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Chapter 3

ACUTE KIDNEY INJURY DUE TO RHABDOMYOLYSIS

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ABSTRACT

Rhabdomyolysis is a clinical syndrome caused by injury to skeletal muscle fibres with release of their breakdown products, especially myoglobin into the circulation. The condition is associated with traumatic injury, crush syndrome, extreme exercise, drugs, toxins and malignant hyperthermia, among others. The most severe complications are acute kidney injury (AKI), shock, disseminated intravascular coagulopathy and acute compartment syndrome. The three mechanisms of the renal toxicity due to myoglobin are: intratubular casts, renal vasoconstriction, and

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direct toxicity to kidney tubule cells. A number of pathological pathways are involved in the toxicity, including release of reactive oxygen species and cytokines and, lipid peroxidation of mitochondrial and kidney tubule cell membranes. The diagnosis and treatment of acute kidney injury following rhabdomyolysis are a challenge for intensive care physicians.

Keywords: acute kidney injury, rhabdomyolysis, renal replacement therapy

INTRODUCTION

Rhabdomyolysis (RM) is a condition caused by the disintegration of skeletal muscle with leakage of electrolytes, myoglobin, and other sarcolemmal proteins into the circulation. The syndrome is most often associated with traumatic injury, crush syndrome, extreme physical activity, drugs, toxins and malignant hyperthermia but autoimmune diseases, endocrine and metabolic disorders, infections including severe sepsis and adverse drug effects (e.g., statins, anesthetics, immuno-supressives) are included. The most severe complications of RM are acute kidney injury (AKI) requiring renal replacement therapy, hypovolemic or traumatic shock, disseminated intravascular coagulopathy and acute compartment syndrome (ACS).

EPIDEMIOLOGY AND ETIOLOGY OF ACUTE KIDNEY INJURY (AKI)

The reported incidence of RM in patients with traumatic injury is 31.1% [40], and RM induced AKI represents around 7 to 10% of all cases of AKI in the USA. However, the overall incidence of RM induced AKI is difficult to establish, but estimates range from 13 to 50% [7].

Acute kidney injury is a common and serious medical issue especially in critically ill patients in hospital intensive care units (ICU). It can be

divided, according to pathophysiological pathway into: 1) pre-renal, 2) renal and 3) post-renal AKI. Epidemiologically, AKI may be 1) community acquired 2) hospital acquired or 3) occur in critically ill hospitalized patients. AKI in RM can be considered pre-renal, community acquired or in the critically ill, in specific situations (sepsis, metabolic disorders, drugs). Thus, AKI associated with critical illness may be caused by an underlying disease or induced by diagnostic and/or therapeutic interventions with nephrotoxic effects e.g., iodine contrast agent and some antibiotics. The etiology of community acquired AKI, varies according to country. Industrial and venom poisonings, infectious diseases and obstetric complications are the most serious factors for AKI in developing countries. Pneumonia, urosepsis as in infectious diseases and nephrotoxic therapeutic agents are the most frequent in developed countries [38]. However, crush syndrome due to earthquakes, avalanches, floods and wars, is common to both developed and developing countries.

Clinical Presentation

There are historical descriptions of the clinical picture of RM, especially those relating to wars and natural disasters. Injured persons suffer from myalgia, edema and/or swelling of damaged muscles, weakness, muscle stiffness and dark brown urine, the color of tea. Fever, nausea and vomiting are nonspecific and common to other clinical situations. Oliguria and anuria, due to secondary kidney failure, accompanying the most severe cases are one of the leading causes of the high mortality. Common complications of AKI and RM are dysrhythmia, tachycardia and cardiac arrest, due to hyperkalemia and other metabolic disorders such as severe metabolic acidosis with a $pH < 7.1$. Potassium is an intracellular cation which is released into the circulation from injured muscle cells. The situation in RM is aggravated by anuria, decrease in potassium removal and by metabolic acidosis which enhances release of potassium from the intracellular space.

Table 1. AKI classification according to KDIGO 2012 [22]

AKI stage	Serum creatinine	Urine output
1.	1.5-1.9 times baseline or ≥ 0.3 mg/dL (≥ 26.5 μmol/L) increase	< 0.5 mL/kg/hour for 6-12 hours
2.	2.0-2.9 times baseline	< 0.5 mL/kg/hour ≥ 12 hours
3.	3.0 times baseline To 4.0 mg/dL (≥ 353.6 μmol/L) increase or initiation of RRT	< 0.3 mL/kg/hour ≥ 24 hours or Anuria for ≥ 12 hours

AKI- Acute Kidney Injury **RRT**- Renal Replacement Therapy.

Diagnosis and Classification of Acute Kidney Injury

Diagnosis of acute kidney injury is based on two fundamental markers: 1) increase in serum creatinine and/or 2) decreased urinary output which reflects reduced glomerular filtration. According to the KDIGO (Kidney Disease Improving Global Outcomes) in 2012, patients with AKI were divided into three groups (Table 1).

According to the duration of the renal injury, with loss of function, three clinical syndromes can be recognized: 1) AKI, 2) acute kidney disease (AKD) and 3) chronic kidney disease (CKD) where AKI is defined as an abrupt reduction in kidney function occurring over 7 days or less. AKD describes acute or subacute damage and/or loss of kidney function for a duration of between 7 and 90 days following exposure to insult and CKD is defined as persistence of renal damage or kidney disease for a period of > 90 days [8]. The diagnosis of AKI due to RM is based on biochemical/laboratory findings, in combination with clinical presentation [32]:

- *Biochemical results:* increase in serum creatinine, blood urea nitrogen, myoglobin, creatine phosphokinase, potassium, calcium, phosphorus, lactate dehydrogenase, transaminases, metabolic

acidosis, urine myoglobin or positive dipstick test for blood without any erythrocytes.

- *Clinical status and presentation:* myalgia, muscular weakness, swelling of injured muscle groups, tenderness, stiffness, fever, nausea or vomiting, tachycardia, hypotension or shock, decreased urinary output or anuria.

