Morphological diagnosis of hyperthermia-related deaths

Mihai Ceausu*1, Sorin Hostiuc1, Dan Dermengiu1, George Cristian Curcă2

Abstract: Hyperthermia, characterized by a core temperature of over 40°C, occurs when the body’s thermoregulatory mechanisms are no longer able to dissipate heat. From a clinical point of view hyperthermia can be of three main types: (1) a consequence of an imbalance between anti and pro-inflammatory agents (usually in septic conditions), (2) heatstroke and (3) malignant hyperthermia. Death caused by hyperthermia, either heatstroke or malignant hyperthermia, is diagnosed at the autopsy mainly using seric, histopathological and immunohistochemical methods. Even though unspecific morphological lesions are found in almost every organ, the most affected are skeletal muscles, gut, kidneys and brain. We present here a case of severe hyperthermia leading to heatstroke and discuss the main diagnostic methods available to the forensic pathologist.

Key words: hyperthermia, heatstroke, malignant hyperthermia, morphology, immunohistochemistry

Hyperthermia, characterized by a core temperature of over 40°C, occurs when the body’s thermoregulatory mechanisms are no longer able to dissipate heat, and is associated with variable clinical manifestations, depending on its type, degree and duration. From a clinical point of view hyperthermia can be of three main types: (1) a consequence of an imbalance between anti and pro-inflammatory agents (usually in septic conditions), (2) heatstroke and (3) malignant hyperthermia.

Heatstroke is defined by a body temperature above 40.6 degrees Celsius, associated with mental abnormalities like delirium, convulsions, or coma, caused by overexposure to environmental heat, is often associated with ethanol intake [1-3], and is characterized pathophysiologically by a systemic inflammatory response determined by the heat, with subsequent multi organ failure. A less severe form of heatstroke is heat exhaustion, characterized mainly by cardiovascular manifestations. Heatstroke can be associated or not with intense labor; if not it is considered a classic type of heatstroke, occurs mainly in the elderly or the very young, and is highly dependent on environmental conditions.[4] Exercise induced heatstroke appears often in workers, sportsmen or soldiers, and is often associated with other aggravating conditions – for example in workers a frequent aggravating condition is concomitant ethanol intake, in sportsmen is often associated with substance abuse, etc.

Malignant hyperthermia is a pharmacogenetic disease characterized by increased metabolic response to various drugs (usually volatile anesthetics or succinylcholine) or various stresses [5], usually associated with inherited myopathies (central core disease, multi-minicore disease, etc.). Almost all patients with a susceptibility for MH have no phenotypic changes in the absence of a trigger, making it almost impossible to diagnose it especially as, even when the trigger has acted, it has a highly variable onset and initial clinical symptoms. As cited triggers are, besides the type of anesthetic, age, degree of stress, mitigating drugs administered together, and environmental temperature [6, 7].

*1) Corresponding author; MD, PhD, National Institute of Legal Medicine “Mina Minovici”, Sos. Vitan Birzesti 9, Sector 4, 042122Bucharest, MD, Tel. +4021.332.50.08, Fax: +4021 334 62 60, E-mail: ceausu_mihai@yahoo.com
2) Associate Professor, MD, PhD, Chair of Legal Medicine, University of Medicine and Pharmacy “Carol Davila”

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Clinically, MH is mainly characterized by hyperthermia/hyperpyrexia, unexplained elevation of end-tidal carbon dioxide concentration, muscle rigidity, tachycardia, acidosis, hyperkalemia, highly increased CK, cola-coloured urine. In order to estimate the likelihood of a MH during anesthesia and the likelihood of MH susceptibility when a subject has a family susceptibility for MH, a consortium of experts developed a scale, presented in Table 1 [8]. According to these guidelines there are six MH ranks, as follows: 1 (score=0) – almost never associated with MH, 2 (score 3-9) – unlikely to be associated with MH, 3 (score = 10 – 19), somewhat less than likely to be associated, 4 (score = 20 – 34) – somewhat greater than likely, 5 (score = 35 – 49) – very likely, and 6 (score >50) – almost certain.

