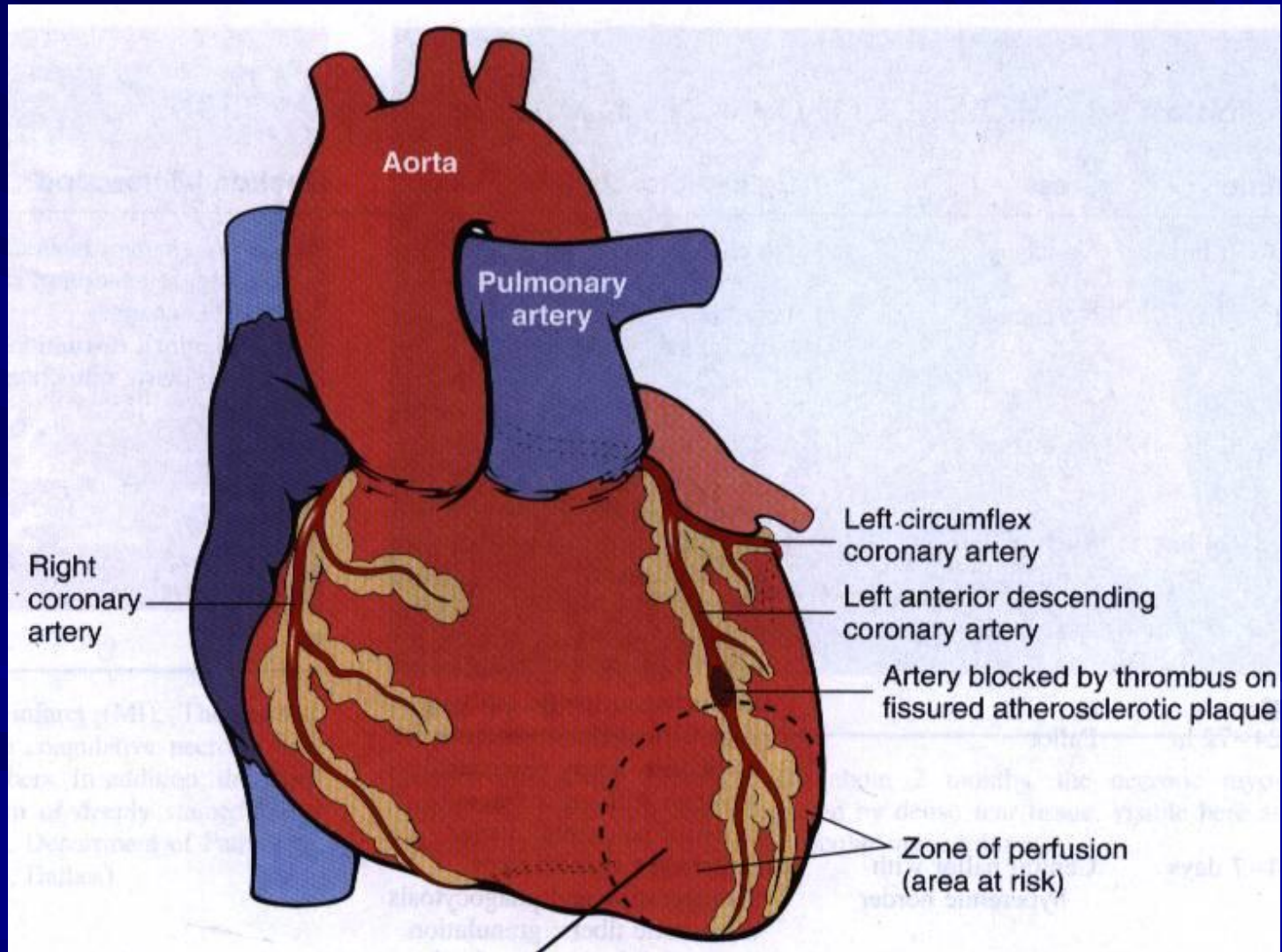# LABORATORY EVALUATION OF THEROSCLEROSIS AND ACUTE CORONARY SYNDROME

R. Mohammadi
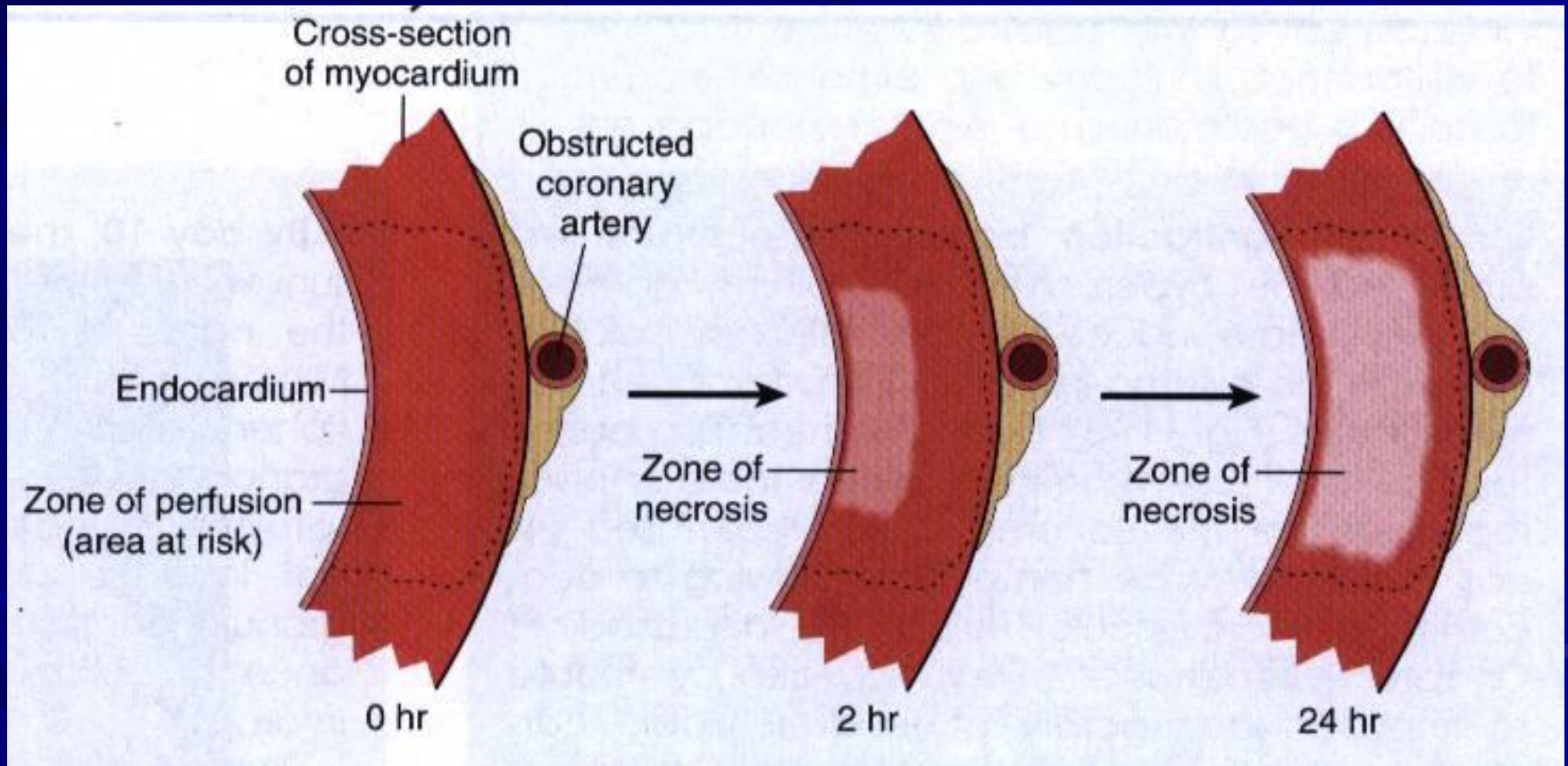
Biochemist (Ph.D.)

Faculty member of Medical Faculty
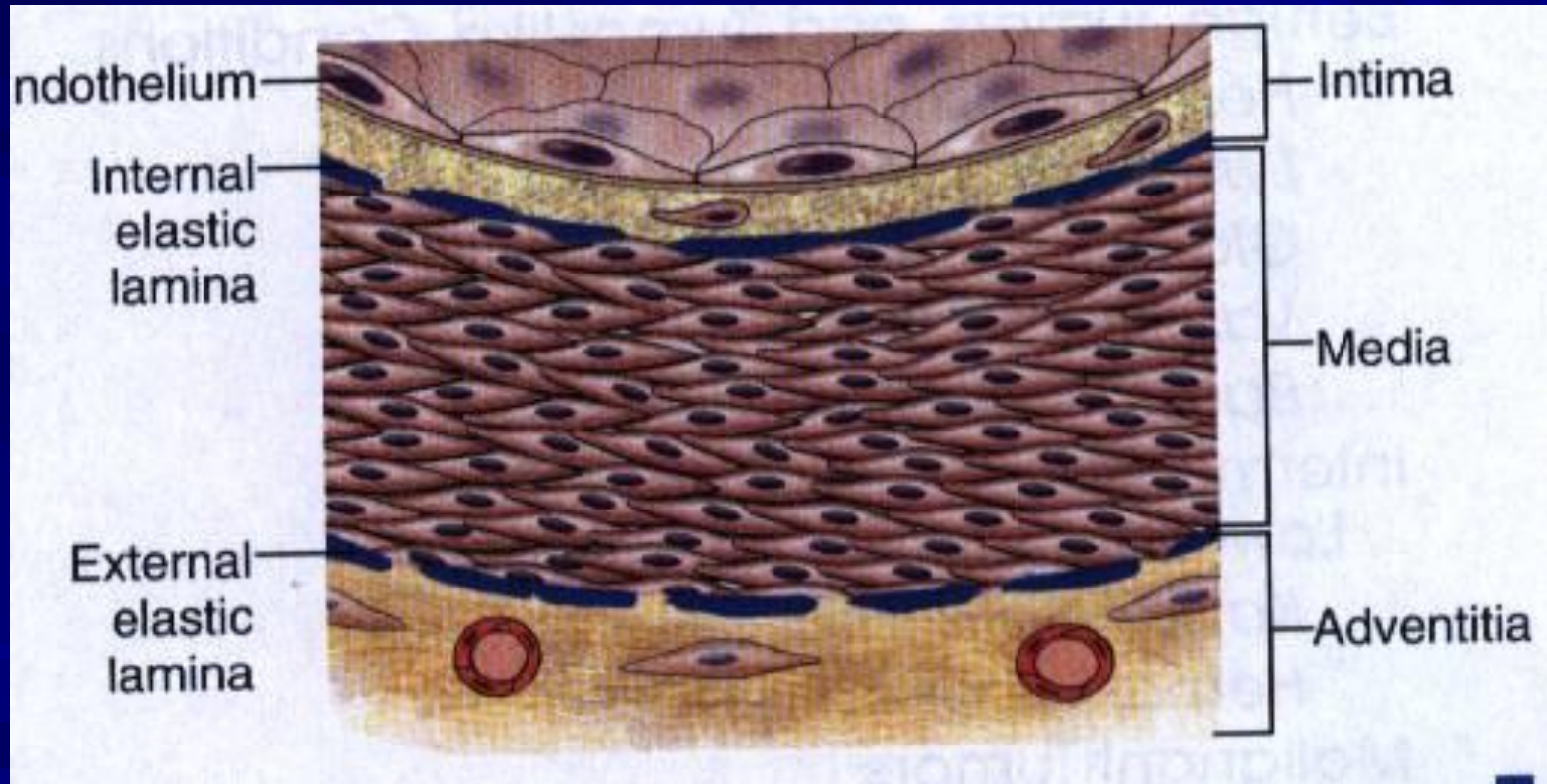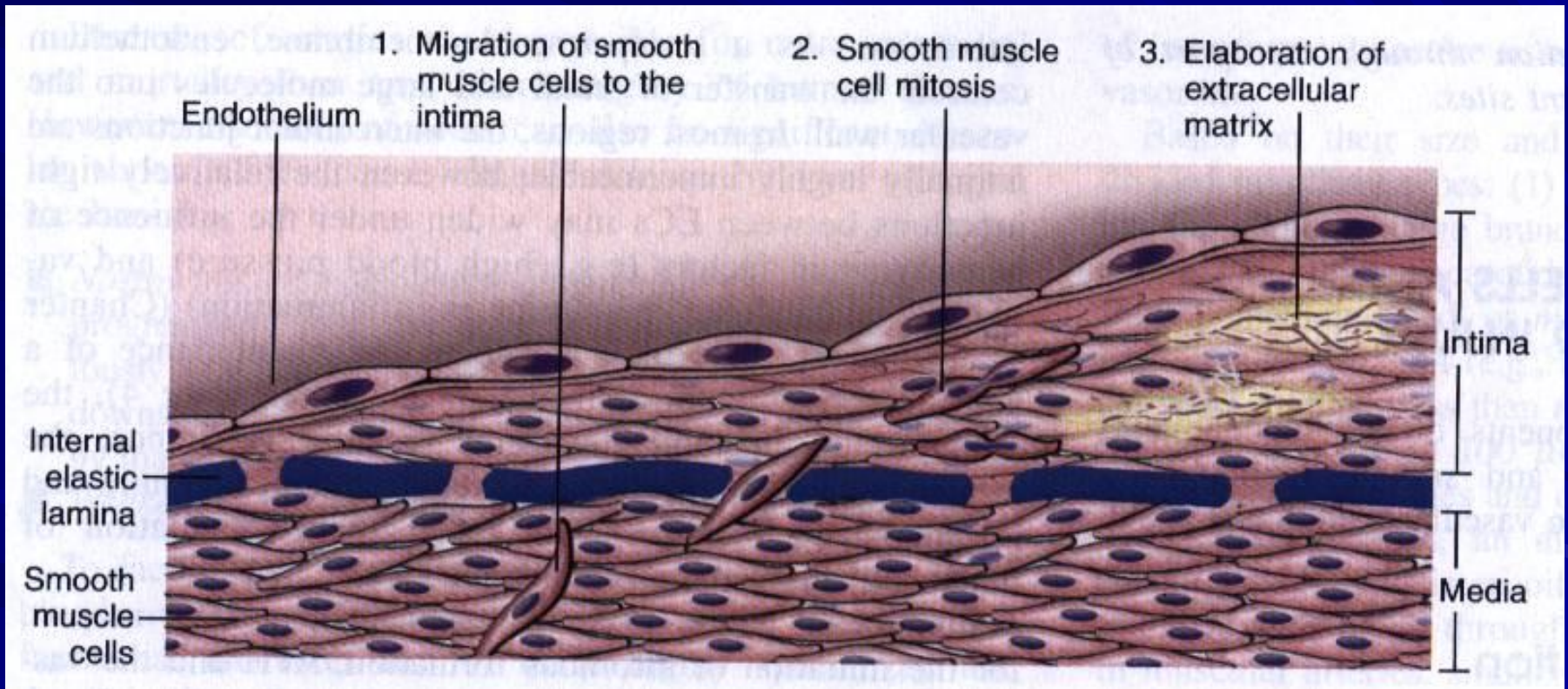
CORONARY HEART DISEASE

CORONARY ARTERY OBSTRUCTION

MAIN COMPONENTS OF VASCULAR WALL

INTIMAL THICKENING

1. Migration of smooth muscle cells to the intima

2. Smooth muscle cell mitosis

3. Elaboration of extracellular matrix

Endothelium

Internal elastic lamina

Smooth muscle cells

Intima

Media

MAJOR COMPONENETS OF ATHEROMATOUS PLAQUE

PATHOGENESIS OF ATHERSCLEROSIS

Endothelium

Intima

Media

Adventitia

1. Chronic endothelial "injury":
 - Hyperlipidemia
 - Hypertension
 - Smoking
 - Homocysteine
 - Hemodynamic factors
 - Toxins
 - Viruses
 - Immune reactions

