Journal of Trace Elements in Medicine and Biology 52 (2019) 247–253

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.com/locate/jtemb

Clinical studies

Circulating levels of selenium-binding protein 1 (SELENBP1) are associated with risk for major adverse cardiac events and death

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ARTICLE INFO

Keywords:
Trace element
Biomarker
Selenoprotein
Metabolism
Redox regulation

ABSTRACT

Objective: Selenium-binding protein 1 (SELENBP1) is an intracellular protein with variable expression in response to cellular stress. As the selenium (Se) status is affected by inflammation and hypoxia, we hypothesized that SELENBP1 contributes to disease-specific Se metabolism. To test this hypothesis, a quantitative assay was developed and used to monitor SELENBP1 in patients with acute coronary syndrome (ACS).

Materials and methods: SELENBP1 was expressed, antibodies were generated and a luminometric immuno assay (LIA) was established and characterized. Serum samples were collected from controls (n = 37) and patients (n = 85) admitted to the Chest Pain Unit with suspected ACS. Blood samples were available from time of first medical contact in the ambulance, at admission to hospital, and after 2, 4, 6 and 12–36 h.

Results: Circulating SELENBP1 was close to limit of detection in healthy controls and elevated in patients with suspected ACS. SELENBP1 was unrelated to other biomarkers of myocardial damage such as troponin T or aspartate aminotransferase. Serum SELENBP1 enabled a categorization of patients on first medical contact as either high-risk or low-risk for major adverse cardiac events (MACE) or death, when using 0.8 nmol/l as threshold. The odds-ratios (OR) for MACE and death were OR = 11 (95% CI: 2–49, p = 0.0022) and OR = 12 (2–74, p = 0.014), respectively.

Conclusions: Until now, SELENBP1 was mainly considered as an intracellular protein involved in Se metabolism and redox control. Our data indicate that SELENBP1 constitutes a circulating biomarker for cardiac events categorizing patients with suspected ACS at first medical contact into high-risk or low-risk for MACE and death, independent from and complimentary to current biomarkers.

1. Introduction

Selenium (Se) is an essential trace element for mammals [1], with high importance for redox signaling [2] in, *e.g.*, the endocrine [3] and immune system [4]. In blood, Se is found in different forms, mainly as constituent of circulating proteins containing either selenomethionine or selenocysteine (Sec) [5]. The latter are called selenoproteins and are characterized by the co-translational insertion of the 21st proteinogenic amino acid Sec. Two circulating selenoproteins account for more than 50% of serum or plasma Se, i.e., the extracellular glutathione peroxidase (GPX3) and the Se-transporter selenoprotein P (SELENOP) [6]. SELENOP reflects Se status and intake [7], and responds to different pathogenic stimuli including proinflammatory cytokines [8]. Hepatic selenoprotein expression is redirected under hypoxia [9], contributing to reduced circulating SELENOP and Se status in critical illness [10,11]. Se deficiency was also demonstrated in cardiac patients already prior to intervention, which was further aggravated during surgery [12]. Low postoperative Se levels were associated with risk for organ dysfunction and a poor mid- and long-term outcome, potentially necessitating supplementation [13].

Abbreviations: SELENBP1, selenium-binding protein 1; ACS, acute coronary syndrome; LIA, luminometric immuno assay; MACE, major adverse cardiac event(s); RLU, relative light units; (N)STEMI, (Non-) ST elevation myocardial infarction; ASAT, aspartate aminotransferase; MTO, methanethiol oxidase; mAb, monoclonal antibody; FAS, functional assay sensitivity; hsTnT, high-sensitivity Troponin T

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https://doi.org/10.1016/j.jtemb.2019.01.005
Received 16 October 2018; Received in revised form 30 December 2018; Accepted 7 January 2019
0946-672X/

Beside GPX3 and SELENOP, other Se-binding proteins may contribute to disease-related alterations of Se metabolism. Selenium-binding protein 1 (SELENBP1) is one of two intracellular Se-binding proteins with largely unknown function [14]. It was characterized based on its affinity for radioactive Se, followed by protein purification and DNA cloning [15]. A 3D model has been proposed which supports Cys57 as a binding site for selenite at neutral pH [16]. SELENBP1 has been studied in tumor tissues and cell culture models, and evidence is accumulating for a dynamic regulation of intracellular SELENBP1 expression and function as a tumor suppressor [17–19]. High expression levels have been associated with reduced tumor cell proliferation or increased survival in, e.g., prostate [17] or breast cancer [20], characterizing it as a potential prognostic marker in clinical practice. A recent study reported up-regulated SELENBP1 in renal tubular cells in response to chemical stress, indicating its potential as urinary marker for renal damage [21]. Mechanistically, a direct interaction of SELENBP1 with GPX1 has been described, potentially affecting intracellular Se metabolism and redox regulation [22]. Recently, an enzymatic activity of SELENBP1 as a methanethiol oxidase (MTO) was identified [23].

Collectively, the data available implicate the involvement of SELENBP1 in Se homeostasis, protection from redox-active toxic substances and adversely smelling thiols, tumorigenesis, oxidative stress and response to hypoxia. The latter two stimuli are of particular relevance for patients exposed to myocardial ischemia/reperfusion (I/R), who undergo profound alterations of their Se metabolism [12]. Therefore, we decided to investigate the concentrations of circulating SELENBP1 in patients with acute coronary syndrome (ACS).

2. Material and methods

2.1. Patients

Patients (n = 85) were consecutively enrolled in this prospective, observational study and provided written informed consent, as reported earlier [24]. A small group of random controls (n = 37) from a recent case-control study [25] were included to test for normal distribution in healthy subjects. Both studies had been approved by the institutional review board of the Charité -Berlin and were performed in adherence to the Declaration of Helsinki. The analyses were conducted by personnel blinded to the clinical findings. Sample acquisition and handling in both studies were comparable, sample collections were both stored at −80 °C, and time intervals at room temperature were minimal.

