

## Appendix.

*Case 1.* This young boy developed severe muscle cramps and aching during school sport; after approximately 30 min of sports training he had signs of rhabdomyolysis indicated by dark-colored urine. Furthermore, he developed recurrent elevations (1 – 2 times per month) of body temperature up to 40.0°C. Therefore, he was investigated by pediatricists and neurologists, but no pathological findings regarding the muscular symptoms and the temperature increases were found. A muscle biopsy for evaluation of susceptibility to MH and histology was performed in 1991. With the IVCT the boy was diagnosed as susceptible to MH. Furthermore, this patient carried a C487T mutation, which is associated with the MH phenotype. After diagnosis of MH susceptibility we recommended him to reduce duration and intensity of exercise. In the following years his condition improved, but he had to avoid extreme physical exercise up to now.

*Case 2.* The patient took part in a strong march of 8-km during his military service. He collapsed during the march followed by reduced consciousness for several hours. He was immediately transferred to the next hospital. The clinical examination showed signs of dehydration and neurological disorders: heart rate was 160 bpm, blood pressure was 85/60 mm Hg, stretching cramps and eye deviation, and a body temperature of 41°C. Laboratory investigation on admission showed the following values: leucocytes 12.4/nl, serum glutamic oxaloacetic transaminase (SGOT) 27 U/l, serum glutamic pyruvic transferase (SGPT) 18 U/l, gamma-glutamyl transpeptidase (GGT) 65 U/l, lactate dehydrogenase (LDH) 375 U/l, alpha-hydroxybutyrate dehydrogenase (HBDH) 222 U/l, CK 642 U/l and K<sup>+</sup> 5.4 mmol/l. The urine was dark colored. Control of laboratory investigation on the next day showed increasing values of SGOT 1565 U/l, SGPT 1335 U/l, GGT 962 U/l, LDH 2870 U/l, HBDH 280 U/l, CK 19.040 U/l and myoglobin in urine. During the following days the patient revealed completely, the elevated laboratory results came back to normal values and no further complications occurred. Cerebrospinal fluid diagnosis, computed tomographic scan of the head and electromyography (EMG) did not show any pathological results. The patient was discharged 10 days later without any neurological or other impairment. Six months later we investigated him in our MH laboratory and he was diagnosed as MHEh according to the European MH Group criteria.<sup>13</sup>

*Case 3.* Patient No. 3 complained of recurrent episodes of myalgia following physical exercise over several years. Three days after a bicycle tour of approximately 10-km distance he had bilateral muscle pain and cramps in the thighs and the CK value was increased up to 7.000 U/l. The patient was examined by a neurologist, who found pseudohypertrophy of the calf and strong thigh muscles. EMG of the M. gastrocnemius showed pathological potentials, whereas the M. vastus lat. showed normal findings. Under suspicion of a metabolic myopathy or a dystrophinopathy a muscle biopsy was performed. The results of the histological examinations were normal, but all test substances produced abnormal contractures in the IVCT indicating MH susceptibility.

*Case 4.* This patient had made a bicycle tour of 20-km distance. After this tour he complained of muscle aching of his lower extremities and his urine became dark-colored. Two days later a CK value in serum of 1.792 U/l was measured suggesting a higher value directly after the bicycle training. The patient was admitted to the neurologic department of our hospital. However, neither the neurological examination nor the EMG showed pathological signs. Therefore, he was admitted to our MH consultation hour and a muscle biopsy was performed. Histological examination showed only internal nuclei within the muscle fibers, in the IVCT the muscle specimen developed abnormal contracture responses.

*Case 5.* Following training in a body building center in the year 1989, the patient developed dark-colored urine for several days indicating myoglobinuria. However, at this time no laboratory investigations such as CK or myoglobin were measured. His past medical history was uneventful and he had two general anesthetics for rhinoplasty without any complications. Prior to an anesthesia in our hospital, this patient reported of this event and therefore a neurological examination was indicated, which showed no pathological results. The neurologist recommended histological examination to exclude a myopathy, and it was decided to perform additionally an IVCT on this muscle probe. This patient was tested as MHS, muscle histology revealed non-specific alterations (hypertrophy of muscle fibers type II).

