

v1: 2 December 2024

Case Report

Case Report: Urinary Proteomic Analysis of Exercise-Induced Rhabdomyolysis with Acute Kidney Injury

Peer-approved: 2 December 2024

© The Author(s) 2024. This is an Open Access article under the CC BY 4.0 license.

Qeios, Vol. 6 (2024)
ISSN: 2632-3834

Andréia Carneiro da Silva¹, Janaina Macedo da Silva², Verônica Feijoli Santiago², Priscila Robertina dos Santos Donado², Gilberto Santos de Oliveira², Zeinab Ghasemishahrestani³, Josino Costa Moreira⁴, Cíntia Verdan Lucena⁵, Diego Viana Gomes⁵, Gianna Mastroianni Kirsztajn⁶, João Bosco Pesquero⁷, Marcos Dias Pereira⁸, Giuseppe Palmisano²

1. Brazilian Navy, Center of Physical Education Admiral Adalberto Nunes, Rio de Janeiro, Brazil; 2. Department of Parasitology, Institute of Biomedical Sciences, Universidade de São Paulo, Brazil; 3. Division of Dermatology, Department of Medicine, MRC-SA Wound Healing Unit, Grootte Schuur Hospital, University of Cape Town, South Africa; 4. Center for Human Ecology and Worker's Health, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil; 5. Department of Running, School of Physical Education and Sport, Universidade Federal do Rio de Janeiro, Brazil; 6. Department of Medicine (Nephrology), Universidade Federal do Rio de Janeiro, Brazil; 7. Department of Biophysics, Universidade Federal do Rio de Janeiro, Brazil; 8. Department of Biochemistry, Universidade Federal do Rio de Janeiro, Brazil

Exertional rhabdomyolysis (ER) is a frequently observed consequence following sustained strenuous exercise. The incidence of exercise-induced rhabdomyolysis has risen in the healthy population in recent decades, posing potential systemic, life-threatening complications like acute kidney injury (AKI). Early diagnosis requires prompt identification and management to prevent morbidity. This case report details the presentation of a 24-year-old male military member from the amphibious command specialization course, who attended the hospital emergency room with symptoms of nausea and dark brown urine 24 hours after strenuous military physical training. Laboratory results revealed a significant elevation in serum creatine kinase (CK) and creatinine (Cre) levels, reaching 9300 IU/L and 5.7 mg/dL, respectively. Concurrently, liver enzymes and urea levels were elevated, leading to the diagnosis of both ER and AKI. The individual exhibited a polygenic risk profile for ER, increasing susceptibility to inflammation and muscle damage. Further investigation through urine proteomic analysis unveiled the presence of various proteins associated with muscle damage, including creatine kinase M (CKM), myoglobin (MB), carbonic anhydrase (CA1), titin (TTN), as well as proteins linked to AKI, such as alpha-2-macroglobulin (A2MG), beta-2-microglobulin (B2MG), insulin-like growth factor-binding protein 7 (IBP7), metalloproteinase inhibitor 1 (TIMP1), and uromodulin (UROM), among others. Following a 12-day intensive care unit (ICU) treatment, a notable reduction in CK and MB levels was observed, accompanied by the restoration of renal function to normal levels. Subsequent laboratory tests during outpatient follow-up, two weeks after discharge, confirmed the normalization of relevant markers. The utilization of urinary proteomics emerged as a non-invasive method for monitoring

pathophysiological changes, offering valuable insights into the mechanisms underlying ER and associated AKI.

Andréia Carneiro, João Macedo-da-Silva, and Vinícius Santiago equally contributed to this work and share first authorship.

Corresponding authors: Giuseppe Palmisano, palmisano.gp@usp.br; Andréia Carneiro, andreia.carneiro@marinha.mil.br

1. Introduction

Exertional rhabdomyolysis (ER) is characterized by the degradation of muscle cells, resulting in the subsequent release of their contents into the bloodstream following intense physical activity^[1]. Different studies have reported various factors associated with rhabdomyolysis. These include cocaine use, exercise, immobilization, drugs such as statins, alcohol abuse, and intense eccentric exercise^{[1][2][3][4]}. Furthermore, the most common metabolic myopathy causing recurrent ER is associated with defect in fatty acid β -oxidation^[1]. Amongst them, the incidence of exercise-induced rhabdomyolysis has demonstrably risen in recent decades within the healthy population, necessitating an increased understanding of its implications^[5]. ER can lead to potentially life-threatening systemic complications, with acute kidney injury (AKI) being a particularly concerning sequel^[1]. The importance of early diagnosis cannot be overstated, as prompt identification and management are imperative to prevent morbidity associated with these complications.

The most common manifestations of rhabdomyolysis include muscle pain and myoglobinuria. In the context of ER, this is expressed as the production of dark urine alongside an increase in circulating creatine kinase (CK) levels^[1]. The severity of the injury can range from elevated serum concentrations of muscle proteins in asymptomatic patients to extremely high in severe cases. Severe cases particularly predisposed to complications such as acute kidney injury (AKI), electrolyte imbalances, and, in extreme cases, may culminate in fatality^[2].

Myoglobinuria is a consequential aspect of rhabdomyolysis, reflecting the release of myoglobin from damaged muscle cells into the bloodstream^[1]. This myoglobin is subsequently filtered and excreted

by the kidneys into the urine. The liver quickly metabolizes myoglobin, and it undergoes filtration by the glomeruli. As water is gradually reabsorbed, the myoglobin concentration increases until it binds to the Tamm-Horsfall protein, obstructing cylinders and inducing AKI^[2]. Myoglobin release is typically related to an increase in CK levels. Since myonecrosis progresses gradually, and the half-life of serum CK is high (approximately 36 hours, compared to myoglobin's shorter half-life of 2-3 hours), the concentration of serum CK remains elevated for a more prolonged duration and with greater consistency than myoglobin^{[1][2]}. Therefore, serum CK appears to be more reliable indicator than myoglobin for assessing both the presence and the intensity of muscle damage over an extended period^[2].

The classic triad of presentation, consisting of muscle pain, weakness, and dark urine, is seen in less than 10% of patients with rhabdomyolysis^[1]. AKI associated with myoglobinuria represents the most severe complication of both traumatic and non-traumatic rhabdomyolysis, with the potential for fatality^[4]. Rhabdomyolysis-associated AKI accounts for about 7-10% of all AKI cases in patients^[6].

