Biomarkers of myocardial infarction

What is myocardial infarction?
- Basically MI is an injury to heart muscle that occurs due to rapid development of myocardial necrosis caused by prolonged ischemia and hypoxia (critical imbalance between the oxygen supply and demand of the myocardium)
- Usually histological cell death starts in as little as 20 min and can have complete necrosis after 2-4 hours and that can eventually lead to death if untreated.
- A healed infarction usually takes at least 5-6 weeks depending on the severity of the injury itself (severity of necrosis)
  
  - What happens is that we have a clog in the blood vessel → obstruction of bloodstream → ischemic region → necrosis
  - **Ischemia to myocardial muscles (with low O\textsubscript{2} supply) → anaerobic glycolysis → increased accumulation of Lactate → decrease in p → activate lysosomal enzymes → disintegration and degradation of myocardial proteins → cell death & necrosis** (to be discussed next week)

Cell death and necrosis can be diagnosed in a variety of ways such as clinical manifestations (chest pain... etc) or ECG changes. But the most common way to diagnose MI is by BIOCHEMICAL MARKERS.

“Now” **What is a molecular biomarker?** Basically it’s a molecular alteration (a change in a level of a certain protein or expression of a certain gene) that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes like MI, or pharmacologic responses to a therapeutic intervention (diagnosis and prognosis)

(similar to liver enzyme aspartate transaminase test, which is an indicator for liver dysfunction. you do a blood test and a liver enzyme test and depending on how much aspartate transaminase is present in serum it can tell you if there is liver disease or not. It does not mean is this protein marker is a cause of that condition it just mean is it indicates its presence)

**Criteria for ideal markers for MI**

- **Specific:** (it has to be specific for that condition only or very few conditions that we can exclude later on to have only one) e.g. present in the myocardium absent in nonmyocardial tissues
- **Sensitive:** this marker should not be at very low level that can’t be detected weather the patient at normal condition or pathological condition.
- **Prognostic:** that can tell me something about severity of the disease. Is it severe or not?
- **Persists longer:** if a marker increases for 30 min and then goes down u can’t catch it, so it has to be present for long time
- **Reproducible:** if u do a test now and u repeat it after min should give u the same reading
Simple, inexpensive if its really expensive you/ insurance may not afford it
Quick for some thing like MI it has to be diagnose right now and can't wait for 2 day like a bacterial culture it has be right now
Acceptable (by both patient and clinician – not embarrassing or intrusive)

What are biomarkers good for?
- Diagnostic (there is pathologic condition or not ; infarct vs. reinfarct)
- Differentiating (mean if thre is MI or renal failure)
- Risk-stratifying (low- vs. high-risk) if this person at high risk to developing a disease or at low risk to developing a disease - just like a measuring cholesterol level if high so this person at risk to developing a heart attack.
- Prognostic (how severe the condition is)

There is a number MI biomarker and they develop as a result from different stages of a disease:
They are MI RISK Factors: **LDL/HDL ratio** and inflammatory biomarkers like (C-reactive proteins) and we have **plaque rupture** (clog in blood vessel) , we have other biomarker like **Myeloperoxidase** in unstable angina , and we have **biomarker for myocardial ischemia** (modified albumin) and for **myocardial injury**.

In this chart, 0 time is the onset of infarction, and then we have the levels of these biomarkers (how much biomarkers in serum?) There is a release of a no. of biomarkers.

- **In the beginning**: there is release of the myoglobin protein, notice how it declines really fast. Then we have a release of troponin which is very specific biomarker for infarction and we have a release of Creatine Kinase and ck isoform, and they last for some time. Their persistence in serum is different, so some of them we use to later on diagnosis other can use be used right now.

  so they are different in terms of when they are released and for how long
(Troponin) Is the goal standard for diagnosing myocardial infarction. Troponin is a cellular protein and its associated with tropomyosin and tropomyosin associated with actin, so this protein is important in muscle contracion (cardiac function).

Now there are three types of these protein TN-T, TN-C, TN-I

TN-C is a Ca binding subunit, its present in almost every single cell in the body so it's not really specific to a cardiac tissue.
Then we have T and I so (T) is tropomyosin binding subunit and (I) is an inhibitory subunit. These can be cardiac specific.
Typically troponin I subunit is more specific than troponin T.

