIP-1β, sIL2Rα, and sCD30 as compared with ICCs derived from all study participants. Evaluating this result, it is important to consider that the time between blood draw was in median (SD) 10.0 (1.8) years and 6.1 (4.1) years in men and women, respectively (**Table 4**). This difference is explained by the generally shorter interval between blood draw in MA (4 years) as compared with VIP (10 years). However, similar results were obtained when repeating sex-stratified analyses restricted to participants with >8 years between blood draw,

Table 3. Median and median percent change of immune marker concentrations assessed in repeated blood samples collected in median 9.4 years apart.

Marker	First sample Median (5th–95th percentile)	Repeated sample Median (5th–95th percentile)	Median % change[a] (5th–95th percentile)
IL10[b]	12.1 (1.3–41.4)	11.3 (1.4–46.3)	−3% (−76% to 336%)
IL13[b]	19.7 (3.4–73.4)	19.5 (3.9–68.4)	0% (−50% to 120%)
TNFα[b]	12.7 (5.2–27.7)	11.9 (4.8–31.1)	−2% (−56% to 106%)
CXCL13[b]	66.3 (32.5–144.8)	66.4 (31.0–150.4)	1% (−41% to 77%)
MCP-3[b]	56.7 (18.4–137.5)	55.6 (23.1–155.8)	0% (−50% to 120%)
MIP-1α[b]	9.0 (2.7–22–7)	8.3 (2.5–22.4)	−7% (−64% to 108%)
MIP-1β[b]	39.5 (18.5–88.5)	38.2 (16.8–92.4)	−3% (−52% to 71%)
Fractalkine[b]	328 (155–653)	296 (141–665)	−6% (−62% to 80%)
VEGF[b]	362 (132–1,100)	346 (119–1,173)	−1% (−60% to 131%)
FGF-2[b]	245 (100–554)	235 (98–512)	−7% (−55% to 94%)
TGFα[b]	3.9 (0.6–11.8)	3.9 (0.6–15.1)	−3% (−81% to 392%)
sIL2Rα[b]	400 (192–807)	426 (188–1,021)	6% (−44% to 152%)
sIL6R[b]	15,261 (8,679–25,079)	15,308 (9,301–26,102)	0% (−39% to 69%)
sTNF-R1[b]	3,393 (2,342–5,056)	3,688 (2,450–6,009)	7% (−21% to 66%)
sTNF-R2[b]	5,055 (3,250–7,815)	5,316 (3,397–9,493)	4% (−29% to 75%)
sVEGF-R2[b]	12,474 (7,719–19,431)	12,331 (7,823–21,107)	0% (−38% to 74%)
sCD23[b]	2,194 (1,031–4,219)	2,314 (1,048–4,970)	3% (−25% to 66%)
sCD27[c]	18.2 (7.2–35.8)	18.9 (7.5–42.8)	2% (−33% to 80%)
sCD30[d]	2.5 (1.5–5.2)	2.5 (1.4–6.0)	0% (−46% to 86%)

[a]Calculated by [(Marker $\text{level}_{\text{Repeated sample}}$ − Marker $\text{level}_{\text{First sample}}$)/Marker $\text{level}_{\text{First sample}}$] × 100.
[b]Marker concentration in pg/mL.
[c]Marker concentration in U/mL.
[d]Marker concentration in ng/mL.

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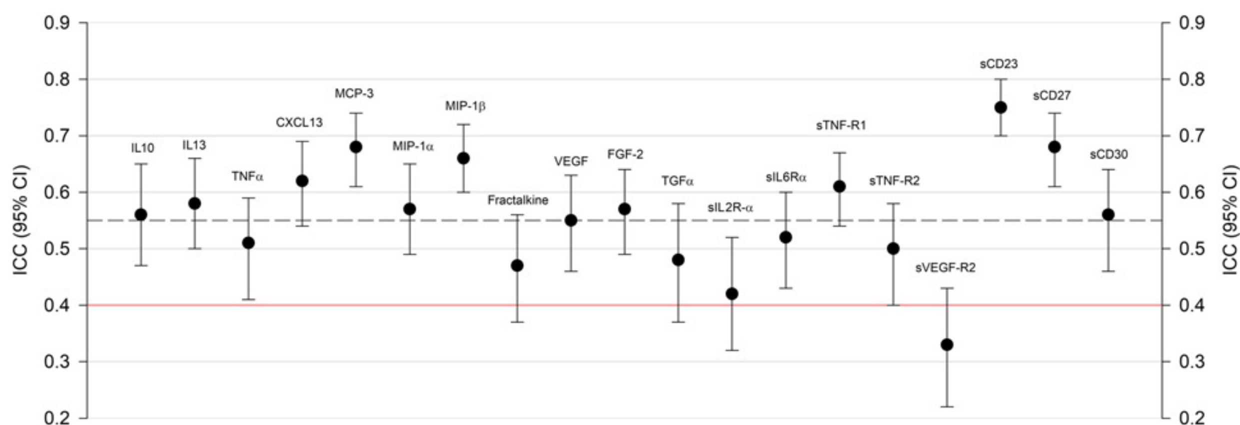

Figure 1.

