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Microheterogeneity and preanalytical stability of protein biomarkers of inflammation and renal function

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ABSTRACT

Protein biomarker microheterogeneity has attracted increasing attention in epidemiological and clinical research studies. Knowledge concerning the preanalytical stability of proteins is paramount to assess the biological significance of their proteoforms. We investigated the stability of the inflammatory markers C-reactive protein (CRP), serum amyloid A (SAA), and calprotectin (S100A8/9), and the renal function marker, cystatin C (CnC). In total 16 proteoforms were quantified by immuno-MALDI-TOF MS in EDTA plasma and serum samples from 15 healthy volunteers. Prior to analysis blood samples were stored at either room temperature from 1 h up to 8 days, or underwent up to 9 consecutive freeze/thaw cycles. Pearson's correlation coefficient and *t*-test, intra-class correlation coefficient (ICC), and Autoregressive Integrated Moving-Average (ARIMA) models were used to investigate the stability of proteoform concentrations and distributions in blood. Plasma and serum concentrations of CRP and SAA proteoforms were highly stable during room temperature exposure and repeated freeze/thaw cycles, demonstrating excellent reproducibility (ICC > 0.75), no serial dependency in ARIMA models, and stable distribution of proteoforms. Stability analyses for proteoforms of S100A8/9 and CnC identified only minor preanalytical changes in concentrations and distributions, and none of the proteoforms were produced during prolonged exposure to room temperature or repeated freezing/thawing. The four proteins and their proteoforms are stable during sub-optimal sample handling, and represent robust biomarker candidates for future biobank studies aimed at investigating the microheterogeneity of SAA, S100A8/9, and CnC in relation to inflammation, renal dysfunction and various clinical outcomes.

1. Introduction

Interest in protein biomarkers for diagnosis, risk assessment, and treatment of diseases has grown during the last decade. Novel analytical technologies have been established for protein biomarker discovery and validation, many of them based on LC MS and MALDI MS. With the development of targeted approaches [1,2], the use of immuno-affinity enrichment and multiple (MRM), selected (SRM), and parallel reaction monitoring (PRM), determination of protein microheterogeneity has become an option in clinical proteomics [3–5]. Furthermore, recent improvements in protein sample preparation by multi-well robotic platforms enable high-throughput assay formats for clinical applications [6].

Proteoforms, largely formed by post-translational modifications (PTMs) and truncations, can play important roles in biological processes even if the structural changes are minor [7,8]. Since protein microheterogeneity is related to many pathologies and varies between different individuals, investigation of proteoforms may become an important feature of personalized medicine [9,10]. Stability data exist for numerous protein biomarkers [11,12], but information concerning storage effects on proteoform concentrations is lacking. Knowledge of preanalytical stability to exclude the possibility that microheterogeneity is related to sample handling and storage is a prerequisite for the utilization of proteoforms in clinical diagnostics and epidemiological research. While protein instability is generally a minor problem at very low temperatures, storage time at room temperature and repeated freeze/thaw cycles are known to cause protein degradation [13–15]. In addition, biomarkers concentrations and stability may vary depending on the chosen sample matrix [16,17].

We have developed an immuno-MALDI-TOF MS assay for the simultaneous quantification of the inflammatory markers C-reactive protein (CRP), serum amyloid A (SAA), calprotectin (S100A8/9), and the renal function marker cystatin C (CnC) [18]. Various proteoforms

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Abbreviations

CRP	C-reactive protein
SAA	Serum amyloid A
S100A8/9	Calprotectin
CnC	Cystatin C
ICC	Intraclass correlation coefficient
ARIMA	Autoregressive Integrated Moving-Average
RT	Room temperature

have been reported for SAA, S100A8/9, and CnC, and several of these proteoforms have been linked to common diseases such as diabetes mellitus and renal dysfunction [19–22]. Here, we report on the stability of these biomarkers and their 16 proteoforms in EDTA plasma and serum samples, which underwent prolonged storage at room temperature or multiple freeze/thaw cycles.

2. Methods

2.1. Sample collection and experimental design

Analyte stability was assessed using EDTA plasma and serum samples from 15 healthy volunteers. For comparison of biomarker levels in plasma versus serum over a wide concentration range, anonymized blood samples from 83 patients with elevated levels of CRP were provided by the central laboratory at Haukeland University Hospital (Bergen, Norway). Under the current Norwegian regulations concerning quality control investigations, the study was exempt from review by the institutional ethical research board. Blood for plasma and serum samples were collected in 10 mL EDTA and 10 mL silica-coated vacutainer tubes, respectively. Blood was centrifuged at 3100 × G for 12 min at 4 °C. Plasma and serum samples from the 15 healthy volunteers were divided into 14 aliquots (Fig. 1). One aliquot was chosen as reference sample for both series, i.e. storage at room temperature and repeated freezing/thawing. Nine aliquots were stored at room temperature (24 °C in darkness) for 1, 2, 4, 8, and 12 h or 1, 2, 4, and 8 days, while the remaining 4 aliquots underwent 1, 2, 4, and 8 cycles of freezing/thawing. In order to analyse all samples simultaneously in one batch, all samples were stored at −80 °C until analysis. Consequently, the total numbers of freezing/thawing cycles were 2, 3, 5 and 9.