2.2. Expression and purification of recombinant SELENBP1

Recombinant human SELENBP1 (rhSELENBP1) was expressed in baculovirus-infected insect cells. The cDNA sequence encoding rhSELENBP1 was amplified by PCR from hepatic cDNA using primers P1 (atcggatccaccatggctacgaaatgtgggaattgtg) and P2 (atcaagcttcagtgatggtgatggtgatgaatccagatgtcagagctacaatcgcc) containing a BamHI and HindIII restriction site, respectively (Invitrogen, Thermo Fischer Scientific, Dreieich, Germany). The pFastBac1 plasmid was digested with BamHI and HindIII, the plasmid fragment was removed and replaced with the PCR sequence giving rise to pFastBac1- SELENBP1-His6 plasmid. DH10Bac *E. coli* cells were transformed and Bacmid-positive cells were identified, cultivated and recombinant bacmid was isolated. Sf9 insect cells were transfected with bacmid DNA by Cellfectin (Thermo Fischer Scientific) for obtaining a recombinant virus stock that was used to initiate rhSELENBP1biosynthesis in “High Five” insect suspension cells. 72 h after infection, cells were harvested, lysed and rhSELENBP1-His6 was isolated from cell extract using affinity chromatography on Ni-NTA agarose according to the manufacturer’s instructions (Qiagen GmbH, Hilden, Germany). The concentration of purified rhSELENBP1 was determined using a commercial bicinchoninic acid protein assay kit (Pierce BCA, Thermo Fischer Scientific).

2.3. Immunization, isolation of monoclonals and purification of antibodies

Monoclonal antibodies (mAb) were generated essentially as described [26] by a commercial service provider (UNICUS Karlsburg OHG, Greifswald, Germany). In brief, BALB/c mice were immunized with an emulsion of purified rhSELENBP1 in TiterMax® Gold adjuvant (Sigma-Aldrich Corp., St. Louis, U.S.A.), followed by additional injections for boosting the immune response. Antibody-titers were determined by an indirect ELISA with immobilized rhSELENBP1 in combination with polyclonal rabbit anti-mouse Ab. Positive mice were sacrificed, the splenocytes isolated, fused with myeloma cells and hybridomas were selected. Positive cultures were propagated and recloned by limiting dilution to obtain homogenous cell clones. Secreted antibodies were purified using protein A chromatography by a commercial service supplier (InVivo Biotech Services, Hennigsdorf, Berlin, Germany).

2.4. Assay development and characterization

Several mAb combinations recognizing SELENBP1 were compared, essentially as described [26]. Two suitable mAb (anti-SELENBP1-mAb1 and -mAb2) were chosen and a two-site non-competitive immunoassay (sandwich assay) was established. Phosphate buffered saline (PBS) with 0.05 M K_2HPO_4, 0.1 M NaCl, adjusted to pH 6.5, was used as a basis. For coating purposes, flat-bottomed, white high binding 96-well plates (Greiner Bio-One, Frickenhausen, Germany) were incubated with 100 µl PBS containing 2.1 µg/ml anti-SELENBP1-mAb1 per well for 12 h at + 4 °C. Calibration standards were generated by diluting rhSELENBP1 in PBS containing 1.0 mmol/l bovine serum albumin (BSA). Positive standards were prepared by adding rhSELENBP1 to human serum.

The assay parameters were optimized for improving the signal/noise ratio, and the following protocol was routinely used. The plates were washed four times using PBS with 1% (v/v) Triton X-100. Next, 85 µl PBS with 10% (v/v) glycerol and 1% (w/v) BSA and 15 µl sample per well were added, plates were sealed and placed on a microplate shaker for 1 h at RT. Thereafter, the plates were washed four times with PBS/BSA and incubated with 100 µl PBS with 0.05% (v/v) MACN-labelled anti-SELENBP1-mAb2, 0.1% (v/v) Triton X-100 and 5% (w/v) skim milk per well and placed on a shaker for another 1 h at RT. Finally, the plates were washed four times with PBS/BSA and placed into a luminometer (Mithras LB 940, Berthold, Bad Wildbad, Germany). Detection of SELENBP1 was accomplished photometrically at 430 nm, after injecting 75 µl of 0.06% (v/v) H_2O_2 and 0.2 M NaOH. Relative light units (RLU) were recorded for 1 s. Six calibration standards with increasing concentrations of rhSELENBP1 were included in each plate, and SELENBP1 concentrations were calculated by linear regression as stated below. In addition, each plate contained a standard serum with 4.8 nmol/l rhSELENBP1. This standard allowed a comparison of the accuracy of the luminometric immunoassay (LIA) across different plates (inter assay variation). The standards used for analyzing clinical serum samples ranged from 0.3 to 38.2 nmol/l rhSELENBP1. Concentrations below the linear range were extrapolated. Quantification was based on linear regression of the RLU as a function of SELENBP1 concentration, with m and b being plate-specific parameters, calculated using calibrators on each plate:

$$SELENBP1 = e^{\frac{ln(RLU)-b}{m}}$$

2.5. Sensitivity

Different quantities of rhSELENBP1 were analyzed to assess functional assay sensitivity (FAS) of the LIA. Each concentration was measured in triplicate. All samples were prepared with 1.2 mmol/l BSA to mimic regular serum protein concentrations. FAS was defined as the

range of concentrations allowing reliable measurements with coefficients of variations (CV) below 20%.

