Value of SERCA2a as a Biomarker for the Identification of Patients With Advanced Heart Failure Requiring Circulatory Support

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Research

Keywords: Heart failure, Nucleocytoplasmic transport, Heart transplantation, Mechanical circulatory support, SERCA2a

Posted Date: March 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-293368/v1

Abstract

Background: Heart failure (HF) alters the nucleo-cytoplasmic transport of cardiomyocytes and reduces SERCA2a levels, essential for intracellular calcium homeostasis. We consider in this study whether the molecules involved in these processes can differentiate those patients with advanced HF and the need for mechanical circulatory support (MCS) as a bridge to recovery or urgent heart transplantation from those clinically stable and who are transplanted in an elective code.

Material and method: Blood samples from patients with advanced HF were analyzed by ELISA and the plasma levels of Importin5, Nucleoporin153 kDa, RanGTPase-Activiting Protein 1 and sarcoplasmic reticulum Ca2 + ATPase were compared among patients that need MCS and patients without MCS.

Results: SERCA2a showed significantly lower levels in patients who had MCS compared to those who did not require it (0.501 ± 0.530 ng / mL and 1,123 ± 0.661 ng / mL p = 0.01, respectively). By constructing the ROC curve with the SERCA2a values (area under the curve of 0.812 ± 0.085, with a p of 0.004 and a 95% confidence interval between 0.646 and 0.979), we have established a cut-off point of 0.84 ng / mL with sensitivity of 92%, specificity of 62%, negative predictive value of 91% and positive predictive value of 67%.

Conclusion: Patients with advanced HF and need for MCS have significantly lower levels of SERCA2a than stable patients without need for MCS. More studies are needed to validate these results.

Trial registration: retrospectively registered

Introduction And Objective:

Heart transplantation (HT) is currently the gold standard for the treatment of patients with advanced heart failure (HF) because it improves survival, functional status, and quality of life[1]. However, the number of heart donors is naturally very low and the waiting times for recipients can be very long. This, added to the growing number of unstable patients, has fostered the development of mechanical circulatory support (MCS) systems which can act as a bridge to recovery, transplantation or decision. This means that there is a need for robust risk stratification and patient selection tools to aid in MCS mediated intervention planning and strategy. Currently, these decisions are based on both clinical and hemodynamic criteria, and so far, there has been little evidence supporting the use of conventional biomarkers in decision-making.

HF has been associated with changes at the molecular level in the mitochondria, cytoskeleton, and nuclei of cardiomyocytes[2–4], and identifying these changes has helped in understanding the process of ventricular remodeling and its pathophysiology[5].

The transport of macromolecules between the nucleus and the cytoplasm is facilitated by the nuclear pore complex (NPC) in cardiomyocytes with nuclear pores comprising of channels made up of

multiprotein complexes (nucleoporins) that cross the nuclear envelope, with each NPC being made up of multiple copies of approximately 30 different nucleoporins[6]. The process of importing and exporting molecules requires the participation of importins (IMPs) and exportins (EXPs), in addition to Ran, a GTPase from the Ras family, which interacts with the IMPs and EXPs, and is responsible for generating the energy gradient, between the nucleus and cytoplasm, required to support this transport process[7]. This machinery alters its conformation and function in response to internal or external factors[8,9], with our group describing specific alterations in their function during HF[10–13].

Sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a) is an enzyme involved in calcium homeostasis (Ca^{2+}), a fundamental process in the myocardial contraction-relaxation cycle[14]. Specific inhibition of the transport activity of SERCA2a, or reductions in its expression, has been shown to result in changes in contractile function[15] and, in the same way, administration of SERCA2a via lentiviral vector has been shown to improve contraction in damaged cardiac tissues[16]. This means that SERCA2a is one of the most important pathophysiological substrates for HF[17–19] and makes it a particularly interesting therapeutic target[20,21].