The outcome of rhabdomyolysis is generally benign unless renal failure occurs. However, mortality data vary widely according to the study population, environmental conditions, and the number and severity of coexisting diseases^{[7][7][7][7]}. Specifically, among intensive care unit (ICU) patients, the reported mortality rate is 59% when AKI is present. In contrast, this rate decreases to 22% in the absence of AKI^{[8][9]}^[7].

This study lays the foundation for a case report detailing the presentation of a 24-year-old male military individual from the amphibious command specialization course, who exhibited symptoms of ER and AKI following strenuous military physical training. Laboratory findings, including elevated serum creatine kinase (CK) and creatinine (Cre) levels, underscore the severity of the condition. Importantly, the individual manifested a polygenic risk profile for ER, shedding light on the genetic susceptibility to inflammation and muscle damage.

Urine proteomic analysis emerged as a valuable tool for unraveling the molecular underpinnings of ER and associated AKI^[10]. The identification of specific proteins, such as creatine kinase M (CKM), myoglobin (MB), carbonic anhydrase (CA1), titin (TTN), alpha-2-macroglobulin (A2MG), beta-2-microglobulin (B2MG), insulin-like growth factor-binding protein 7 (IBP7), metalloproteinase inhibitor 1 (TIMP1), and uromodulin (UROM), among others, provides insights into the intricate network of molecular events associated with muscle damage and renal dysfunction.

The subsequent sections of this case report explore the clinical course, management, and outcomes of the presented case, with a particular emphasis on the role of urinary proteomics as a non-invasive methodology for monitoring pathophysiological changes. By elucidating the molecular mechanisms underlying ER-related AKI, this case report contributes to the advancement of knowledge essential for the effective diagnosis, management, and prevention of exertional rhabdomyolysis and its complications. The insights gained from this study have the potential to inform future research and clinical practice, paving the way for the development of targeted interventions and improved patient care. Moreover, we aim to raise awareness of the possibility, especially in healthy and fit individuals, of developing ER-related AKI, which may necessitate ICU admission and dialysis.

2. Case description

The military individual was in the initial phase of the Special Amphibious Commandos Course (SACC) as part of a group of 39 participants. The course included intensive exercises with a 35kg backpack and equipment, followed by a final test consisting of a 24,00m run. SACC aims to physically prepare them for subsequent missions. Blood samples were collected at baseline (D0) for biochemical monitoring by our research group, in which we observed laboratory values within normal limits (CK 78 U/L; Urea 37 mg/dL and creatinine 1,1 mg/dL).

The patient, a 24-year-old male military individual, presented to the hospital with symptoms of nausea, vomiting, myalgia, loss of appetite and anuria for 12 hours, following strenuous military physical training 20 hours prior. Laboratory findings on the first day of hospitalization (day 4, after enrollment in the operative military course) included elevated serum CK (9300 U/L), urea (147 mg/dL) and creatinine (5.7 mg/dL). Significant myoglobinuria (> 900 ng/mL) was observed on day 6 (D6). The patient underwent

hemodialysis on multiple days D6, D7, D8, D10, D11 and D14, leading to normalization of laboratory parameters, and he was discharged after 16 days without any symptoms, supplementary Figure 1 shows the evolution of biochemical exams and urinary output over the course of these days.

Post-discharge follow-up on day 17 showed normal levels of CK (100 U/L), urea (20 mg/dL) and creatinine (0.7 mg/dL). Subsequently, genetic testing revealed a polygenic profile associated with susceptibility to inflammation and muscle damage shown as follows: ACTN3 XX, ACE II, AGT MM, and BDKRB2-9-9.

The case emphasizes the potential severity of ER-related AKI, especially in healthy and fit individuals, and highlights the role of urinary proteomics in understanding molecular mechanisms and monitoring pathophysiological changes.

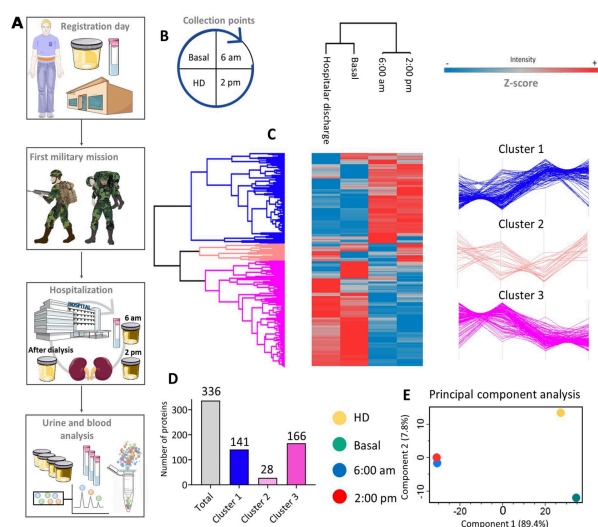


Figure 1. Workflow adopted for blood and urine sample analysis and proteomics results. Analytical workflow for blood and urine sample analysis. (A) Urine samples were collected at four time points: before training, during registration in the SACC course (Day 0, Do); on Day 7, the soldier was hospitalized, after first mission and two urine collections were made (D7, at 6:00 AM and 2:00 PM); and finally, at hospital discharge (D16). After these collections, the samples were analyzed using an LTQ Orbitrap Velos system, and the data obtained were mapped using MaxQuant software, utilizing the reviewed Homo sapiens database. (B) Flowchart of the collection time points: Baseline, 6:00 AM, 2:00 PM, and hospital discharge (HD). (C) Heatmap of identified proteins, showing the organization of clusters. (D) Summary of the total number of proteins: 336 proteins in total, distributed among clusters, with 141 in cluster 1, 28 in cluster 2, and 166 in cluster 3. (E) Principal component analysis.

3. Diagnostic assessment/Results

A 24-year-old man (Military 3201) presented symptoms after strenuous military physical training, prompting a diagnostic assessment. The adopted workflow (Figure 1A) guided the collection of blood and urine samples at various time points, allowing for a comprehensive analysis. On the day of enrollment in the operative military course (Do), basal blood and urine samples were collected from 39 participants as part of a research Project. The study was evaluated and approved by the Research Ethics Committee (CEP) of Hospital Naval Marcilio Dias, with opinion number 2.219.303.