<table>
<thead>
<tr>
<th>Processes (green background) and Parameters</th>
<th>Score</th>
</tr>
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<tbody>
<tr>
<td><strong>Rigidity</strong></td>
<td></td>
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<tr>
<td>Generalized muscular rigidity (not associated with hypothermia or immediately following emergence from inhalational general anesthesia)</td>
<td>15</td>
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<tr>
<td>Masseter spasm shortly after succinylcholine administration</td>
<td>15</td>
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<tr>
<td><strong>Muscle Breakdown</strong></td>
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<td>CK&gt;20000 U/L (associated with succinylcholine administration)</td>
<td>15</td>
</tr>
<tr>
<td>CK&gt;10000 U/L (associated with anesthetics without addition of succinylcholine)’</td>
<td>15</td>
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<tr>
<td>Cola coloured urine in the perioperative period</td>
<td>10</td>
</tr>
<tr>
<td>Myoglobin in urine (&gt;60µg/L)</td>
<td>10</td>
</tr>
<tr>
<td>Myoglobin in serum (&gt;170 µg/L)</td>
<td>5</td>
</tr>
<tr>
<td>Blood/plasma/serum K+&gt;6mEg/L (in absence of renal failure)</td>
<td>3</td>
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<tr>
<td><strong>Respiratory acidosis</strong></td>
<td></td>
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<tr>
<td>PETCO2&gt;55 mmHg (appropriately controlled respiration)</td>
<td>15</td>
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<tr>
<td>Arterial paCO2&gt;60 mmHg (appropriately controlled respiration)</td>
<td>15</td>
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<tr>
<td>PETCO2&gt;60 mmHg (spontaneous respiration)</td>
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<tr>
<td>Arterial paCO2&gt;65 mmHg (spontaneous respiration)</td>
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<tr>
<td>Inappropriate hypercarbia (according to the anesthesiologist)</td>
<td>15</td>
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<tr>
<td>Inappropriate tachypnea</td>
<td>10</td>
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<tr>
<td><strong>Temperature increase</strong></td>
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<tr>
<td>Inappropriate rapid temperature increase (according to the anesthesiologist)</td>
<td>15</td>
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<tr>
<td>Inappropriate temperature increase over 38.8 degrees Celsius (according to the anesthesiologist)</td>
<td>10</td>
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<tr>
<td><strong>Cardiac involvement</strong></td>
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<tr>
<td>Inappropriate sinus tachycardia</td>
<td>3</td>
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<tr>
<td>VT or VF</td>
<td>3</td>
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<tr>
<td><strong>Family history of MH</strong></td>
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<tr>
<td>Positive in first degree relatives</td>
<td>15</td>
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<tr>
<td>Positive in other relatives</td>
<td>5</td>
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<tr>
<td><strong>Other indicators</strong></td>
<td></td>
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<tr>
<td>Arterial base excess more negative than -8 mEg/L</td>
<td>10</td>
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<tr>
<td>Arterial pH&lt;7.25</td>
<td>10</td>
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<tr>
<td>Positive family history+ another positive parameter (except elevated resting serum CK)</td>
<td>10</td>
</tr>
<tr>
<td>Resting elevated serum CK + patient history of MH</td>
<td>10</td>
</tr>
<tr>
<td>Rapid reversal of MH symptoms with dantrolene</td>
<td>5</td>
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</table>

*If more than one parameter is from the same process only count the one with the highest score (except the other process)*
*Orange indicators are only for MH susceptibility; they are added to the highest score obtained from the other parameters.*

MH is associated with a rapid increase of body temperature (1-2 degrees every five minutes), until 43-44°C, which leads to a marked oxygen consumption, increased CO₂ production, increased anionic gap, rhabdomyolysis with increased myoglobin and potassium serum levels, metabolic acidosis with subsequent MOSF.[9]. In almost all MH cases either a defective calcium channel (ryanodine receptor – RYR) on the sarcoplasmic reticulum membrane (most often) or a closely associated RYR protein (triadin, FK-506 bp) were identified [10-25].

Hyperthermia is mainly caused by increased heat retention or decreased heat dissipation. Decreased heat dissipation is associated with chronic alcoholism, elderly/very young, obesity, burns, drugs, absent heat acclimatization, humidity levels of more than 75%, drugs, etc. Increased heat retention
is associated with bed confinement, living on the top floor, thyrotoxicosis, malignant hyperthermia, drugs. Various classes of licit and illicit drugs have been associated with hyperthermia, either alone or in combination with other factors, by either increasing heat retention, decreasing heat dissipation or both: anticholinergic, succinylcholine, gaseous anesthetics, antihistamines, antidepressants (MAO, tricyclic), anti-parkinsonian, antipsychotics (phenothiazine, butyrophenone, thioxanthenes), cocaine, alcohol [26], amphetamines, phencyclidine, LSD, diuretics, etc [27].

