Muscle Enzymes & Serum Markers

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Muscle Biopsy

If patient is free of muscle weakness, muscle biopsy is unlikely to show any significant changes!

Muscle Selection

*Myopathic processes do not affect all skeletal muscles equally!* → risk of sampling error

1. **degree** of muscle involvement
   * avoid clinically ***unaffected*** muscle (may not be involved pathologically).
   * avoid ***severely affected*** muscle (may only show *endstage* features - atrophy, fat, fibrosis).

Muscles that are ***moderately weak*** should undergo biopsy

* use Medical Research Council (MRC) strength grading and/or electrodiagnostic testing.
* best is muscle with MRC grade 4/5 strength vs. muscle with MRC grade 3/5 strength is often too severely affected, with extensive non-specific end-stage changes (up to lack of muscle fibers).

1. **rapidity** of onset of disease process
   * slowly progressive disorder - use moderately affected muscle.
   * acute disorder – use more severely affected muscle.
2. **muscle's history** - chosen muscle should neither be involved with *another disease process* (e.g. neuropathy) nor have suffered recent (i.e. within 1 month) *injection* or *needle EMG*.

needle electrode examination should be performed on only one side of body (and this should be clearly labeled in chart!) - homologous muscle in contralateral extremity is sampled.

1. **pathological familiarity** of muscle
2. **accessibility** of muscle
3. **EMG** findings.
4. **MRI** findings.

MRI of the lower limbs in the case of a toxic myopathy. T2 images show asymmetric involvement affecting only the left lower limb. Had a muscle biopsy been taken from the right gastrocnemius, the pathologic tissue would have been missed.

An external file that holds a picture, illustration, etc.
Object name is nihms398121f2.jpg

Most frequently biopsied muscles:

*lower extremity* - **quadriceps** (e.g. vastus lateralis), **tibialis anterior**; avoid gastrocnemius (type 1 muscle fiber predominance, greater susceptibility to random pathological changes, pennate nature\*); **peroneus brevis**, located in close proximity to the superficial peroneal nerve, is a favored biopsy site when a nerve biopsy is also indicated.

\*inadvertent sampling near myotendinous junction can occur (tends to have more central nucleation, muscle fiber size variability, and split muscle fibers); however, **gastrocnemius** and **tibialis anterior** muscles are appropriate choices in diseases with distal limb signs and symptoms

*upper extremity* - **deltoid** and **biceps** **brachii**; deltoid muscle normally has 60-80% predominance of type 1 fibers.

N.B. pathologist will need to be informed about biopsy site - muscles vary in their normal ratio of type I to type II fibers making this information necessary

Technique

Open Biopsy

* carefully *avoid muscle infiltration* during local anesthesia.
* small incision in belly region (i.e. away from myotendinous junction) along long axis of muscle; incision is extended only to fascia.
* **quadriceps** has a thick fascia.
* sectioned fascia should be sutured to prevent muscle herniation - chronic nuisance for the patient.
* site is wrapped with an elastic wrap, a light pressure for a few hours.
* no follow-up visits after the muscle biopsy are normally necessary.

**Specimens**

**Fixed** specimens should be shipped separately from **frozen** specimens!

include: patient's name, sampled muscle, procurement time, brief note (detailing clinical presentation and workup findings to date + list of pending studies).

1. Unclamped specimen for **histochemistry** (most important piece)
   * 2-3 cm in length; about as round as pencil.
   * handle gently by its ends using tweezers.
   * place in cool, normal saline-moistened piece of gauze to prevent drying out (soaked gauze may interfere with freezing and produce artifacts).
   * gauze-wrapped specimen is placed in screw-cap vial.
   * transported rapidly to pathology laboratory (otherwise it may lose enzymatic activity) → ***freezing*** by immersion in liquid nitrogen-cooled isopentane → immediately placed in previously cooled specimen container → sent to outside reference laboratory.
2. Clamped specimen for **electron microscopy**
   * can be slightly smaller.
   * gently raised (e.g. with Metzenbaum scissors) just high enough to permit placement of muscle clamp.
   * clamp is locked → muscle specimen cut just *outside* clamped sites.

Clamping helps avoid contraction artifact.