On the 4th day (D4), Military 3201 was admitted to the hospital with nausea, vomiting, myalgia, loss of appetite and anuria, after 12 hours of strenuous military physical training. Blood samples were collected during hospitalization and submitted to biochemical analysis to determine the levels of classic biomarkers of muscle damage and kidney damage.

Seven sessions of hemodialysis were conducted over approximately four hours each, using heparin as anticoagulation and the F8 and ELI20 filter from Fresenius Medical Care (Bad Homburg, Germany). These sessions were administered on the following days: Day 6 (D6) with CK: 9426U/L, Cre:4,3 mg/Dl, and urine output (UO) in 12 hours: 900mL; Day 7 (D7) with CK: 8336 U/L, Cre: 6,1 mg/dL, and UO in 12 hours: 200mL; Day 8 (D8) with CK: 2419.8 U/L, Cre: 6.6 mg/dL, and UO in 12 hours: 800ml, in the ICU. In Day10 (D10) the patient (Military 3201) was discharged from ICU to medical ward, where made three more session of hemodialysis, on days: 10th (D10) and the exams shows CK: 933 U/L, Cre: 4,5 mg/dL and UO in 24 hours: 1800ml; 11th (D11) with CK:309.3 U/L, Cre: 3.3mg/dL and UO in 12 hours: 900mL, and 14th (D14), last hemodialysis, the Cre was 1.9 mg/dL and UO in 24 hours: 6500mL. On the last day of hospitalization (D16), the urinary output was 8200 mL (Supplementary figure 2).

In addition to the basal collection on Do, urine samples were also collected on the Day 7 (D7) at 6:00 am and 2:00 pm, as well as on the Day16 (D16) for proteome analysis.

The patient presented elevated levels of CK and aspartate transaminase (AST) (U/L), two classic biomarkers of muscle damage since the admission to the hospital with emergency interventions. These levels remained above the reference values for several days, reaching a peak of 9300 U/L and 348 U/L, respectively. Serum creatinine and urea levels remained high throughout the hospitalization period, with peaks of 7.7 and 180 mg/dL, respectively, indicating kidney injury (Supplementary figure 1). There was a gradual return of these biomarkers to basal levels.

Urine samples collected on Do, D7 and D16 were processed and applied to quantitative mass spectrometry-based proteomic analysis. Hierarchical clustering analysis revealed the segregation of the four collection points into two groups: Hospital discharge (D16)/Basal (Do) vs. 6:00 am/2:00 pm (D7) (Figure 1B).

The segregation on day D7 (6:00 am and 2:00 pm) reflects changes in the urinary proteome following strenuous physical exercise. The greater similarity between D0 and D16 suggests a return of protein abundance to basal levels after the military personnel recovered from AKI and muscle injury. The identified proteins were divided into three clusters according to changes in their abundance: proteins whose abundance increased or decreased after strenuous physical activity (clusters 1 and 3, respectively) and proteins that peaked in abundance on day D7 at 2:00 pm (cluster 2).

Figure 1C displays the total protein profiles in each cluster. Principal component analysis revealed that the most significant variation exists between the Hospital discharge/Basal vs. 6:00 am/2:00 pm groups, thus confirming the aforementioned results (Figure 1E).

A gene ontology analysis of the proteins pertaining to each cluster was conducted to identify the biological processes impacted after strenuous physical exercise. Across all clusters, enriched biological processes related to the immune system, extracellular matrix, and peptidases were observed (Figure 2A-C). The top 10 proteins with the highest fold change among collection times are highlighted in Figure 2D-H. Notably, among the most identified proteins, CD99, DEFA3, CD7, LCAT, and HABP2 exhibited the highest increase in urine after strenuous exercise on day D7 (6:00 am and 2:00 pm) compared to the Basal (D0) and Hospital discharge (D16) (Figure 2E-H).

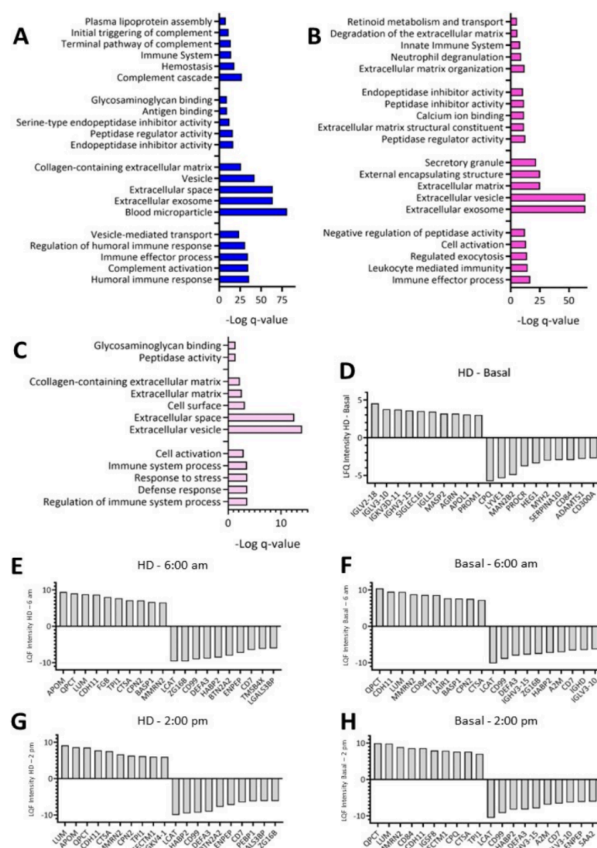


Figure 2. Exploring gene ontology and protein dynamics: analysis of clusters 1, 2, and 3 across time points. The figure presents the gene ontology analysis of the clusters, highlighting the results for (A) cluster 1, (B) cluster 3, and (C) cluster 2. The changes in proteins between the analyzed time points are shown in the comparisons: (D) hospital discharge versus baseline, (E) hospital discharge versus 6:00 AM, (F) baseline versus 6:00 AM, (G) hospital discharge versus 2:00 PM, and (H) baseline versus 2:00 PM. The analyses were performed using an LTQ Orbitrap Velos system, with the data processed by MaxQuant software, based on the reviewed Homo sapiens database.