2.6. Stability of SELENBP1 in serum

Stability of SELENBP1 was analyzed by exposing serum samples to prolonged storage at RT or at + 4 °C, and by repeated cycles of freezing and thawing. Two individual serum samples were drawn using BD Vacutainer systems with serum separation tubes (SST, Becton Dickinson GmbH, Heidelberg, Germany), left at RT for 30 min to allow coagulation, and centrifuged at 900 × *g* (2600 rpm) for 10 min at RT to separate serum from the clotted material. Three preparations were made from the supernatant of the first serum sample: one left as is (E), and a second and third sample supplemented with rhSELENBP1 to 5.7 nmol/l (C) and 15.3 nmol/l (D), respectively. The supernatant of the second serum sample was left as is (A) or supplemented with rhSELENBP1 to 5.7 nmol/l (B). Six aliquots were prepared from each of the preparations A–E and placed at + 4 °C or RT. Aliquots were taken after 1 h, 3 h, 7 h, 1 d, 3 d and 7 d, respectively. The aliquots were stored at −20 °C until analysis. Ten more aliquots were taken of the preparations B, D and E for the determination of stability upon repeated freeze-thawing cycles.

2.7. Intra- and inter-assay variability, comparison of matrices

A duplicate of samples from a standard serum with added rhSELENBP1 was analyzed with each 96 well plate in technical duplicates and used to calculate the CV between plates (inter-assay CV). To assess variability within duplicates the quantiles of the CVs were calculated. rhSELENBP1 was added to freshly drawn serum, as well as to citrated, heparin- and EDTA-containing plasma samples. After incubation at RT, SELENBP1 concentrations were determined by the LIA as described above.

2.8. Statistical analysis

All statistical analyses were performed using freely available statistical software (R 3.4.1, The R Foundation for Statistical Computing). When testing for differences between groups, Wilcoxon's rank-sum test was used. Confidence intervals (CI) and significance were reported for an interval of 0.95 and accordingly an α of 0.05.

A multivariable logistic regression with risk of death until discharge was performed. For each variable the odds-ratio (OR) and CI were calculated to demonstrate the effect of the variable on the model.

3. Results

3.1. Recombinant expression of SELENBP1 and generation of antibodies

An expression vector for rhSELENBP1 was generated and sequence verified. Insect cells were transfected, and a protein band of the predicted size (65 kDa) was identified by SDS PAGE from transfected but not from control cells. The protein was purified by metal ion affinity chromatography exploiting the recombinant histidine tag and used for infecting mice in order to induce an immune response. After verifying high Ab concentrations directed against rhSELENBP1, hybridomas were generated, tested and isolated by limited dilution. From the positive clones, a pair of SELENBP1-specific mAb were selected and produced. Both mAb yielded specific signals with rhSELENBP1 in Western blot analyses (Fig. 1A).

3.2. Establishment of a LIA for SELENBP1

Two SELENBP1-specific mAb (anti-SELENBP1-mAb1 and -mAb2) were selected for establishing a luminometric immuno assay (LIA), using the recombinant protein for standardization. By preparing a dilution series of rhSELENBP1, a calibration curve was generated. All chosen concentrations of rhSELENBP1 yielded RLU above background and collectively allowed to construct a dose-response curve (Fig. 1B). The straight line indicates the range of concentrations (0.3–38.2 nmol/l) for which a linear function of signal intensity to SELENBP1 concentrations could be deduced after logarithmic transformation of the signals. The 95% CI for the points in the linear range do not overlap, supporting the suitable choice of calibrators. The average variation for each measured triplet was 9% (6%–13%), and functional assay sensitivity (as defined by a CV < 20%) extends down to 0.1 nmol/l.

3.3. SELENBP1 in serum of healthy controls

High quality serum samples from a small group of control adults (n = 37) were available and used to test for SELENBP1 levels in healthy subjects (Table 1). Only marginal concentrations were detectable, mainly below the linear range of the SELENBP1 assay. The values obtained were normally distributed with a mean of 0.3 nmol/l, and 95% of concentrations were within the range of 0.2–0.4 nmol/l (Fig. 1C).

3.4. Stability of SELENBP1

Handling and storage of serum samples are major issues in daily hospital routine as well as during scheduled analyses of blood samples from clinical trials. The stability of the analyte in a given matrix was therefore determined at different temperatures and upon freezing and thawing. As judged by the novel LIA, storage of samples at + 4 °C for up to 7 h had no effect on SELENBP1 in serum, whereas a 7 h storage period at RT reduced immunoreactive SELENBP1 to 73% (58–88%) of control (Fig. 2A). In contrast to prolonged incubation times at RT, freezing had little influence, and a marginal decline to 81% (72–90%) of initial SELENBP1 concentrations was observed after 10 cycles of freezing and thawing (Fig. 2B).

3.5. Variability of the SELENBP1 LIA and comparison of analytes

The inter-assay CV was determined with a set of standards of rhSELENBP1 in a total of 32 analyses. The average concentration of the standards was 4.6 nmol/l (4.4–4.7 nmol/l). The average inter-assay CV was 9.9%. To assess intra-assay variability, 1394 duplicates were analyzed and the 5^{th}, 50^{th} and 95^{th} quantile of the CVs were calculated, yielding a CV of 0.2%, 3% and 12%, respectively. Different matrices (EDTA, heparin and citrated plasma as well as serum) were tested for their impact on the measurement and its precision. Variability of the results between the matrices was low (mean CV: < 12.5%), indicating that all four matrices tested are suitable for SELENBP1 quantification.