Urine Dipstick Test

For early detection of patients at high risk for RM and AKI development, a convenient urine dipstick test (UDT) with orthotoluidine was developed. This turns blue in the presence of hemoglobin or myoglobin. Positive UDT for blood, with no red blood cells in freshly spun urine sediment, can be used as a surrogate marker for myoglobin [36]. In one Iranian study, on 1,821 rescued victims of the Bam earthquake in 2003, the accuracy of the UDT to detect patients at risk of crush-induced RM and AKI was evaluated. The results showed that a urine red blood cell count of ≤ 5 in blood positive UDT, as a surrogate marker for myoglobinuria, had a sensitivity and a specificity of 83.3% and 56.6%, respectively, for identifying high risk patients. In addition, blood-positive UDTs, had a 92.5% sensitivity for creatine kinase, with a cut-off of 15, 000 IU/L [1].

Creatine Kinase

Creatine kinase (CK) is one of the key enzymes in cellular energy metabolism. It catalyzes the conversion of creatine (Ck) to creatine phosphate (CkP) with close connection to ATP production or consumption depending on Ck/CkP action. Cellular CkP serves as an energy source and is highly expressed in cells with high energy requirements e.g., skeletal muscle, myocardium, nervous tissue, smooth muscle tissue, retinal photoreceptor cells and spermatozoa. In cytoplasmatic CK, three isoforms can be distinguished in the combination of Muscle and Brain subunits (e.g., MM, MB and BB forms). This aside, mitochondrial CK may occur in ubiquitous or sarcommeric form. The physiological range of serum CK

concentration depends on gender (female 0.48 – 2.80 μkat/L; 28.24 – 164.71 IU/L and male 0.50 – 3.33 μkat/L; 29.41 – 195.88 IU/L).

In the case of rhabdomyolysis and myopathies, serum CK concentration reflects the severity of muscle membrane (sarcolemma) damage [42, 43]. Serum CK levels, especially the CK-MM fraction, increases in muscle damage between 2 to 12 hours after the insult, with a peak within 24 to 72 hours and a decline over 7 to 10 days. For a diagnosis of RM, a CK level **higher than fivefold** the physiological serum concentration, is documented as one of the diagnostic criteria [20]. The value of serum CK in predicting the risk of RM induced AKI has been investigated in traumatic and non-traumatic RM cases. A significant correlation was found between mean CK level and risk of crush-induced AKI development with the cut-off ranging from 1,000 to 15,000 IU/L. The correlation was higher in cases of traumatic injuries. However, an accurate cut point still needs further study [35]. According to a 10-year retrospective study, carried out in Norway, on patients treated for RM, a better predictor for AKI was myoglobin. The risk of developing AKI increased with a higher myoglobin/CK ratio (> 0.2) [41].

Myoglobin

Myoglobin is a dark red, oxygen binding metalloprotein – monomeric cytoplasmic hemoprotein consists of 154 amino acids with a molecular weight of 17,800 Da stored in muscle. The biological half-life of myoglobin is short - 1- 6 hours in a healthy organism but elimination can be significantly prolonged in patients with acute renal failure. Myoglobin can be monitored by chemoimmunofluorescence immunoassay. If the plasma myoglobin concentration exceeds – 1.5 mg/dL, myoglobin appears in the urine [5]. Physiological blood serum concentrations of myoglobin depend on gender: the male range is from 19-92 μg/L and the female 12-76 μg/L, with minor differences between different biochemical laboratories and techniques. The possibility of using the serum level of myoglobin, as a marker of myoglobinuric AKI was investigated in one study of 484 patients with RM. The median peak of myoglobin was 7,163 μg/L. Myoglobin levels above 15 mg/L, were most significantly related to the

development of AKI and the need for hemodialysis treatment. A significant percentage of patients (51%) experienced RM induced AKI, whose frequency increased in relation to serum myoglobin concentrations [34]. However, myoglobin blood concentration on hospital admission, did not predict AKI development. The peak level had greater predictive value but this must be reliably established with renal protective therapy in patients at high risk for renal failure [18].

Biomarkers

The new generation of biomarkers which can be measured in serum or urine with high predictive value for AKI, are neutrophil gelatinase associated lipocalin (NGAL) and kidney injury molecule - 1 (KIM-1). These reflect renal tubular injury. A second type is the two cycle cell arrest biomarkers: tissue inhibitor of metalloproteinase 2 (TIMP-2) and insulin - like growth factor - binding protein 7 (IGFBP-7). Variation in serum and urinary NGAL in humans, after strenuous physical exercise has been described in one Italian study involving 16 trained male athletes after a 60 km ultramarathon. 38% of the athletes met the criteria for AKI and serum and urinary creatinine as a marker of glomerular damage, had increased considerably. A significant correlation was found between pre- and post - exercise serum creatinine and serum NGAL but not with either urinary NGAL or the urinary NGAL/creatinine ratio. Urinary NGAL and serum creatinine probably reflect two different pathways of renal impairment - tubular and glomerular. The urinary NGAL appeared to be relatively independent of creatinine levels and extra-renal sources. Thus, it may be more reliable for monitoring renal impairment in this situation [27]. Urinary TIMP-2 and IGFBP-7 for risk stratification of AKI, were discovered and validated in more than 1,000 critically ill patients including those with traumatic injury. The markers of cell-cycle arrest in G_1 phase mentioned, may signal that the renal epithelium has been stressed and functionally impaired but capable of recovery without permanent damage to the organ. Both biomarkers are sufficient for predicting moderate and severe AKI development in the 12 hours following injury or sample collection. The new biomarkers of cell cycle arrest are promising,

especially in patients with polytrauma and sepsis [19]. In relation to this frequent severe clinical situation, also investigated are altered expression of microRNAs (miRNAs). These are non-coding, single-stranded RNA, containing 18-24 nucleotides [2]. Mature miRNAs act as posttranscriptional regulators, by binding mRNAs, with the main function - the degradation of their target mRNAs [14]. Under stress conditions as in cellular injury and depending on nuclear signals, specific miRNAs are released. This enables their use as biomarkers for diagnosis, evaluation and monitoring of critically ill patients. The expression of miRNAs may be usable in the future as a non-invasive method for the evaluation and monitoring of AKI patients [16].