Response to injury

2. Endothelial dysfunction (e.g., increased permeability, leukocyte adhesion) Monocyte adhesion and emigration.
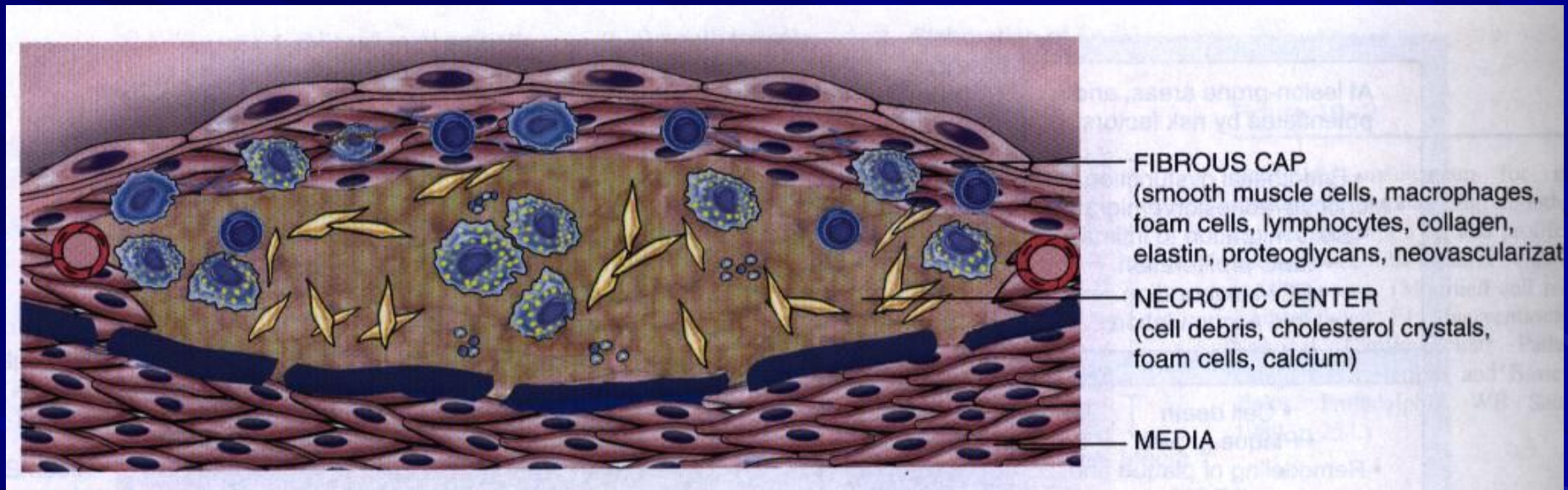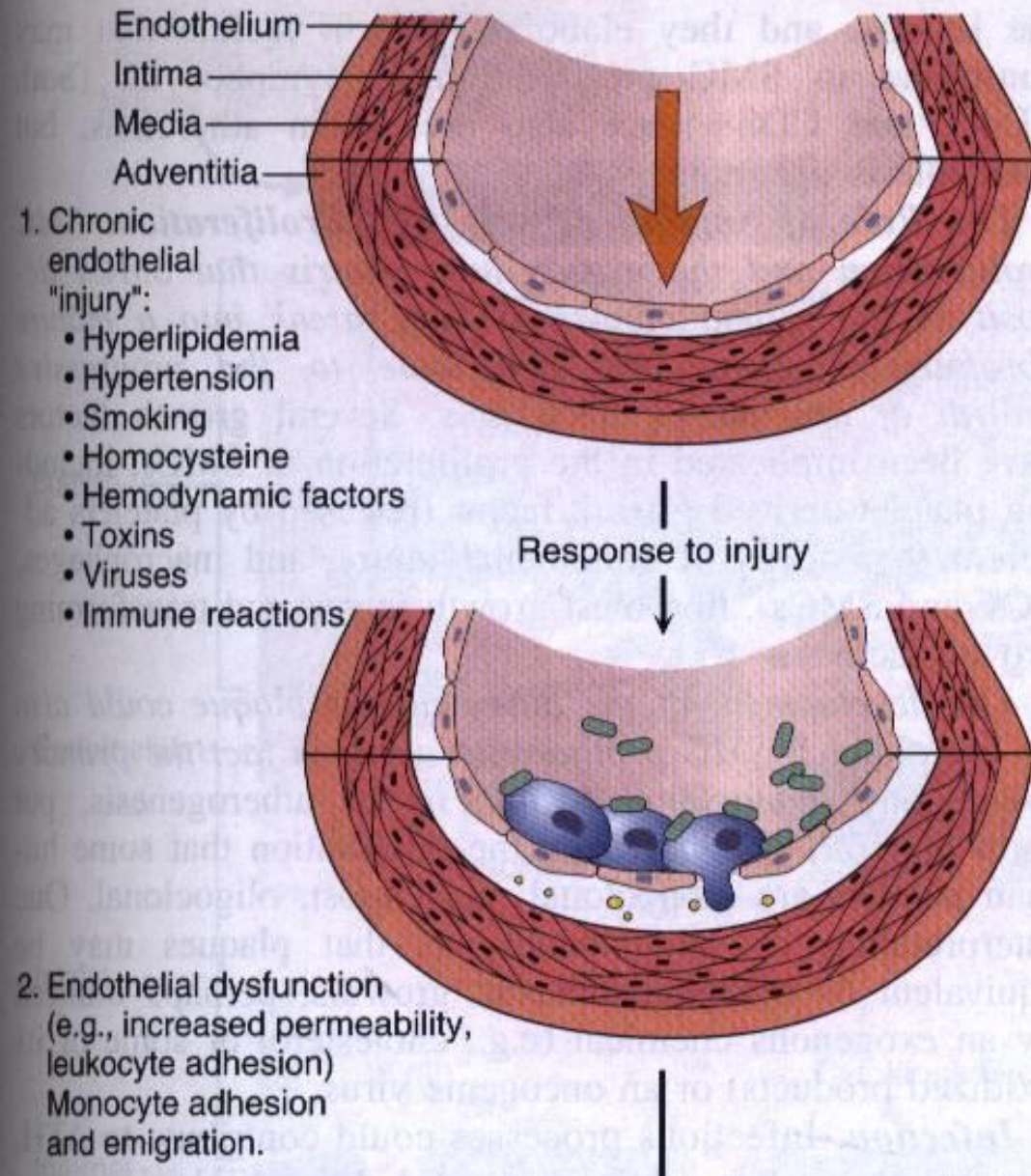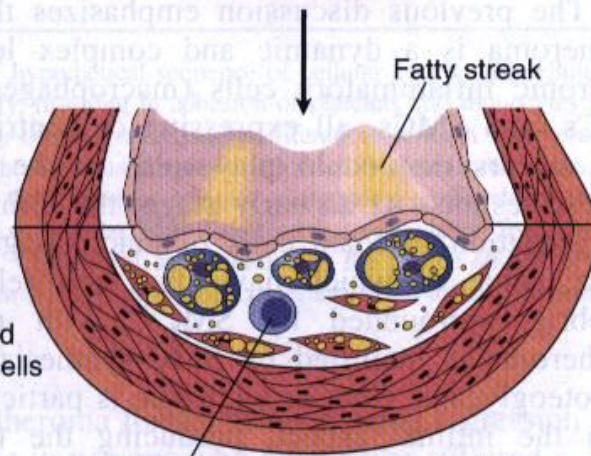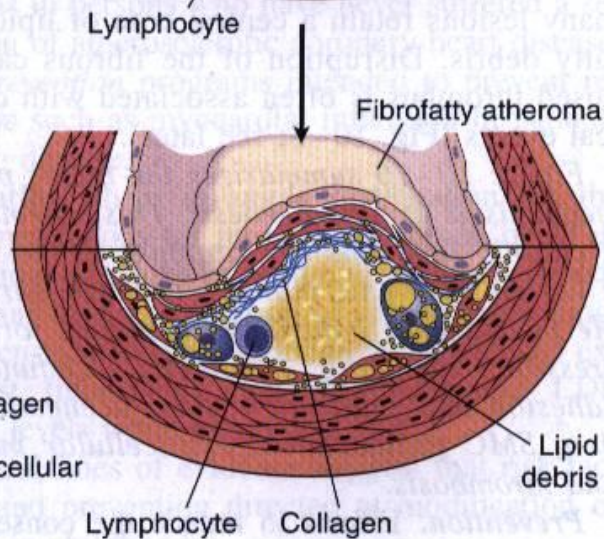
3. Smooth muscle emigration from media to intima. Macrophage activation.