So in normal people, these proteins (troponins) have all of their subunits present inside the cell and there are hardly any troponins in the serum.

Some having cell damage, cell rupture and release of the cellular content → troponin starts to be released. So a person with MI with have higher level of troponin, more specifically troponin (I). This is not specific for MI so we can have someone with renal failure and have release of troponin I and T, but again its very specific for MI. (Positive results: MI or chronic disease)

Now the release of troponin start after 5 hours and it peaks after 24 hours and then it can last for up to 14 days.

REMEMBER diagnosis of MI not only depend on biomarker but also depend on changes in ECG and clinical manifestations:
If someone comes in with chest pain and there is ECG changes → immediately test for troponin. AND serial sampling should be taking after 3 to 6 hours, the idea behind it is to see if troponin level will continue to increase or decrease. Because that can tell you something about when the infarction took place → if it is decreasing it indicates it's a later event and if it still increasing it indicates it is an early event.

Why is troponin release prolonged?
Because we have 2 pools of troponin we have a free cytosolic protein and we have an actin or a tropomyosin bound protein, so whenever there is a rapture in the cell we have release of the cytosolic pool, this is followed by release of the bound protein, so there is elevation in the release of troponin.

There are a number of advantages of measuring troponin: It's very very sensitive (its the highest biochemical markers) there are a few false positives. It's prognostic for death, so a person with higher amount of troponin level in serum this is an indication that this person is at a high risk of dying, because the more troponin released, there is the more damage to the tissue.
The one problem with it is that release of troponin can be elevated as a result of a number of conditions and it's NOT a good marker for early event, so if someone lives near the hospital and comes in with chest pain in very little time and you perform a troponin level test and there is nothing happening! This doesn’t mean that this person does not have MI This means that the patient has come earlier than time of the higher proportion of troponin levels. So you need to do sampling and repeat the test after a few hours just to make sure this person have MI.

There is another thing that you need to pay attention to: What happens if someone with myocardial infarction → the troponin level should go up and then should go down What about if the person has a chronic high level of troponin! This means that this person may have a condition like renal failure and has continuous release of troponin. You can’t say this person does not have MI because we have to do other tests: ECG and take history and so on. But at the same time you have to know if there is some increase or not because when someone has MI there may be an increase above the high level that results from renal failure, so again they should be rise and fall to make sure there is a good diagnosis of MI.

If you are at a place where there is no troponin test anymore (just like the university here because there is no money) Troponin test doesn’t exist anymore in hospital we need to look to alternative.

- An example is Creatine Kinase (CK).
  CK is an enzyme that phosphorlates creatine, this creatine can be converted into creatinine which is eliminated by kidneys. So creatine when phosphorlated forms phosphocreatine. What is the use of phosphocreatine? it stored in muscle cells and it can be use as a source of energy

- Let’s say a person sees a lion what that happens → the first thing that muscles depend on is free ATP in the tissues which will be in 5 to 15 sec. Then muscle tissue turns to phosphocreatine to use as a source of phosphate to make ATP, it can last for a minute or so
  - That’s reason of people run 100 m depend mainly on ATP and phosphocreatine. If a person run for longer time this person depend on anaerobic metabolism (glucose, lactic acid). Let’s say a person runs in marathon and this person after finishing ATP, creatinephosphate, and anaerobic glycolysis .. this person depends on aerobic glycolysis as a source of ATP
**so** phosphocreatine is produced from the enzyme creatine kinase

- there is 3 different isoenzyme for “ck”
- it’s a dimer and its present as → BB, MB, or MM subunits
- m : muscle , b : brain
- In brain tissue mainly what present is BB subunit. In skeletal muscle tissues what present is mainly MM.
- But in cardiac muscle we have more MB than in skeletal muscle tissue. There is more MM also but there is a high level of MB as well. So they look more for MB.
- In other word if there is tissue damage in the cardiac muscle then it will a release of MB at a higher level than what normally exists.