Pathophysiology of Acute Kidney Injury Due to Rhabdomyolysis

For better understanding of the pathophysiology of RM induced AKI, pivotal, are appropriate animal and/or cellular models. The animal model for myoglobinuric AKI is best achieved by a single i.m. injection of 50% glycerol (8 mL/kg, i.m) in rats. Intramuscular injection of glycerol (10 mg/kg) in the rabbit, induces acute renal failure, resembling the AKI caused by massive release of myoglobin in rhabdomyolysis in humans. The pathophysiological basis of glycerol induced AKI, involves three pathological pathways: 1) ischemic injury due to vasoconstriction, 2) tubular toxicity caused by myoglobin and cast formation and 3) release of cytokines in RM with renal action – inflammation and lipid peroxidation [39]. However, the pathophysiology of RM induced AKI is complex. According to recent data, there are a number of molecular mechanisms involved. The potential of these discoveries is in the wider range of preventive and therapeutic measures that will be available.

Renal Vasoconstriction

Rhabdomyolysis, as a pathophysiological process, is associated with high volume depletion due to enhanced leakage of extracellular fluids into the damaged muscle cells with increased swelling of affected tissues and

muscles. In the blood circulation, decreased volume of extracellular fluids leads to hypotension and shock, with diminished renal blood flow and activation of the renin-angiotensin-aldosterone system (RAAS). The latter, causes the production of angiotensin 2 (ANG 2), a well-known renal vasoconstrictor. In an ischemic situation, ANG 2 can antagonize itself by release of nitric oxide (NO), a major vasodilator, in renal vessels. However, the second aspect of RM is release of myoglobin with its oxidation in the redox cycle to ferrylmyoglobin which interacts with NO. One animal study on RM induced AKI, found enhanced afferent arteriolar reactivity to ANG 2 due to myoglobin induced increase in superoxide and associated reduction in NO bioavailability [28]. However, myoglobin effects on the renal microcirculation may be more specific as reported by Heyman et al. in another animal study. Outer medullary blood flow and P_{O2} in RM induced AKI are reduced, while renal blood flow and cortical P_{O2} are unaffected [12]. Medullary vasoconstriction is an important factor in the global pathophysiology of RM induced AKI. The other contributing factor to vasoconstriction, is activation of endothelial inflammation with a number of vasoactive compounds, including the intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM) with effect on local hemodynamics [6].

Formation of Intratubular Casts

Myoglobin in low pH urine is precipitated with Tamm-Horsfall proteins to form intratubular casts. Urine acidification due to metabolic acidosis together with volume depletion and renal vasoconstriction plays a significant role in myoglobin intratubular cast formation. Under light microscopy, the myoglobin casts appear translucent or refractile, with colors ranging from light pink to slightly brown to dark red by hematoxylin and eosin.

Renal biopsies in RM induced AKI, display acute tubular injury associated with intratubular debris, thinning and vacuolization of the tubular epithelium. Approximately 10% of myoglobin positive biopsies were found to be associated with calcium oxalate or phosphate deposition [26].

Proximal Tubular Myoglobin Uptake via Endocytosis and Direct Cytotoxic Effects

From animal studies, it has been established that the renal uptake of myoglobin is mediated by megalin and cubilin, the multiligand endocytic receptors found in the membrane of proximal tubular cells [11]. These membrane receptors are different in their basic structure and not specific for myoglobin uptake. Megalin is a large glycosylated receptor of 600 kDa molecular weight and a member of the low density lipoprotein receptor family. This receptor is expressed in the apical membranes of the proximal tubule, endocytic vesicles and to some degree, in lysosomes. Cubilin is a 460 kDa glycosylated extracellular protein which interacts with other membrane proteins to enhance endocytosis. A number of substances can be reabsorbed by the proximal tubular cell membrane. These include: vitamin D binding protein, hemoglobin, apolipoproteins, albumin, lactoferin, drugs and toxins [30].

The first step in myoglobin degradation after cellular uptake is proteolytic cleavage. Free heme released from myoglobin is then catalyzed by heme-oxygenase 1 (HO-1) to biliverdin, iron and carbon monoxide (CO).

Heme-oxygenase, as an enzyme has a substantial cytoprotective effect especially in low amounts of filtered myoglobin. Overexpression, leads to heme degradation, iron efflux, antioxidant generation, ferritin upregulation and CO production [13].

Another situation in RM, is when large amounts of myoglobin in high concentration are presented for proximal tubular cell metabolism. Oxidative stress is a key process in tubular cell injury by induction of membrane lipid peroxidation. The iron released from the heme, may convert from ferrous (Fe^{2+}) to ferric (Fe^{3+}) form and back, depending on the intracellular redox state, creating a redox cycle as a result of heme oxidation and reduction [47].

In redox cycling, ferric myoglobin is then metabolized to ferryl Fe^{4+} form to maintain its stability. Hydrogen peroxide (H_2O_2) is critically involved in this process. It is generated in tubular cells, from direct stimulation by iron [45]. Membrane lipid peroxidation of tubular cells and

mitochondria can be induced as a result of the generation of hydroxyl radicals via the Fenton reaction (Figure 1). During rhabdomyolysis, oxidation of ferric myoglobin by endogenous lipid hydroperoxide (LOOH) or hydrogen peroxide, yields ferryl myoglobin and the alcoxyl radical (LO°). LOOH also reacts with ferryl myoglobin to form ferric myoglobin and the lipid peroxyl radical (LOO°) [29]. The consequence of membrane lipid peroxidation, mainly fatty acids, DNA and proteins, is production of F2 – isoprostanes and malondialdehyde.