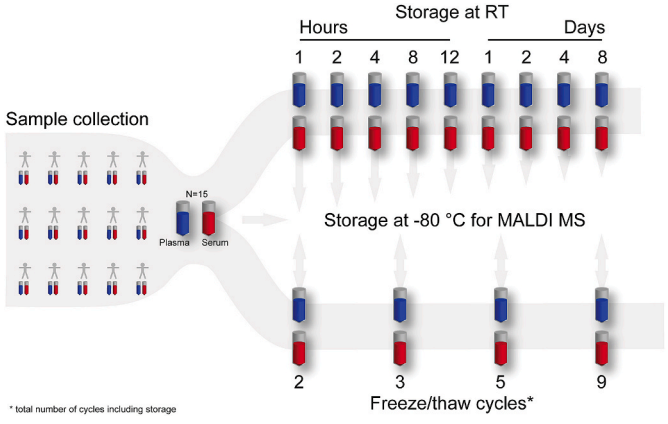

Fig. 1. Design of the stability experiment. EDTA plasma (blue) and serum (red) samples from 15 healthy subjects were divided into 14 aliquots. One aliquot was used as reference sample, 9 aliquots were incubated at room temperature (RT), and 4 aliquots underwent repeated freezing/thawing. At last, all sample were stored at −80 °C to be analysed as one batch by MALDI MS. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.2. Laboratory analyses

Proteoforms were quantified by a novel immuno-MALDI-TOF MS assay [18]. All steps of the method including antibody immobilization on paramagnetic beads, preparation of internal standards, immuno-affinity purification, MALDI MS analysis, and proteoform quantification are described in Supplementary Material. Briefly, 20 µL EDTA plasma or serum were spiked with 20 µL internal standards and incubated with antibody-immobilized paramagnetic beads for 1 h. After repeated washing of the beads, proteins were eluted and prepared for MALDI-TOF MS. Liquid handling was fully automated using a 96-microtiter plate format.

2.3. Statistical analyses

Protein concentrations were presented as geometric means with standard errors and were log-transformed prior to statistical analyses. Correlations between EDTA plasma and serum concentrations were analysed by Pearson's correlation coefficient, using the patients' samples and first aliquots of samples from the healthy volunteers. Proteoform stability was investigated by four different methods: 1. Profiles of the room temperature exposure time and freeze/thaw cycles were plotted as line and bar plots, respectively. Significant changes between segments of the time- or cycle-series were determined by the *t*-test. 2. Changes in proteoform distributions between the first and last sample of the time series were illustrated by radar plots and deviations were analysed by the *t*-test. 3. Reproducibility was assessed by the intra-class correlation coefficient (ICC), defined as the ratio of between-sample variance to total variance. Calculations included all room temperature exposure time points or freeze/thaw cycles and was performed using the ICC (1,1) method as defined by Shrout and Fleiss [23]. ICCs were classified according to Rosner as poor (< 0.4), fair to good (0.4–0.75), and excellent (≥ 0.75) [24]. 4. Biomarker concentrations and ratios according to room temperature exposure were analysed by Autoregressive Integrated Moving-Average (ARIMA), a widely used method for modelling and forecasting time series. ARIMA identifies three kinds of serial dependencies in a time series, which are described by autoregressive (AR), integrated (I), and moving-average (MA) processes [25, 26]. The AR process describes the value of a variable at time t as a regression upon an earlier value at time $t - p$, where p is the time lag. The MA component indicates the regression error by random jumps, which cannot be predicted by AR. The parameter q represents the number of lags in which the effect of random jumps persists in the time series. The integrated process I represents a linear or non-linear trend in the series and the parameter d is the number of differentiating transformations to receive stationarity. The calculated ARIMA models are denoted as (p, d, q). During modelling serial dependency is analysed by each of the three processes and removed from the series until the remaining dependency can be described by a random "white noise" process. In this process each value in the series is statistically independent from others and the series is characterised by a mean of 0 and constant variance. Therefore, biomarker concentrations were determined to be stable during storage, if modelling delivered (0, 0, 0). The packages "ICC", "psych", "forecast" and "stat" of R version 3.5.3 was used for statistical analyses.

3. Results and discussion

3.1. Correlation of plasma and serum for total biomarker concentrations

Total plasma and serum concentrations of the four proteins were compared in both the 15 healthy volunteers, using the first aliquot, and the 83 clinical patients. Plasma and serum concentrations of CRP and SAAt were highly correlated in the healthy volunteers (CRP: $r = 0.97$, $p < 0.001$; SAAt: $r = 0.97$, $p < 0.001$) and patients (CRP: $r = 0.97$,

$p < 0.001$; SAAt: $r = 0.99$, $p < 0.001$). These findings are consistent with earlier reports for these major acute phase proteins [27–29]. However, comparable SAA data only exist for equine SAA in serum and heparin plasma [29].

A strong association between values in plasma versus serum was also found for S100At in the clinical patients ($r = 0.95$, $p < 0.001$), but no correlation was observed among healthy volunteers with very low concentration ($r = -0.37$, $p = 0.16$). Interestingly, for the clinical patients serum S100At concentrations were 40% higher than those observed for plasma, whereas in healthy participants serum concentrations were approximately 300% higher than plasma levels. Similar discrepancies when comparing blood matrices have been described earlier [30,31]. Recently, Nordal et al. speculated that *in-vitro* activation of neutrophils during serum clotting may raise calprotectin concentrations. They therefore recommended EDTA plasma as preferred sample matrix [32]. Consequently, *in-vitro* activation would attenuate the expected correlation between levels in plasma versus serum and the effect would be most pronounced at the low concentrations typically observed among healthy subjects.

Plasma and serum concentrations of CnCt were strongly correlated in the group of healthy volunteers ($r = 0.73$, $p < 0.001$) and patients ($r = 0.95$, $p < 0.001$). These findings are in agreement with earlier reports demonstrating no significant difference according to these matrices [33,34].

3.2. Preanalytical stability graphs of total biomarker concentrations

Total concentrations during room temperature exposure (line plots) and repeated cycles of freezing and thawing (bar plots) of the four biomarkers in EDTA plasma and serum samples from 15 healthy volunteers are presented in Fig. 2. CRP and SAAt showed excellent stability in both plasma and serum samples even at room temperature exposure for up to 8 days. Blood concentrations of both proteins were also stable during 9 cycles of freezing/thawing. Our observations are in agreement with recent data describing the stability of CRP [28] and SAA [35].