3.6. SELENBP1 in patients with acute coronary syndrome

To characterize the role of SELENBP1 in inflammatory diseases, circulating SELENBP1 in patients with suspected ACS (n = 85) were measured. Of those patients, 34 would eventually be diagnosed with an ST-Elevation Myocardial Infarction (STEMI), 18 with Non-ST-Elevation Myocardial Infarction (NSTEMI), 19 with unstable angina pectoris, while 14 were diagnosed with a different condition. Patients were predominantly male (81 %) with an average age of 62 years (see Table 2). The blood samples had been drawn at several time points after first onset of symptoms of ACS. Average SELENBP1 concentrations of patients were higher than in controls, and the concentrations showed a dynamic time course with patient-specific characteristics (Supplemental Fig. 1). No correlation was observed between concentrations of SELENBP1 in the serum and classical risk factors, like: age, body mass index (BMI), smoking, diabetes mellitus, previous myocardial infarctions, hypercholesterinemia, arterial hypertension, known coronary artery disease (CAD) or CAD in the family history (results not shown).

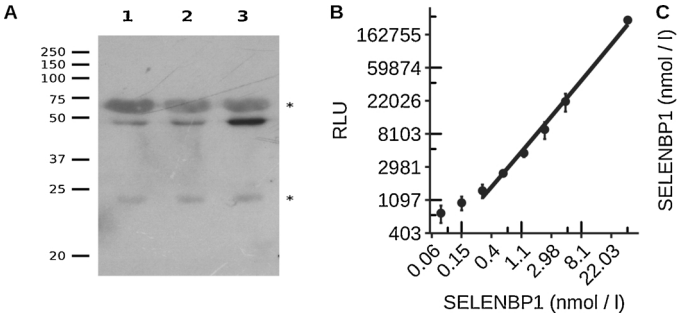

Fig. 1. Western blot, measuring range and quantile-quantile analysis.
A) Western Blot of three sera from patients with different concentrations of SELENBP1. The signal at 54 kDa corresponds to SELENBP1. The star (*) denotes an unspecific band at the relative position of human albumin. B) Different rhSELENBP1 concentrations ranging from 0.1 to 38.2 nmol/l were measured with the novel SELENBP1 LIA, and relative light units (RLU) were recorded (n = 3, mean and 95% CI). Axes are transformed using the natural logarithm (ln). The line marks the interval where SELENBP1 was calculated using a straight line fitted to the ln-ln transformed values (Pearson's product-moment correlation: $r = 1.0$, $p < 0.0001$). C) Quantile-quantile plot of random adult controls (n = 37) from a small case-control study in Germany (non-medical contact). The analysis indicates that SELENBP1 concentrations in control subjects are nearly normally distributed.

Table 1
Demographics of the Control Subjects.

	all Patients	Female	Male
n	37	22	15
Age, years	52 (47–57)	53 (47–58)	52 (48–55)
Weight, kg	84 (76–90)	78 (67–90)	85 (82–90)
Height, cm	176 (170–183)	171 (167–176)	183 (178–186)
BMI, kg/m^2	26 (24–29)	28 (25–29)	26 (24–29)
Lifestyle			
Smoking	19 % (7)	8 % (3)	11 % (4)
Alcohol	76 % (28)	46 % (17)	30 % (11)
Sports	59 % (22)	32 % (12)	27 % (10)
Vegetarian	0 % (0)	0 % (0)	0 % (0)
Supplements	65 % (24)	55 % (12)	80 % (12)
Chemistry			
SELENBP1, nmol/l	0.28 (0.26–0.31)	0.27 (0.24–0.29)	0.28 (0.27–0.31)

Data are % (n) or median (IQR), as described before in detail [25].

3.7. Relation of SELENBP1 to traditional biomarkers of coronary syndrome

As SELENBP1 rapidly increased after onset of first symptoms of ACS in patients with myocardial ischemia, SELENBP1 may constitute a novel and diagnostically helpful biomarker for risk stratification in ACS. Dynamics and specificity of SELENBP1 concentrations were compared to Troponin T (high-sensitivity assay, hsTnT) as a specific marker for myocardial damage, and to ASAT (aspartate aminotransferase) indicative of muscle, liver, or heart damage. None of these established biomarkers showed a significant correlation to SELENBP1 concentrations, indicating that the release of SELENBP1 into the circulation is unrelated to the extent of myocardial necrosis (Fig. 3). Similarly, no correlation was found between circulating SELENBP1 and creatinine, potassium, glucose, haemoglobin, fibrinogen, prothrombin time, cholesterol, platelet or white blood cell counts (Supplemental Figs. 2 and 3).

3.8. SELENBP1 concentrations are associated with risk of MACE

Next, the clinical significance of serum SELENBP1 concentrations in ACS was assessed. SELENBP1 was measured at first medical contact in 85 patients (Fig. 4). Of those, 52 (61%) were diagnosed with AMI, and 18 (21%) suffered a MACE until discharge. Six of the patients experiencing a MACE (7% of all) died. A receiver-operator-curve analysis (Fig. 5) indicates two sensible prognostic thresholds for SELENBP1, at 0.45 and 0.80 nmol/l, respectively. Either of these SELENBP1 threshold concentrations at first medical contact enabled a categorization of patients with suspected AMI as being either at a high-risk or at low-risk for MACE and ultimately death. Patients categorized as high-risk had a 6- to 11-fold higher odds for suffering a MACE depending on the cutoff (0.45 or 0.80 nmol/l), and an 11- to 12-fold higher odds for ultimately dying before discharge (Table 3). The SELENBP1-based analysis is characterized by high specificity and a negative predictive value (NPV) suitable for risk stratification. To control for confounding factors, we calculated a logistic regression to predict the risk of death before discharge based on measurements of SELENBP1 at first medical contact (binary) and other findings (Table 4).