Fatty streak
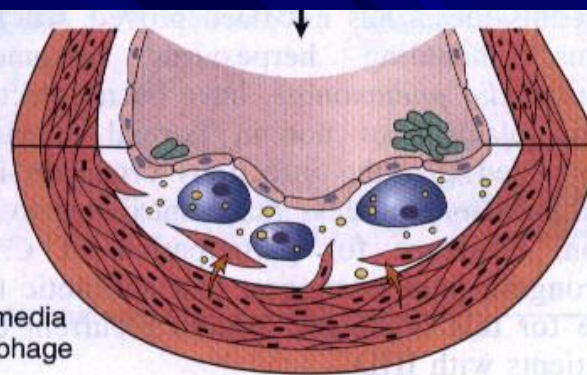
4. Macrophages and smooth muscle cells engulf lipid

Lymphocyte

Fibrofatty atheroma

5. Smooth muscle proliferation, collagen and other ECM deposition, extracellular lipid

Lymphocyte   Collagen

Lipid debris

Adventitia
Media
Intima

NORMAL

Atherosclerosis →

Adventitia
Media
Intima
Lipids
Atherosclerotic plaque

FIXED CORONARY OBSTRUCTION
(Typical angina)

Platelet aggregate

PLAQUE DISRUPTION

Healing →

SEVERE FIXED CORONARY OBSTRUCTION
(Chronic ischemic heart disease)

Thrombus

Thrombus

MURAL THROMBUS WITH VARIABLE OBSTRUCTION / ? EMBOLI
(Unstable angina or acute subendocardial myocardial infarction or sudden death)

OCCLUSIVE THROMBUS
(Acute transmural myocardial infarction or sudden death)

ACUTE CORONARY SYNDROMES

CELLULAR EVENTS & CELLULAR INTERACTIONS
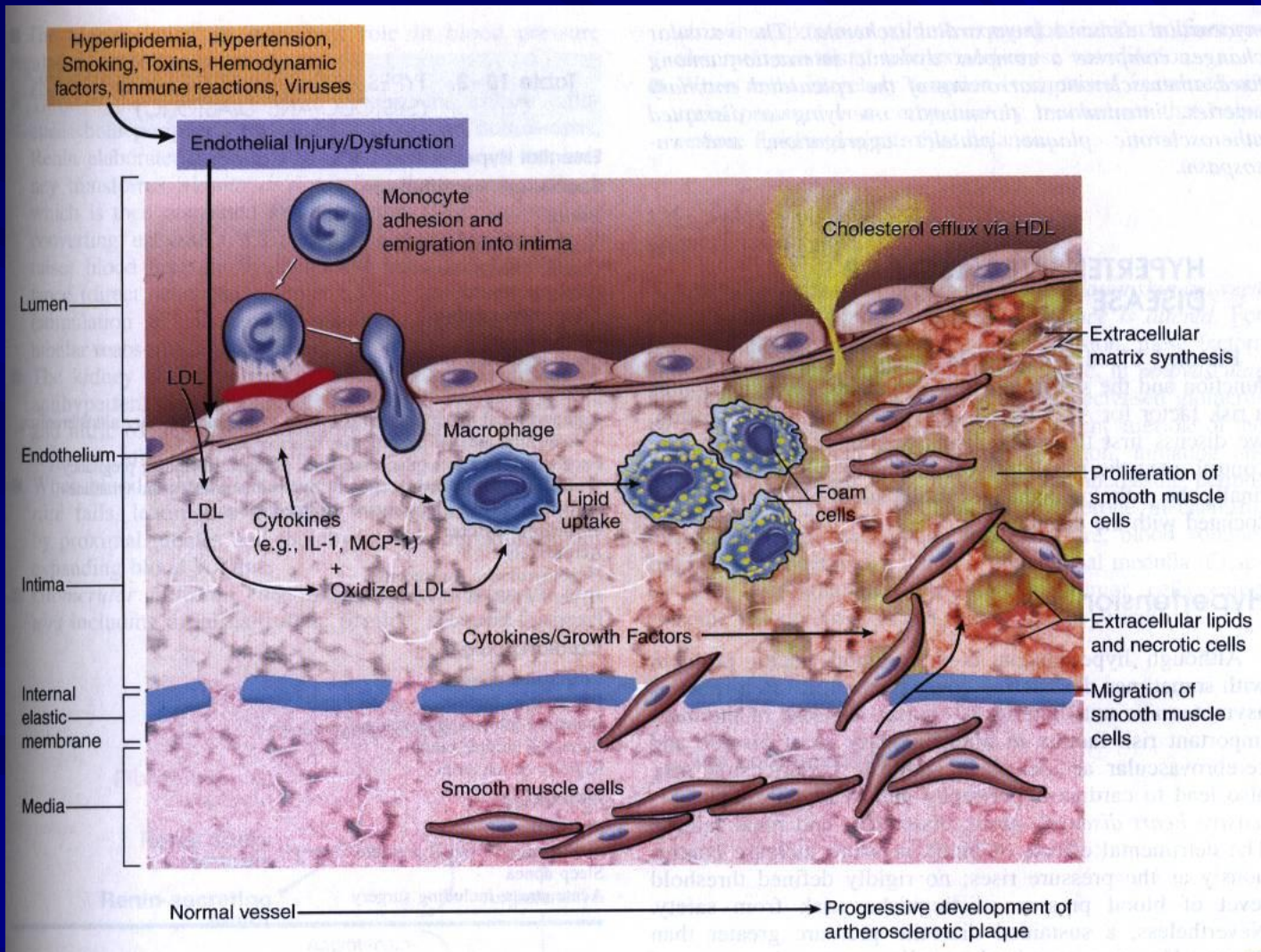
RISK FACTORS FOR CHD

- **Clinical Risk Factors**
- **Laboratory Risk Factors**

CLINICAL RISK FACTORS

جدول ۱. فاکتورهای خطر (غیرآزمایشگاهی) برای بیماری کرونری قلب (CHD)

- استعمال دخانیات (هر نوع استعمال در ماه گذشته)
- فشار خون بالا (فشار خون بیش از ۱۴۰/۹۰ mmHg یا تحت درمان فشار خون)
- سابقه خانوادگی CHD زودرس (CHD در خویشاوند مرد درجه اول ≥ ۵۵ سال یا در خویشاوند زن درجه اول ≥ ۶۵ سال
- سن (مرد ≤ ۴۵ سال و زن ≤ ۵۵ سال)
- چاقی
- دیابت قندی
- شیوه زندگی بی‌تحرک

LABORATORY RISK FACTORS

- Common Lipid Markers Including TC, HDL-C, LDL-C & TG

- Uncommon Lipid Markers Including Lp(a), beta-VLDL, Apo A-I & Apo B-100

- Nonlipid Markers Including Homocysteine & hsCRP

SOURCES OF RESULT VARIABILITY IN LIPID MEASURMENT

- Analytical Error
- Physiologic Variation
- Fasting
- Diseases
- Drugs

- Posture
- Venous vs. Capilary Samples
- Plasma vs. Serum
- Storage

ANALYTICAL ERROR

■ %Total Error = % Bias + 1.96 (%CV)

جدول ۲. رهنمودهای NCEP برای خطای اندازه‌گیری قابل‌قبول

ضریب تغییرات	تورش	خطای کل	آنالیت
۳ ≥	۳ ≥	۹ ≥	کلسترول
۵ ≥	۵ ≥	۱۵ ≥	تری‌گلیسرید
۴ ≥ *	۵ ≥	۱۳ ≥	HDL–کلسترول
۴ ≥	۴ ≥	۱۲ ≥	LDL–کلسترول

❋ معیارهای دقت در مورد مقادیر HDL–کلسترول ۴۲ md/dL و بیشتر می‌باشد. در مقادیر کمتر، CV به کار نمی‌رود. در عوض، انحراف معیار نباید از ۱/۷ mg/dL تجاوز کند.