Plasma concentrations of S100At were stable at room temperature for up to 4 days, while serum concentrations were stable for up to 2 days before gradually declining by 45%. Repetitive freezing and thawing had no effect on S100At levels in either plasma or serum. Stability of S100A8/9 has been tested previously in other matrices and at different temperatures. Nilsen et al. investigated purified calprotectin in buffer solutions and showed high stability at 30 °C for over 106 days, but a 20% decline after 4 freeze/thaw cycles [36]. Another report from Biovendor demonstrated stable levels both in plasma and serum for 7 days at 2–8 °C, and after 5 cycles of repeated freezing/thawing [37].

Total concentrations of CnC were stable in plasma at room temperature for up to 8 days. Serum levels of CnCt were stable for up to 2 days at room temperature, but declined by 7.5% during the following 6 days. Freeze/thaw cycles did not affect CnCt concentrations in either matrix. Our data agree with earlier findings demonstrating CnC concentrations were stable at room temperature for between 2 and 7 days [33,38,39] and up to 10 consecutive freeze/thaw cycles [38].

3.3. Preanalytical stability graphs of proteoform concentrations

Plasma and serum concentrations of SAA, S100A8/9 and CnC proteoforms after room temperature exposure (line plots) and repeated cycles of freezing and thawing (bar plots) are illustrated in Fig. 3. For each protein, graphs were grouped according to non-truncated and truncated proteoforms, and selected ratios.

Plasma and serum concentrations of SAA proteoforms did not differ, and proteoforms were stable at room temperature for 8 days in both matrices. Ratios of proteoforms were also constant during 8 days of room temperature exposure. Repeated freezing and thawing of samples did not affect stability of the SAA proteoforms or selected ratios in plasma and serum.

As for S100At, concentrations of S100A8/9 proteoforms were higher in serum than plasma. Plasma concentrations of S100A8, S100A9 and S100A9dm were stable for 4-day at room temperature, while serum concentrations of these proteoforms decreased after 2 days under such conditions. Ratios of S100A8 to S100A9t in plasma and serum remained stable during 8 days at room temperature, whereas the ratio in serum was about 60% higher than in plasma. This suggests that the complex stoichiometry S100A8/9 is matrix-dependent and may explain the

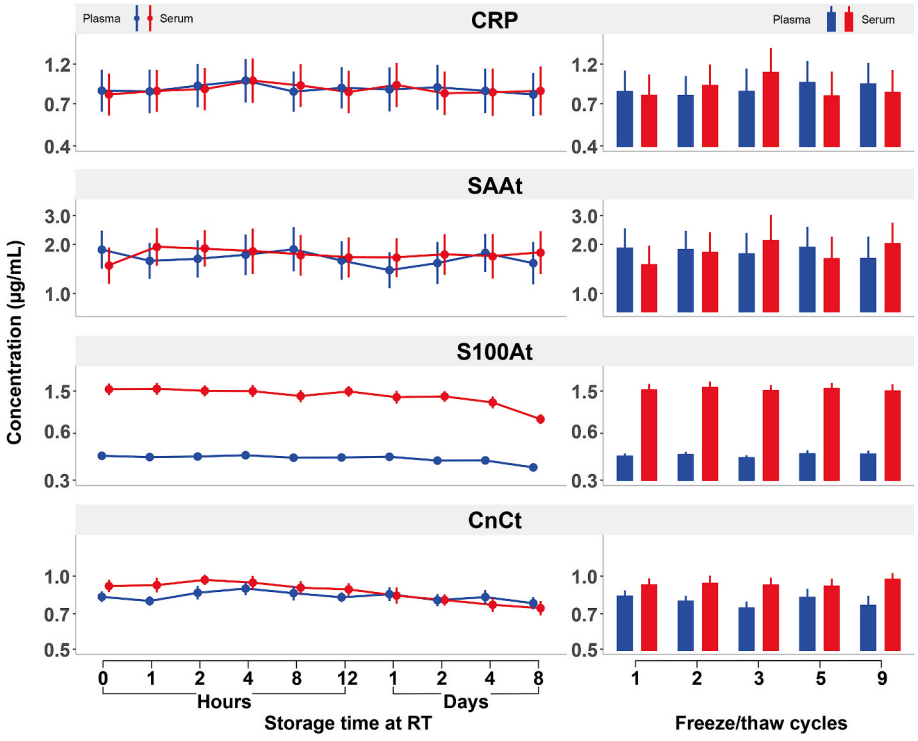

Fig. 2. Preanalytical stability graphs of total concentrations of CRP, serum amyloid A (SAAt), calprotectin (S100At), and cystatin C (CnCt) in EDTA plasma (blue) and serum (red) samples from 15 healthy volunteers. Samples were incubated at room temperature (RT) for 8 days (line plots) or underwent 9 freeze/thaw cycles (bar plots). Data are plotted as geometric means with standard errors. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