4. Discussion

This study describes the generation of a SELENBP1-specific LIA and reports circulating SELENBP1 in cardiac patients. The assay showed a wide measuring range with high precision and good reproducibility. SELENBP1 was low in healthy subjects supporting the notion that it is an intracellular protein under normal circumstances [14], and that it is unlikely to contribute to Se transport. Importantly, even in the patients,

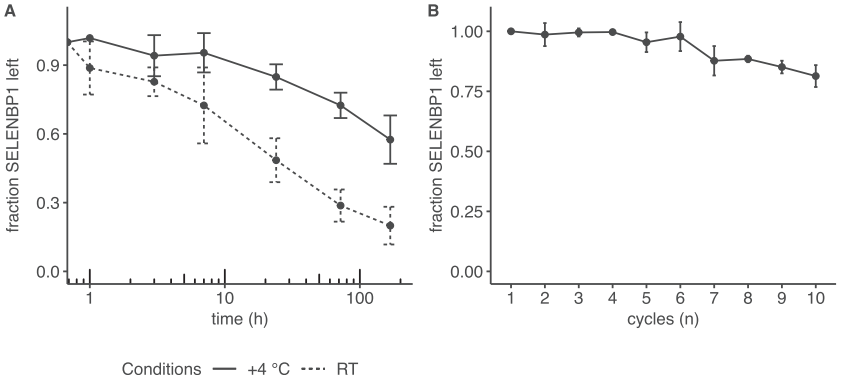

Fig. 2. Stability of SELENBP1 with respect to incubation time and thawing.
A) SELENBP1 concentrations were measured after 1 h, 3 h, 7 h, 24 h, 72 h and 168 h at 4 °C and 37 °C, respectively, without detecting a major decline after 7 h at 4 °C, and after 1 week of storage (n = 3 sera, mean and 95% CI). B) SELENBP1 concentrations remained fairly constant after repeated cycles of freezing and thawing (n = 2 sera, mean and SD). One cycle consisted of placing the sample at −20 °C for at least 20 min and then bringing it back to room temperature (RT).

Table 2
Demographics of the Study Population.

	all Patients	STEMI	NSTEMI	UAP	Other
n	85	34	18	19	14
Male sex	81 % (69)	32 % (27)	19 % (16)	19 % (16)	12 % (10)
Age, years	62 (54–71)	60 (53–68)	64 (54–74)	64 (54–75)	60 (56–68)
Risk Factors					
Smoking	59 % (50)	25 % (21)	16 % (14)	12 % (10)	6 % (5)
Diabetes mellitus	22 % (19)	8 % (7)	4 % (3)	6 % (5)	5 % (4)
Hypertension	51 % (43)	16 % (14)	12 % (10)	12 % (10)	11 % (9)
Hyperlipoproteinemia	49 % (42)	25 % (21)	9 % (8)	12 % (10)	4 % (3)
Family History	41 % (35)	16 % (14)	11 % (9)	8 % (7)	6 % (5)
Known CAD	44 % (37)	13 % (11)	12 % (10)	14 % (12)	5 % (4)
Prior MI	29 % (25)	9 % (8)	7 % (6)	9 % (8)	4 % (3)
Symptoms					
SOT, min	150 (94–300)	155 (118–240)	120 (75–440)	172 (94–545)	248 (109–356)
Coronary Arteries					
Coronary Angiography	73 % (62)	36 % (31)	16 % (14)	13 % (11)	7 % (6)
LAD Stenosis > 50 %	42 % (36)	21 % (18)	11 % (9)	9 % (8)	1 % (1)
LCX Stenosis > 50 %	35 % (30)	20 % (17)	7 % (6)	7 % (6)	1 % (1)
RCA Stenosis > 50 %	46 % (39)	22 % (19)	11 % (9)	12 % (10)	1 % (1)
Therapy					
Lysis	25 % (21)	18 % (15)	7 % (6)	0 % (0)	0 % (0)
PCI	16 % (14)	12 % (10)	2 % (2)	1 % (1)	1 % (1)
Conservative	54 % (46)	11 % (9)	8 % (7)	21 % (18)	14 % (12)
Resuscitation	5 % (4)	0 % (0)	4 % (3)	0 % (0)	1 % (1)

Data are % (n) or median (IQR), adapted from data described before in detail [24]. UAP: unstable angina pectoris; SOT: symptom onset time; CAD: Coronary artery disease; MI: Myocardial infarction; LAD: left anterior descending; LCX: left circumflex artery; RCA: right coronary artery; PCI: percutaneous coronary intervention.

we observed SELENBP1 concentrations of 10 nmol/l or less, again a concentration range unlikely to contributing to circulating Se concentrations, as the molar ratio of Se and SELENBP1 has been determined to be 1:1 [16]. In patients with ACS, SELENBP1 concentrations vary dynamically potentially reflecting cellular stress and tissue injury. This interpretation is supported by the association of SELENBP1 with risk of ACS-related MACE and MACE-related death. Unexpectedly, it was possible to stratify chest pain patients on first contact as high- or low-risk subjects for MACE and death by using serum SELENBP1 concentrations of 0.45 or 0.80 nmol/l as threshold. Notably, elevated SELENBP1 in ACS patients was unrelated to routine biomarkers of heart disease, suggesting a specific release mechanism of SELENBP1. Elevated SELENBP1 was not correlated to cardiac TnT which is released during loss of myocardial tissue reflecting the extent of necrosis [27]. The