The objective of this study was to evaluate whether changes in certain molecules involved in nucleocytoplasmic transport [Importin5 (IPO5), Nucleoporin153 kDa (NUP153), and RanGTPase-Activiting Protein 1 (RanGAP1) and intramyocardial calcium homeostasis (SERCA2a)], could be used to differentiate patients with advanced HF and MCS carriers from those with greater clinical stability in whom elective transplantation could be performed without prior MCS intervention.

Material And Methods:

This was a descriptive prospective cohort study in which patients with advanced HF studied as potential candidates to HT were selected, who then underwent elective or urgent HT at our medical center (La Fe University and Polytechnic Hospital) between 2016 and 2018. These patients were divided into two groups: (1) patients who received MCS as a bridge to HT or recovery and (2) those without an urgent need for MCS intervention and undergoing an elective transplant.

Patients who received a cardiopulmonary transplant, those who were under 18 years of age, or those who did not sign the informed consent for inclusion and/or extraction of peripheral blood samples were excluded from the study. In the MCS group, only those with a short-term assist implant, extracorporeal membrane oxygenator (ECMO), or continuous-flow DAV (Levitronix®) were included in the recovery, decision, or HT groups. While patients who received a long-term or short-term care after their cardiac surgery were also excluded.

Demographic, clinical, echocardiographic, and hemodynamic data were collected from all the enrolled patients (in a situation of clinical and hemodynamic stability). In addition, peripheral blood samples were collected from these patients and stored in the Biobank at our medical center (Biobanco La Fe). Blood

samples were taken just before implantation in the patients receiving circulatory support or before HT in the patients without MCS.

Sample processing and subsequent analysis

Blood samples were obtained using peripheral venipuncture via a 10 mL glass vacuum extraction tube, treated with 15% EDTA anticoagulant (0.12 mL) (BD Vacutainer K3E; REF 368480). The tubes were centrifuged (Eppendorf Model 5415R Centrifuge) at 1300 rpm for 10 min at 4°C and the supernatant was collected and aliquoted into 500 µl screen-printed plastic cryotubes that were stored in the Biobank at -80°C until further analysis.

For analysis, each aliquot was brought to 4°C, thawed and then centrifuged (Eppendorf Centrifuge model 5702R) at 1300 rpm for 10 min at 4°C. The supernatant was collected and allowed to equilibrate to room temperature for 30 min.

The concentrations of each of the target biomarkers were determined using appropriate enzyme-linked immunosorbent assays (ELISAs). The SEG374Hu ELISA from Cloud-Clone Corp. (Katy, TX, USA) was used to evaluate SERCA2a and had a detection range of 0.312–20 ng/mL with a detection limit of 0.115 ng/mL; the intra- and inter-assay precision was < 10% and < 12%, respectively. NUP153 was assayed using MBS011353, which had a detection range of 3.12–100 ng/mL with a detection limit of 1.0 ng/mL. IPO5 was assayed using MBS9311906, which had a detection range of 0.625–20 ng/mL and the detection limit was 0.1 ng/mL. Finally, RANGAP1 was assayed using MBS9321016 which had a detection range of 3.12–100 ng/mL with a detection limit of 1.0 ng/mL. Intra- and inter-assay precision for all three assays was < 15%, with all three kits sourced from MyBioSource.com (San Diego, CA, USA).

Statistical analysis:

We used the Kolmogorov–Smirnov method to evaluate the normality of the data generated in these assays. Data are reported as the mean ± standard deviation for continuous variables with normal distributions, the median ± interquartile range for continuous variables that did not follow a normal distribution, and as a percentage for discrete variables. The Chi-square test was used for the comparison of categorical variables; the Student's t-test or ANOVA were used for the comparison of continuous variables with normal distributions and the Mann–Whitney test was used for parameters without a normal distribution. Sensitivity, specificity, and predictive values were evaluated using an ROC curve. Statistical significance was set to a P value of < 0.05. All statistical analyses were carried out using SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA).