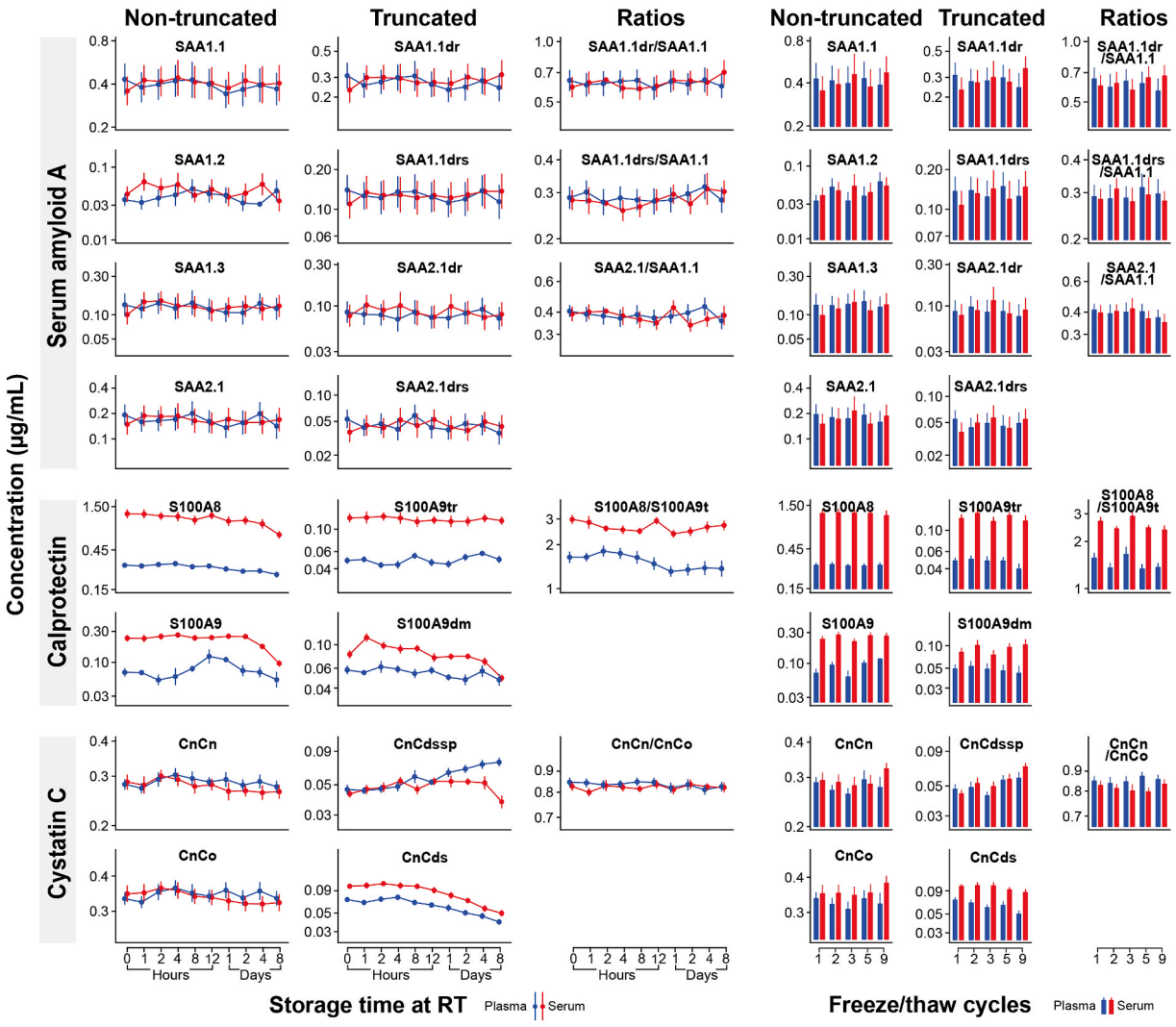

Fig. 3. Preanalytical stability graphs of serum amyloid A, calprotectin, and cystatin C proteoforms in EDTA plasma (blue) and serum (red) samples from 15 healthy volunteers. Samples were incubated at room temperature (RT) for 8 days (line plots) or underwent 9 freeze/thaw cycles (bar plots). Profiles are grouped (non-truncated, truncated and ratios) and data are plotted as geometric means with standard errors. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

variation among proposed structures of S100A8/9 in the literature [21,40,41]. Repeated freezing and thawing had no impact on S100A8/9 proteoform concentrations and ratios in plasma or serum.

Concentrations of the major CnC proteoforms CnCn and CnCo, including the ratio between the two, were highly stable at room temperature and during freeze/thaw cycles irrespective of sample matrix. Small changes were observed for the minor forms, CnCdssp and CnCds. Plasma concentrations of CnCdssp increased slightly after 2 days. CnCds concentrations were 40% higher in serum than plasma, and declined in both matrices after exposure to room temperature for 12 h. The decline of CnCds accompanied by a parallel increase of CnCdssp suggested that degradation of CnCds was caused by loss of the two N-terminal amino acids leading to the formation of CnCdssp. Similar relationships were obtained in plasma and serum samples during repeated freezing and thawing. A truncated variant of CnC, recently identified as product of inadequate storage conditions [42], was not identified by our analyses.

3.4. Changes in proteoform distributions after 8 days at room temperature

Proteoform distributions of SAA, S100A8/9 and CnC are illustrated as patterns of radar chart plots in Fig. 4. Proteoform distributions in the first (day 0, blue/red colour) and last aliquots (day 8, grey) were overlaid, and significant changes by the *t*-test were indicated.

Distributions of SAA proteoforms were similar in both sample matrices and changes were not significant. The distribution of most S100A8/9 proteoforms was stable in plasma samples except S100A8. However, in serum the distribution changed significantly after 8 days, largely explained by the decrease in S100A8 and S100A9 concentrations. Proteoform distribution differed between blood matrices, with S100A8 present in higher concentrations in serum than plasma. The distributions of CnC proteoforms were essentially the same in plasma and serum, and only minor changes were observed for CnCds and CnCdssp following exposure to room temperature for 8 days.