CD99 is involved in T-cell adhesion processes, DEFA3 is an antimicrobial peptide, and CD7 is a T-cell antigen CD7. LCAT is a central enzyme in the extracellular metabolism of plasma lipoproteins, and HABP2 is an extracellular protease that may play a role in the coagulation and fibrinolysis systems. Regarding the proteins with the greatest decrease in urine after strenuous physical exercise, six proteins (QPCT, LUM, MMRN2, CDH11, CTSA, TPI1) exhibited decreased abundance on day D7 (6:00 am and 2:00 pm) compared to the Basal (D0) and Hospital

discharge (D16) (Figure 2E-H). LUM and MMRN2 are extracellular matrix structural constituents, CDH11 mediates cell-cell adhesion, CTSA is a lysosomal enzyme, TPI1 is an enzyme in the glycolysis and gluconeogenesis pathways, and QPCT is a secreted enzyme responsible for the biosynthesis of pyroglutamyl peptides.

As mentioned earlier, the blood biochemical analysis identified the presence of muscle and kidney injury biomarkers after strenuous physical exercise (Supplementary table 1). In our Urinary Proteome dataset, we sought protein markers of muscle and kidney damage. Notably, we observed an increase in urinary abundance of kidney damage markers, such as A1AG1, A1AT, A2MG, ANGT, CERU, A2GL, and NGAL (Figure 3A-B), as well as an increase in muscle damage markers, including CKM, TTN, MB, CA1, and TTR (Figure 3C). In a previous study, we identified a panel of proteins whose abundance in urine after strenuous physical exercise correlated well with the levels of plasma biomarkers of muscle damage^[10]. Additionally, some of the identified proteins were positively correlated with three biomarkers of muscle damage, suggesting their potential as new urinary markers of muscle damage. Herein, we observed an increase in the abundance of FABP3 and HBA1, along with a reduction of PIK3IP1, DEFB1, COL1A1, SLURP1, UTER, HMCN1, and DSC2 in the urine (Figure 3D-E). These results align with our previous findings, indicating the potential use of these proteins as urinary biomarkers of muscle damage^[10].

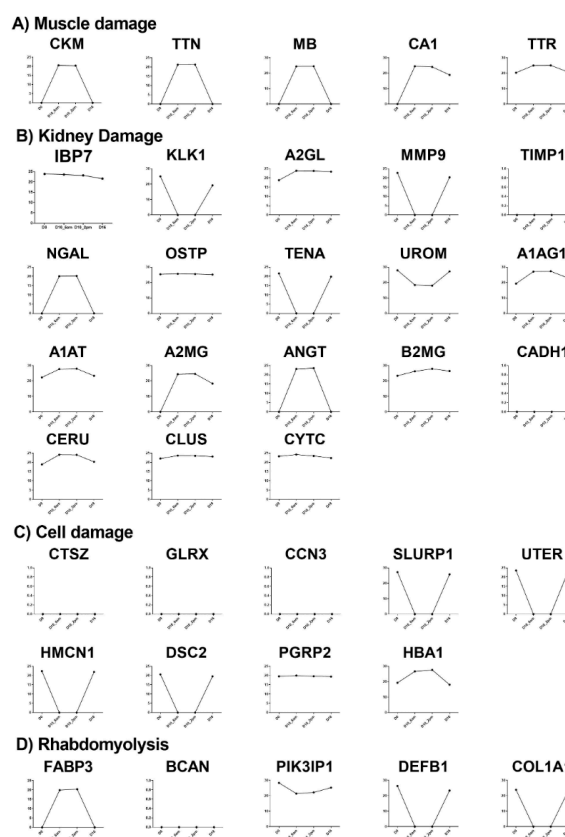


Figure 3. Urinary proteomic biomarkers of muscle and kidney damage across collection time points.

(A) Proteins related to muscle damage include creatine kinase, muscle type (CKM), titin (TTN), myoglobin (MB), carbonic anhydrase 1 (CA1), and transthyretin (TTR). (B) Proteins associated with kidney damage are insulin-like growth factor-binding protein 7 (IBP7), kallikrein-1 (KLK1), alpha-2-glycoprotein (A2GL), matrix metalloproteinase 9 (MMP9), tissue inhibitor of metalloproteinases 1 (TIMP1), neutrophil gelatinase-associated lipocalin (NGAL), osteopontin (OSTP), tenascin (TENA), uromodulin (UROM), alpha-1-acid glycoprotein 1 (A1AG1), alpha-1-antitrypsin (A1AT), alpha-2-macroglobulin (A2MG), angiotensinogen (ANGT), beta-2-microglobulin (B2MG), cadherin-1 (CADH1), ceruloplasmin (CERU), clusterin (CLUS), and cytochrome C (CYTC). (C) Proteins indicative of cell damage include cathepsin Z (CTSZ), glutaredoxin (GLRX), cellular communication network factor 3 (CCN3), secreted Ly-6/uPAR-related protein 1 (SLURP1), uteroglobin (UTER), hemicentin 1 (HMCN1), desmocollin-2 (DSC2), peptidoglycan recognition protein 2 (PGRP2), and hemoglobin subunit alpha 1 (HBA1). (D) Proteins associated with rhabdomyolysis include fatty acid-binding protein 3 (FABP3), brevican (BCAN), phosphoinositide-3-kinase-interacting protein 1 (PIK3IP1), beta-

defensin 1 (DEFB1), and collagen type I alpha 1 chain (COL1A1).

Finally, we performed a correlation analysis between plasma CK levels and the abundance of identified proteins in the Urinary Proteome (Figure 4). Several proteins showed a positive or negative correlation with CK levels, suggesting their potential as novel urinary biomarkers of muscle damage.