ICCs and 95% CIs of all investigated immune markers. Immune markers investigated were cytokines (IL10, IL13, and TNFα), chemokines (CXCL13, MCP-3, MIP-1α, MIP-1β, and fractalkine), growth factors (VEGF, FGF-2, and TGFα), and soluble cell surface receptors (sIL2Rα, sIL6R, sVEGF-R2, sTNF-R1, sTNF-R2, sCD23, sCD27, and sCD30). ICCs were estimated by linear mixed effects modeling adjusted for age, sex, and analysis plate. Fair immune marker reproducibility (ICC ≥ 0.40) is indicated by the red solid line. A more stringent criterion for acceptable marker reproducibility (ICC ≥ 0.55) is indicated by the black dashed line.

supporting true sex-specific differences of the long-term marker stability in MIP-1α, MIP-1β, sIL2Rα, and sCD30. Analyses stratified by BMI into healthy weight (BMI, 18.5–24.9 kg/m^2) and overweight or obese (BMI ≥ 25.0 kg/m^2) individuals displayed ICCs of similar size in most markers (Supplementary Table S1). The ICC for sCD27 was lower in overweight or obese individuals (ICC = 0.41) compared with healthy weight participants (ICC = 0.78). Analyses stratified by smoking status showed that current or former smokers had somewhat lower ICCs for TGFα, sVEGF-R2, and sCD27 than never smokers (Supplementary Table S1).

Sensitivity analyses, restricted to a data set with all participants having their first and repeated blood sample on the same analysis plate ($N = 286$), did not provide indication of a significant plate-mediated effect on estimated intraindividual marker stability. In addition, we restricted analyses to a dataset with all participants donating their first and repeated blood sample to VIP ($N = 135$) with the majority of the

Table 4. Long-term immune marker reproducibility as evaluated by ICCs and 95% CIs in all participants and stratified by the time between blood draws and sex.

Marker	All participants $N = 304$ ICC[a] (95% CI)	>3 to 10 years[b] $N = 168$ ICC[a] (95% CI)	>10 to 20 years[c] $N = 82$ ICC[a] (95% CI)	Females[d] $N = 212$ ICC[e] (95% CI)	Males[f] $N = 92$ ICC[e] (95% CI)
IL10	0.56 (0.47–0.65)	0.61 (0.50–0.71)	0.52 (0.31–0.67)	0.54 (0.43–0.63)	0.59 (0.43–0.72)
IL13	0.58 (0.50–0.66)	0.53 (0.41–0.64)	0.68 (0.52–0.79)	0.56 (0.45–0.65)	0.62 (0.46–0.75)
TNFα	0.51 (0.41–0.59)	0.57 (0.44–0.68)	0.36 (0.13–0.56)	0.53 (0.42–0.63)	0.45 (0.27–0.60)
CXCL13	0.62 (0.54–0.69)	0.57 (0.46–0.67)	0.66 (0.50–0.77)	0.62 (0.53–0.69)	0.61 (0.45–0.73)
MCP-3	0.68 (0.61–0.74)	0.70 (0.62–0.78)	0.64 (0.47–0.76)	0.69 (0.60–0.76)	0.66 (0.53–0.77)
MIP-1α	0.57 (0.49–0.65)	0.63 (0.53–0.72)	0.44 (0.24–0.62)	0.66 (0.57–0.74)	0.44 (0.24–0.61)
MIP-1β	0.66 (0.60–0.72)	0.69 (0.60–0.77)	0.53 (0.32–0.69)	0.73 (0.66–0.79)	0.53 (0.38–0.67)
Fractalkine	0.47 (0.37–0.56)	0.52 (0.40–0.63)	0.37 (0.14–0.55)	0.48 (0.35–0.58)	0.44 (0.25–0.59)
VEGF	0.55 (0.46–0.63)	0.65 (0.54–0.73)	0.45 (0.25–0.62)	0.54 (0.43–0.63)	0.56 (0.39–0.69)
FGF-2	0.57 (0.49–0.64)	0.60 (0.49 -0.69)	0.46 (0.25 -0.62)	0.59 (0.49 -0.69)	0.51 (0.34 -0.65)
TGFα	0.48 (0.37–0.58)	0.57 (0.44–0.68)	0.35 (0.10–0.59)	0.53 (0.41–0.64)	0.40 (0.19–0.59)
sIL2Rα	0.42 (0.32–0.52)	0.44 (0.30–0.57)	0.23 (0.01–0.46)	0.47 (0.36–0.58)	0.29 (0.07–0.47)
sIL6R	0.52 (0.43–0.60)	0.48 (0.34–0.61)	0.53 (0.35–0.67)	0.55 (0.44–0.64)	0.44 (0.24–0.59)
sTNF-R1	0.61 (0.54–0.67)	0.60 (0.47–0.70)	0.53 (0.33–0.68)	0.63 (0.54–0.71)	0.56 (0.41–0.70)
sTNF-R2	0.50 (0.40–0.58)	0.47 (0.33–0.59)	0.48 (0.30–0.65)	0.50 (0.38–0.59)	0.48 (0.31–0.63)
sVEGF-R2	0.33 (0.22–0.43)	0.24 (0.08–0.39)	0.42 (0.20–0.61)	0.31 (0.17–0.44)	0.38 (0.18–0.55)
sCD23	0.75 (0.70–0.80)	0.70 (0.61–0.78)	0.75 (0.62–0.84)	0.76 (0.70–0.82)	0.72 (0.61–0.81)
sCD27	0.68 (0.61–0.74)	0.79 (0.72–0.84)	0.44 (0.21–0.63)	0.74 (0.67–0.80)	0.63 (0.48–0.75)
sCD30	0.56 (0.46–0.64)	0.59 (0.48–0.69)	0.54 (0.38–0.68)	0.63 (0.54–0.70)	0.35 (0.12–0.53)