3.5. Reproducibility of total biomarker and proteoform concentrations in terms of intraclass correlation coefficients (ICCs)

The reproducibility of total biomarkers and the proteoforms during room temperature exposure and repeated freezing/thawing is presented in Fig. 5. Excellent ICCs (≥ 0.75) [24] were obtained for plasma/serum concentrations of CRP and total and all proteoforms of SAA. The ICCs for S100At and the most abundant proteoform, S100A8, are good to excellent ($0.65 < \text{ICCs} < 0.89$), but varied markedly ranging from poor (< 0.4) to excellent (> 0.75) for the other proteoforms. The ICCs were

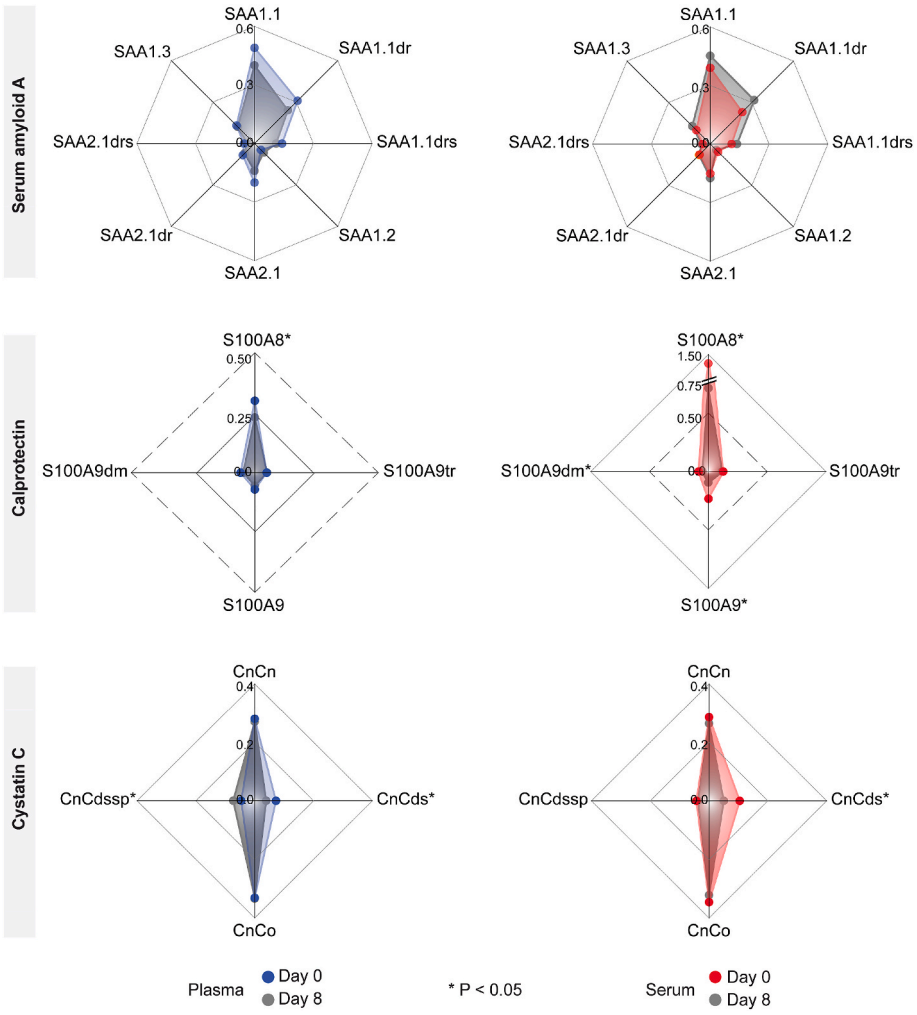

Fig. 4. Changes in proteoform distributions of SAA, S100A8/9 and CnC between day 0 (blue/red colour) and day 8 (grey), illustrated as radar plots. Distributions in EDTA plasma and serum samples are shown in the left and right panels, respectively. Significant changes by the *t*-test ($P < 0.05$) are indicated (*). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

comparable for storage and repeated freeze/thaw cycles, but large variations were observed when comparing reproducibility in plasma versus serum, and higher ICCs in serum for most proteoforms. The latter observations could be explained by plasma concentrations being closer to the assay LOQ. The ICCs observed for CnCt and the major proteoforms, CnCn and CnCo, ranged between fair to good and excellent ($0.59 < ICCs < 0.79$). Again, the least abundant proteoforms, CnCds and CnCdssp, showed lower ICCs, ranging between poor and fair to good ($0.34 < ICCs < 0.68$).

3.6. Autoregressive integrated moving-average modelling of total biomarker and proteoform concentrations

Time series of biomarker concentrations at room temperature were analysed by ARIMA and results are shown in Table 1. Time series of CRP and SAA, including all proteoforms, showed no serial dependency, in either plasma or serum, which indicated high stability during storage in both blood matrices.

ARIMA modelling of S100A8/9 identified a linear trend for S100At, S100A8 and S100A9dm in plasma and serum. S100A9 showed a trend in serum, while the time series of S100A9tr demonstrated no serial dependency. A linear trend in plasma was observed for the ratio of S100A8/S100A9t.

The profiles of CnCt, CnCn and CnCo showed no serial dependency in plasma, but a linear trend was observed in serum samples. No dependency was found for the ratio of CnCn/CnCo. ARIMA modelling of CnCdssp and CnCds showed linear trends for both proteoforms in plasma. In serum, the profile of CnCdssp showed no dependency across the time series, whereas the time series of CnCds was the only proteoform following a quadric trend.

In conclusion, results from ARIMA models were consistent with the shown profiles of total biomarker and proteoform concentrations.

3.7. Limitations

This study, however, is not without limitations. We did not investigate the effects of room temperature exposure on whole blood during sample collection. In addition, protein stability at 4 °C was not investigated, a commonly recommended temperature for short-term storage. Nevertheless, it is expected that protein stability increases with lower temperature.