Case Report

A 58 years old male was found on a shipway (working as a dyer in a summer day) in coma (GCS=3) with hyperthermia and was transferred to the emergency hospital with initial diagnosis of coma with unknown etiology. At admission patient was hyperpyretic (temperature of 41-42 degrees Celsius), with tonico-clonic convulsions, TA=130/85 mmHg, HR=85/min, O2 saturation=98-99%, pH=7.32, cBase=−2.3mmol/L, White cell count=6800mm³, haemoglobin= 13.9g/dl, Ht=42, platelet count=16000mm³, Na=130.2mmol/L, K=3.61mmol/L, BUN=34.6mg/dl, creatinin=0.66mg/dl, proteins=6.42g/dl, albumin=3.73g/dl, bilirubin (total)=2.45mg/dl, CK-MB=56U/L, ALT=175U/L, AST=465U/L, LDH=1885U/L, CK=306 U/L, PALK=92U/L, glycemia=190mg/dl, APTT=22.1 sec, NRI=1.9. Toxicological screening positive for bensodiazepines. After 13 hours ALT=510U/L, AST=1213U/L, INR=1.6, APTT=23.6, PT=17.8 sec, platelet count=19000/mm³, K=3.01 mmol/L, Na=135 mmol/L). Patient died the next day with a final diagnosis of asystole, hemodynamic insufficiency, acute hepatic insufficiency, haematological insufficiency, metabolic insufficiency, acute respiratory insufficiency, hyperthermia, coma of unknown origin.

Fig. 1 Common lesions in various organs (HE, 20x): a) lung with haemorrhagic focus and inflammatory exudate, b) focal hepatocytic necrosis on a steatotic background, c) mild fibrosis of the renal medulla accompanied by hyaline globules in convoluted tubules, d) microhaemorrhagic focus in adrenal medulla
Forensic autopsy was performed the next day and found during the macroscopical examination diffuse myocardial fibrosis, acute hepatitis, shock kidneys.

Microbiological examination found Acinetobacter and Cinetobacter sp. in blood and CSF with positive Pandy reaction (+++), white cell count = 350/mm³, 100% PMN’s, negative aerob and anaerob cultures, negative Gram stain. Toxicological examination was negative for drugs or alcohol.

Tissue samples of brain, lung, myocardium, liver, kidney, pancreas, spleen, adrenal glands and stomach were taken for histopathology investigation. The selected specimens were formalin-fixed and paraffin-embedded. Sections were cut at 3 μm and stained using the standard H&E and van Gieson. Special stains such as PTAH and PAS have been carried out. Immunohistochemistry (IHC) was done for myoglobin (DAKO, polyclonal, 1:50). An indirect bisladial technique with hydro soluble polymerized dextran, according to manufacturer specifications (Dako EnVision Systems) was used. To ensure the reliability of the experimental study, internal quality control of histopathological and IHC techniques were performed as a part of an implemented and certified quality assurance system (ISO 9001/2008). All slides were examined and photographed on a Zeiss AxioImager A1 microscope. Digital images acquired with Zeiss Axio Vision program have been processed and analyzed with ACDSee Pro Photo Manager 3.0, running under Windows Vista.

Classical microscopic examination has shown diffuse alveolar haemorrhage associated to bronchopneumonic foci in lung, moderate sclero-lipomatosis of the heart and coronary atherosclerosis, hepatocytic medio-lobular necrosis foci on a steatosis background, and micro-haemorrhagic foci in adrenal medulla (fig. 1). In kidney were found moderate nefroangiosclerosis, mild sclerosis of the renal medulla, hyaline globules in renal convoluted tubules and rare tubular necrosis. Frequent granular proteic aggregates staining intense to myoglobin were noticed in quite many convoluted renal tubules (fig. 2). PTAH stain was negative in lung, kidney and liver.

Fig. 2 Proteic aggregates in convoluted tubes staining intense for myoglobin, IHC, 40x
Discussions

Prolonged exposure to elevated ambient temperatures can result in heat cramps, heat exhaustion and heat stroke. Heat stroke is associated with high ambient temperatures and high humidity. Thermoregulatory mechanisms fail, sweating ceases and core body temperature rises. Arrhythmias, disseminated intravascular coagulation, necrosis of the muscles and myocardium may occur[28].