* + alternative - suturing muscle tissue specimen (e.g. with 3-0 silk) to piece of tongue depressor before excising it (i.e. it is sutured in situ).
  + once removed, specimen is placed in 4% ***buffered glutaraldehyde*** (or ***Karnovsky fixative***) → embedded in plastic for electron microscopy.

N.B. EM is important in only certain diseases - congenital myopathies, mitochondrial disorders.

1. Clamped specimen for **histopathology** - similar to specimen for electron microscopy, with exception that it is fixed in ***formalin*** → embedded in ***paraffin*** for light microscopy.
2. Fourth specimen is ***frozen*** in event further studies are deemed necessary.

When **specialized studies** are planned (e.g. mitochondrial DNA studies), larger tissue specimens may be necessary!

Needle Biopsy

Advantages of open biopsies - larger specimen can be obtained, specimen can be fixed at its in situ length (preventing contraction artifact).

Advantages of needle biopsy - limited scarring, ability to sample multiple sites (in either same or different muscles) in single session.

Disadvantages of needle biopsy - smaller specimen size, greater orientation difficulty.

Diagnostic Staining Methods

|  |  |
| --- | --- |
| **STAINS** | |
| Hematoxylin & eosin (H & E) | Hematoxylin: nuclei, cross-striations (purple)  Eosin: cytoplasm (red), connective tissue (darker red) |
| ***general morphology*** |
| Modified Gomori trichrome | Nuclei, mitochondria, T-tubules, sarcoplasmic reticulum (red); myocytes (blue-green) |
| identifying ***ragged-red fibers***. |
| Periodic acid-Schiff (PAS) | **Glycogen** (purple; type 1 > 2; ***glycogen storage disorders***) |
| Oil red O | **Lipid** (orange; type 1 > 2; ***lipid storage disorders***) |
| Sulfonated Alcian blue | stains ***amyloid*** ("sea foam" green), **mast cells** (red). |
| Alkaline Congo red | stains ***amyloid*** (red; apple green birefringence under polarized light). |
| **REACTIONS** | |
| NADH-TR (NAD dehydrogenase-tetrazolium reductase) | oxidative enzyme - reflects concentration of **mitochondria**; also **T-tubules**, **sarcoplasmic reticulum** - sarcoplasm appears granular. |
| Type 1 (dark); type 2A (intermediate); type 2B (light)  N.B. atrophied type 2 fibers appear darker! |
| Succinate dehydrogenase | Krebs' cycle enzyme - selective stain for **mitochondria**; tubular elements are not highlighted. |
| Cytochrome-c oxidase | respiratory chain enzyme (orange-brown; type 1 > 2) - selective stain for **mitochondria**; tubular elements are not highlighted. |
| Myofibrillar ATPase | - most accurate method of muscle fiber typing: |
| ATPase (at pH 4.3)  ATPase (at pH 4.6)  ATPase (at pH 9.4) | type 1 (dark); type 2A, B (light); type 2C (intermediate)  type 1 (dark); type 2A (light); type 2B, C (intermediate)  type 1 (light); type 2 (dark) |
| Acid phosphatase | lysosomal enzyme- ***degeneration*** (stains red; background fir green), inflammatory cells, ***lysosomal storage disorders*** |
| Alkaline phosphatase | ***regeneration*** (stains black; background yellow) |
| Nonspecific esterase | acetylcholinesterase (yellow-red; type 1 >2) - endplates, lysosomes, macrophages, recently (i.e. within 6 months) denervated muscle fibers (appear smaller and darker). |
| Immunologic techniques | stain proteins that are deficient in some ***muscular dystrophies***. |

Normal findings

* cross section - muscle fibers appear polygonal and their diameters vary (within given section, they are somewhat uniform).
* ***intermyofibrillar pattern*** (best demonstrated with reactions for oxidative enzymes) should appear uniform.
* muscle fibers of different motor units are interspersed - normal muscle shows *checkerboard pattern* of light and dark fibers.