4. Conclusions

We investigated the stability of the inflammatory markers C-reactive protein (CRP), serum amyloid A (SAA), and calprotectin (S100A8/9), and the renal function marker, cystatin C (CnC) in response to prolonged room temperature exposure and repeated freeze/thaw cycles. Blood concentrations of the major acute-phase reactants CRP and SAA, including all proteoforms were determined to be highly stable in both

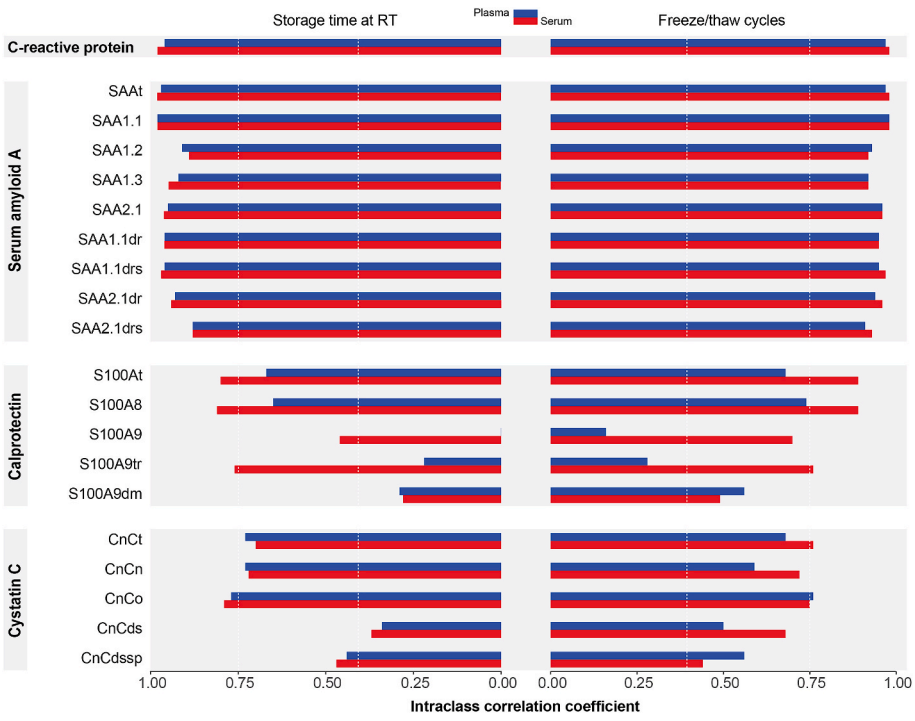

Fig. 5. Intraclass correlation coefficients (ICCs) for total biomarker and proteoform concentrations during incubation at room temperature (RT) for 8 days (left panel), and 9 freeze/thaw cycles (right panel). EDTA plasma and serum concentrations are illustrated as blue and red bar plots, respectively. Thresholds of ICCs (<0.4: poor; 0.4–0.75: fair to good; ≥ 0.75: excellent) [24] are marked by dashed lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Autoregressive integrated moving average (ARIMA) models of time series of the biomarkers and their ratios during storage at RT.

Biomarkers and ratios	EDTA plasma	Serum
CRP	(0, 0, 0)	(0, 0, 0)
SAA		
SAAt	(0, 0, 0)	(0, 0, 0)
SAA1.1	(0, 0, 0)	(0, 0, 0)
SAA1.2	(0, 0, 0)	(0, 0, 0)
SAA1.3	(0, 0, 0)	(0, 0, 0)
SAA2.1	(0, 0, 0)	(0, 0, 0)
SAA1.1dr	(0, 0, 0)	(0, 0, 0)
SAA1.1drs	(0, 0, 0)	(0, 0, 0)
SAA2.1dr	(0, 0, 0)	(0, 0, 0)
SAA2.1drs	(0, 0, 0)	(0, 0, 0)
SAA2.1/SAA1.1	(0, 0, 0)	(0, 0, 0)
SAA1.1dr/SAA1.1	(0, 0, 0)	(0, 0, 0)
SAA1.1drs/SAA1.1	(0, 0, 0)	(0, 0, 0)
S100A8/9		
S100At	(0, 1, 0)	(0, 1, 0)
S100A8	(0, 1, 0)	(0, 1, 0)
S100A9	(0, 0, 0)	(0, 1, 0)
S100A9tr	(0, 0, 0)	(0, 0, 0)
S100A9dm	(0, 1, 0)	(0, 1, 0)
S100A8/S100A9t	(0, 1, 0)	(0, 0, 0)
CnC		
CnCt	(0, 0, 0)	(0, 1, 0)
CnCn	(0, 0, 0)	(0, 1, 0)
CnCo	(0, 0, 0)	(0, 1, 0)
CnCds	(0, 1, 0)	(0, 2, 0)
CnCdssp	(0, 1, 0)	(0, 0, 0)
CnCn/CnCo	(0, 0, 0)	(0, 0, 0)

EDTA plasma and serum. Significant changes in S100A8/9 and CnC concentrations were either minor or appeared late in the time series. Consequently, proteoforms of all four biomarkers assessed in the present investigation should be considered as stable during standard procedures for handling and storage in most biobanks, where blood sample processing occurs within hours, with storage at +4 °C for less than 24 h [43], and the number of freeze/thaw cycles are minimized.

The stability data presented here will enable future studies to investigate the associations of micro-heterogeneity of SAA, S100A8/9, and CnC with various clinical outcomes.

Nomenclature of proteoforms

Total blood levels of serum amyloid A, calprotectin and cystatin C were denoted as SAAt, S100At, and CnCt, respectively. N-terminal truncations of SAA, S100A8/9 and CnC were labelled with a "d" and the one-letter codes of the missing amino acids. Proteoforms of SAA were abbreviated according to the isoforms expressed by the *SAA1* or *SAA2* gene. Monomers of the S100A8/9 complex were assigned as S100A8 and S100A9. The shortest truncation of S100A9, missing 5 amino acids, was abbreviated as S100A9tr. The sum of S100A9, S100A9tr and S100A9dm was defined as S100A9t. The native and the hydroxylated forms of CnC were abbreviated as CnCn and CnCo, respectively.

Credit author statement

Jie Gao: Writing – Original Draft, Software, Visualization; **Arve Ulvik**: Software, Formal analysis, Writing – Review & Editing; **Adrian McCann**: Conceptualization, Methodology, Writing – Review & Editing; **Per Magne Ueland**: Supervision, Writing – Review & Editing; **Klaus Meyer**: Supervision, Writing – Review & Editing, Resources.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.121774.