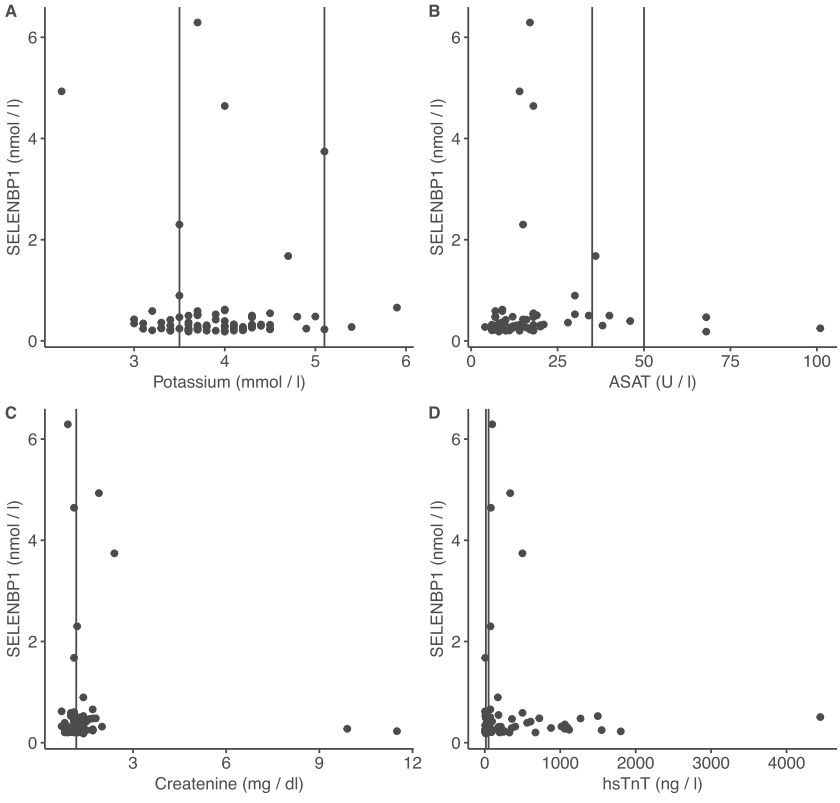

Fig. 3. SELENBP1 at time of arrival in the Emergency Department in comparison to routine biomarkers.
SELENBP1 concentrations do not correlate to A) potassium, B) ASAT, C) creatinine or D) hsTnT concentrations. Vertical bars mark the reference ranges for A) potassium, B) ASAT; upper reference limits for women (left) and men (right), respectively, C) creatinine and D) hsTnT, where the vertical bars from left to right indicate an observation range and the cut-off for acute myocardial infarction (AMI).

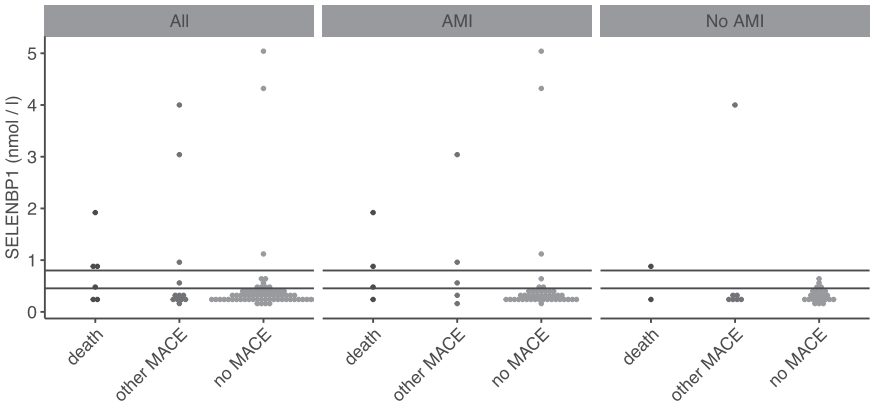

Fig. 4. SELENBP1 in relation to outcome until discharge or death.
Circulating SELENBP1 concentrations were determined at first medical contact. Horizontal lines mark the two cut-offs used in Table 3 (0.45 and 0.80 nmol/l, respectively). Patients are grouped according to clinical events, i.e., MACE (death or other major cardiac events) or no MACE, and results are presented for all patients (n = 85, left), and separated into patients with AMI (center) or without AMI (right).

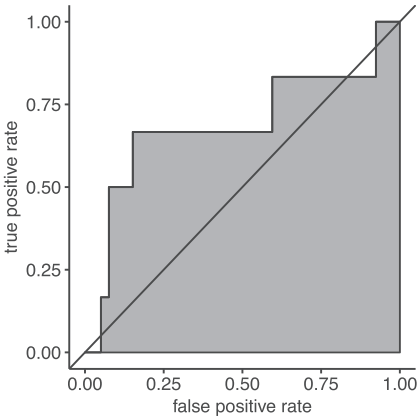

Fig. 5. Receiver-Operator-Curve (ROC) analysis for the association of SELENBP1 and death. Circulating SELENBP1 concentrations at first medical contact are associated with death risk, yielding an AUROC (area under the curve) of 0.7. The false positive rate (fpr) is given on the X- and the corresponding true positive rate (tpr) is given on the Y-axis, respectively.

Table 3
Odds Ratio and Test Metrics for MACE incl. death or death alone.

	SELENBP1 > 0.45 nmol/l		SELENBP1 > 0.80 nmol/l	
	MACE	Death	MACE	Death
Odds Ratio	6	11	11	12
CI	2–19	2–68	2–49	2–74
p	0.0036	0.0098	0.0022	0.014
Sensitivity	0.44	0.67	0.33	0.50
Specificity	0.88	0.85	0.96	0.92
PPV	0.50	0.25	0.67	0.33
NPV	0.86	0.97	0.84	0.96

Table 4
Logistic Regression for Risk of Death until Discharge.

	OR	95 % CI	p
Intercept	2.62	1.6–4.2	0.00025
hsTnT > 50 ng/L	0.98	0.9–1.0	0.45
SELENBP1 > 0.8 nmol/l	1.73	1.5–2.0	< 0.0001
Hb	0.97	0.9–1.0	0.019
blood glucose	1.00	1.0–1.0	0.010
Potassium	0.95	0.9–1.0	0.11
Creatinine	1.09	1.1–1.1	< 0.0001
Sex (male)	1.03	0.9–1.1	0.48

n = 51, adjusted R2: 0.8, SELENBP1 at first medical contact, hsTnT at first medical contact, other laboratory findings at arrival at emergency department.

missing relation between circulating SELENBP1 and hsTnT indicates that SELENBP1 release does not result from necrosis, but may rather dynamically reflect disease activity, cell stress and intracellular redox imbalances.