BIOLOGICAL VARIATIONS

■ Due to Age, Gender, Diet, Season

جدول ۳ . تغییرپذیری آنالیت‌های متداول

CV (%) روش CV (٪.)	CV (%) درون-فردی	CV (%) بین-فردی	آنالیت
۲/۳	۸/۲	۲۲/۳	کلسترول، تام
۲/۵	۱۲/۴	۲۸/۳	HDL–کلسترول
۴/۷	۲۸/۸	۵۶/۸	تری‌گلیسرید
۴/۸	۷/۰	۱۷/۸	آپولیپوپروتئین A
۲/۷	۹/۵	۲۷/۶	آپولیپوپروتئین B

FASTING

- Fasting for at Least 9 Hours Is Necessary for TG
- Fasting Is Not Necessary for TC
- Slight Decrease of LDL-C & HDL-C After Eating

DIEASES

- Myocardial Infarction
- Shock
- Trauma
- Surgery
- Weight lose
- Fever
- Thyroid Disease
- Liver Disease
- Kidney Disease

DRUGS

- Oral Contraceptive Increases VLDL
- Anabolic steroids Increase VLDL & Decrease HDL

POSTURE & OCCLUSION

- TG, TC & Lipoproteins Increase in Standing Position

- Prolonged Venous Occlusion Has Similar Effect

Venous vs. Capilary

- Measurements in Capilary Blood Sample Seem to Be a Little Lower Than Venous Sample

PLASMA vs SERUM

- Serum & Plasma Can be Used
- Plasma Is Preferred for Electrophoresis & Ultracentrifugation
- EDTA Is Preferred, But the Results Are Lower
- Heparin Can Also Be Used
- Protein Aggregation Occurs In Plasma

STORAGE

- It Is Recommended to Analyze at Day of Sample Collection
- Samples Can Be Stored in Refrigerator or Freezer

ANALYTICAL APPROACH IN LIPIDS ABNORMALITIES

1) LIPIDS DETERMINATIONS
2) LIPOPROTEIN ANALYSIS

LIPIDS DETERMINATION

- *Cholesterols*
- *Triglycerides*
- *Phospholipids*
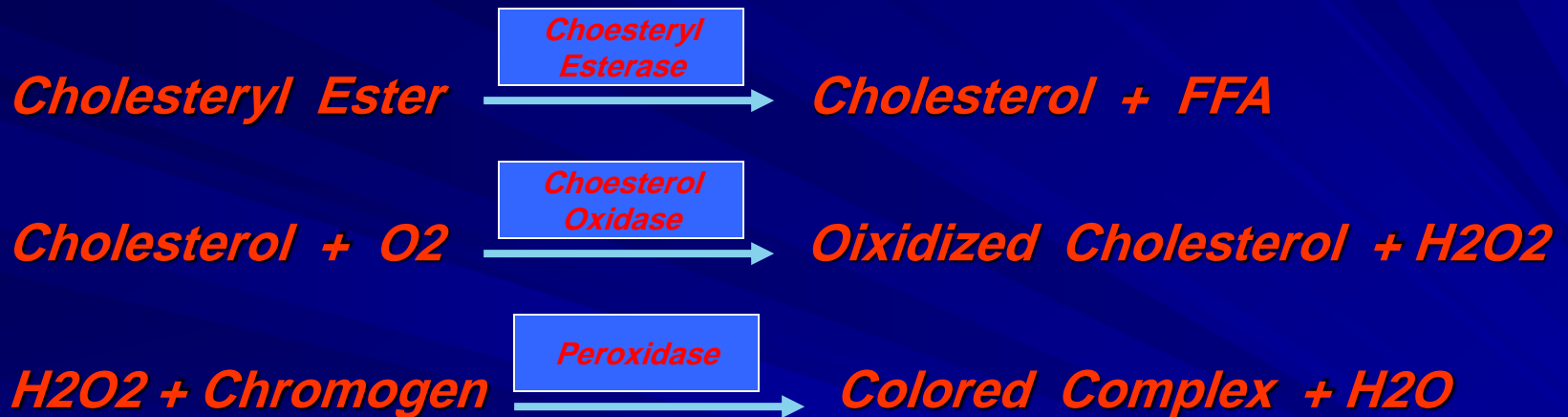- *Free Fatty Acids (FFAs)*
- *Total Serum Lipids*

CHOLESTEROL DETERMINATIOM

Chemical

- *Liberman-Burchard*
- *Schoenheimer-Sperry*
- *Abell-Kendal*

CHOLESTEROL DETERMINATIOM

- Enzymatic

Cholesteryl Ester $\xrightarrow{\text{Choesteryl Esterase}}$ **Cholesterol + FFA**

Cholesterol + O2 $\xrightarrow{\text{Choesterol Oxidase}}$ **Oixidized Cholesterol + H2O2**

H2O2 + Chromogen $\xrightarrow{\text{Peroxidase}}$ **Colored Complex + H2O**

CHOLESTEROL DETERMINATIOM

Sample

- *Fasting Is Not Necessary*
- *Effect of Posture & Venous Stasis*
- *Variation in Body*
- *Stable for 4 d, 3 m, and Some years at 4°C, -20°C and -70°C Respectively*

TRIGLYCERIDE DETERMINATION

Chemical

- *Extraction*
- *Hydrolysis*
- *Glycerol Determination*

TRIGLYCEROIDE DETERMINATIOM

- Enzymatic

Triglyceride $\xrightarrow{\text{Lpiase}}$ Glycerol + FFA

Glycerol + ATP $\xrightarrow{\text{Glycerol kinase}}$ Glycerol 3-p + ADP

UV Spectrophotometry

Glycerol 3-p + NAD$^+$ $\xrightarrow{\text{Glycerol phosphate Dehydrogenase}}$ DHAP + NADH

Colorimetry

Glycerol 3-p + O2 $\xrightarrow{\text{Glycerol phosphate Oxidase}}$ DHAP + H2O2

H2O2 + Chromogen $\xrightarrow{\text{Peroxidase}}$ Colored Complex + H2O

TRIGLYCERIDE DETERMINATION

Interference by

- *Glucose*
- *Phospholipids*
- *Glycerol*
- *Oxidants & Reductants*

TRIGLYCERIDE DETERMINATION

Specimen

- *Fasting Is Necessary*

- *Affected by Posture & Venous Stasis*

- *Oxidants & Reductants*

- *Testing in the Same Day*

- *If Necessary, Storage at 4°C for a few days, -20°C for 3 m and -70°C for Years*

LIPOPROTEIN ANALYSIS

- *Ultracentrifuge*
- *Electrophoresis*
- *Serum Appearance*
- *Precipitation Methods*
- *Calculation*
- *Apolipoprotein Derermination*