Atrophy

* 1. **Denervation atrophy**:
     + ***decrease in cell size*** (down-regulation of myosin and actin synthesis, resorption of myofibrils), but cells remain viable.
     + atrophic fibers in cross-section have roughly triangular shape ("***angulated***").
     + some fibers develop cytoskeletal reorganization - rounded zone of disorganized filaments ("***target fiber***").
     + while Acch receptors are normally located in center of length of muscle fibers, after denervation, fibers develop supersensitivity throughout their course.
     + during reinnervation, *checkerboard pattern* of type 1 and type 2 fibers is altered - fibers of same staining type are grouped (due to collateral sprout-related reinnervation); adjacent atrophied myocytes are of same fiber type ("**fiber type grouping**").
     + with reinnervation, motor fibers reform neuromuscular junctions at original end plates.
     + if fibers are not reinnervated within ≈ 20 months, they will be replaced by connective tissue.
  2. **Disuse atrophy** - *checkerboard arrangement* is maintained; mostly affected are type 2 fibers.

Preferential atrophy:

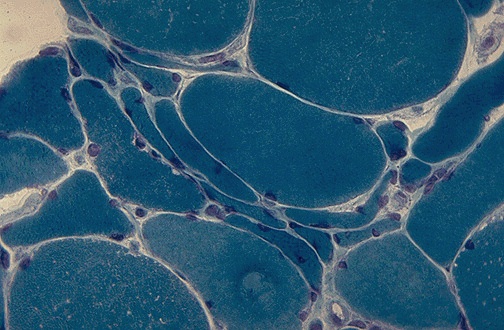
**Type 1 fibers**: myotonic dystrophy (prominent), nemaline myopathy, centronuclear myopathy, congenital fiber type disproportion.

**Type 2 (especially 2B) fibers**: disuse, corticosteroid excess (exogenous, endogenous).

**Perifascicular atrophy** (fibers near edges of fascicle are atrophied) - hallmark of ***dermatomyositis***.

**Panfascicular atrophy** - indicative of ***Werdnig-Hoffmann disease*** (spinal muscular atrophy type I).

Typical "grouped atrophy" with denervation:



[Source of picture: “WebPath - The Internet Pathology Laboratory for Medical Education” (by Edward C. Klatt, MD) >>](http://library.med.utah.edu/WebPath/webpath.html" \t "_blank)

Cytoarchitectural Abnormalities

Preferential involvement:

**Type 1 fibers**: target fibers, central cores (central core disease), rod bodies (nemaline myopathy), mitochondrial abnormalities.

**Type 2 fibers**: tubular aggregates.

**Target fibers** (cardinal feature of ***neurogenic disorders***)

* + predominant among type 1 fibers.
  + composed of three "rings":
    1. central light-staining ring
    2. intermediate dark-staining ring
    3. peripheral normal-staining ring.

**Central cores** (***central core disease***)

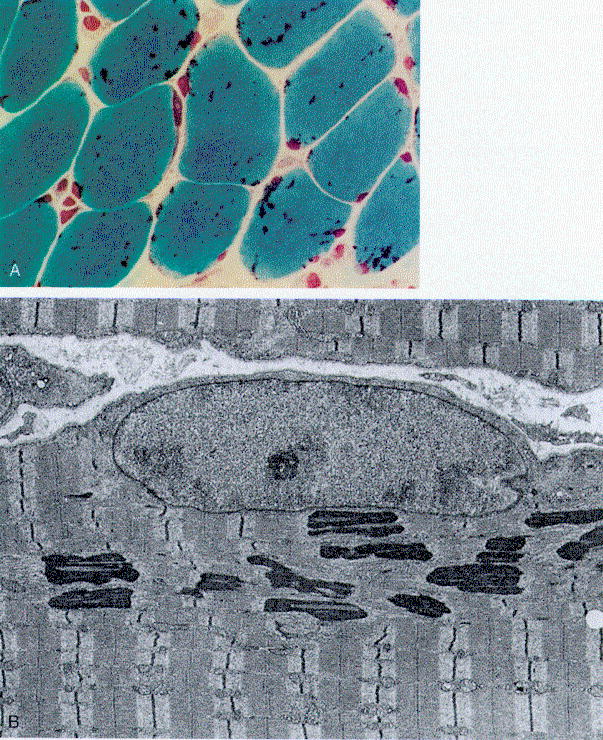
* + only in type 1 fibers, which usually predominate.
  + central core - amorphous area in center\* of fiber – ***devoid of enzymatic activity***, ***lacks myofibrils and mitochondria*** - does not stain for NADH-TR, glycogen, but sometimes stains with ATPase; stains blue with Gomori trichrome stain.