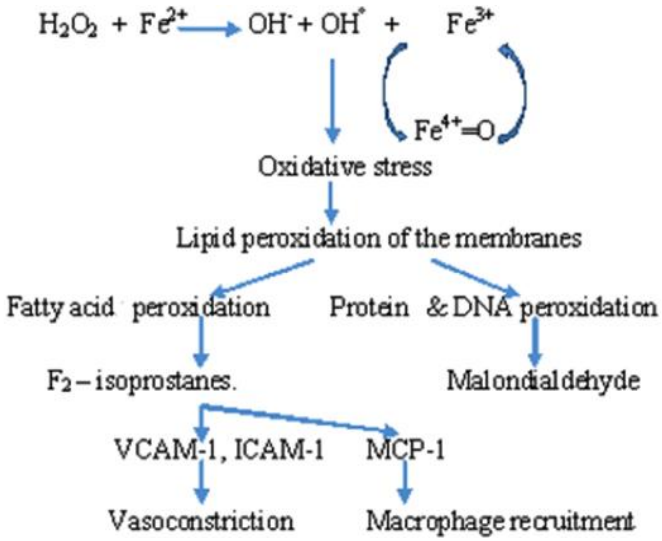

Abb: ICAM – 1 – intercellular adhesion molecule-1, **VCAM-1** – vascular cell adhesion molecule-1, **MCP-1** -monocyte chemoattractant protein - 1.

Figure 1. Membrane lipid peroxidation and tubular cell injury by heme [29, 31, 47].

Mitochondrial Dysfunction

The deleterious effects of myoglobin on mitochondrial metabolism result from lipid peroxidation of mitochondrial membranes, reactive oxygen species (ROS) production with oxidative stress due to damage of the inner mitochondrial membrane. Mitochondrial dysfunction itself involves excessive production of ROS (superoxide O_2^-, peroxynitrite $ONOO^-$ and NO). Under pathological conditions, such as RM with myoglobin toxicity, the uncoupling of oxidative phosphorylation and loss

of mitochondrial membrane integrity, induce excessive ROS production from the respiratory chain [15].

The suggestion that the failure of the permeability barrier by the inner mitochondrial membrane after myoglobin exposure in kidney mitochondria, is due to lipid peroxidation of mitochondrial membranes, and following reduction in respiratory control, uncoupling of oxidative phosphorylation and stimulated NO synthesis has been shown in one in *vitro* study. Lipid peroxide induced distortion of the ion permeability of the inner mitochondrial membrane can be explained by low respiratory control and the relative isolation of incubated mitochondria with myoglobin [33].

Programmed Cell Death in AKI due to Rhabdomyolysis

Besides apoptotic and necrotic cell death, new forms of programmed cell death in association with acute kidney injury have been discovered and are now accepted by pathologists – regulated necrosis, including ferroptosis, parthanatos, necroptosis, pyroptosis and mitochondrial permeability transition-mediated regulated necrosis. Some publications designate the type of cell death in RM induced AKI, as apoptosis due to release of pro-apoptotic cytokines from injured mitochondria after myoglobin incubation. However, a better description of the lethal process in tubule cells is ferroptosis - an iron and ROS – dependent form of regulated cell death with inclusion of all the mechanisms of heme oxidation, the membrane lipid peroxidation mentioned and the formation of extra mitochondrial ROS. Depletion of glutathione and glutathione-dependent gluthatione peroxidase 4 (GPX-4) is the crucial factor in the antioxidant capacity of cells and development of ferroptosis [21].

Inflammation and Endoplasmatic Reticulum Stress

Rhabdomyolysis is a complex clinical syndrome with activation of the inflammatory process and induction of AKI by release of proinflammatory cytokines from injured muscle cells: interleukin - 6 (IL-6), tumor necrosis factor α (TNF- α) and nuclear factor κB (NF-κB) as well as reactive oxygen species. Inflammatory mediators, recruit leukocytes and upregulate adhesion molecules e.g., ICAM-1, VCAM. Activated leukocytes produce

pro-inflammatory cytokines such as interleukin 1 (IL-1) and TNF- α which cause a number of injuries to the proximal tubule cells [6].

More recently added to whole inflammatory process in RM induced AKI, is endoplasmic reticulum (ER) stress. In this case, thioredoxin-interacting protein (TXNIP) is rapidly induced by inositol-requiring protein 1α (IRE1α) activation. Increased TXNIP proteins trigger expression of the nucleotide-binding oligomerization domain (NOD)-like receptor containing the pyrin domain 3 (NLRP3) inflammasome. TXNIP then acts as the link between ER stress and NLRP3 inflammasome activation and is a critical node in a chain of destruction leading from the ER to programmed cell death – apoptosis - activation. In addition, the ER stress associated TXNIP/NLRP3 cooperation, has influence on procaspase-1 cleavage and interleukin secretion (e.g., IL-1β and IL-18) [25, 44].

Treatment of Acute Kidney Injury Due to Rhabdomyolysis

Therapeutic measures for AKI due to RM are preventive, conservative and invasive using renal replacement therapy (RRT). In general, the therapeutic approach depends on the patient's clinical status especially hemodynamic stability. In hemodynamically stable patients, the preferred invasive methods are intermittent (IRRT) and in unstable patients, continuous (CRRT). The deterioration of renal function is important but not the only determining factor in the decision making cascade for initiation of RRT. Anuria or oliguria (urine output < 0.5 mL/kg/hour) in a period of more than 12 hours is usually the criterion for RRT application.

Preventive and Conservative Therapeutic Measures in Acute Kidney Injury

Conservative therapeutic measures in critically ill patients with RM induced AKI are: 1) specific for patients with RM induced AKI and 2) general for all critically ill patients with AKI.

Treatment of any underlying disease is, undoubtedly the first step in the therapeutic approach in patients with RM with the exception of primary

crush syndrome in cases of accidents and disasters. Excessive hydration or forced diuresis, hemodynamic stabilization, use of diuretic agents, urine alkalization and acid-base balance or ion correction are necessary in the treatment of RM. However, as indicated above, significant decrease in urine output or anuria as a marker of advanced renal injury are contraindications for these procedures [7, 32].