[a]ICCs were estimated by linear mixed effects modeling adjusted for age at blood draw, sex, and analysis plate.
[b]Analyses including individuals with >3 to 10 years between first and repeated blood draws ($N_{Females} = 114$; $N_{Males} = 54$).
[c]Analyses including individuals with >10 to 20 years between first and repeated blood draws ($N_{Females} = 44$; $N_{Males} = 38$).
[d]Analyses including female participants (median time ± SD between first and repeated blood draws was 6.1 ± 4.1 years).
[e]ICCs were estimated by linear mixed effects modeling adjusted for age at blood draw and analysis plate.
[f]Analyses including male participants (median time ± SD between first and repeated blood draws was 10.0 ± 1.8 years).

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samples collected in the morning after overnight fasting. Results from these analyses remained similar for most markers with exception of MIP-1α and sCD30 which slightly decreased in ICCs from 0.57 to 0.48 and from 0.56 to 0.45, respectively.

Discussion

Improved cancer control could be achieved by using blood-based screening strategies allowing the detection of tumor-related molecules (35). Because tumor development and progression are affected by inflammatory processes (36), the identification of immune marker changes has a promising role for early cancer detection. Identification of tumor-specific changes in immune marker levels requires high intraindividual long-term stability of potential biomarker candidates in cancer-free individuals (6, 7). Using repeated blood samples from NSHDS, we were able to collect data on intraindividual long-term immune marker stability in 304 cancer-free individuals.

Our results suggest fair reproducibility (ICC ≥ 0.40) in repeated blood samples collected up to 20 years apart for 18 of 19 investigated immune markers. Using a more stringent reproducibility criterion (ICC ≥ 0.55; ref. 19), IL10, IL13, CXCL13, MCP-3, MIP-1α, MIP-1β, VEGF, FGF-2, sTNF-R1, sCD23, sCD27, and sCD30 showed acceptable reproducibility (**Fig. 1**). Reproducibility of this magnitude (ICC ≥ 0.55) is consistent with high intraindividual marker stability over the long term (20). High long-term marker stability in cancer-free individuals suggests a potential use for the identification of tumor-associated inflammatory processes by IL10, IL13, CXCL13, MCP-3, MIP-1α, MIP-1β, VEGF, FGF-2, sTNF-R1, sCD23, sCD27, and sCD30. Our results also suggest that the effect of fasting status on ICCs is small for most of the studied markers.

Interestingly, several markers displaying intraindividual long-term stability in this study, have been suggested to be of potential early diagnostic value in different cancers such as gastric cancer (IL10; ref. 37), ovarian cancer (IL13; ref. 38), lung cancer (IL10, CXCL13, and VEGF; refs. 39–41), multiple myeloma (MCP-3, MIP-1α, VEGF, and FGF-2; ref. 29), nasopharyngeal carcinoma (MIP-1α and MIP-1β; ref. 42), colon cancer (MIP-1α; ref. 43), and B-cell lymphoma (CXCL13, sCD23, sCD27, and sCD30; refs. 28, 44).