\*surrounded by normally staining periphery.

* + central cores resemble target fibers, but cores run whole length of fiber.

**Rod bodies** (***nemaline myopathy*** - numerous subsarcolemmal rod bodies in many muscle fibers; small numbers of rod bodies may be found in muscular dystrophy, polymyositis, HIV-related myopathy, muscle injured by tenotomy).

* + spindle-shaped threadlike appearance (Gr. *nema* – thread).
  + predominantly, but not exclusively, in type 1 fibers.
  + reddish purple in modified Gomori trichrome stain; difficult to demonstrate with conventional H&E stain.
  + electron microscopy shows that rods represent abnormal deposition of ***Z-band material*** (α-actinin).



**Ragged-red fibers** (***mitochondrial myopathies***)

* subsarcolemmal\* collections of ***mitochondria*** (enlarged, bizarrely shaped, with paracrystalline “parking-lot” inclusions).

\*with severe involvement, may extend throughout fiber.

* mitochondria distort muscle fiber contour (irregular on cross-section – “ragged”).
* stain red with modified Gomori trichrome stain.



**Tubular aggregates** (frequently seen with ***hyperkalemic periodic paralysis***) - faintly basophilic deposits in both interior and periphery of muscle fibers.

* + ***sarcoplasmic reticulum***-derived collections.
  + ultrastructure - fascicular arrays of parallel double-walled 60-90 nm tubules with hexagonal array in transverse section.
  + stain red with modified Gomori trichrome stain.
  + demonstrated with NADH-TR but not highlighted by succinate dehydrogenase (vs. mitochondrial aggregates)!

**Rimmed vacuoles** (inclusion body myopathy, oculopharyngeal muscular dystrophy, distal myopathy, denervation)

* blue margins with H&E; red margins with modified Gomori trichrome stain.

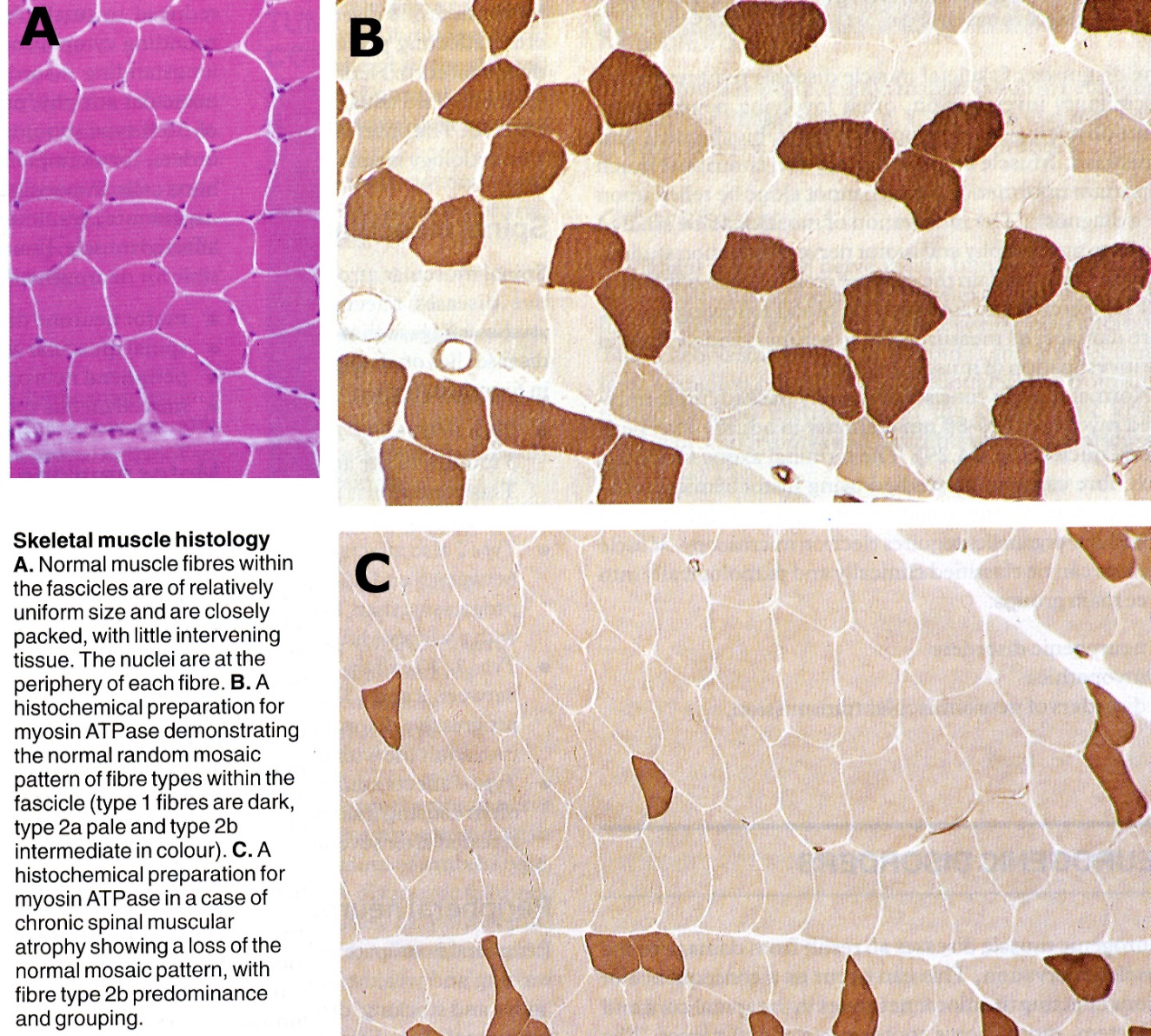
Differentiating MYOPATHIC and NEUROpathic changes