Results:

A total of 29 plasma samples were analyzed, 13 of which were obtained prior to MCS implantation (2 ECMO and 11 Levitronix ®) and 16 directly prior to elective HT. Nine of the patients with MCS underwent

emergency HT, 3 died awaiting transplant, and one patient recovered without a transplant or other assistance.

The mean age of the patients was 51 ± 12 years, with the majority of the cohort being men (83%) with ischemic heart disease (45%), the vast majority of patients could be characterized as functional class NYHA III-IV to IV (90%). In the MCS group the patients were in INTERMACS class II-III, except for two who were in class I; while in the non-MCS group they were in class IV-V and three of them were in class III. When we separated the patients into two groups (with and without MCS), we observed that the baseline characteristics of age, sex, weight, underlying pathology, previous history, previous cardiovascular surgery, ICD / CRT implantation, echocardiographic and hemodynamic data were similar between both groups. Table 1 summarizes the variables for this cohort.

Table 1
Baseline characteristics of patients with and without mechanical circulatory support (MCS).

	MCS: Yes (n = 13)	MCS: No (n = 16)	P
Age (years)	52 ± 10	50 ± 14	0.60
Gender (%Men)	85	81	1.00
BMI (Kg/m^2)	26.4 ± 4	26.4 ± 3	0.90
ICM (%)	54	37	0.50
INTERMACS (%)	I-II: 15.4	III: 18,8	0.00
	II-III: 84,6	IV-V: 81,3	
RI (%)	31	25	0.90
PHT (%)	90	81	0.90
Pr.Infection (%)	31	12	0.40
DM (%)	15	12	1.00
HBP (%)	23	37	0.40
COPD (%)	8	0	0.40
AF (%)	54	50	1.00
Smoking (Yes/Ex) (%)	8/54	12/37	0.70
Pr.VascD (%)	15	12	1.00
MV (%)	23	0	0.08
Pr.CVS (%)	8	12	0.90
ICD (%)	77	94	0.30
CRT (%)	15	31	0.40
LVEF (%)	20 ± 7	27 ± 18	0.20

Values for continuous variables with a normal distribution are represented as the mean ± standard deviation while continuous variables with a paranormal distribution are represented by the median ± interquartile range. Discrete variables are described using percentages. MCS: mechanical circulatory support. BMI: body mass index kg/m2. ICM: ischemic cardiomyopathy. RI: renal insufficiency (defined as creatinine ≥ 1,4 mg/dL). PHT: pulmonary hypertension (defined as mPAP > 25 mmHg). Pr. Infection: previous infection. DM: diabetes mellitus. HBP: high blood pressure. COPD: chronic obstructive pulmonary disease. AF: atrial fibrillation or atrial flutter. Pr.VascD: previous vascular disease. MV: mechanial ventilation. Pr.CVS: previous cardiovascular surgery. ICD: implantable cardioverter-defibrillator. CRT: cardiac-resynchronization therapy. LVEF: ejection fraction of the left ventricle. mPAP: mean pulmonary artery pressure. PCWP: pulmonary capilary wedge pressure. CO: cardiac output. PVR: pulmonary vascular resistance.

	MCS: Yes (n = 13)	MCS: No (n = 16)	P
mPAP (mmHg)	41 ± 12	34 ± 10	0.10
PCWP (mmHg)	28 ± 10	24 ± 8	0.40
CO (l/Min)	3.3 ± 0.4	3.6 ± 0.8	0.40
PVR (dyn/s/cm^2)	3.7 ± 2.4	2.8 ± 1.1	0.20

Values for continuous variables with a normal distribution are represented as the mean ± standard deviation while continuous variables with a paranormal distribution are represented by the median ± interquartile range. Discrete variables are described using percentages. MCS: mechanical circulatory support. BMI: body mass index kg/m2. ICM: ischemic cardiomyopathy. RI: renal insufficiency (defined as creatinine ≥ 1,4 mg/dL). PHT: pulmonary hypertension (defined as mPAP > 25 mmHg). Pr. Infection: previous infection. DM: diabetes mellitus. HBP: high blood pressure. COPD: chronic obstructive pulmonary disease. AF: atrial fibrillation or atrial flutter. Pr.VascD: previous vascular disease. MV: mechanial ventilation. Pr.CVS: previous cardiovascular surgery. ICD: implantable cardioverter-defibrillator. CRT: cardiac-resynchronization therapy. LVEF: ejection fraction of the left ventricle. mPAP: mean pulmonary artery pressure. PCWP: pulmonary capilary wedge pressure. CO: cardiac output. PVR: pulmonary vascular resistance.