**CK-MB Release:**
Starts after 3 hours and peaks after 24 h (like troponin) then goes down very quickly, so after around 3 days there is no more ck-mb.
So what do they is compare ck-mb to the total ck in serum (all isoenzymes of CK) and they do what's known as: cardiac relative index; they compare amount of ck-mb there in serum v.s. total ck
- If the ratio is less than 3% → so main source of ck-mb comes from the skeletal muscle so there injury in skeletal muscle
- if the ratio is above 5% → that means there is injury of the cardiac muscle and it may be an indication for MI
- if the ratio between 3 and 5 → so here between yes or no we need more tests, they do troponin test ECG and take history and so on

ONE THING GOOD ABOUT CK-MB: since it peaks after 24 h and after 3 days it's gone
⇒ it's an indicator for **Reinfarction**: so let's say person develop another infarction after 3 day, if you do troponin level it still high, but if you do ck-mb it will be high as well (increase in ck-mb) so both troponin an ckmb can be markers for reinfarction

**Total CK can be elevated in other conditions and may lead to false-positive results:**
- Significant skeletal muscle injury
- Significant CNS damage (Stroke/Trauma)
- Occasionally from GI, renal failure, urologic disease Others: i.m. injection, hypothermia, exercise, intoxication and drug abuse
- Statins

**CK-MB isoforms:**
Two isoforms called MB1 (in plasma) and MB2 (cellular). Therefore in a normal person ckmb1 should be higher than ckmb2, but if there is damage in the tissue → here we have flipping of the ratio (more of 2 than 1). Except this test still under development it requires a skilled technician and it could give some false (+ve) result so test need optimization.
Types of infarction:

- **Incident MI**: If someone has MI at a first time we call it “Incident MI”
- **Reinfarction**: MI occurring within the first 28 days after an incident event.
- **Recurrent MI**: MI occurring after 28 days following an incident MI.

**HOW do you diagnose MI?** If a person is having a clinical symptoms then u need to do troponin test and u need to do serial measurement for troponin. If troponin is high but without change and does not decrease → take second sample. **If it goes up by 20% this is an indication of reinfarction.**

- **Important things that you have to focus on:** when does the biomarker appear? When does the biomarker peak and goes down? How long does it persist?

  - **MYOGLOBIN (Mb)**

Notice that for Troponin and ckmb the increase is after around 5 hours of the incidence of MI, **LET’S SAY** if someone has chest Pain and troponin levels as well as CK are normal, this does not mean this person not have MI. IT MAY BE detected at an early event. **One of the markers that we can depend on is myoglobin.** And myoglobin increases after an hour of the event of MI, so it can be a good marker for early detected MI especially if it double **AFTER few hours.** That means there is early damage AND can be use to exclude MI as well: If a person has a high troponin level and low myoglobin level → this person may not have MI!

- The thing about myoglobin → it’s not specific alone because it’s present in all muscle tissues. But it can be useful for ruling out but not for confirming the diagnosis of MI.
- And it has to be used in a combination with other biomarker

- **Troponen its highly specific followed by ckmb and last one myoglobin**
There are some old biomarkers used years ago:

- **Aspartate aminotransferase**: increase in event of MI „, BUT not very specific „, and not used any more

- **Lactate dehydrogenase**: still used but a lot less than CK and Troponin. Increases after the onset of MI, it peaks after 5 days, and it can last for 2 weeks. LETS SAY a person is really negligent of his health and comes to the doctor saying he had chest pain a week ago! We can’t measure troponin and ck_mb, but we can do lactate dehydrogenase which can tell me something about if a person have MI or not

it exists in different isoforms because it’s a tetramer, it’s made of 4 polypeptide. One of them is called M polypeptide and the other one is called H (heart) polypeptide, and each isoform has a different combination of these polypeptides:

SO a protein containing (4 H) polypeptide is heart specific. We can also have (3 to 1 H3M1), (2 to 2 H2M2), (1 to 3 H1M3) and we can have (4 M). So we have lactate dehydrogenase 1, 2, 3, 4 and 5.