- Fluid resuscitation with initial bolus of crystalloid (isotonic saline or glucose 5%), e.g., 10-20 mL/kg (or l 000 mL IV) with 100 mmol/L (mEq/L) bicarbonate and maintaining fluids 3-6 L per day IV, need to be followed by forced diuresis 1-2 mL/kg/hour.
- To force diuresis and reduce the edema of injured muscle, the diuretic agent 20% mannitol can be used intravenously in a bolus of 100 mL following by a dose of 10 mL per hour according to clinical need. Physicians must be careful on cessation of therapy, as, due to osmotic activity there may be decrease in urine output or deterioration in renal function. As the second diuretic agent can be used a loop diuretic – Furosemide (1 mg/kg IV, initially).
- Proper use of bicarbonate (4,2% solution), (e.g., 30 to 40 mEq/L of isotonic saline at a run 100 mL/hour) for urine alkalization (pH > 6.5) is based on its preventive effects on myoglobin cast formation.

General recommendation for conservative treatment and prevention of AKI development are based on [17, 22]:

- Controlled fluid resuscitation in volume depletion with avoidance of fluid overload.
- Correction of hypovolemia or dehydration using isotonic crystalloids.
- Regular monitoring of the acid-base balance and chloride levels when chloride rich solutions are used.
- Use of diuretics to control or avoid fluid overload in a patient with mild or moderately severe AKI.

- Use of a vasopressor – norepinephrine - to maintain mean arterial pressure 65-70 mmHg.
- Correction of blood glucose levels using insulin at least below 10 mmol/L to prevent hyperglycemia.
- In situations where nephrotoxic antibiotics are needed (e.g., aminoglycosides, vancomycin), therapeutic drug monitoring is necessary. With aminoglycosides, a once daily dose (every 24 hours) is recommended.

However, conventional therapy has its limitation especially in patients with oliguria and hyperhydration where there is no hesitation in initiating RRT. Thus, regardless, RRT therapies have their indications [3, 22]:

- Hyperhydration with no reaction to diuretics (Furosemide IV) with clinical presentation of fluid overload – pulmonary edema, encephalopathy, lethargy.
- Anuria or urinary output < 0.5 mL/kg/hod for more than 12 hours.
- Uremic symptomathology – pericarditis, encephalopathy.
- Severe hyperkalemia with serum potassium > 6.5 mmol/L precluding continuation with the conservative approach, ECG changes
  (Peak T-waves, bradykardia, AV block).
- Severe metabolic acidosis with pH < 7.1, when conservative approaches are inapplicable.
- Clinical and laboratory presentation of multiple organ dysfunction syndrome.
- Increase in serum creatinine according to the KDIGO (see Table 1) or blood urea > 28 mg/dL.

Severe metabolic disorders and clinical criteria are the determining factors for RRT initiation, regardless of myoglobin or creatine kinase serum concentration. However, severe rhabdomyolysis with high myoglobin and CK serum concentration, is usually accompanied by some degree of renal injury.

Invasive Therapeutic Measures with Renal Replacement Therapy and Blood Purification Techniques

Renal replacement therapy is the treatment of choice in cases of severe renal injury with metabolic disorders, hyperhydration/fluid overload with no response to diuretic administration, decreased urine output or anuria. There are two types of RRT based on duration: 1) intermittent RRT and 2) continuous RRT. When the procedures are performed for non-renal indications, they are called blood purification techniques, with wide-spread use. Special clinical indications for CRRT are sepsis, septic shock, hepatorenal syndrome, cardiorenal syndrome and intoxication with dialyzable toxin.

Intermittent Hemodialysis and Hemodiafiltration

In hemodynamically stable AKI patients, intermittent hemodialysis with high cut-off and high permeability membranes which allow elimination of myoglobin, can be considered. However, this therapeutic approach is mostly preferred and has been investigated in patients with chronic kidney disease. Two recent prospective randomized studies (ClinicalTrials.gov NCT02377570 and NCT02377622) were carried out to compare hemodialysis with novel medium cut-off (MCO) dialyzers (cut off >25 kDa) and conventional high-flux hemodialysis and hemodiafiltration in patients with chronic kidney disease. Medium cut-off membranes were developed to increase the removal of middle molecules during a hemodialysis session and, in contrast to more permeable high cut-off membranes, are intended for routine use in chronic hemodialysis patients. The reported mean myoglobin clearance by MCO hemodialysers ranged from 52 mL/min to 68 mL/min. By high-flux hemodialyser, it was significantly lower 11-19 mL/min and in hemodiafitration with high-flux hemodialyser, 35 mL/min. There was significantly higher albumin removal with MCO (median 2.9 to 7.3 g) compared to high flux hemodialysis (median 0.2 g) and to hemodiafiltration (0.4 g), respectively [24]. However, excessive albumin loss especially in critically ill patients is undesirable. Another retrospective analysis of 10 chronic hemodialysis patients treated by on-line hemodiafiltration and converted to MCO

hemodialysis over a one year period, showed no difference in the removal of ß2-microglobulin and myoglobin. Moreover, there was no significant difference in median serum albumin and prealbumin levels for the therapeutic approaches used [4].

CRRT Techniques

Continuous renal replacement therapy techniques mainly in venovenous form, are often used in critically ill patients with hemodynamic instability and need for vasopressor treatment initiation, to improve blood pressure and circulation. According to basic physiological principles: diffusion or convection, these are continuous venovenous hemodialysis (CVVHD) based on diffusion, continuous venovenous hemofiltration (CVVH) based on convection or a combination of the two - hemodiafiltration (CVVHDF). The latter is the most effective in removing solutes, depending on type of membrane or hemofilter used, on filtration, dialysis and blood flow rate. Due to the common clinical presentation of RM as severe crush syndrome, CRRT is more often used in injured patients. Myoglobin can be removed from the circulation by convective hemofiltration or by the dialysis principle, when highly permeable membranes are used with a cut-off > 50 kDa (high cut-off membranes). Myoglobin reduction by high cut-off CVVHD (dose and dialysate flow rate 35 mL/kg/hour) in patients with RM and increased risk for AKI in comparison with high flux hemofiltration CVVH (ultrafiltration rate 35 mL/kg/hour) is the subject of an ongoing German study (ClinicalTrial. govNCT01467180) [10]. The meta-analysis of three studies on the efficacy of CRRT in removing myoglobin and the effect on mortality and renal function, was inconclusive. In all three studies, conventional therapy for RM was included as a control. In patients with rhabdomyolysis, no significant differences in mortality rate were found in comparison with conventional therapy. However, due to the major differences in techniques and results, the renal outcome was unclear [46]. The most undesirable albumin loss, in more effective CRRT therapy is a debated issue in intensive care. New high-permeability membranes and diffusive transport with a preference for removing middle molecules less than 67 kDa, which

is the molecular weight of albumin, may be a prospective measure for supplementary treatment of RM induced AKI.