When we compared the plasma levels of SERCA2a, NUP153, RanGAP1, and IPO5 we noted that SERCA2a was significantly lower in the patients with advanced HF and MCS intervention when compared to the patients without MCS (0.501 ± 0.530 ng/mL and 1,123 ± 0.661 ng/mL, p = 0.01, respectively). However, IPO5, NUP153, and RanGAP1 did not show statistically significant differences between these two groups, although NUP153 did show a trend toward significance (P = 0.07) (Fig. 1).

Taking these data into account, we used the SERCA2a values to construct an ROC curve and used this to evaluate its predictive value for identifying patients with advanced HF who will require circulatory support. We obtained an area under the curve of 0.812 ± 0.085, with a p of 0.004 and a 95% confidence interval between 0.646 and 0.979. Furthermore, we established a cutoff point for SERCA2a of 0.84 ng/mL, and a sensitivity of 92%, specificity of 62%, negative predictive value of 91%, and a positive predictive value of 67% for this assay (Fig. 2).

Discussion:

In recent years, the use of MCS has grown exponentially, with these devices predominantly being applied as a bridge to transplantation. The data from the 2018 Spanish HT registry indicated that 43.5% of transplants that year were performed in patients with prior circulatory support[23]. Internationally, this percentage has been around 50% since 2017[24]. In fact, the latest clinical practice guidelines establish a class I recommendation with a B evidence level for the implantation of a left or biventricular assistance device in patients with advanced HF despite optimal treatment[25].

Currently patients are selected for circulatory support based on fundamental clinical criteria including abrupt clinical deterioration, frequent hospitalizations due to decompensation and inotropic dependence

amongst others. Monitoring of transaminase, creatinine, and lactate levels is used to evaluate the function of the target organs, since their progressive deterioration is also a criterion for MCS implantation. However, these tests and criteria are merely indicative without an established cutoff point, and high levels of these molecules usually indicate an ongoing failure in these organs.

The Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) classification is used to establish the implantation device for patients needing an MCS, with this document supplying some prognostic and clinical criteria for the evaluation of the need, type, and duration of the MCS[26]. For example, those patients classified as INTERMACS 1 should be treated with peripheral venoarterial ECMO support, while INTERMACS 1–2 patients who are not in a critical condition should be treated with a short-term continuous flow DAV (such as Levitronix®), as this instrument can provide support for a longer time, with fewer long-term complications[27].

The major determining factors for the success of MCS are patient selection and the timing of device implantation. Clinicians should remain acutely aware of the dual effect of early use, and find a balance between efficacy and device-derived complications. Both factors are often based on subjective criteria. The latest guidelines recommend the use of these devices early to limit the prolonged use of catecholamines and avoid the development of right ventricular dysfunction and/or multi-organ failure. Only five of the patients in the MCS group demonstrated any dysfunction in one or more of the target organs, with no statistically significant changes in any of the commonly assayed biomarkers (creatinine, transaminases, bilirubin, or lactate) when compared to the group without MCS.

Given these limitations it is critical to develop tools for the accurate assessment of patients and produce adequate stratification protocols describing their clinical situation based not only on their clinical, echocardiographic and hemodynamic data, but also considering more objective criteria. Thus, the purpose of our study was to define biochemical markers that can help us identify early patients with advanced HF whose short-term clinical evolution may lead to the need for circulatory support as a bridge to recovery, transplantation, or decision. To date, there are no other studies that have evaluated the usefulness of NUP153, IPO5, RanGAP1, and SERCA2a in the plasma in this clinical setting.