Apolipoproteins

B-48

C-III

C-II

Cholesterol

Phospholipids
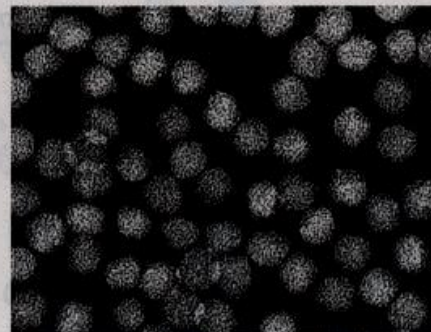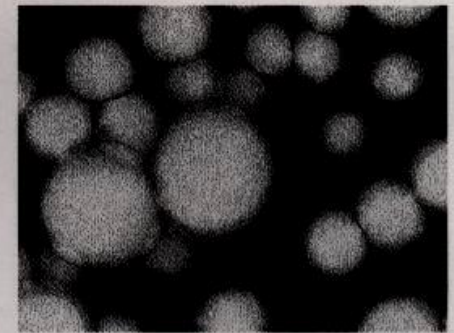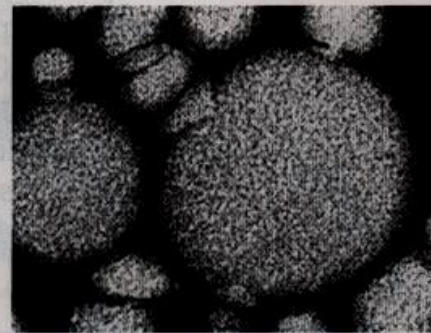
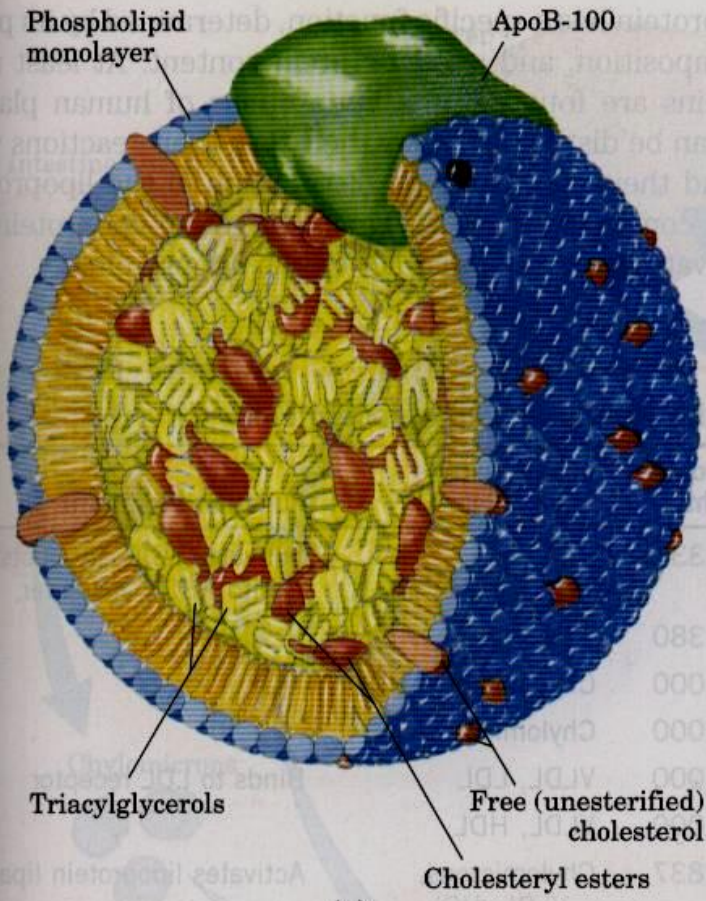Triacylglycerols and
cholesteryl esters
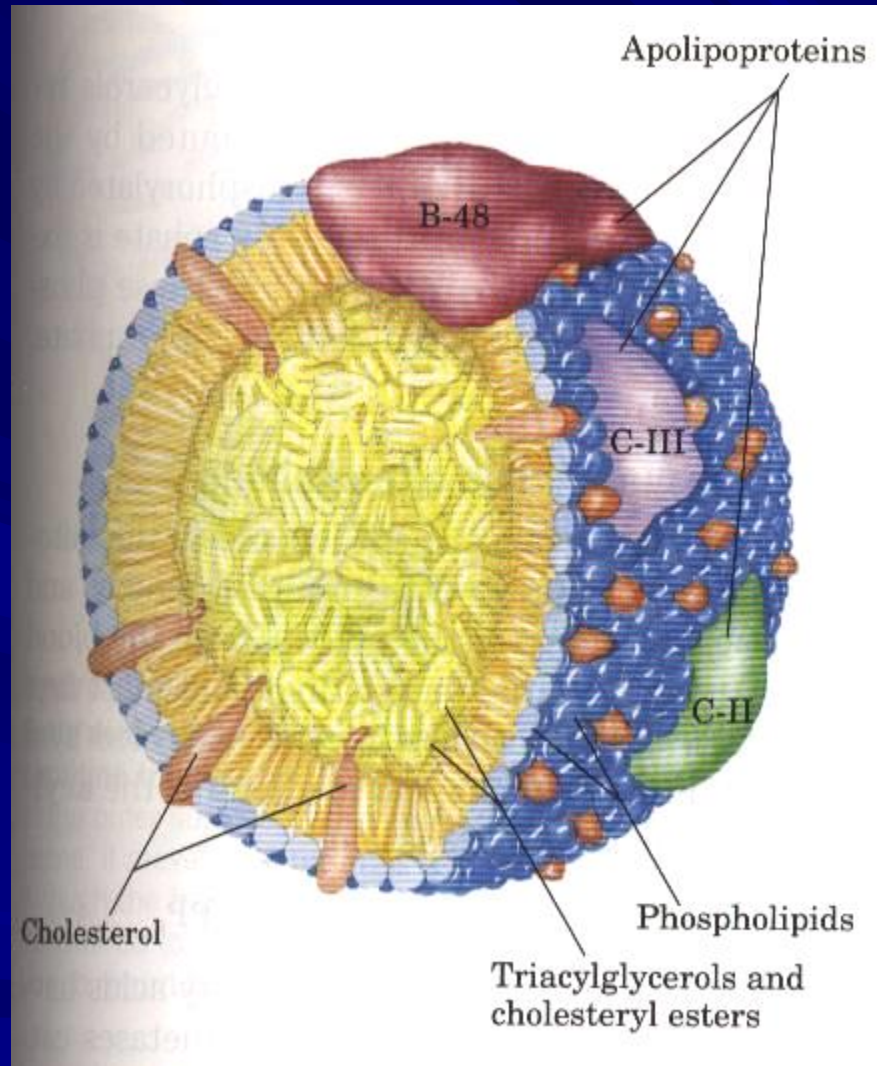
MAIN LIPOPROTEINS

- *CHYLOMICRON*
- *VERY LOW DENSITY LIPOPROTEINS (VLDL)*
- *LOW DENSITY LIPOPROTEINS (LDL)*
- *HIGH DENSITY LIPOPROTEINS (HDL)*

MAJOR LIPOPROTEINS

LIPOPROTEINS	DENSITY (g/ml)	DIAMETER (nm)	ELECTROPH ORESIS	PROTEIN (%)	TRIGLYCE RIDE (%)	CHOLESTER OL (%)	PHOSPHOLI PID (%)
Chylomicron	*<0.950*	*75-1200*	*Origin*	*1-2*	*86*	*4*	*8*
VLDL	*0.950-1.006*	*25-75*	*Pre-β*	*10*	*50*	*20*	*20*
LDL	*1.019-1.063*	*20-25*	*β*	*20*	*11*	*46*	*22*
HDL	*1.063-1.210*	*7.5-20*	*α*	*50*	*3*	*27*	*30*

(a)

- Phospholipid monolayer
- ApoB-100
- Triacylglycerols
- Free (unesterified) cholesterol
- Cholesteryl esters

(b)

Chylomicrons (×60,000)

VLDL (×180,000)