Plasmapheresis

Therapeutic plasma exchange with human albumin or fresh frozen plasma substitution is a blood purification method which can be effective in myoglobin removal under specific conditions accompanying RM: sepsis, dermatomyositis or in hematological diseases such as sickle cell anemia. Plasmapheresis is not the primary therapeutic method for AKI but it can lead to successful treatment of the underlying disease leading to RM with myoglobin release. It can be considered in the clinical situations referred to.

Hemoadsorption with Cytosorb

An advanced type of extracorporeal treatment is hemoadsorption with CytoSorb, invented to decrease pro-inflammatory and anti-inflammatory cytokine overproduction in severe sepsis. CytoSorb (Cytosorbents corp., USA) is a hemoadsorption device containing hemocompatible, porous, polymeric beads efficient for removing cytokines and middle molecular substances from the circulation by surface adsorption and size exclusion [37]. Use of the CytoSorb device in a CVVH circle has been recently studied in one American prospective randomized study carried out on the feasibility of the CytoSorb as an adjunct to the standard care in patients with rhabdomyolysis requiring RRT. The efficacy of the device was measured by change in serum myoglobin (https://clinicaltrials.gov/ct2/show/NCT02111018) [9]. However, the final results are not yet published.

Other Experimental Therapeutic Options

Knowledge of the basic pathophysiological processes leading to AKI in rhabdomyolysis with production of free radicals, reactive oxygen species, redox cycling of iron, membrane lipid peroxidation and DNA damage, has led to a wide range of medical approaches in recent years. To avoid the deleterious effects of the above substances, the human body has a system of endogenous antioxidant cell protectants: 1) superoxide

dismutase, 2) catalase and 3) glutathione peroxidase. N-acetylcysteine is a source of glutathione and sulfhydryl groups and due to its interactions with ROS, is a scavenger of cellular free radicals. In one animal study with glycerol induced AKI and RM, renal protection in rats with N-acetylcysteine pretreatment (150 mg/kg) was found, with a decrease in pro-apoptotic pathway activation and increased expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL. Bcl-2 proteins are known to regulate cellular apoptosis by controlling mitochondrial permeability [23].

Ascorbic acid and vitamin E in the prevention of myoglobinuric AKI have produced conflicting results.

CONCLUSION

Acute kidney injury due to rhabdomyolysis is a common diagnostic and therapeutic issue in critically ill patients. Rhabdomyolysis is associated with a range of clinical conditions and acute kidney injury is a complicated and advanced situation. Better and deeper understanding of the pathophysiology and new approaches in diagnosis can substantially contribute to improving current and future therapeutic options for the preservation and recovery of renal function.

REFERENCES

[1] Alavi-Moghaddam, M., Safari, S., Najafi, I. and Hosseini, M. **(**2012). Accuracy of urine dipstick in the detection of patients at risk for crush-induced rhabdomyolysis and acute kidney injury. *Eur J Emerg Med*, 19 (5): 329-332.

[2] Bedreag, O. H., Papurica, M., Rogobete, A. F., Sandesc, D., Dumache, R., Cradigati, C. A., Sarandan, M., Bratu, L. M., Popovici, S. E. and Sima, L. V. (2016). Using Circulating miRNAs as Biomarkers for the Evaluation and Monitoring of the Mitochondrial

Damage in the Critically Ill Polytrauma Patients. *Clin Lab*, 62 (8): 1397-1403.

[3] Bellomo, R. (2011). Acute renal failure. *Semin Respir Crit Care Med*, 32: 639-650.

[4] Belmouaz, M., Diolez, J., Bauwens, M., Duthe, F., Ecotiere, L., Desport, E. and Bridoux, F. (2018). Comparison of hemodialysis with medium cut-off dialyzer and on-line hemodiafiltration on the removal of small and middle-sized molecules. *Clin Nephrol*, 89 (1): 50-56.

[5] Berrige, B. R., Van Vleet, J. F. and Herman, E. (2013). Cardiac, Vascular and Sceletal Muscle Systems. In: Haschek, W. M., Rousseaux, C. G., Wallig, M. A., Bolon, B. and Ochoa, R. Editors. *Haschek and Rousseaux's Handbook of Toxicologic pathology*. 3rd Edition. Waltham, San Diego, USA, Elsevier.

[6] Bonventre, J. V. (2010). Pathophysiology of AKI: injury and normal and abnormal repair. *Contrib Nephrol*, 165: 9-17.

[7] Bosch, X., Poch, E. and Grau, J. M. (2009). Rhabdomyolysis and acute kidney injury. *N Engl J Med*, 361 (1): 62-72.

[8] Chawla, L. S., Bellomo, R., Bihorac, A., Goldstein, S. L., Siew, E. D., Bagshaw, S. M., Bittleman, D., Cruz, D, Endre, Z., Fitzgerald, R. L., Forni, L., Kane-Gill, S. L., Hoste, E., Koyner, J., Liu, K. D., Macedo, E., Mehta, R., Murray, P., Nadim, M., Ostermann, M., Palevsky, P. M., Pannu, N., Rosner, M., Wald, R., Zarbock, A., Ronco, C. and Kellum, J. A; Acute Disease Quality Initiative Workgroup 16. (2017). Acute Disease Quality Initiative Workgroup 16: Acute kidney disease and renal recovery: consensus report of the Acute Disease Quality Initiative (ADQI) 16 Workgroup. *Nat Rev Nephrol*, 13 (4): 241-257.

[9] ClinicalTrial.Gov. *Extracorporeal therapy for the removal of myoglobin Using the CytoSorb in patients with Rhabdomyolysis*. https://clinicaltrials.gov/ct2/show/NCT02111018.

