Inflammatory markers in cardiovascular disease

Dr. Mona Soliman, MBBS, MSc
Department of Physiology
College of Medicine
King Saud University
Content

- Scientific Basis of Inflammation in Atherogenesis
- Inflammation and ACS
  - Inflammatory markers and cardiovascular events: C-Reactive Protein (CRP)
Scientific Basis of Inflammation in Atherogenesis

- The role of inflammation has been well established over the past decade in describing the atherosclerotic disease process.
- Experimental studies showed that signs of inflammation occur with lipid accumulation in the artery wall.
“Inflammation”
Inflammation...cont.

- The process of:
  - Setting on fire
  - Arousing to strong emotions
  - Making more violent

Webster’s Dictionary
Inflammation...cont.

As a result of:
- Irritation
- Injury
- Infection

Calor
Rubor
Tumor
Dolor
Inflammation...cont.

- “The reaction of blood vessels leading to the accumulation of fluid and leukocytes in the extravascular tissue”

“Atherosclerosis”
Triggers for Inflammation in Atherogenesis
Risk Factors of atherosclerosis and cardiovascular diseases

- Oxidized lipoproteins
- Dyslipidemia
- Hypertension
- Diabetes
- Obesity
- Infection

Atherosclerosis & Inflammation

Cardiovascular Diseases
Triggers for Inflammation in Atherogenesis...cont.

- Oxidation hypothesis:
  - LDL in intima + proteoglycan
  - Oxidative modification
    - (modified lipids: lipid hydroperoxides, lysophospholipids)
  - Expression of adhesion molecules, chemokines, cytokines and other inflammatory mediators

?? Relevance to human atherosclerosis
Triggers for Inflammation in Atherogenesis...cont.

**Dyslipidemia and inflammation**

- **VLDL**
  - Oxidative modification
    - Activate inflammation in endothelial cells

- **HDL** protect against atherosclerosis??
  1. Reverse cholesterol transport
  2. Transport antioxidant enzymes (platelet-activating factor) that break down oxidized lipids
Increasing evidence supports the view that inflammation participate in hypertension and thus providing a link between atherosclerosis

Angiotensin II

\[ \downarrow \]

Superoxide anion (ROS)
From ECs and SMCs

\[ \uparrow \]

Cytokines (IL-6, MCP-1) & VCAM-1

Clinical benefits of ACEI

Proinflammatory pathways
Hyperglycemia

↓

AGE (advanced glycation end products)

↓

Bind to surface receptors (RAGE)

Augment the production of cytokines and other inflammatory pathways
Obesity predispose to:
1. Insulin resistance
2. Diabetes
3. Atherogenic dyslipidemia?

Free fatty acids from visceral fat reach liver and stimulate synthesis of VLDL → ↓ HDL

Adipose tissue synthesize cytokines (TNF-\(\gamma\) and IL-6) → promote inflammation
Triggers for Inflammation in Atherogenesis...cont.

**Infection**

*Extravascular infection*
- (gingivitis, bronchitis)
- Augment production of cytokines

*Intravascular infection*
- Local inflammatory stimulus
- Accelerate atherosclerotic lesion
Triggers for Inflammation in Atherogenesis...cont.

**Infection**

- Many human plaques show signs of infection by *Chlamydia pneumoniae*↓
  - Lipopolysaccharide and heat shock proteins↓
  - Proinflammatory mediators by ECs and SMCs↓

- Studies showed that antibodies directed against *Chlamydia pneumoniae* predict cardiovascular risk

*J Clin Invest, 1999;103:571-577*
What are the Signs of Inflammation in Atherosclerosis?
Scientific Basis of Inflammation in Atherogenesis...cont.

1. **Leukocyte Binding to arterial wall**
2. **Leukocyte penetration into intima**
3. **Local inflammatory response**
4. **Acute thrombotic complications**
Leukocyte Binding
Scientific Basis of Inflammation in Atherogenesis... cont.

Leukocyte Binding
1. ↑ expression of adhesion molecules (VCAM-1)

- VCAM-1 are endothelial adhesion molecules
- The normal endothelium does not support binding of WBCs
- VCAM-1 plays an important role in atherogenesis by promoting monocyte accumulation in the arterial intima... *Atheroscler Thromb.* 1993;13:197-204

- Increase adhesion molecule expression within sites prone to atheroma... *Proc Natl Acad Sci USA.* 1996;93:10417-10422
VCAM-1 deficient mice were produced by genetic studies.

Mice were fed cholesterol-enriched diet for 8 weeks.

Atherosclerotic lesions were identified by en face oil red O staining.

The atherosclerotic lesions were reduced in VCAM-1 deficient mice.
Scientific Basis of Inflammation in Atherogenesis...cont.