*Case 6.* Patient No. 6 had an uneventful medical history, there was no history of muscle diseases or of anesthetic complications within the family of the patient. The patient himself has had no anesthesia prior to the exercise-induced rhabdomyolysis. Following body building with weights of approximately 40 kg and duration of 45 – 60 min the young man developed recurrent episodes of muscle aching. Following one of these episodes a CK of 1.470 U/l was measured and the patient was transferred in our hospital for examination of a neuromuscular disease. Neurological examinations showed overall normal results, therefore the patient was biopsied for histological investigations and IVCT. Whereas muscle histology was normal, the IVCT showed abnormal contracture responses to halothane, caffeine and ryanodine.

*Case 7.* This patient was admitted to the neurologic consultation hour because of recurrent muscle aching and cramping after mild exercise like walking or ball playing. After a bicycle tour of a short distance two years before admission, he developed extreme muscle pain and cramping, which recurred three to four times a day for the next two weeks. During this time the urine was dark-red colored.

Measurement of laboratory parameters showed increased calcium and phosphate values in the urine, and decreased calcium values in serum indicating a hypoparathyroidism. Neurologic examination showed a pathological EMG and an abnormal lactate increase in a standardized forearm muscle exercise test. Histological tests showed only minimal muscle fiber type II atrophy. Therefore, it was decided to perform also an IVCT, which resulted in abnormal contracture responses to all test agents.

*Case 8.* After jogging of approximately 5-km distance, the patient had muscle aching and dark-colored urine. One day later CK was 2.644 U/L and lactate dehydrogenase 327 U/l. Two months after this event the patient came to the neurologic department, however, all investigations showed normal findings. Therefore, muscle biopsy was performed for histological and in vitro contracture tests. Results of the histological examination were without any pathological findings. Furthermore, the IVCT showed overall normal results. Therefore, the reason for rhabdomyolysis remained unclear in this case.

*Case 9.* In this patient elevated CK values of 200 – 300 U/l were found during a routine examination. Two months later he complained of muscle cramping and aching following mild sports training. After one training hour he developed more severe muscle problems and a CK was measured which showed a value of 800 U/l. The neurological examination and EMG showed no pathological findings and a muscle biopsy for histological examinations and the IVCT was recommended. Prior to his admission the patient had three uneventful general anesthetics. Muscle histology showed hypertrophy of skeletal muscle fibers type I and II, the IVCT showed pathological contracture responses, indicating MH susceptibility.

*Case 10.* Following working on weights in a fitness studio this patient complained about muscle cramping and aching, the urine became dark-colored and the CK in serum was 1.450 U/l. Neurologic examination showed a normal EMG, the results of a standardized forearm muscle exercise test were also normal. Additionally, a magnetic resonance tomography of the lower extremities was performed without any pathological findings. Muscle histology showed normal results, whereas the patient was diagnosed as MHS with the IVCT.

*Case 11.* This 34-year-old male manifested recurrent elevations in body temperature up to 40°C, and fatigue associated with muscle cramping and aching in response to mild exercise or emotional stress. These symptoms disappeared spontaneously after several hours of rest. The patient had noted first signs of a reduced stress tolerance 10 years earlier. Since that times the above exercise or emotional stress associated symptoms have increased in severity and occurred more frequently. Prior examinations from internists, neurologists, psychiatrists and dermatologists did not reveal pathological findings. All blood parameters were within normal limits and no signs for autoimmune diseases were found. Allergies and cardiac disorders were ruled out. There were no signs for chronic or acute infections. The patient did not take any medication, drugs and/or alcohol on a regular basis. There was no history of muscle diseases or of anesthetic complications within the family of the patient. Because his clinical episodes resembled exercise-induced MH, a muscle biopsy for IVCT and muscle histology was taken. IVCT showed abnormal contracture responses, whereas muscle histology was normal. Furthermore, genetic mutation screening led to the discovery of the G7297A mutation, which is associated with MH.<sup>28</sup>

*Case 12.* The young boy exhibited signs of severe muscle aching first during school sport and soccer training. The episodes of these symptoms occurred more frequent and more intense over a time period of three years, and he suffered from rhabdomyolysis indicated by dark-colored urine after an exhausting training practice. However, CK values as well as myoglobin were not measured at this time. He was admitted to the neurological department, but none of the investigations (EMG, etc.) showed pathological results. Therefore, it was decided to perform a muscle biopsy for histological examination and an IVCT. Histology revealed a muscle fiber type II hypertrophy, the IVCT was positive indicating MH susceptibility. Furthermore, this patient carried a G1021A mutation, which is associated with the MH phenotype.