Recently, an enzymatic activity of SELENBP1 as an MTO has been shown. It has been proposed that SELENBP1 generates hydrogen peroxide (H_2O_2) and hydrogen sulfide (H_2S), enabling a direct participation in redox signaling [23]. Intracellularly, a link between SELENBP1 expression and GPX1 activity has been demonstrated [22]. The described interaction may affect Se metabolism, redox regulation and oxidative stress response, with potential relevance to both acute and chronic myocardial damage [28]. On a molecular level, hypoxia has been identified as a modulator of SELENBP1, and HIF1-alpha efficiently increases its expression [21,29]. This type of regulation appears physiologically meaningful for coping with oxidative stress and decreased oxygen supply. As both biochemical pathways are highly activated in the initial phase of an ACS, the choice of patients enrolled in this study has been fortunate, but may not represent the most meaningful application of SELENBP1 as a diagnostic biomarker. There are other diseases with even more wide-spread hypoxia, tissue destruction or redox imbalance that should be analyzed next for verifying the hypothesized role of circulating SELENBP1.

Given the exploratory nature of the present study, some limitations need to be acknowledged. Firstly, only relatively few patients and ACS-related complications were available for analysis, restricting the extent of subgroup analyses. Secondly, the dynamics of circulating SELENBP1 concentrations after the insult are only incompletely covered, and the exact time interval between ACS and first medical contact is not always known, which collectively may have led to an underestimation of the extent of disease-associated SELENBP1 release into the circulation.

The particular strengths of our study are given by the quality and specificity of the newly generated mAbs, the high precision and robustness of the novel LIA, and the first identification and characterization of circulating SELENBP1 in a disease setting. Moreover, having an additional risk marker for discriminating between low- and high-risk for MACE and MACE-related death at admittance of ACS patients to the CPU constitutes a promising perspective for future studies and for improving the initial diagnosis on the emergency ward.

5. Conclusions

In this report, increased serum SELENBP1 in patients with suspected ACS was characterized. Elevated concentrations indicated an increased risk for cardiac events including death and may enable a meaningful stratification of patients with acute symptoms.

Sources of funding

The collection of serum samples from controls was financially

supported by the FOP e. V., Germany, and research by the Deutsche Forschungsgemeinschaft (DFG Research Unit 2558 TraceAge, Scho 849/6-1 to LS, DFG; STO 1099/-2 to CS, and DFG; 388962813 to AS).

Disclosures

AS, ECDK-H, JS, CS, WBM, CS and MM have nothing to declare in relation to this study. LS holds shares in selenOmed GmbH, a company involved in Se status assessment and supplementation. ECK and LS are named as inventors on a related patent application.

Acknowledgements

We thank Vartitér Seher, Katja Schreiber and Fabian Holert for excellent technical support, Sandra Hybsier and Torsten Schulz for highly appreciated counselling during assay development, and patients and controls for participating in this study.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2019.01.005.