Neuropathic Processes

Most typical attribute is atrophy!

1. small **angulated** (in cross section) **fibers** - may be earliest sign!
   * not selective for fiber types, scattered throughout specimen.
   * denervated fibers appear darker (e.g. nonspecific esterase).
2. **fiber type grouping** (sine qua non of reinnervation) - enlarging groups of contiguous fibers of same type due to collateral sprouting reinnervation → diminished normal checkerboard staining pattern.
   * must be distinguished from fiber type predominance.
3. **grouped atrophy** (hallmark of ***chronic denervation***) - atrophy of these reinnervation groups.
   * extreme version of grouped atrophy is panfascicular atrophy (in ***Werdnig-Hoffmann disease***).
4. target fibers
5. nuclear bags
6. minimal interstitial fibrosis.

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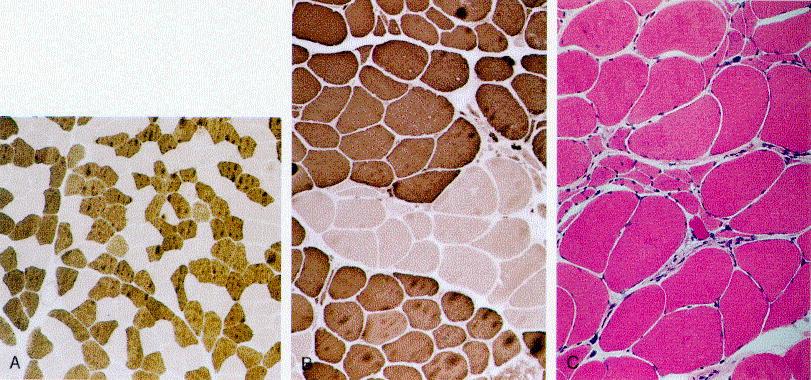


ATPase histochemical staining, at pH 9.4:

**A**. **normal muscle** showing checkerboard distribution of intermingled type 1 (light) and type 2 (dark) fibers.

**B**. **muscle reinnervation** - fibers of either histochemical type are grouped together.

**C**. **group atrophy** - cluster of atrophic fibers in center.



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[see p. A46 (5a) >>](http://www.neurosurgeryresident.net/A.%20Neuroscience%20Basics\A45-50.%20Spinal%20Cord\A46%20(5a).%20Spinal%20Cord%20-%20somatic%20motor%20system%20(motor%20units).pdf)

Myopathic Processes

1. **random fiber loss** (vs. loss of whole motor unit territories).