[10] ClinicalTrial.Gov. *Myoglobin Reduction by High Cut-off (HCO) Continuous Veno- Venous Hemodialysis (CVVHD) in Patients With*

Rhabdomyolysis and an Increased Risk for Acute Kidney Injury. https://clinicaltrials.gov/ct2/show/NCT01467180.

[11] Gburek, J., Birn, H., Verroust, P. J., Goj, B., Jacobsen, C., Moestrup, S. K., Willnow, T. E. and Christensen, E. I. (2003). Renal uptake of myoglobin is mediated by the endocytic receptors megalin and cubilin. *Am J Physiol Renal*, 285 (3): 451-458.

[12] Heyman, S. N., Rosen, S., Fuchs, S., Epstein, F. H. and Brezis, M. (1996). Myoglobinuric acute renal failure in the rat: a role for medullary hypoperfusion, hypoxia, and tubular obstruction. *J Am Soc Nephrol*, 7 (7): 1066-1074.

[13] Hill-Kapturczak, N., Chang, S. H. and Agarwal, A. (2002). Heme oxygenase and the kidney. *DNA Cell Biol*, 21 (4): 307-332.

[14] Ichii, O. and Horino, T. (2018). MicroRNAs associated with the development of kidney diseases in humans and animals. *J Toxicol Pathol*, 31 (1): 23-34.

[15] Ishimoto, Y. and Inagi, R. (2016). Mitochondria: a therapeutic target in acute kidney injury. *Nephrol Dial Transplant*, 31(7): 1062-1069.

[16] Ivan, M. V., Rogobete, A. F, Bedreag, O. H, Papurica, M., Popovici, S. E., Dinu, A., Sandesc, M., Beceanu, A., Bratu, L. M., Popoiu, C. M., Sandesc, D., Boruga, O. and Fulger, L. (2018). New Molecular and Epigenetic Expressions as Novel Biomarkers in Critically Ill Polytrauma Patients with Acute Kidney Injury (AKI). *Clin Lab*, 64 (5): 663-668.

[17] Joannidis, M., Druml, W., Forni, L. G., Groeneveld, A. B. J., Honore, P. M., Hoste, E., Ostermann, M., Oudemans-van Straaten, H. M. and Schetz, M. (2017). Prevention of acute kidney injury and protection of renal function in the intensive care unit: update 2017: Expert opinion of the Working Group on Prevention, AKI section, European Society of Intensive Care Medicine. *Intensive Care Med*, 43 (6): 730-749.

[18] Kasaoka, S., Todani, M., Kaneko, T., Kawamura, Y., Oda, Y., Tsuruta, R. and Maekawa, T. (2010). Peak value of blood myoglobin predicts acute renal failure induced by rhabdomyolysis. *J Crit Care*, 25 (4): 601-604.

[19] Kashani, K., Al-Khafaji, A., Ardiles, T., Artigas, A., Bagshaw, S. M., Bell, M., Bihorac, A., Birkhahn, R., Cely, C. M., Chawla, L. S., Davison, D. L., Feldkamp, T., Forni, L. G., Gong, M. N., Gunnerson, K. J., Haase, M., Hackett, J., Honore, P. M., Hoste, E. A., Joannes-Boyau, O., Joannidis, M., Kim, P., Koyner, J. L., Laskowitz, D. T., Lissauer, M. E., Marx, G., McCullough, P. A., Mullaney, S., Ostermann, M., Rimmelé, T., Shapiro, N. I., Shaw, A. D., Shi, J., Sprague, A. M., Vincent, J. L., Vinsonneau, C., Wagner, L., Walker, M. G., Wilkerson, R. G., Zacharowski, K. and Kellum, J. A. (2013). Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. *Crit Care* [On-line serial], 17 (1): R25. doi: 10.1186/cc12503.

[20] Keltz, E., Khan, F. Y. and Mann, G. (2014). Rhabdomyolysis. The role of diagnostic and prognostic factors. *Muscles Ligaments Tendons J*, 3 (4): 303-312.

[21] Kers, J., Leemans, J. C. and Linkermann, A. (2016). An Overview of Pathways of Regulated Necrosis in Acute Kidney Injury. *Semin Nephrol*, 36: 139-152.

[22] Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO Clinical Practice Guideline for Acute Kidney Injury. *Kidney Inter Suppl*, 2012, 2: 1–138.

[23] Kim, J. H., Lee, S. S., Jung, M. H., Yeo, H. D., Kim, H. J., Yang, J. I., Roh, G. S., Chang, S. H. and Park, D. J. (2010). N-acetylcysteine attenuates glycerol-induced acute kidney injury by regulating MAPKs and Bcl-2 family proteins. *Nephrol Dial Transplant*, 25 (5): 1435-1443.

[24] Kirsch, A. H., Lyko, R., Nilsson, L. G., Beck, W., Amdahl, M., Lechner, P., Schneider, A., Wanner, C., Rosenkranz, A. R. and Krieter, D. H. (2017). Performance of hemodialysis with novel medium cut-off dialyzers. *Nephrol Dial Transplant*, 32 (1): 165-172.

[25] Lerner, A. G., Upton, J. P., Praveen, P. V., Ghosh, R., Nakagawa, Y., Igbaria, A., Shen, S., Nguyen, V., Backes, B. J., Heiman, M., Heintz, N., Greengard, P., Hui, S., Tang, Q., Trusina, A., Oakes, S. A. and Papa, F. R. (2012). IRE1α induces thioredoxin - interacting protein

to activate the NLRP3 inflammasome and promote programmed cell death under irremediable ER stress. *Cell Metab*, 16 (2): 250-264.

[26] Liapis, H., Boils, C., Hennigar, R. and Silva, F. (2016). Myoglobin casts in renal biopsies: immunohisochemistry and morphologic spectrum. *Hum Pathol*, 54: 25-30.

[27] Lippi, G., Sanchis-Gomar, F., Salvagno, G. L., Aloe, R., Schena, F. and Guidi, G. C. (2012). Variation of serum and urinary neutrophil gelatinase associated lipocalin (NGAL) after strenuous physical exercise. *Clin Chem Lab Med*, 50 (9): 1585-1589.