References

[1] A.V. Lobanov, D.L. Hatfield, V.N. Gladyshev, Eukaryotic selenoproteins and selenoproteomes, Biochim. Biophys. Acta 1790 (11) (2009) 1424–1428.
[2] R. Brigelius-Flohé, L. Flohé, Selenium and redox signaling, Arch. Biochem. Biophys. 617 (2017) 48–59.
[3] J. Köhrle, F. Jakob, B. Contempré, J.E. Dumont, Selenium, the thyroid, and the endocrine system, Endocr. Rev. 26 (7) (2005) 944–984.
[4] Z. Huang, A.H. Rose, P.R. Hoffmann, The role of selenium in inflammation and immunity: from molecular mechanisms to therapeutic opportunities, Antioxid. Redox Signal. 16 (7) (2012) 705–743.
[5] R.F. Burk, K.E. Hill, A.K. Motley, Plasma selenium in specific and non-specific forms, Biofactors 14 (1–4) (2001) 107–114.
[6] G.F. Combs Jr., J.C. Watts, M.I. Jackson, L.K. Johnson, H. Zeng, A.J. Scheett, E.O. Uthus, L. Schomburg, A. Hoeg, C.S. Hoefig, C.D. Davis, J.A. Milner, Determinants of selenium status in healthy adults, Nutr. J. 10 (2011) 75.
[7] K. Ashton, L. Hooper, L.J. Harvey, R. Hurst, A. Casgrain, S.J. Fairweather-Tait, Methods of assessment of selenium status in humans: a systematic review, Am. J. Clin. Nutr. 89 (6) (2009) 2025S–2039S.
[8] I. Dreher, T.C. Jakobs, J. Köhrle, Cloning and characterization of the human selenoprotein P promoter. Response of selenoprotein P expression to cytokines in liver cells, J. Biol. Chem. 272 (46) (1997) 29364–29371.
[9] N.P. Becker, J. Martitz, K. Renko, M. Stoedter, S. Hybsier, T. Cramer, L. Schomburg, Hypoxia reduces and redirects selenoprotein biosynthesis, Metallomics 6 (5) (2014) 1079–1086.
[10] B. Hollenbach, N.G. Morgenthaler, J. Struck, C. Alonso, A. Bergmann, J. Köhrle, L. Schomburg, New assay for the measurement of selenoprotein P as a sepsis biomarker from serum, J. Trace Elem. Med. Biol. 22 (1) (2008) 24–32.
[11] X. Forceville, D. Vitoux, R. Gauzit, A. Combes, P. Lahilaire, P. Chappuis, Selenium, systemic immune response syndrome, sepsis, and outcome in critically ill patients, Crit. Care Med. 26 (9) (1998) 1536–1544.
[12] A. Stevanovic, M. Coburn, A. Menon, R. Rossaint, D. Heyland, G. Schalte, T. Werker, W. Wonisch, M. Kiehntopf, A. Goetzenich, S. Rex, C. Stoppe, The importance of intraoperative selenium blood levels on organ dysfunction in patients undergoing off-pump cardiac surgery: a randomised controlled trial, PLoS One 9 (8) (2014) e104222.
[13] C. Benstoem, A. Goetzenich, S. Kraemer, S. Borosch, W. Manzanares, G. Hardy, C. Stoppe, Selenium and its supplementation in cardiovascular disease—what do we know? Nutrients 7 (5) (2015) 3094–3118.
[14] A.M. Diamond, The subcellular location of selenoproteins and the impact on their function, Nutrients 7 (5) (2015) 3938–3948.
[15] M.P. Bansal, T. Mukhopadhyay, J. Scott, R.G. Cook, R. Mukhopadhyay, D. Medina, DNA sequencing of a mouse liver protein that binds selenium: implications for selenium's mechanism of action in cancer prevention, Carcinogenesis 11 (11) (1990) 2071–2073.
[16] R. Raucci, G. Colonna, E. Guerriero, F. Capone, M. Accardo, G. Castello, S. Costantini, Structural and functional studies of the human selenium binding protein-1 and its involvement in hepatocellular carcinoma, Biochim. Biophys. Acta 1814 (4) (2011) 513–522.
[17] E. Ansong, Q. Ying, D.N. Ekoue, R. Deaton, A.R. Hall, A. Kajdacsy-Balla, W. Yang, P.H. Gann, A.M. Diamond, Evidence that selenium binding protein 1 is a tumor suppressor in prostate cancer, PLoS One 10 (5) (2015) e0127295.
[18] W. Yang, A.M. Diamond, Selenium-binding protein 1 as a tumor suppressor and a prognostic indicator of clinical outcome, Biomark. Res. 1 (1) (2013) 15.
[19] C. Zhang, Y.E. Wang, P. Zhang, F. Liu, C.J. Sung, M.M. Steinhoff, M.R. Quddus, W.D. Lawrence, Progressive loss of selenium-binding protein 1 expression correlates with increasing epithelial proliferation and papillary complexity in ovarian serous borderline tumor and low-grade serous carcinoma, Hum. Pathol. 41 (2) (2010) 255–261.
[20] S. Zhang, F. Li, M. Younes, H. Liu, C. Chen, Q. Yao, Reduced selenium-binding protein 1 in breast cancer correlates with poor survival and resistance to the antiproliferative effects of selenium, PLoS One 8 (5) (2013) e63702.
[21] E.K. Lee, Y.J. Shin, E.Y. Park, N.D. Kim, A. Moon, S.J. Kwack, J.Y. Son, S. Kacew, B.M. Lee, O.N. Bae, H.S. Kim, Selenium-binding protein 1: a sensitive urinary biomarker to detect heavy metal-induced nephrotoxicity, Arch. Toxicol. 91 (4) (2017) 1635–1648.
[22] W.F. Fang, M.L. Goldberg, N.M. Pohl, X.L. Bi, C. Tong, B. Xiong, T.J. Koh, A.M. Diamond, W.C. Yang, Functional and physical interaction between the selenium-binding protein 1 (SBP1) and the glutathione peroxidase 1 selenoprotein, Carcinogenesis 31 (8) (2010) 1360–1366.
[23] A. Pol, G.H. Renkema, A. Tangerman, E.G. Winkel, U.F. Engelke, A.P.M. de Brouwer, K.C. Lloyd, R.S. Araiza, L. van den Heuvel, H. Omran, H. Olbrich, M. Oude Elberink, C. Gilissen, R.J. Rodenburg, J.O. Sass, K.O. Schwab, H. Schäfer, H. Venselaar, J.S. Sequeira, H.J.M. Op den Camp, R.A. Wevers, Mutations in SELENBP1, encoding a novel human methanethiol oxidase, cause extraoral halitosis, Nat. Genet. 50 (1) (2018) 120–129.
[24] A. Slagman, J. Searle, C. Müller, M. Möckel, Temporal release pattern of copeptin and troponin T in patients with suspected acute coronary syndrome and spontaneous acute myocardial infarction, Clin. Chem. 61 (10) (2015) 1273–1282.
[25] L. Hildebrand, T. Gaber, P. Kühnen, R. Morhart, H. Unterbörsch, L. Schomburg, P. Seemann, Trace element and cytokine concentrations in patients with Fibrodysplasia Ossificans Progressiva (FOP): a case control study, J. Trace Elem. Med. Biol. 39 (2017) 186–192.
[26] S. Hybsier, T. Schulz, Z. Wu, I. Demuth, W.B. Minich, K. Renko, E. Rijntjes, J. Köhrle, C.J. Strasburger, E. Steinhagen-Thiessen, L. Schomburg, Sex-specific and inter-individual differences in biomarkers of selenium status identified by a calibrated ELISA for selenoprotein P, Redox Biol. 11 (2017) 403–414.
[27] J. Hallen, Troponin for the estimation of infarct size: what have we learned? Cardiology 121 (3) (2012) 204–212.
[28] E.J. Jun, J.S. Ye, I.S. Hwang, Y.K. Kim, H. Lee, Selenium deficiency contributes to the chronic myocarditis in coxsackievirus-infected mice, Acta Virol. 55 (1) (2011) 23–29.
[29] C. Huang, G. Ding, C. Gu, J. Zhou, M. Kuang, Y. Ji, Y. He, T. Kondo, J. Fan, Decreased selenium-binding protein 1 enhances glutathione peroxidase 1 activity and downregulates HIF-1alpha to promote hepatocellular carcinoma invasiveness, Clin. Cancer Res. 18 (11) (2012) 3042–3053.