References

[1] N.P. Manes, A. Nita-Lazar, Application of targeted mass spectrometry in bottom-up proteomics for systems biology research, J. Proteomics 189 (2018) 75–90.
[2] T.K. Toby, L. Fornelli, N.L. Kelleher, Progress in top-down proteomics and the analysis of proteoforms, Annu. Rev. Anal. Chem. 9 (1) (2016) 499–519.
[3] C.E. Parker, T.W. Pearson, N.L. Anderson, C.H. Borchers, Mass-spectrometry-based clinical proteomics - a review and prospective, Analyst 135 (8) (2010) 1830–1838.
[4] C.E. Parker, C.H. Borchers, The special issue: clinical proteomics for precision medicine, Proteonomics Clin. Appl. 12 (2) (2018) 1600144.
[5] V. Greco, C. Piras, L. Pieroni, M. Ronci, L. Putignani, P. Roncada, A. Urbani, Applications of MALDI-TOF mass spectrometry in clinical proteomics, Expert Rev. Proteomics 15 (8) (2018) 683–696.
[6] M. Alexovič, P.L. Urban, H. Tabani, J. Sabo, Recent advances in robotic protein sample preparation for clinical analysis and other biomedical applications, Clin. Chim. Acta 507 (2020) 104–116.
[7] R. Aebersold, J.N. Agar, I.J. Amster, M.S. Baker, C.R. Bertozzi, E.S. Boja, C. E. Costello, B.F. Cravatt, C. Fenselau, B.A. Garcia, Y. Ge, J. Gunawardena, R. C. Hendrickson, P.J. Hergenrother, C.G. Huber, A.R. Ivanov, O.N. Jensen, M. C. Jewett, N.L. Kelleher, L.L. Kiessling, N.J. Krogan, M.R. Larsen, J.A. Loo, R.R. O. Loo, E. Lundberg, M.J. MacCoss, P. Mallick, V.K. Mootha, M. Mrksich, T. W. Muir, S.M. Patrie, J.J. Pesavento, S.J. Pitteri, H. Rodriguez, A. Saghatelian, W. Sandoval, H. Schluter, S. Sechi, S.A. Slavoff, L.M. Smith, M.P. Snyder, P. M. Thomas, M. Uhlen, J.E. Van Eyk, M. Vidal, D.R. Walt, F.M. White, E.R. Williams, T. Wohlschlager, V.H. Wysocki, N.A. Yates, N.L. Young, B. Zhang, How many human proteoforms are there? Nat. Chem. Biol. 14 (3) (2018) 206–214.
[8] A. Bogaert, E. Fernandez, K. Gevaert, N-terminal proteoforms in human disease, Trends Biochem. Sci. 45 (4) (2020) 308–320.
[9] D. Nedelkov, U.A. Kiernan, E.E. Niederkofler, K.A. Tubbs, R.W. Nelson, Investigating diversity in human plasma proteins, Proc. Natl. Acad. Sci. U. S. A 102 (31) (2005) 10852–10857.
[10] G. Walsh, R. Jefferis, Post-translational modifications in the context of therapeutic proteins, Nat. Biotechnol. 24 (10) (2006) 1241–1252.
[11] J.E. Lee, S.Y. Kim, S.Y. Shin, Effect of repeated freezing and thawing on biomarker stability in plasma and serum samples, Osong. Public Health Res. Perspect. 6 (6) (2015) 357–362.
[12] M. Insenser, M.Á. Martínez-García, R.M. Nieto, J.L. San-Millán, H.F. Escobar-Morreale, Impact of the storage temperature on human plasma proteomic analysis: implications for the use of human plasma collections in research, Proteomics. Clin. Appl. 4 (8-9) (2010) 739–744.
[13] B. Mitchell, Y. Yasui, C. Li, A. Fitzpatrick, P. Lampe, Impact of freeze-thaw cycles and storage time on plasma samples used in mass spectrometry based biomarker discovery projects, Canc. Inf. 1 (2005) 98–104.
[14] E.H. Cao, Y.H. Chen, Z.F. Cui, P.R. Foster, Effect of freezing and thawing rates on denaturation of proteins in aqueous solutions, Biotechnol. Bioeng. 82 (6) (2003) 684–690.
[15] S. Pasella, A. Baralla, E. Canu, S. Pinna, J. Vaupel, M. Deiana, C. Franceschi, G. Baggio, A. Zinellu, S. Sotgia, G. Castaldo, C. Carru, L. Deiana, Pre-analytical stability of the plasma proteomes based on the storage temperature, Proteome Sci. 11 (2013) 10.
[16] Z. Yu, G. Kastenmüller, Y. He, P. Belcredi, G. Möller, C. Prehn, J. Mendes, S. Wahl, W. Roemisch-Margl, U. Ceglarek, A. Polonikov, N. Dahmen, H. Prokisch, L. Xie, Y. Li, H.E. Wichmann, A. Peters, F. Kronenberg, K. Suhre, J. Adamski, T. Illig, R. Wang-Sattler, Differences between human plasma and serum metabolite profiles, PloS One 6 (7) (2011), e21230.
[17] S. Barelli, D. Crettaz, L. Thadikkaran, O. Rubin, J.D. Tissot, Plasma/serum proteomics: pre-analytical issues, Expert Rev. Proteomics 4 (3) (2007) 363–370.
[18] J. Gao, K. Meyer, K. Borucki, P.M. Ueland, Multiplex immuno-MALDI-TOF MS for targeted quantification of protein biomarkers and their proteoforms related to inflammation and renal dysfunction, Anal. Chem. 90 (5) (2018) 3366–3373.
[19] T.O. Yassine HN, H. He, C.R. Borges, D. Nedelkov, W. Mack, et al., Serum amyloid a truncations in type 2 diabetes mellitus, PloS One 10 (2015), e0115320.
[20] M. De Buck, M. Gouwy, J.M. Wang, J. Van Snick, G. Opdenakker, S. Struyf, J. Van Damme, Structure and expression of different serum amyloid A (SAA) variants and their concentration-dependent functions during host insults, Curr. Med. Chem. 23 (17) (2016) 1725–1755.