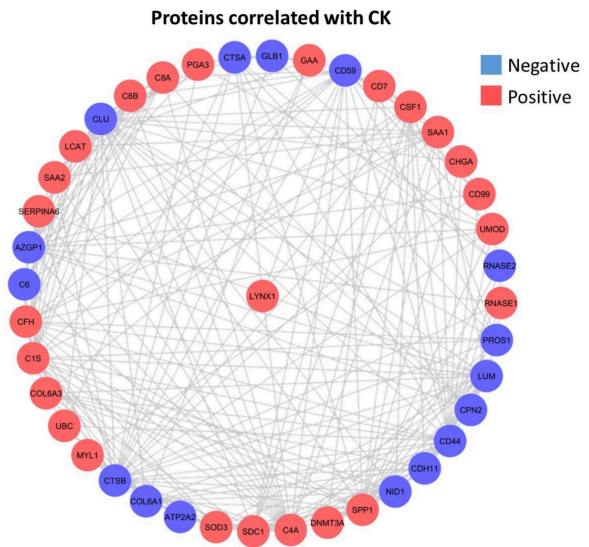


Figure 4. Proteins statistically correlated with CK level. Proteins that show negative correlations with CK include: ATPase Na⁺/K⁺ transporting subunit alpha 2 (ATP2A2), cadherin 11 (CDH11), cathepsin A (CTSA), cathepsin B (CTSB), CD44 molecule (adhesion receptor for hyaluronate) (CD44), CD59 molecule (complement regulatory protein) (CD59), clusterin (CLU), collagen type VI alpha 1 chain (COL6A1), complement component 6 (C6), carboxypeptidase N subunit 2 (CPN2), galactosidase beta 1 (GLB1), lumican (LUM), nidogen 1 (NID1), ribonuclease A family member 2 (RNASE2), and vitamin K-dependent protein S (PROS1), zinc-binding (AZGP1).

Proteins that show positive correlations with CK include: CD7 molecule (CD7), CD99 antigen (CD99), chromogranin A (CHGA), complement C1s subcomponent (C1S), complement C4-A (C4A), complement factor H (CFH), complement component 8 alpha chain (C8A), complement component 8 beta chain (C8B), colony-stimulating factor 1 (CSF1), collagen type VI alpha 3 chain (COL6A3), DNA (cytosine-5)-methyltransferase 3 alpha (DNMT3A), lecithin-cholesterol acyltransferase (LCAT), lymphocyte antigen 6 complex locus protein 1 (LYNX1), myosin light chain 1 (MYL1), N-acetylneuraminase (GAA), pepsinogen 3, Group I (Pepsinogen A) (PGA3), ribonuclease A family member 1 (RNASE1), secreted phosphoprotein 1 (SPP1), serum amyloid A-1 protein (SAA1), serum amyloid A-2 protein (SAA2), serpin family A member 6 (SERPINA6), superoxide dismutase 3 (SOD3), syndecan-1 (SDC1), uromodulin (UMOD), and ubiquitin C (UBC). The red and blue colors indicate a positive and negative correlation, respectively.

4. Discussion

Exertional rhabdomyolysis is a condition occasionally seen after strenuous exercise. Progression to compartment syndrome or AKI are rare complications that require prompt recognition and treatment to prevent morbidity^[1]. The progression of renal failure is well-associated with ER-induced myoglobinuria in 13%-50% of patients^{[11][12]}. In this case report, the patient presented classic signs of ER, such as muscle pain, myoglobinuria, and myonecrosis, the latter confirmed by CK (U/L) values above five times the upper limit of normality^[4]. The patient, initially healthy and in good physical condition, was hospitalized for the first time due to ER. He performed strenuous exercises on Day 3 of exercise and immediately sought medical treatment 20 hours after strenuous activity, with the onset of primary symptoms of ER, including nausea and vomiting.

Despite the lack of consensus in the literature, there is no defined threshold value of serum CK above which the risk of AKI is markedly increased. Nevertheless, it has been described that the risk of AKI in rhabdomyolysis is generally low when admission CK levels are <20,000 U/L^[4]. Although recent studies have shown AKI can be associated with CK values from 5,000 U/L^[13]. In our patient's case, the CK level on admission to the hospital was 9,300 U/L.

The incidence of AKI in rhabdomyolysis is challenging to establish due to its various definitions and clinical scenarios. The reported incidence of AKI related to rhabdomyolysis ranges from 13% to approximately 50%^[14]. AKI associated with rhabdomyolysis typically leads to a faster rise in plasma creatinine than other forms of AKI^[15], as observed in our patient. Likewise, a low ratio of urea nitrogen to creatinine in the blood is often noted in patients with rhabdomyolysis. Rhabdomyolysis-induced AKI frequently causes oliguria and occasionally causes anuria^[16].

The patient exhibited known genetic risk factors for ER, as indicated by his polygenic profile (ACTN3 XX, ACE II, AGT MM, BDKRB2-9-9). These genetic factors may increase susceptibility to inflammation and muscle damage after resistance exercise, and they can be utilized to predict the development of clinical conditions associated with muscle damage and myocardial injury^{[17][18][1]}. Polymorphic variants in ACTN3, ACE, AGT and BDKRB2 genes have been one of the determinants of the clinical manifestations

associated with the increased susceptibility to developing ER.

ACTN3 XX genotype is characterized by changes in the structural and metabolic function of muscles, including a decreased percentage of fast-twitch fibers, resulting in lower muscle strength^[19] and an increased susceptibility to muscle damage during eccentric exercises^{[20][21]}. It is noteworthy that individuals with the XX homozygote genotype have a higher propensity to develop exertional rhabdomyolysis compared to RR or RX genotypes^[22].

ACE insertion (I)/deletion (D) polymorphism might influence ER through various pathways associated with exercise-induced muscle hypertrophy, muscle injury, and inflammatory processes^[23]. Previously published data demonstrated the association between the I-allele of ACE and elevated CK levels after eccentric exercises, increasing the risk of developing ER^[24]. Additionally, AGT MM and BDKRB2 -9-9 polymorphisms might increase the susceptibility to inflammation and muscle damage after endurance exercises^[18], potentially contributing to the development of ER. Therefore, we suspect that this genetic profile may have been one of the determinants of the ER clinical condition presented by the patient.