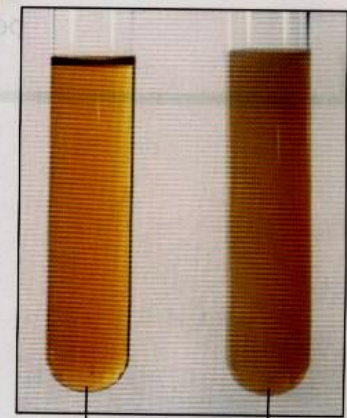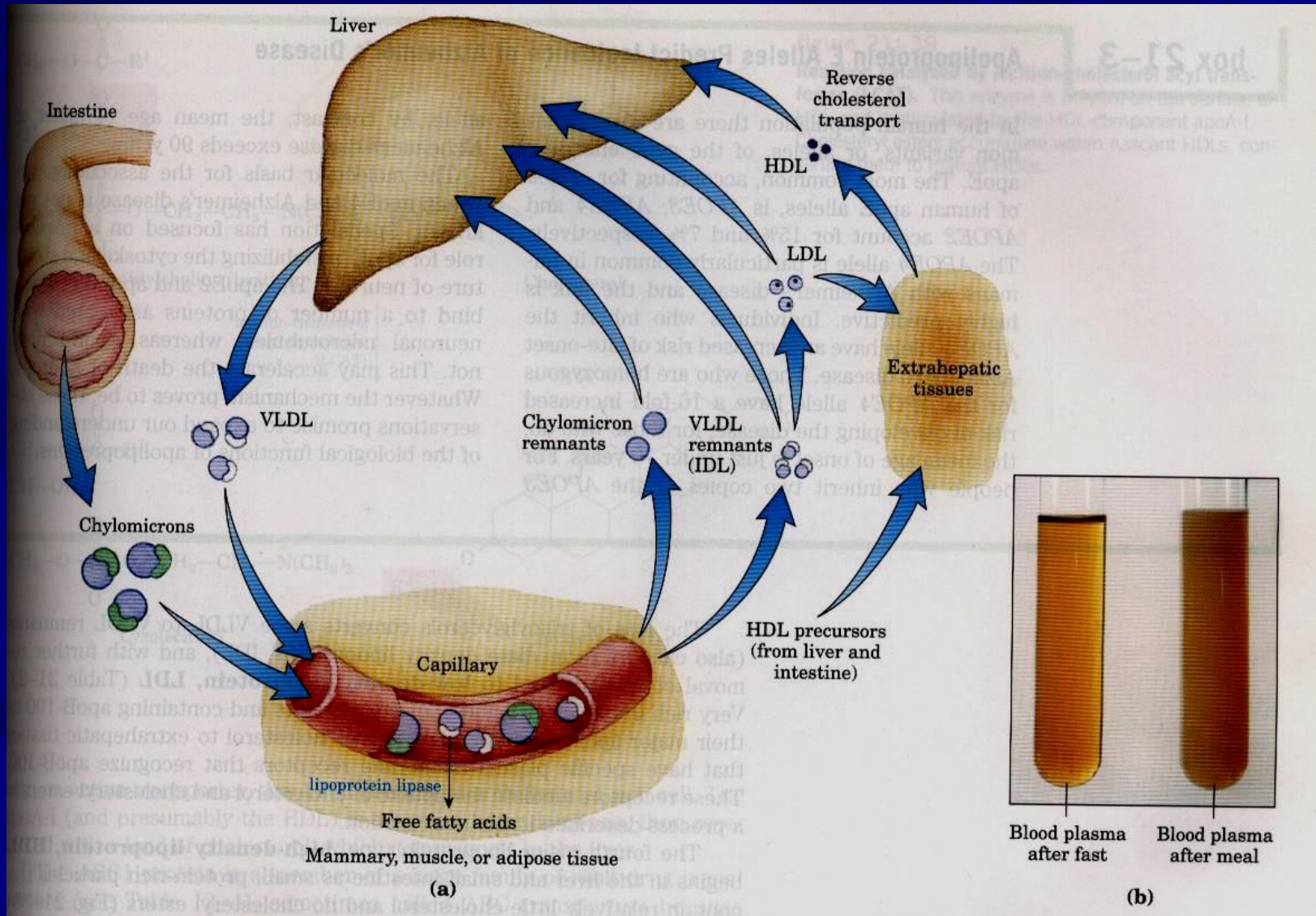LDL (×180,000)

HDL (×180,000)

SERUM APPEARANCE

- **Increased Chylomcron** → *Turbidity, Creamy Layer*
- **Increased VLDL** → *Turbidity*
- **Increased LDL** → *Clear*
- **Increased HDL** → *Clear*

REVIEW OF LIPOPROTEIN METABOLISM

APOLIPOPROTEINS DETERMINATION

- *Apo AI*
- *Apo B*
- *Apo CII*

HDL-C DETERMINATION

- **Precipitation of Apo B Containing Lipoproteins (VLDL, IDL, LDL) by**
- **Polyanions & Bivalent Cations**

Heparine Sulfate & Mn^{2+}

Dextran Sulfate & Mg^{2+}

Sodium Tungstate & Mg^{2+}

جدول ۴. سیستم‌های رسوب‌دهنده برای اندازه‌گیری HDL-C

سیستم	مقایسه نتایج با اولتراسانتریفوژ	نکات
هپارین سولفات / Mn^{2+}	برابر	تداخل Mn^{2+} در بعضی از روش‌های اندازه‌گیری کلسترول
سولفات دکستران / Mg^{2+}	۵٪کمتر	رسوب بهتر نمونه‌های لیپمیک، پایداری معرف‌ها، تغییرپذیری در اثر تغییر معرف و درجه حرارت
فسفوتنگستات / Mg^{2+}	۵٪کمتر	رسوب بهتر نمونه‌های لیپمیک، حساس به تغییر غلظت معرف‌ها
هپارین / Ca^{2+}	۱۰٪ بیشتر	–
پلی‌اتیلن گلیکول ۶۰۰۰	۲۰٪کمتر	–

LDL-C DETERMINATION

- **Ultracentrifugation**
- **Immunochemical**
- **Calculation with *Friedwald Equation***

$$Total\text{-}C = HDL\text{-}C + LDL\text{-}C + VLDL\text{-}C$$

$$LDL\text{-}C = Total\text{-}C - (HDL\text{-}C + \frac{TG}{5}$$

CRITERIA FOR DIAGNOSIS OF AMI

- Chest Pain
- Electrocardiogram (ECG)
- Cardiac Markers

Diagnosis Requires at Least Two of These Criteria

- **Diagnostic Specificity of ECG Is about 100%**

- **But Its Diagnostic Sensitivity Is 63-82%**

FEATURES OF AN IDEAL CARDIAC MARKERS

They Should
- *Be Heart Specific*
- *Be Highly Sensitive for Cardiac Damage*
- *Undetectable in Patients without Myocardial Damage*
- *Be Able to Differentiate Reversible from Irreversible Damage*
- *Allow The Monitoring of Reperfusion*
- *Be Able to Estimate Infarct Size And Prognosis*
- *Easy to Use And Cost Effective*

جدول ۵ . مارکرهای قلبی که به دنبال آسیب میوکارد آزاد می‌شوند

مارکر	نکات
ایزوآنزیم CK-MB	طـــی حـــدود ۶ تـــا ۷۲ ســـاعت بـــعد از حـــمله قـــلبی بـــالا مـــی‌باشد. مـحدودیت ویـــژگی تشـــخیصی، حساسیت تشخیصی و مشکلات تکنیکی دارد. اندکس نسبی معیار بهتری است.
ایزوفرم‌های CK-MB و CK-MM	۳ تا ۴ بعد از حمله، نسبت بالای MB_2/MB_1 یا MM_3/MM_1 وجود دارد.
ایزوآنزیم LD1 و LD2	برای تشخیص دیررس MI؛ ویژگی پایینی دارد؛ استفاده از وارونگی LD.
تروپونین قلبی T	ویژگی بافتی و حساسیت تشخیص بالا، برای تشخیص زودرس و دیررسی MI
تروپونین قلبی I	ویژگی بافتی و حساسیت تشخیص بالا، برای تشخیص زودرس و دیررسی MI
میوگلوبین	فاقد ویژگی بافتی، برای تشخیص زودرس AMI و آنفارکتوس مجدد
کربنیک انیدراز III	برای افزایش ویژگی بافتی میوگلوبین
گلیکوژن فسفریلاز BB	برای تشخیص زودرس AMI و حتی تشخیص آسیب قابل‌برگشت میوکارد
پروتئین قلبی اتصال به اسید چرب	تشخیص زودرس AMI
آلبومین تغییریافته به طریق ایسکمی	جستجوی ایسکمی قبل از آسیب سلولی، فاقد ویژگی بافتی
زنجیرهای سنگین میوزین	برای تشخیص دیررس AMI و ارزیابی وسعت آنفارکتوس، ولی کاربرد زیادی ندارد.