* if portion of muscle fiber is degenerated (segmental necrosis), muscle fiber functions as two separate fibers - portion with motor endplate (i.e. innervated fiber) and portion without it (i.e. denervated fiber).
* precursor (satellite) cells can regenerate destroyed portion.
* denervated portion can be adopted by collateral sprouting → small foci of fiber type grouping

N.B. small patches of fiber type grouping should not be considered synonymous with neuropathic process!

* not reinnervated fibers undergo degeneration → extensive collagen deposition and fatty infiltration.

1. **central nucleation** - centrally located nuclei (normally observed in < 3% normal muscle fibers).

* especially prominent in ***myotonic dystrophy***.
* *single* (para)central nucleus in every myocyte - ***centronuclear myopathy***.

1. **rounded fibers**
2. **fiber size variability** - combination of atrophy and hypertrophy.
3. **fiber necrosis** (degeneration)
4. cellular **infiltration with myophagocytosis**
5. ***perivascular*** collections - collagen vascular disorder, dermatomyositis
6. most pronounced ***intracellularly*** - facioscapulohumeral dystrophy.
7. **fiber regeneration** - basophilic sarcoplasm (rich RNA content), large internalized nuclei with prominent nucleoli.
8. **fiber splitting** (normally occurs near myotendinous junctions - muscle biopsies from this region may appear myopathic!) - large fibers divide along segment so that, in cross-section, single large fiber contains cell membrane traversing its diameter, often with adjacent nuclei.
9. various **structural changes** (e.g. rod bodies, central cores, ragged-red fibers, vacuoles).
10. **microorganisms** (e.g. toxoplasmosis, trichinosis).

Active myopathic process - muscle fiber necrosis, basophilia, myophagocytosis.

Chronic myopathy - muscle fiber splitting and fibrosis.

Serum Markers

Many diseases of motor unit may not cause elevated enzymes!

**Creatine phosphokinase** (CPK or CK)

- lysosomal enzyme released by damaged / degenerating muscle fibers.

* found in only three organs – different isozymes:

**MM** for skeletal muscle

**MB** for cardiac muscle

**BB** for brain.

N.B. in differential diagnosis, *isoenzyme study is not helpful* - appearance of "cardiac isoenzyme" MB does not necessarily implicate heart when there is limb weakness!

* normal maximum is 50 units.
* characteristically elevated in certain diseases and magnitude of CK increase is characteristic for particular diseases:

1. very high levels (at least 20 times normal) – ***dystrophinopathies***; attacks of ***myoglobinuria***.
2. high levels - interictal ***phosphorylase deficiency*** or ***acid maltase deficiency***; men with ***nonvacuolar form distal myopathy***, dermatomyositis, polymyositis,
3. some ***spinal muscular atrophies*** (esp. Werdnig-Hoffmann disease, Kugelberg-Welander syndrome, ALS) – usually < 500 U.
4. **normal people**:
   1. *idiopathic hyperCKemia*, some African individuals
   2. for days *after strenuous voluntary exercise*!
   3. generalized motor *seizure or tetany*
   4. minor muscle *trauma* (e.g. EMG).

**Other sarcoplasmic enzymes** (AST or SGOT, ALT or SGPT, LDH) – increased in myogenic disorders together with CK, but less sensitive than CK.

Elevated AST and ALT → differentiate between:

**hepatic disease** → liver-specific enzyme GGT

**muscle disease** → muscle-specific enzyme CK

**Creatinine**↓ - useful indicator of diseased muscle mass.

Serum **myoglobin** has same diagnostic significance as serum **CK**.

Urinary Markers

**3-methyl His** - quantitative measurement of ***muscle breakdown***.

* some of His residues of actomyosin complex are methylated after their incorporation.

Quantitative **creatinine** excretion – index of ***muscle mass***.

* requires meat-free diet.
* must be done over period of ≥ 72 hours.

Bibliography for ch. “Diagnostics” → follow this [link >>](http://www.neurosurgeryresident.net/D.%20Diagnostics\D.%20Bibliography.pdf)

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