The analysis of the urinary proteome at D7 revealed the expression of different proteins related to muscle injury, including creatine kinase M (CKM), myoglobin (MB), carbonic anhydrase (CA1), titin (TTN), transthyretin, as well as proteins linked to kidney injury such as Alpha-1-acid glycoprotein 1 (A1AG1), Alpha-1-antitrypsin (A1AT), Alpha-2-macroglobulin (A2MG), Angiotensinogen (ANGT), Beta-2-microglobulin, B2MG, Cadherin-1 (CADH), Ceruloplasmin (CERU), Clustering (CLUS), Cystatin-C (CYTC), Insulin-like growth factor-binding protein 7 (IGFBP7), Kallikrein-1(KLK1), Leucine-rich alpha-2-glycoprotein (A2GL), Matrix metalloproteinase-9 (MMP9), Metalloproteinase inhibitor 1 (TIMP1), Neutrophil gelatinase-associated lipocalin (NGAL), Osteopontin (OSTP), Tenascin (TENA), and Uromodulin (UROM). A prior study by Carneiro et al. ^[10] established urinary profiles through proteomic analysis in patients with ER. This study presents a severe clinical case of rhabdomyolysis that progressed to acute kidney injury, diagnosed using proteomics as a precision medicine tool, in clinical practice.

The current study emphasizes the importance of the genetic and proteomic profile of individuals engaged in regular strenuous physical exercises, particularly as

part of their professional activities. This knowledge can facilitate the implication of preventive measures, keep the individual and their team vigilant to the possibility of ER, and enable an earlier diagnosis of ER and its complications, such as AKI.

Likewise, it would be advantageous if early urinary markers for ER, easily monitored during exercise, could be identified, and used in clinical practice to support decision-making, aiming to intervene before the clinical condition worsens.

5. Patient perspective

Upon hospital discharge, the military personnel (3201) was advised to engage only in low-impact activities and was exempted from all activities requiring intense effort. Furthermore, he continued to receive medical follow-up for three months, undergoing blood tests and imaging to monitor renal function. After this period, the military personnel resumed physical activities normally, without any renal sequelae.