[21] D. De Seny, M. Fillet, C. Ribbens, R. Marée, M.-A. Meuwis, L. Lutteri, J.-P. Chapelle, L. Wehenkel, E. Louis, M.-P. Merville, Monomeric calgranulins measured by SELDI-TOF mass spectrometry and calprotectin measured by ELISA as biomarkers in arthritis, Clin. Chem. 54 (6) (2008) 1066–1075.
[22] O. T, Hussein N. Yassine, The association of plasma cystatin C proteoforms with diabetic chronic kidney disease, Proteome Sci. 25 (14) (2016) 7.
[23] P.E. Shrout, J.L. Fleiss, Intraclass correlations: uses in assessing rater reliability, Psychol. Bull. 86 (2) (1979) 420–428.
[24] B. Rosner, One-way ANOVA-The Random-Effects Model. Fundamentals of Biostatistics, seventh ed., 2006.
[25] P.J. Brockwell, Time series, in: M. Lovric (Ed.), International Encyclopedia of Statistical Science, Springer Berlin Heidelberg, Berlin, Heidelberg, 2011, pp. 1601–1605.
[26] C.A. VanLear, Modeling and analyzing behaviors and the dynamics of behavioral interaction, in: C.A. VanLear, D.J. Canary (Eds.), Researching Interactive Communication Behavior: A Sourcebook of Methods and Measures, SAGE Publications, Inc, 2017, pp. 246–248.
[27] E. Brindle, M. Fujita, J. Shofer, K.A. O'Connor, Serum, plasma, and dried blood spot high-sensitivity C-reactive protein enzyme immunoassay for population research, J. Immunol. Methods 362 (1) (2010) 112–120.
[28] N. Aziz, J.L. Fahey, R. Detels, A.W. Butch, Analytical performance of a highly sensitive C-reactive protein-based immunoassay and the effects of laboratory variables on levels of protein in blood, Clin. Diagn. Lab. Immunol. 10 (4) (2003) 652–657.
[29] J. Howard, C. Graubner, Comparison of paired serum and lithium heparin plasma samples for the measurement of serum amyloid A in horses using an automated turbidimetric immunoassay, Vet. J. 199 (2013).
[30] L. Pedersen, E. Birkemose, C. Gils, S. Safi, M. Nybo, Sample type and storage conditions affect calprotectin measurements in blood, J. Appl. Lab. Med. 2 (2018) 851–856.
[31] M. Malham, K. Carlsen, L. Riis, A. Paerregaard, I. Vind, M. Fenger, V. Wewer, Plasma calprotectin is superior to serum calprotectin as a biomarker of intestinal inflammation in ulcerative Colitis, Scand. J. Gastroenterol. 54 (10) (2019) 1214–1219.
[32] H.H. Nordal, M.K. Fagerhol, A.K. Halse, H.B. Hammer, Calprotectin (S100A8/A9) should preferably be measured in EDTA-plasma; results from a longitudinal study of patients with rheumatoid arthritis, Scand. J. Clin. Lab. Invest. 78 (1–2) (2018) 102–108.
[33] E.J. Erlandsen, E. Randers, J.H. Kristensen, Evaluation of the dade behring N latex cystatin C assay on the dade behring nephelometer II system, Scand. J. Clin. Lab. Invest. 59 (1) (1999) 1–8.
[34] J. Kyhse-Andersen, C. Schmidt, G. Nordin, B. Andersson, P. Nilsson-Ehle, V. Lindstrom, A. Grubb, Serum cystatin C, determined by a rapid, automated particle-enhanced turbidimetric method, is a better marker than serum creatinine for glomerular filtration rate, Clin. Chem. 40 (10) (1994) 1921–1926.
[35] A. Hillstrom, H. Tvedten, I. Lilliehook, Evaluation of an in-clinic Serum Amyloid A (SAA) assay and assessment of the effects of storage on SAA samples, Acta Vet. Scand. 52 (2010) 8.
[36] T. Nilsen, S.H. Haugen, A. Larsson, Extraction, isolation, and concentration of calprotectin antigen (S100A8/S100A9) from granulocytes, Health Sci. Rep. 1 (5) (2018) e35.
[37] Biovendor Product Brochure, 2014.
[38] H. Finney, D.J. Newman, W. Gruber, P. Merle, C.P. Price, Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the behring nephelometer systems (BNA, BN II), Clin. Chem. 43 (6) (1997) 1016–1022.
[39] E.M. Spithoven, S.J. Bakker, J.E. Kootstra-Ros, P.E. de Jong, R.T. Gansevoort, D. C. Investigators, Stability of creatinine and cystatin C in whole blood, Clin. Biochem. 46 (15) (2013) 1611–1614.
[40] I.P. Korndorfer, F. Brueckner, A. Skerra, The crystal structure of the human (S100A8/S100A9)(2) heterotetramer, calprotectin, illustrates how conformational changes of interacting alpha-helices can determine specific association of two EF-hand proteins, J. Mol. Biol. 370 (5) (2007) 887–898.
[41] T. Vogl, A.L. Gharibyan, L.A. Morozova-Roche, Pro-inflammatory S100A8 and S100A9 proteins: self-assembly into multifunctional native and amyloid complexes, Int. J. Mol. Sci. 13 (3) (2012) 2893–2917.
[42] O. Carrette, Truncated cystatin C in cerebrospinal fluid: technical artefact or biological process? Proteomics 5 (12) (2005) 3060–3065.
[43] W. Paskal, A.M. Paskal, T. Debski, M. Gryziak, J. Jaworowski, Aspects of modern biobank activity - comprehensive review, Pathol. Oncol. Res. 24 (4) (2018) 771–785.