**Leukocyte Binding**

Augment the production of certain leukocyte adhesion molecules (intracellular adhesion molecule-1 [ICAM-1])

*J Invest, 1994; 94:885-891*
Leukocyte Binding

- ICAM-1 deficient mice were produced by genetic studies
- The atherosclerotic lesions were not reduced in ICAM-1 deficient mice
2. ↓ local production of endothelium-derived NO

- Leukocyte Binding
  - Vasodilator
  - Anti-inflammatory properties
  - ↓ expression of VCAM-1

NO

Scientific Basis of Inflammation in Atherogenesis...cont.

J Invest, 1995;96:60-68
3. **Promote production by arterial smooth muscle cells (SMCs) of proteoglycans**

- Proteoglycans regulate cell adhesion, migration and proliferation

- Proteoglycans bind and retain lipoprotein particles

  ➔ Oxidative modification

  ➔ Inflammatory response

*Scientific Basis of Inflammation in Atherogenesis... cont.*

*Leukocyte Binding*

*J Biol Chem. 2001; 276: 13847-13851*
1. Leukocyte Binding to arterial wall
2. Leukocyte penetration into intima
3. Local inflammatory response
4. Acute thrombotic complications

Scientific Basis of Inflammation in Atherogenesis...cont.
Recent research has identified chemoattractant molecules responsible for leukocyte penetration.

- **Leukocyte penetration**

- **Monocyte chemoattractant protein-1 (MCP-1)**

- **Immunological role**

  - **Chemoattractants**
  - **Lymphocytes**

*References:* 
- J Clin Invest. 1999;104:1041-1050
Leukocyte penetration

• The mechanism of T-lymphocyte recruitment within the atherosclerotic lesion is not clear

???

• Multiple process

Chemokines:

• IL-8, IP-10, Mig and I-TAC
Scientific Basis of Inflammation in Atherogenesis...cont.

T-cell chemoattractants attract lymphocytes

- IP-10, Mig and I-TAC in human atherosclerotic lesion
T-cell chemoattractants attract lymphocytes

- **Western blot analysis** of vascular tissue extracts from normal human aortas and human carotid atherosclerotic lesions
- **Analyzed for IP-10 and Mig expression**

_J Clin Invest. 1999;104:1041-1050_
1. Leukocyte Binding to arterial wall
2. Leukocyte penetration into intima
3. Local inflammatory response
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Scientific Basis of Inflammation in Atherogenesis...cont.

A. Leukocyte Binding

B. Leukocyte penetration

C.
Scientific Basis of Inflammation in Atherogenesis...cont.

**Local inflammatory response**

- **Macrophages** express scavenger receptors for lipoproteins → ingest lipid → foam cells
- **Macrophages colony-stimulating factor (M-CSF)** → differentiation of monocyte into foam cells
- **T cells** → inflammatory cytokines (γ-interferon and TNF-β) → stimulate macrophages, endothelial cells and SMCs
Scientific Basis of Inflammation in Atherogenesis...cont.

1. Leukocyte Binding to arterial wall
2. Leukocyte penetration into intima
3. Local inflammatory response
4. Acute thrombotic complications
Activated macrophages → proteolytic enzyme → degrade collagen of the plaques fibrous cap → thin, weak cap → rupture

T lymphocytes → γ-interferon → prevent collagen synthesis in the plaque

Macrophages → tissue factor: procoagulant and trigger to thrombosis
Content

- Scientific Basis of Inflammation in Atherogenesis
- Inflammation and ACS
  - Inflammatory markers and cardiovascular events:
    - C-Reactive Protein (CRP)
Inflammation and ACS

Thrombolytic
Anticoagulant
Antiplatelet

Nitrates

Atherosclerosis

Thrombosis

Vaso-constriction

Serotonin
Thromboxane A2
Thrombin
Inflammation and ACS...cont.

Yet, even with aggressive thrombolytic, anticoagulant, and/or antiplatelet agents or interventional therapy, patients with ACS have 12-16% incidence of major cardiac events 4-6 months after hospital discharge... *N Engl J Med.* 2001;344:1879-1887
"We have one and a half million heart attacks in the U.S. each year. About 50 percent have normal cholesterol. Clearly, current guidelines are not adequate to identify all those individuals who are at increased risk," said Nader Rifai, shown at right with Paul Ridker.
Inflammation and ACS...cont.

- Increased understanding of the underlying mechanisms of atherosclerosis and inflammation and the pathophysiological mechanisms of the ACS should yield further improvements in **Outcome**

and may lead to new target for **Therapy**
Could the serum levels of markers of inflammation be used to predict the risk of cardiovascular events
Inflammatory Markers

Cytokines
- Interleukin-1β and 6
- Tumor necrosis factor α

Liver

C-reactive protein
Fibrinogen
Serum amyloid A

Rader NEJM. 2000; 343 (16):1179
Inflammatory Markers

- ICAM-1
- VCAM-1
- E-selectin
- P-selectin

Cytokines:
- Interleukin-1β and 6
- Tumor necrosis factor α

Liver:
- C-reactive protein
- Fibrinogen
- Serum amyloid A

Rader NEJM. 2000; 343 (16):1179
Inflammatory Markers

Vessel wall

- ICAM-1
- VCAM-1
- E-selectin
- P-selectin

Macrophages

- Lipoprotein-associated phospholipase A2
- Secretory phospholipase A2

Cytokines
- Interleukin-1β and 6
- Tumor necrosis factor α

Liver

- C-reactive protein
- Fibrinogen
- Serum amyloid A

Rader NEJM. 2000; 343 (16):1179
Cytokines
- Interleukin-1β and 6
- Tumor necrosis factor α