**Normal: LD1:LD2 = 0.5-0.75**

**MI: LD1:LD2 > 1**

- LDH1 and LDH 2 are more specific for myocardiac tissue but LDH2 it also present at higher level in serum.
- How can we take advantage of lactate dehydrogenase? we know that in a normal person if you looked at the ratio of 1 to 2 → the ratio will be **less than 1** because there is more LDH2 that is present in serum than LDH1
- If someone has an MI then you have release of LDH1 → there is flipping in the ratio and becomes **above 1**, so this indication for an injury for a cardiac tissue

There are other conditions which cause this, just read them don’t memorize.
These GELS for LDH and other one for CPK, we can see the different isoforms.

- For a normal person: this person with have a high level of LDH 2 than 1
- Someone is having MI $\rightarrow$ flipping: 1 more than 2
- Person having liver disease $\rightarrow$ more 2 to 1 but there is more release of 5 therefore someone having liver disease and heart failure: there is high release of 5 and there is flipping.

- Look at the level of CPK:
  - normal person having some mm in tissue but NOT mb or bb
  - someone is having MI and then release in MB
  - If Someone is having liver disease so more release of the mm but there is no release of mb or bb
  - if some one is having liver disease and heart failure then there is release of mb ((in the case of injury in the cardiac tissue)) but more release of mm ((in the case of liver failure))
- This may come in the exam so study them well.

Now there are other biomarkers that can increase specificity and sensitivity of MI diagnosis:

- Natriuretic peptides: s released from different tissues including the heart (specifically BMP) it is released from the a heart as a result of muscle stretching or cardiac overload. So we have a release BMP.
  - The main function of these hormone is homeostasis of Na and water retention, so what happens is $\rightarrow$ if there is more pressure or over load on the heart so there is release of BMP
  - BMP effects the kidneys to reduce blood pressure and the cardiac load, and it can also act as a vasodilator
  - This hormone is synthesized as a (propeptide) prohormone BMP and it's cleaved into a mature form, and the end terminal region is also released. So they measure how much of this BMP there is and how much of this peptide exists as well in the sample. And they noticed that it's actually a prognostic indicator of death and heart failure. So the more BMP is released or exists in serum $\rightarrow$ mean that there is a good indication there is a high risk of MI and even dying
- There is still question marks on the utilization of this marker especially that there are gender and age differences: older people release BMP more than younger AND women release more than men
- Release of this protein is **biphasic**: whenever there is MI there is release within 24 hours and then levels go down. Then there is another release after 5 days, so it can be use as indication for **when an infarction take place**. It is unknown why this happens
- People with higher level of BMP have higher chance of dying

  o **Glycogen phosphorylase BB (GPBB)**
    - Present in heart and brain tissue
    - It can raise as a result of MI and unstable angina
    - It’s elevated 1 to 3 hours after ischemia

  o **Fatty acid binding protein**: again if it used in a combination with troponin it’s an excellent indicator for mortality, so if you have a high level of both so there’s a high chance that this person will die
    - Both the glycogen phosphorylase and fatty acid binding protein are released early on before the release of troponin so can be used as early markers for infarction

  o **C-Reactive Protein** released in cases of inflammation, it’s not really specific for MI, but its high level is used to determine the risk of of the first MI / ischemia stroke / mortality and so on. Varies according to age and gender as well

  o **Albumin** can bind to cobalt, and under ischemia cobalt can’t bind to albumin anymore. So they measure how much free cobalt there is in a sample of patient fluid once we add albumin. So in an ischemic sample there will be more free cobalt than in a normal sample.
    - it rises really early on after the onset of ischemia and before MI takes place, so it’s the test that can be done to tell if MI would happen or not.
    - It’s an excellent marker but still under testing

  o **Myeloperoxidase** it’s an inflammatory marker

He read the last slides, a person said that we should all focus on troponin only, but others argued that we need the other markers because one marker may not tell the whole story so we need more markers (a multi-marker strategy) to tell you what the problem is. This person replied that it all comes back to money and this is a whole story (how much many should a country spend, mutli-markers bring a little more benefit but the cost is a lot higher and countries cannot afford it.) → Multi-marker assessment has been shown to be associated with higher Emergency Department, coronary care and cardiac intervention costs but...[it] has not been shown to reduce overall costs despite reducing admissions.”

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