CREATINE KINASE (CK)

- **It Is A Dimer Comprising two Subunit**
  1) B Subunit (Brain Form)
  2) M Subunit (Muscle Form)
- **IT Has Three Isoenzyme:**
  1) CK-BB (CK-1) from Brain
     Is Specific for Brain
  2) CK-MB (CK-2) from Cardiac Muscle
     Is The Most Specific for Heart
  3) CK-MM (CK-3) from Muscle
     Has Low Tissue Specificiy

TOTAL CK

- **After Onset of chest Pain**
  1) It Increases within Few Hours
  2) Peaks within 24 h
  3) Return to Normal Levels within 48 to 72 h
- **It Is Not Specific**

CK-MB ACTIVITY

■ **After Onset of chest Pain**
  1) *It Increases within 4 to 6 h*
  2) *Peaks within 24 h*
  3) *Return to Normal Levels within 48 to 72 h*

■ **It Is Valuable for Diagnosis of AMI, But Have Several Limitations :**
  1) *Low Cardiac Specificity*
  2) *Presence In Normal Serum*
  3) *Low Cardiac Content*
  4) *Its Cardiac Distribution Is Not Uniform*
  5) *Technical Problems*

% CK-MB

- **Is Used for Differentiating Myocardial Damage from Skeletal or Neural Damage**

$$\% \text{ CK-MB} = \frac{\text{CK-MB Activity}}{\text{Total CK activity}} \times 100$$

- **Normally Less Than 1.5%**

CK-MB MASS

- **Measured By Monoclonal Anti-CK2 Antibody**
- **Is Rapid**
- **Is More Specific**
- **Is Detectable Earlier (About 1 h)**

% CK-MB

- **Is Used for Differentiating Myocardial Damage from Skeletal or Neural Damage**

$$\% \text{ CK-MB} = \frac{\text{CK-MB Mass}}{\text{Total CK activity}} \times 100$$

- **Normally Less Than 2%**

CK ISOFORMS

- **Results From Action of Serum Carboxypeptidase to Remove N-terminal Lys from M Subunit**

- **After AMI , CK-MB2 / CM-MB1 and CK-MM3 / CK-MM1 Ratio Increases for Few Hours**

LACTATE DEHYDROGENASE (LD)

- **It Is A Tetramer of Two Subunit**
  - *1) H Subunit (Heart Form)*
  - *2) M Subunit (Muscle Form)*
- **IT Has Five Isoenzyme:**
  - *1) LD1 (HHHH)*
  - *2) LD2 (HHHM)*
  - *3) LD3 (HHMM)*
  - *4) LD4 (HMMM)*
  - *5) LD5 (MMMM)*

LACTATE DEHYDROGENASE (LD)

- **After Onset of AMI**

  1) *It Increases within 12 to 18 h*
  2) *Peaks within 1 to 3 d*
  3) *Return to Normal Levels within 8 to 14 d*

- **It Is Not Specific**

- **LD1 & LD2 Are More Specific**

- **Using LD Flip Is Specific for Myocardial Damage**

- **It Is Helpful for Late Diagnosis of AMI**

- **Determination of LD1 or HBD Activity May Be of clinical Significance for Estimation of The Size of Infarct**
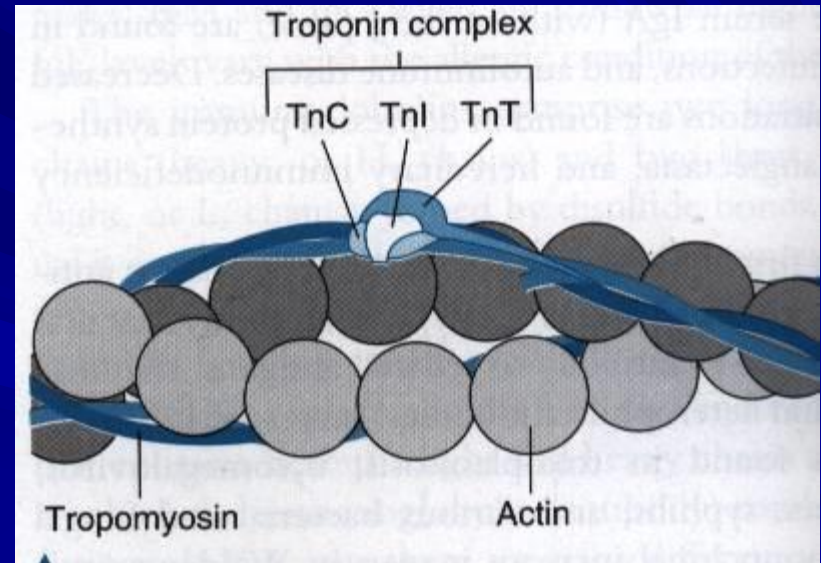
ASPARTATE TRANSAMINASE (AST)

- **Was The first Marker Used for the Laboratory Diagnosis of AMI**
- **It Lacks Cardiac Specificity**
- **Presently Has No Clinical Significance in Diagnosing AMI**

TROPONIN

Thin Filament of Muscle Consist of:

- *Actin*
- *Tropomyosin*
- *Troponin Complex*

1) Troponin C (TnC)
2) Troponin I (TnI)
3) Troponin T (TnT)

CARDIAC TROPONIN T (cTnT)

- **After Onset of AMI**
  1) It Increases within A Few Hours
  2) Peaks within 1 to 2 d
  3) Return to Normal Levels within 5 to 10 d

- **It Is Useful for**
  1) Diagnosis of AMI after 2 to 3 Days
  2) Differential Diagnosis of Myocardial Damage from Skeletal Muscle Damage
  3) Estimation of Infarct Size
  4) Monitoring after Reperfusion

CARDIAC TROPONIN I (cTnI)

- **After Onset of AMI**
  1) *It Increases within A Few Hours*
  2) *Peaks within 1 to 2 d*
  3) *Return to Normal Levels within 5 to 7 d*
- **It Is Highly Specific for Myocardium**
- **It Is A Very Sensitive Marker of Cardiac Damage**

MYOGLOBIN

- Consist of 5-10% Cytoplasmic Proteins of Striated Muscle (Skeletal & Cardiac)
- Earlier Marker for Myocardial Damage
- Mb Increases Within 1 to 2 h after Onset of AMI
- It Is Not Specific for Cardiac Muscle
- It Is Useful for

  1) Rule Out of AMI
  2) Diagnosis of Reinfarction (Rapid Clearance)
- Using CA III to Improve Specificity

CARBONIC ANYDRASE (CA) ISOENZYME III

- **It Is A Soluble Protein That Catalyses Hydration of CO_2 to Bicarbonate**

- **There Are Seven Carbonic Anhydrase Isoenzymes**

- **CA III Is Not Found In Cardiac Muscle, But Presents In Skeletal Muscle**

- **It Can Be Used to Differentiate Skeletal and Cardiac Muscle Damage**

GLYCOGEN PHOSPHORYLASE ISOENZYME BB (GPBB)

- This Enzyme Is Involved in Carbohydrate Metabolism

- It Is Not specific for Heart

- GPBB Increases between 1 to 4 h After Chest Pain Onset and Returns to Normal Levels within 1 to 2 d.

- It Is Significantly More Sensitive Than CK, CK-MB, Mb and TnT during The First 3 to 4 h after Onset of AMI

- May Increase During Reversible Ischemia

HEART FATTY ACID BINDING PROTEIN (H-FABP)

■ **After Onset of chest Pain**

  1) It Increases Rapidly within 2 to 4 h

  2) Peaks within 5 to 10 h

  3) Return to Normal Levels within 24 to 36 h

■ **It Can Be Used**

  1) To Determine Recurrent Infarctions

  2) For Early Confirmation or Exclusion of AMI

ISCHEMIA MODIFIED ALBUMIN

- Is Not Released By Damaged Myocytes
- Results from Ischemia
- Detects Ischemia Before Irreversible Cellular Damage
- It Is Not Specific for Cardiac Ischemia

Myoglobin, or CK-MB isoform ratio

Transaminase, total CK, or CK-MB

LD-1

cTnI or cTnT

Marker Concentration (% of Maximum)

Hours After Onset of Chest Pain