Rhabdomyolysis is characterized by rupture and necrosis of striated muscle cells, which can be caused by trauma or electrolyte abnormalities in hyperthermia conditions. If rhabdomyolysis is extensive, myoglobin released into the circulation can produce acute renal failure[29]. The mortality rate for such patients exceeds 50%. Although direct muscle injury remains the most common cause of muscle injury, other causes include hereditary enzyme disorders, drugs, toxins, endocrinopathies, neuroleptic malignant syndrome, heatstroke, diabetic ketoacidosis, hyperosmolar coma, severe hyperthyroidism and bacterial or viral infections.

Physiopathologically heatstroke has four main types of effects: acute physiological changes, citotoxic effects, generalized inflammatory response, and oxidative damage. In a high temperature environment circulatory flow is redirected towards the skeletal muscle and skin, in order to dissipate heat; if concomitant conditions affecting the cardiocirculatory system or insufficient hidratation are present, acute cardiogenic shock may occur leading to intracranial hypertension, cerebral hypoperfusion, cerebral ischemia and neuronal injury, arterial hypotension, multi organ ischemia, etc, with subsequent increased levels of serum dopamine, tryptophane, glutamine, citokines, NO, glycine, peroxids, etc.[4, 30-37].

At a cellular level heat stress leads to inhibition of DNA synthesis and transcription, RNA splicing and translation, increased protein degradation and aggregation, cell cycle inhibition, inhibition of ribosomal formation, citoskeletal alterations, metabolic changes, altered hsp response, alteration of membrane permeability and ionic equilibrium.[4, 21, 38]. Hyperthermia also leads to an imbalance between proinflammatory and anti-inflammatory factors leading to either inflammatory damage or immunosupression (with a high frequency of infections).

Ethanol consumption is known to exacerbate heat-related pathology as it induces sedation, anesthesia, a hypnotic state, impaires judgement, diminishes thermoregulation, leads to increased lactaacidemia, etc. Even if in our case a toxicological screening for alcohol was negative, possibly determined by the time passed from the incident until the autopsy, the patient was known to be a chronic ethanol addict.

Main cause of death in heatstroke is multi-organ dysfunction, with cerebropathy, acute respiratory distress syndrome (ARDS), dissolution of striated muscle, acute renal failure, cardiac insufficiency, hepatic injury, or hematopenia, (especially thrombocytopenia) pancreatic damage, or hemorrhagic diseases [4, 39], all present in our case.

Death caused by hyperthermia, either heatstroke or malignant hyperthermia, is diagnosed at the autopsy mainly using seric, histopathological and immunohistochemical methods [40]. Tanatochemistry reveals marked increases of creatin-kinase (levels above 12000 U/L are common), especially the MM isoform, catecholamines, and myoglobin (with increased myoglobinuria). Other markers, even if not used in normal circumstance, can bring important additional information, especially if a differential diagnosis is needed. Tryptase, an enzyme mainly stored in mast cell secretory granules, is known to be a very sensitive marker for anaphylaxis [41], but other causes may rise its levels at well – myocardial infarction, heroin intoxication, multiple trauma [42] or hyperthermia [43, 44]. Mean value of postmortem seric tryptase is about 7.5 +/- 4 ng/ml [44] whilst in suspected hyperthermia is over 60 ng/ml – Nishio, in three cases of suspected hyperthermia found the following values – 68.9, 69.4 and 179.68 ng/ml [44]. Its sensibility for hyperthermia is however limited – only three from five presented cases had increased tryptase levels [44]. C Reactive protein were found to be lower in hyperthermia(mean value found was 0.15 mg/dL whilst reference values are (<0.3 mg/dL).[45] Creatinine levels are markedly elevated in hyperthermia (mean value 3.90 mg/dL, reference values 0.61–1.04 mg/dL for adult males, and 0.47–0.79 mg/dL for adult females) [45]

All organs show unspecific gross or histological alterations: skin (hyperemia, edema, hamorrhages), lungs (hyperemia, alveolar lung edema, clots containing myoglobin in small lung vessels [46], alveolar hemorrhages), spleen (red pulp congestion), bone marrow (hyperemia, edema, hemorrhages) [47], liver: edema, fatty degeneration, disseminated liver necrosis [46].