The choice of molecules used in this study was based on the results obtained by our group in previous studies. These studies demonstrated a correlation between the molecules involved in nucleocytoplasmic transport (nucleoporins, IMPs, EXPs, and Ran regulators), and the various parameters of ventricular dysfunction (left ventricular end-systolic diameter, left ventricular end-diastolic diameter, and left ventricular mass index) and advanced HF, as an expression of the remodeling process associated with the restructuring of the cytoskeleton and a series of mitochondrial alterations that result in an increase in the nucleocytoplasmic traffic machinery[10–13]. Additional studies have also shown a reduced expression of SERCA2a in HF and cardiac rejection[18,19].

In our study, the plasma levels of NUP153, IPO5, and RanGAP1 did not show a statistically significant difference between the advanced HF group with MCS and the stable group without MCS, although

NUP153 did demonstrate a trend toward significance (p = 0.07).

SERCA2a is in charge of taking Ca^{2+} back into the sarcoplasmic reticulum during the relaxation phase of the cardiac cycle, allowing enough Ca^{2+} to become available for the next contraction phase. Multiple studies have confirmed the importance of SERCA2a in the pathophysiology of heart disease, since it plays a very important role in regulating the progression of HF, directly contributing to the deterioration of the contraction and relaxation processes of the heart[28,29]. Apart from its role in the pathophysiology of HF and its value as a therapeutic target, SERCA2a has also been investigated as a modulator for other related pathological processes, including rejection after heart transplantation, where it has been shown to be significantly reduced[30]. In addition, a recent study by our group showed that the plasma levels of SERCA2a are an independent predictor of pathological rejection[19].

Given this, we decided to investigate whether the levels of this enzyme can also be used to help clinicians identify patients with advanced HF whose short-term evolution requires MCS implantation. We were able to show that patients with MCS present with significantly lower levels of SERCA2a and that when these levels were represented as an ROC curve, they produced a good area under the curve with an optimal cutoff point of 0.84 ng/mL allowing for a sensitivity of 92% and a negative predictive value of 91% for MCS intervention.

Therefore, our data suggests that the plasma levels of SERCA2a are a highly accurate predictor of advanced HF with an unstable clinical outcome. This means that this biomarker could be applied to identify patients that require temporary support with MCS, allowing for their stratification from patients who electively receive HT without circulatory support. If these preliminary findings are validated in broader cohorts, the determination of SERCA2a could be consolidated as a useful tool in optimizing the selection criteria and determining the appropriate timing of MCS implantation in patients with advanced HF.

Limitations:

Although this study presents some valuable information it does have certain limitations, and the results must be interpreted in this context. This is a preliminary study with a limited number of subjects per group that must be validated in broader prospective cohorts. In addition, the potential variability of the plasma levels of these molecules must be considered in relation to other parameters including pharmacological treatment, stress and diet. However, this is a prospective, single-center study, which provides homogeneity regarding the study protocol and therapeutic strategy of patients with advanced HF who are studied for eventual HT, making it an ideal initial testing ground for novel intervention strategies.

Conclusions:

Patients with advanced HF and the need for MCS implantation as a bridge to recovery or transplantation have significantly lower levels of SERCA2a compared to those in a stable clinical situation receiving elective transplants. If these preliminary findings are validated in larger prospective cohorts, the need for MCS in advanced HF conditions could be more carefully and objectively assessed using SERCA2a analysis, in conjunction with clinical and hemodynamic data.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethical Review Board for Biomedical Research at the La Fe Health Research Institute (Valencia, Spain) and was carried out in accordance with the guidelines for medical ethics established in the Declaration of Helsinki[22]. Furthermore, all patients submitted a signed informed consent prior to their inclusion in the study, this consent included permission to extract and store their peripheral blood samples.

Consent for publication

Not applicable

Availability of supporting data

The datasets generated and/or analysed during the current study are not publicly available due individual privacy can be compromised but are available from the corresponding author on reasonable request.