In the case of lower reproducibility (ICCs between 0.40 and 0.55), attenuated associations between marker levels and cancer are expected (20, 45). However, strong biomarker candidates suitable for early cancer detection are assumed to display blood level changes of large effect size in individuals later diagnosed with cancer (46, 47). Therefore, immune markers with ICCs between 0.40 and 0.55 such as TNFα, fractalkine, TGFα, sIL2Rα, sIL6R, and sTNF-R2 could also be of value for the early identification of tumor-associated inflammatory processes. Fair reproducibility as estimated for fractalkine, TGFα, and sIL2Rα should be interpreted carefully because the lower CI of estimated ICCs was below 0.40 for these markers (**Fig. 1**).

Intraindividual marker variability was evaluated in several studies using repeated blood samples collected ≤ 3 years apart. Compared with our repeated blood samples collection of up to 20 years apart, these studies observed higher ICCs for IL10 (9, 21), IL13 (9), TNFα (9, 19, 21, 22), MIP-1α (17), fractalkine (16, 17), sIL2Rα (9, 21), sTNF-R1 (25), sTNF-R2 (9, 19, 21, 22, 25), and sVEGF-R2 (16). The discrepancies suggest, that high reproducibility in the short-term does not necessarily imply high reproducibility in the long term.

ICCs observed in our cohort were more comparable with long-term ICCs reported by two previous studies investigating immune marker reproducibility in samples collected ≥5 years apart (15, 20). As compared with our study, these studies' ICCs for IL10 (15, 20), CXCL13 (20), and sCD27 (20) were similar. In contrast, ICCs for IL13 (15), sTNF-R2, and sIL2Rα (20) indicated higher reproducibility for these markers in previous studies than observed by us. However, it is important to note that participants in McKay and colleagues study (20) were all young to middle-aged men ($N = 250$) who donated repeated blood samples up to more than 15 years apart. Hofmann and colleagues study (15) included a relatively small number ($N = 28$) of participants who donated repeated blood samples up to 5 years apart. Different time intervals for blood draws and differences in the studied populations might have contributed to the differences in observed ICCs.

To the best of our knowledge, this study is among the largest studies reporting on intraindividual long-term immune marker stability in cancer-free individuals. To evaluate long-term marker stability, blood samples were collected up to 20 years apart. During this period, sample collection and processing did not change within NSHDS. Duplicate analyte measurement (except of sCD30) is another strength of this study. Our study also has some limitations. Because of limited sample size, results of subanalyses should be interpreted with caution. Participants in the study had one repeated blood sample available collected in median 9 years apart. Additional information on the short-term marker variability based on samples collected within a shorter interval would have improved this study. As immuno-oncological mechanisms are complex, a broader evaluation of immune marker levels, including systems immunology analyses allowing to study variations of immune cell components, would have been of high interest (48). Blood immune marker changes likely are influenced by any acute or chronic disease processes that were present at the time of blood draw. The lack of this information is a drawback of the study. However, it might be assumed that general health survey participants (VIP) would have rescheduled appointments in case of acute infections. In addition, it is important to recognize that target populations for early cancer detection programs likely also include individuals with inflammatory processes related to other diseases than cancer.

In conclusion, we observed high intraindividual stability in marker levels in 304 cancer-free individuals over a period of up to 20 years for IL10, IL13, CXCL13, MCP-3, MIP-1α, MIP-1β, VEGF, FGF-2, sTNF-R1, sCD23, sCD27, and sCD30. Given expected cancer latency times (49), changes in blood levels of these markers could help identify early tumor-associated inflammatory processes. On the basis of high-throughput proteomics (50), future studies should assess both intraindividual short- and long-term marker stability in a large cohort of cancer-free individuals. This strategy could provide a broader perspective of the biological variability and the potential utility for early cancer detection for a wide range of markers.

Authors' Disclosures

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Authors' Contributions

F. Späth: Conceptualization, data curation, formal analysis, supervision, funding acquisition, investigation, visualization, methodology, writing–original draft, writing–review and editing. **W.Y.-Y. Wu:** Formal analysis, investigation, methodology, writing–review and editing. **E.J.M. Krop:** Data curation, writing–review and editing, laboratory analyses. **I.A. Bergdahl:** Supervision, methodology, writing–review and editing. **C. Wibom:** Conceptualization, formal analysis, supervision, methodology, writing–review and editing. **R. Vermeulen:** Supervision, methodology, writing–review and editing.

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