The most affected organs are however skeletal muscles, gut, kidneys and brain. CNS is affected directly by the heat and indirectly, secondary to blood redistribution towards skin and muscles, resulting
neuronal necrosis, meningeal edema, and hyperemia. A few markers for hyperthermia lesions in the brain were studied: chromogranin A (CgA) for example, a protein highly distributed in secretory granules of neuroendocrine and endocrine cells, has a positive immunoreaction in the hypothalamus able to differentiate hyperthermia from hypothermia [48]; CgA is also increased in CSF, and seric CgA levels are positively correlated with seric catecholamines (adrenaline, noradrenaline, dopamine). Increased immunopositivity for Ubiquitin in midbrain periaqueductal grey matter was also positively correlated with hyperthermia; the reaction is not specific however as increased immunoreactivity was also determined in delayed head injury deaths, blunt injuries, fire fatalities, and drowning in saltwater (in freshwater nevertheless the immunoreactivity was significantly lower)[49].

Renal examination reveals shock kidneys, myoglobin eosinophilia casts, especially in the distal convoluted and collecting tubules [46]. Extensive muscle damage leads to increased myoglobinemia and myoglobinuria and is known to be associated with immunopositive reaction for myoglobin in renal tubules. In cases of advanced tubular renal damage, often associated with hyperthermia, myoglobin immunopositivity is low, and is associated with a low ubiquitin immunopositivity.[50] If myoglobin immunoreactivity is low due to extensive renal damage, hsp70 can be used as an alternate marker [51, 52].

Skeletal muscle. Gross examination reveals pale and edematous skeletal muscles [53]. Histological examination finds hypercontraction bands, fiber degeneration, focal necrosis [46], variation in muscle fiber diameter [54]. Ultrastructurally hyperthermia associates diffuse nuclear internalization, tubular aggregates, convoluted sarcolemma rolls, swollen mitochondria, dilated cisterns, Z-line streaming [55]. IHC for NADPH-R and ATP-ase exposes a moth-eaten appearance (or central cores) in more than 64% of type I muscle fibers, and less for type II fibers [56].

Desmin, actin and myoglobin staining is less intense than in myocardial fibers [57], whilst a granular pattern of thrombomodulin is sometimes found in the acute phase of malignant hyperthermia [58]. All are however more suggestive for rhabdomyolysis than for MH. Moth-eaten appearance (MEA) and central cores (CC) are however characteristic lesions for malignant hyperthermia; MEA is represented by an irregular area of pallor in type I fibers, determined by a partial loss of mitochondria whilst CC are sharply defined areas of pallor, determined by a complete loss of mitochondria [53, 56]. Both are only detected by histochemistry/IHC for oxidative enzymes and are more obvious in type I than type II fibers as the first ones are less resistant to anaerobiosis [55]. Malignant hyperthermia myopathy is characterized by focal hypertrophy, segmental necrosis, dimensional variation, internal nuclei, MEA or CC, hypercontracted bands.

Susceptibility for MH is diagnosed using the following criteria: myofibrillar necrosis, muscle fiber atrophy, muscle fiber hypertrophy, and diffusely distributed internal nuclei (minimum 3%) [55]. The presence of all four elements is found in 35% of patients with a susceptibility for MH. Myocardial fiber lesions are not characteristic. Usually we can find hypercontraction bands, sarcolemmal disruption, fiber fragmentation, subepicardial, pericardial and subendocardial petechiae, or edema.

Gut lesions. In acute deaths after hyperthermia splanchnic hypoperfusion leads to gut edema with or without petechial hemorrhages, more preeminent on the mucosal than on the serosa layer, and on the iliac and colic regions than on the stomach. In deaths related to hyperthermia not occurring immediately after the traumatic event, splanchnic hypoperfusion is followed by gut ischemia, increased intestinal permeability, bacterial translocation, and subsequent anoxic villi injuries, leading to massive hemorrhages from the mucosal and serosa layer, in all bowel regions (and stomach), sometimes associating hemoperitoneum [59].

Conclusion: hyperthermia/heatstroke can be easily diagnosed when a positive clinical history is associated with signs of focal necrosis in the skeletal muscle, gut ischemia lesions, myoglobin containing clots in small blood vessels, and tubular myoglobin excretion.

References