Funding

This work was supported by the National Institute of Health ‘Fondo de Investigaciones

Sanitarias del Instituto de Salud Carlos III’ [PI16/01627, PI17/01925, PI17/01232, CP18/00145],

‘Consorcio Centro de Investigación Biomédica en Red, M.P” (CIBERCV, under Grant

CB/16/11/00261), and the European Regional Development Fund (FEDER).

Conflict of interest:

None declared

Authors' contributions

ME was a major contributor in writing the manuscript. ER, MP, ET and CG have performed the molecular analyzes. IS and MA have performed the statistical analysis and interpretation. SL and RL contributed to the collection of the study data. LA has supervised the inclusion and collection of patient data. LM has designated the study and monitored its progress.

Acknowledgments

Biobank of La Fe University Hospital (Valencia, Spain).

Abbreviations

HT: Heart transplantation

HF: Heart Failure

MCS: Mechanical Circulatory Support

NPC: nuclear pore complex

IMPs: Importins

EXPs: Exportins

SERCA2a: Sarcoplasmic reticulum Ca^{2+} ATPase

IPO5: Importin5

NUP153: Nucleoporin153 kDa

RanGAP1: RanGTPase-Activiting Protein 1

ECMO: extracorporeal membrane oxygenator

ELISAs: enzyme-linked immunosorbent assays

INTERMACS: Interagency Registry for Mechanically Assisted Circulatory Support

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Figures

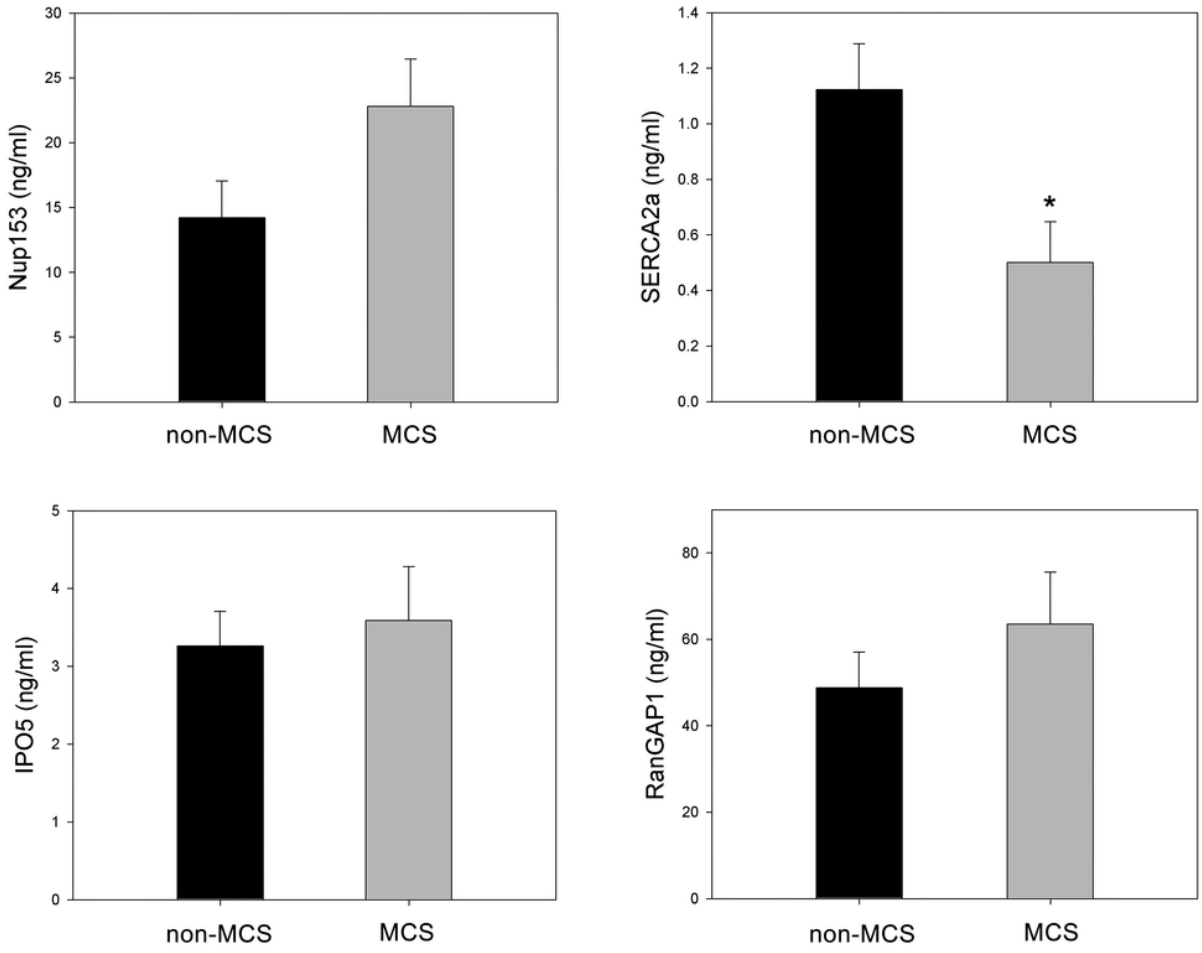

Figure 1

Comparison of the levels of Nup153, SERCA2a, IPO5 and RanGAP1 between patients with and without MCS. The values in the graph represent the mean ± SEM (standard error of the mean) with (P) representing statistically significant differences between the two groups. All values are in ng/mL.

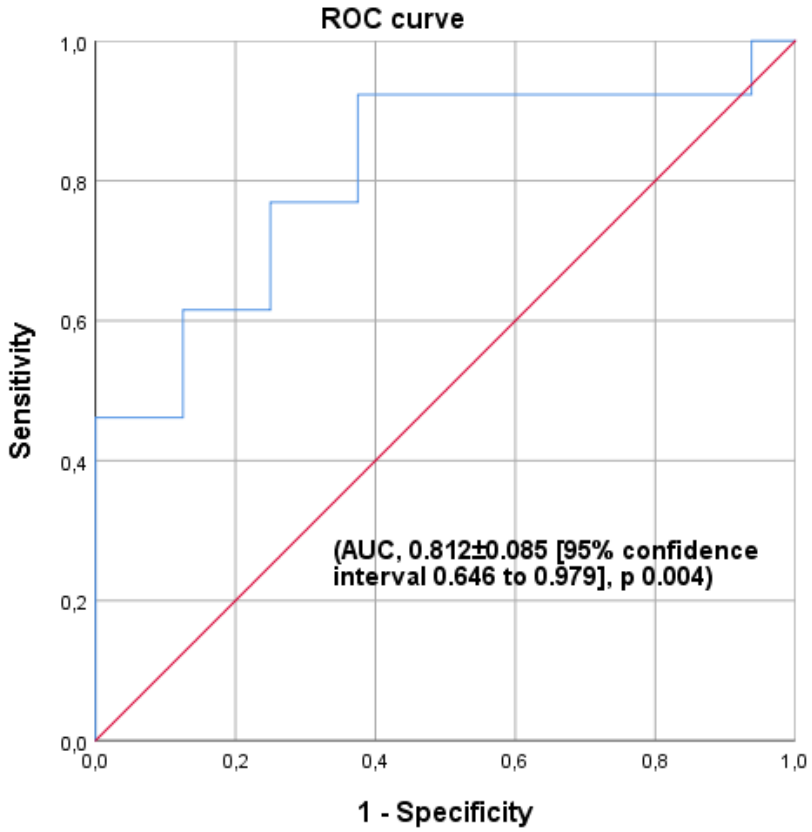

Figure 2

ROC curve for SERCA2a. Area under the curve (0.812 ± 0.085; P=0.004). The 95% confidence interval was set between 0.646 ng/mL and 0.979 ng/mL with a cut-off point at 0.84 ng/mL