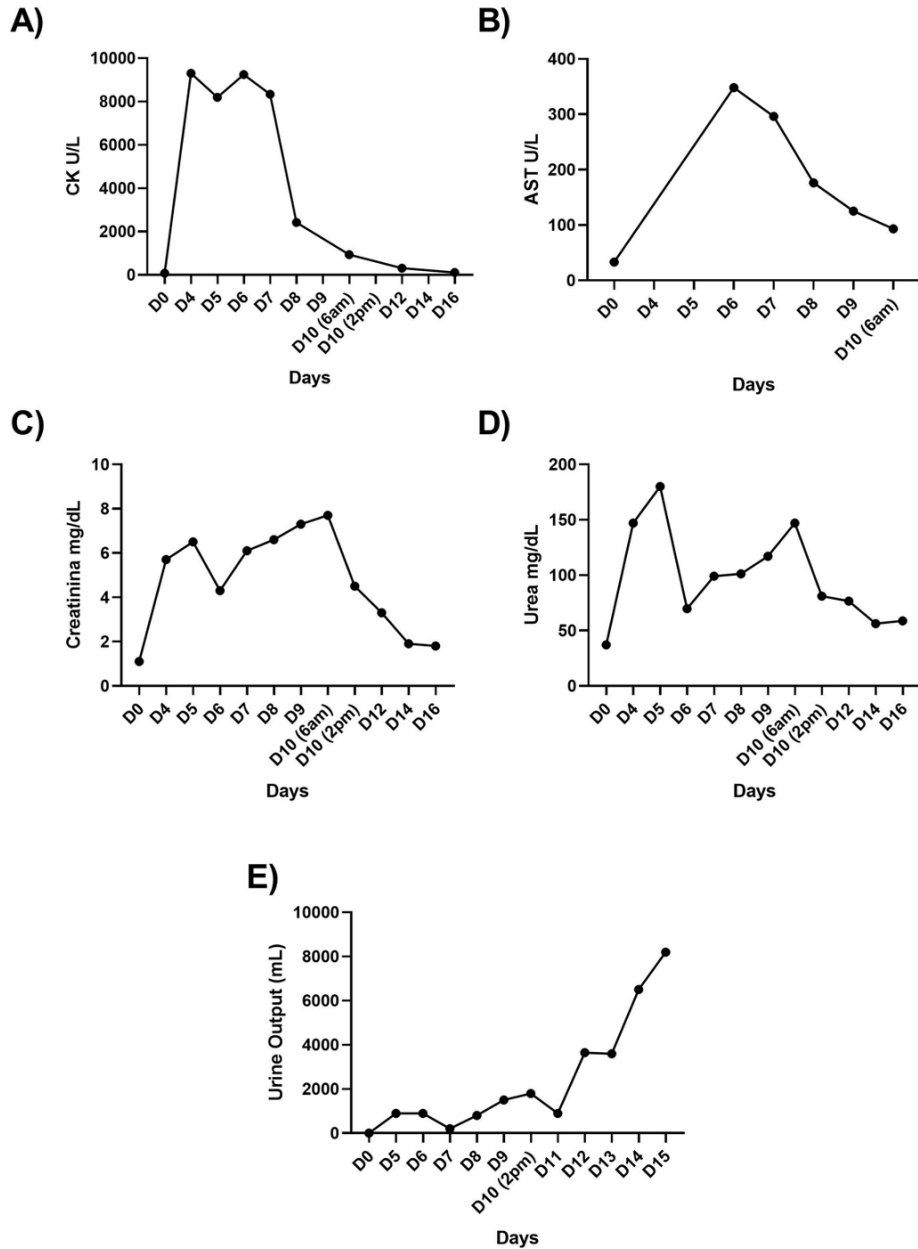
Supplementary Material

Laboratory test	Days of hospitalization												
	D0	D4*	D5*	D6	D7*	D8*	D9	D10 (6:00 am)	D10 (2:00 pm)	D12	D13	D14*	D16
Creatine kinase (U/L)	78.0	9300.0	8200.0	9246.0	8336.0	2419.8		933.0		309.3			109.8
Myoglobin (ng/mL)				> 900.0	> 900.0					335.0			
Urea (mg/dL)	37.0	147.0	180.0	69.8	99.0	101.3	117.0	147.0	81.0	76.5		56.3	58.8
Creatinine (mg/dL)	1.1	5.7	6.5	4.3	6.1	6.6	7.3	7.7	4.5	3.3		1.9	1.8
Sodium (mmol/L)	140.0			140.0	140.0	138.5	134.0	141.0	140.0	140.4	141.3		143.3
Potassium (mmol/L)	4.6			4.4	4.4	4.3	4.8	4.4	4.0	6.2	4.2	3.5	4.2
AST (U/L)	33.0			348.0	298.0	176.0	125.0	93.0					
CRP (mg/dL)				6.9	7.5	7.5	4.0	2.5		2.5			1.6
PT (seg)				15.0	13.8	14.2	14.5	14.4					
PTT (Seg)				180.0	33.0	33.7	33.0	33.0					
ALT (U/L)	28.0			146.0	136.0	105.0	99.0	99.0					
Total bilirubin (mg/dL)				0.8	0.8	0.7	0.7						
Direct bilirubin (mg/dL)				0.2	0.2	0.1	0.1						
Indirect bilirubin (mg/dL)				0.6	0.6	0.6	0.6						
Calcium (mg/dL)	9.3			9.3	8.5	8.2	8.6				8.3		
Magnesium (mg/dL)	2.1			2.2	2.0	2.2	2.3	2.0			1.4		
Phosphorus (mg/dL)	4.1			3.0	4.3	3.9	4.6	4.8			5.2		

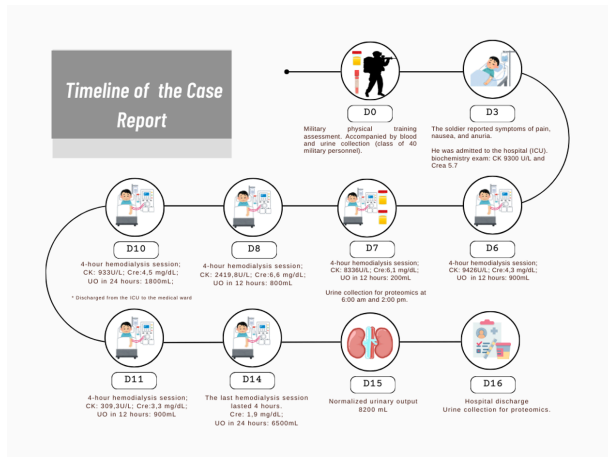
Supplementary Table 1. Blood and urine tests results during patient hospitalization in the intensive care unit (ICU).

*Hemodialysis on days D4, D5, D7, D8, D10, D11 and D14. Aspartate aminotransferase (AST); C-reactive protein (CRP), Prothrombin Time (PT), Activated Partial Thromboplastin Time (PTT), Alanine Aminotransferase (ALT). Unidade de medidas: unit

per liter (U/L), nanogram per milliliter (ng/mL), milligram per deciliter (mg/dL), millimol per 1 (mmol/L), seconds (seg). The analyses were performed through automation using the Vitros 5600 equipment (Ortho Clinical Diagnostics), employing chemiluminescence and immunoassay methods.



Supplementary Figure 1. Biochemical biomarkers of muscle, kidney damage, and urinary output on the days of hospitalization. Evolution of the parameters monitored throughout the days of hospitalization, with (A) the variation in creatine kinase (CK) concentration; (B) the levels of aspartate aminotransferase (AST); (C) the concentration of creatinine; (D) the levels of urea; and (E) the volume of urinary output. The analyses were performed through automation using the Vitros 5600 equipment (Ortho Clinical Diagnostics), employing chemiluminescence and immunoassay methods.



Supplementary Figure 2. Timeline of case report. (D0) Enrollment in the SACC, with the initial obtaining of blood and urine samples. (D3) Hospital admission of the military personnel, presenting symptoms of pain, nausea, and anuria. (D6–D14) Hemodialysis sessions and monitoring of biochemical markers, including Creatine Kinase (CK), Creatinine (Crea), and urinary output. (D15) Normalization of urinary output. (D16) Hospital discharge and new urine collection for proteomic analyses.

Statements and Declarations

Acknowledgments

The authors would like to thank to Brazilian Navy – Marinha do Brasil (Diretoria Geral de Desenvolvimento Nuclear e Tecnológico da Marinha, Comando Geral do Corpo de Fuzileiros Navais, Hospital Naval Marcilio Dias, Instituto de Pesquisas Biomédicas da Marinha, Centro de Instrução Almirante Sylvio de Camargo) for the research support and providing the facilities. We thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – 2021/00140-3, 2018/18257-1, 2018/15549-1, 2018/13283-4, 2020/04923-0 and 2014/27198-8), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) CNPQ 425025/2021-7, 381841/2023-5, Fundação Carlos Chagas de Amparo à Pesquisa do Rio de Janeiro (FAPERJ) APQ1, E-26/010001697/2019, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior for providing financial support for this study.

Conflicts of interest

Andreia Carneiro da Silva, Marcos Dias Pereira and Giuseppe Palmisano declare a conflict of interest due to the filing of a patent in Brazil, part of the data in this article.

Disclaimer

The views expressed are those of the authors and do not reflect the official policies of the Brazilian Navy, the Department of Defense, or the Brazil Government.

References

1. ^{a, b, c, d, e, f, g, h, i, j}Carneiro A, Viana-Gomes D, Mac edo-da-Silva J, Lima GH, Mitri S, Alves SR, et al. (2021). "Risk factors and future directions for preventin g and diagnosing exertional rhabdomyolysis." *Neur omuscular Disorders* 31: 583–595. doi:10.1016/j.nm d.2021.04.007.
2. ^{a, b, c, d, e}Gupta A, Thorson P, Penmatsa KR, Gupta P (2021). "Rhabdomyolysis: Revisited." *Ulster Med J* 9 0: 61. doi:10.1097/md.00000000000011848.
3. ^ΔAttardo S, Musumeci O, Velardo D, Toscano A (202 2). "Statins Neuromuscular Adverse Effects." *Int J M ol Sci* 23. doi:10.3390/IJMS23158364.
4. ^{a, b, c, d}Stanley M, Chippa V, Aeddula NR, Rodriguez BSQ, Adigun R (2023). "Rhabdomyolysis." *StatPearl s*. Available at: <https://www.ncbi.nlm.nih.gov/book s/NBK448168/> (Accessed June 27, 2024).
5. ^ΔAlharbi KF, Alfahmi MZ (2023). "Exercise-induced rhabdomyolysis manifestations and complications: a case report." *Annals of Medicine and Surgery* 85: 6 285. doi:10.1097/MS9.0000000000001479.
6. ^ΔMłynarska E, Krzemińska J, Wronka M, Franczyk B, Rysz J (2022). "Rhabdomyolysis-Induced AKI (RIAK I) Including the Role of COVID-19." *Int J Mol Sci* 23. doi:10.3390/IJMS23158215.
7. ^{a, b, c, d, e}Yang CW, Li S, Dong Y, Paliwal N, Wang Y (2021). "Epidemiology and the Impact of Acute Kidn ey Injury on Outcomes in Patients with Rhabdomyol ysis." *J Clin Med* 10. doi:10.3390/JCM10091950.
8. ^ΔDe Meijer AR, Fikkers BG, De Keijzer MH, Van Engel en BG, Drenth JP (2003). "Serum creatine kinase as predictor of clinical course in rhabdomyolysis: A 5-y ear intensive care survey." *Intensive Care Med* 29: 11 21–1125. doi:10.1007/s00134-003-1800-5.
9. ^ΔCandela N, Silva S, Georges B, Cartery C, Robert T, Moussi-Frances J, et al. (2020). "Short- and long-te rm renal outcomes following severe rhabdomyolysi s: a French multicenter retrospective study of 387 pat