[28] Liu, Z. Z., Mathia, S., Pahlitzsch, T., Wennysia, I. C., Persson, P. B., Lai, E. Y., Högner, A., Xu, M. Z., Schubert, R., Rosenberger, C. and Patzak, A. (2017). Myoglobin facilitates angiotensin II-induced constriction of renal afferent arterioles. *Am J Physiol Renal Physiol*, 312 (5): F908-F916.

[29] Moore, K. P., Holt, S. G., Patel, R. P, Svistunenko, D. A., Zackert, W., Goodier, D., Reeder, B. J., Clozel, M., Anand, R., Cooper, C. E., Morrow, J. D., Wilson, M. T., Darley-Usmar. V. and Roberts, L. J. 2nd. (1998). A causative role for redox cycling of myoglobin and its inhibition by alkalinization in the pathogenesis and treatment of rhabdomyolysis-induced renal failure. *J Biol Chem*, 273 (48): 31731-31737.

[30] Nielsen, R., Christensen, E. I. and Birn, H. (2016). Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease. *Kidney Int*, 89 (1): 58-67.

[31] Panizo, N., Rubio-Navarro, A., Amaro-Villalobos, J. M., Egido, J. and Moreno, J. A. (2015). Molecular Mechanisms and Novel Therapeutic Approaches to Rhabdomyo- lysis-Induced Acute Kidney Injury. *Kidney Blood Press Res*, 40 (5): 520-532.

[32] Petejova, N. and Martinek, A. (2014). Acute kidney injury due to rhabdomyolysis and renal replacement therapy: a critical review. *Crit Care* [On-line serial], 18 (3): 224. doi: 10.1186/cc13897.

[33] Plotnikov, E. Y., Chupyrkina, A. A., Pevzner, I. B., Isaev, N. K. and Zorov, D. B. (2009). Myoglobin causes oxidative stress, increase of

NO production and dysfunction of kidney's mitochondria. *Biochim Biophys Acta*, 1792 (8): 796-803.

[34] Premru, V., Kovač, J. and Ponikvar, R. (2013). Use of myoglobin as a marker and predictor in myoglobinuric acute kidney injury. *Ther Apher Dial*, 17 (4): 391-395.

[35] Safari, S., Yousefifard, M., Hashemi, B., Baratloo, A., Forouzanfar, M. M., Rahmati, F., Motamedi, M. and Najafi, I. (2016). The value of serum creatine kinase in predicting the risk of rhabdomyolysis-induced acute kidney injury: a systematic review and meta-analysis. *Clin Exp Nephrol*, 20 (2): 153-161.

[36] Sauret, J. M., Marinides, G. and Wang, G. K. (2002). Rhabdomyolysis. *Am Fam Physician*, 65 (5): 907-912.

[37] Schädler, D., Pausch, C., Heise, D., Meier-Hellmann, A., Brederlau, J., Weiler, N., Marx, G., Putensen, C., Spies, C., Jörres, A., Quintel, M., Engel, C., Kellum, J. A. and Kuhlmann, M. K. (2017). The effect of a novel extracorporeal cytokine hemoadsorption device on IL-6 elimination in septic patients: A randomized controlled trial. *PLoS One*. [On-line serial], 12 (10): e0187015. doi: 10.1371/ journal.pone. 0187015. eCollection 2017.

[38] Shah, S. H. and Mehta, R. I. (2009). Epidemiology of Community – Acquired Acute Kidney Injury. In: Ronco, C., Bellomo, R., and Kellum, J. A., Editors. *Critical Care Nephrology*. 2nd Edition., Philadelphia, USA, Saunders Elsevier.

[39] Singh, A. P., Junemann, A., Muthuraman, A., Jaggi, A. S., Singh, N., Grover, K. and Dhawan, R. (2012). Animal models of acute renal failure. *Pharmacol Rep*, 64 (1): 31-44.

[40] Stewart, I. J., Faulk, T. I., Sosnov, J. A., Clemens, M. S., Elterman, J., Ross, J. D., Howard, J. T., Fang, R., Zonies, D. H. and Chung, K. K. (2016). Rhabdomyolysis among critically ill combat casualties: Associations with acute kidney injury and mortality. *J Trauma Acute Care Surg*, 80 (3): 492-498.

[41] Vangstad, M., Bjornaas, M. A. and Jacobsen, D. (2017). Rhabdomyolysis: a 10-year retrospective study of patients treated in

a medical department. *Eur J Emerg Med*, Oct 24. doi: 10.1097/MEJ.0000000000000510.

[42] Vohanka, S. (2012). Increased creatine kinase level. *Intern. Med*, 14 (8 and 9): 322-326.

[43] Wallimann, T., Tokarska-Schlattner, M. and Schlattner, U. (2011). The creatine kinase system and pleiotropic effects of creatine. *Amino Acids*, 40 (5): 1271-1296.

[44] Yuan, X., Zheng, Y., Chen, C. and Wang, C. (2017). Anisodamine inhibits endoplasmic reticulum stress-associated TXNIP/NLRP3 inflammasome activation in rhabdomyolysis-induced acute kidney injury. *Apoptosis*, 22 (12): 1524-1531.

[45] Zager, R. A. and Burkhart, K. M. (1998). Differential effects of glutathione and cysteine on Fe2+, Fe3+, H2O2 and myoglobin-induced proximal tubular cell attack. *Kidney Int*, 53 (6): 1661-1672.

[46] Zeng, X., Zhang, L., Wu, T. and Fu, P. (2014). Continuous renal replacement therapy (CRRT) for rhabdomyolysis. *Cochrane Database Syst Rev* [On-line serial] 15 (6): CD008566. doi: 10.1002/14651858. CD008566.pub2.

[47] Zorova, L. D., Pevzner, I. B., Chupyrkina, A. A., Zorov, S. D., Silachev, D. N., Plotnikov, E. Y. and Zorov, D. B. (2016). The role of myoglobin degradation in nephrotoxicity after rhabdomyolysis. *Chem Biol Interact*, 256: 64-70.

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