- ients." *Ann Intensive Care* 10. doi:10.1186/S13613-020-0645-1.
10. ^{a, b, c, d}Carneiro A, Macedo-da-Silva J, Santiago VF, de Oliveira GS, Guimarães T, Mendonça CF, et al. (2022). "Urine proteomics as a non-invasive approach to monitor exertional rhabdomyolysis during military training." *J Proteomics* 258: 104498. doi:10.1016/j.jprot.2022.104498.
 11. ^ΔMelli G, Chaudhry V, Cornblath DR (2005). "Rhabdomyolysis: An evaluation of 475 hospitalized patients." *Medicine* 84: 377-385. doi:10.1097/01.md.0000188565.48918.41.
 12. ^ΔButkus JM, Kramer M, Chan V, Kim E (2022). "Psychosis-Induced Exertional Rhabdomyolysis without Acute Kidney Injury or Myoglobinuria." *American Journal of Case Reports* 23: 0-0. doi:10.12659/AJCR.934943.
 13. ^ΔPark JY, Kim MJ, Lee JG (2019). "Early Predictive Values for Severe Rhabdomyolysis in Blunt Trauma." *Journal of Trauma and Injury* 32: 26-31. doi:10.20408/JTI.2018.029.
 14. ^ΔPetejova N, Martinek A (2014). "Acute kidney injury due to rhabdomyolysis and renal replacement therapy: a critical review." *Crit Care* 18: 224. doi:10.1186/CC13897.
 15. ^ΔBosch X, Poch E, Grau JM (2009). "Rhabdomyolysis and Acute Kidney Injury." *New England Journal of Medicine* 361: 62-72. doi:10.1056/nejmra0801327.
 16. ^ΔShinde V, Shinde S, Mali M (2014). "Exercise-induced rhabdomyolysis with acute kidney injury: A case report with review of literature." *Medical Journal of Dr. D.Y. Patil University* 7: 679-682. doi:10.4103/0975-2870.140498.
 17. ^ΔDel Coso J, Valero M, Salinero JJ, Lara B, Díaz G, Gallo-Salazar C, et al. (2017). "ACTN3 genotype influences exercise-induced muscle damage during a marathon competition." *Eur J Appl Physiol* 117. doi:10.1007/s00421-017-3542-z.
 18. ^{a, b}Sierra APR, Lima GH, Silva ED, Maciel JF, Benetti MP, Oliveira RA, et al. (2019). "Angiotensin-Convertin Enzyme Related-Polymorphisms on Inflammation, Muscle and Myocardial Damage After a Marathon Race." *Front Genet* 10: 1-12. doi:10.3389/fgene.2019.00984.
 19. ^ΔErskine RM, Williams AG, Jones DA, Stewart CE, Degeens H. The individual and combined influence of ACE and ACTN3 genotypes on muscle phenotypes before and after strength training. *Scand J Med Sci Sports*. 2014;24:642-8. doi: 10.1111/sms.12055.
 20. ^ΔLee SY, Kwon MJ, Seo YI, Kim HA. Acute myositis of the tibialis anterior muscle after performance of 108 prostrations. *J Rheum Dis*. 2016;23:382-5. doi: 10.4078/jrd.2016.23.6.382.
 21. ^ΔSeto JT, Lek M, Quinlan KGR, Houweling PJ, Zheng XF, Garton F, et al. Deficiency of α -actinin-3 is associated with increased susceptibility to contraction-induced damage and skeletal muscle remodeling. *Hum Mol Genet*. 2011;20:2914-27. doi: 10.1093/hmg/ddr196.
 22. ^ΔDeuster PA, Contreras-Sesvold CL, O'Connor FG, Campbell WW, Kenney K, Capacchione JF, et al. Genetic polymorphisms associated with exertional rhabdomyolysis. *Eur J Appl Physiol*. 2013;113:1997-2004. doi: 10.1007/s00421-013-2622-y.
 23. ^ΔBaumert P, Lake MJ, Stewart CE, Drust B, Erskine RM. Genetic variation and exercise-induced muscle damage: implications for athletic performance, injury and ageing. *Eur J Appl Physiol*. 2016;116:1595-625. doi: 10.1007/s00421-016-3411-1.
 24. ^ΔYamin C, Amir O, Sagiv M, Attias E, Meckel Y, Eynon N, et al. ACE ID genotype affects blood creatine kinase response to eccentric exercise. *J Appl Physiol* (1985). 2007;103:2057-61. doi: 10.1152/jappphysiol.00867.2007.

Supplementary data: available at <https://doi.org/10.32388/KEBNDT.2>

Declarations

Funding: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) CNPQ 425025/2021-7, 381841/2023-5, Fundação Carlos Chagas de Amparo à Pesquisa do Rio de Janeiro (FAPERJ) APQ1, E-26/010001697/2019, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior.

Potential competing interests: Andreia Carneiro da Silva, Marcos Dias Pereira and Giuseppe Palmisano declare a conflict of interest due to the filing of a patent in Brazil, part of the data in this article.