Macrophages
- Lipoprotein-associated phospholipase A₂
- Secretory phospholipase A₂

Adipose tissue

Liver
- C-reactive protein
- Fibrinogen
- Serum amyloid A

Vessel wall
- ICAM-1
- VCAM-1
- E-selectin
- P-selectin

Heart
- Troponin T and I
- Creatine kinase MB

Rader NEJM. 2000; 343 (16):1179
Sources of Inflammatory Markers and Cytokines

Inflammatory markers and Cardiovascular Diseases

- C-Reactive Protein (CRP)
- Serum amyloid A (SAA)
- Fibrinogen

C- Reactive Protein and risk of Cardiovascular Events

- For clinical purpose, the most promising inflammatory marker is C-reactive protein (CRP)
**C- Reactive Protein and risk of Cardiovascular Events...cont.**

- **C-reactive protein (CRP) is a relatively nonspecific marker of inflammation**
- **Acute phase reactant**
- **C- reactive protein...react with C-polysaccharide of pneumococcus**
- **In 1940s and 1950s, CRP was used as a test for inflammation**
- **CRP concentration:**
  - < 0.05mg/dl is normal
  - 0.06-10 mg/dl moderate increase
  - >10 mg/dl marked increase
C- Reactive Protein and risk of Cardiovascular Events...cont.

- How is CRP measured?
  - Immuno-turbidimetric or immuno-electrophoretic assays
  - High-sensitivity CRP
  - Uses labeled monoclonal or polyclonal anti-CRP antibodies in an enzyme-linked immunosorbent assay (ELISA) or an immuno-fluorescent assay
Growing evidence indicates that in ACS, elevated inflammatory markers, in particular C-reactive protein (CRP), predict unfavourable course.
The Heart Test That Could Save Your Life

An easy, new way to help predict your risk of heart attack and stroke.
"That is a major challenge to our guidelines for heart disease screening," said Ridker, HMS professor of medicine at Brigham and Women's Hospital.
Prospective studies indicate that baseline levels of CRP are associated with increased risk of MI among healthy individuals.

- 22,071 U.S. male healthy physicians
- Age 40 to 84 years in 1982
- Baseline CRP levels
- Report of MI

C- Reactive Protein and risk of Cardiovascular Events...cont.

- Relative risk of future cardiovascular diseases among apparently healthy middle aged men

An Intern Med. 1999
C- Reactive Protein and risk of Cardiovascular Events…cont.

- **CRP** predict risk of future cardiovascular events
  - One year survival free of readmission for MI according to CRP levels at discharge

Incidences of readmission for instability or MI

*Incidence of readmission for instability or MI*

C-Reactive Protein and risk of Cardiovascular Events...cont.

- Elevated CRP levels in after MI, predict risk of future second cardiovascular events

Am J Cardiol. 1999;83:1595-1599.
C- Reactive Protein and risk of Cardiovascular Events...cont.

- After coronary stenting, CRP levels greater than 0.5mg/dl predict future heart attacks

*Am J Cardiol. 1999; 83:1595-1599.*
In 917 patients of unstable angina, elevated levels of CRP, troponin T and fibrinogen are strongly related to risk of death of cardiac cause.

C- Reactive Protein and risk of Cardiovascular Events...cont.

C- Reactive Protein and risk of Cardiovascular Events...cont.

- Incidence of Death from Cardiac Causes at Two Years, According to the Presence or Absence of ST-Segment Depression and the levels of Troponin T and C-Reactive Protein

C-Reactive Protein and risk of Cardiovascular Events...cont.

- Associations of the C-Reactive Protein Level with the Risk of a Coronary Event in 580 men with MI

C- Reactive Protein and risk of Cardiovascular Events...cont.

- Elevated CRP (>3 mg/L) is found in
  - <10% of normals
  - <20% of patients with stable angina
  - >65% of patients with unstable angina
  - >90% of patients with acute infarction

C- Reactive Protein and risk of Cardiovascular Events...cont.

• In 2003, the American Heart Association and the Centers for Disease Control and Prevention issued a scientific statement that suggested the use of C-reactive protein as an optional myocardial infarction risk factor measurement... *Circulation. 2003;107:499-511.*
C- Reactive Protein and risk of Cardiovascular Events...cont.

- **CRP**

1. Provide a simple method for **screening and primary prevention** of myocardial infarction

Provide a novel method of targeting statin therapy

New target for therapy
C- Reactive Protein ...Implications for prevention

- **Statins:**
  - Potent LDL lowering agents
  - Attenuate plaque inflammation and influence stability
  - Reduce macrophage content within atherosclerotic plaques
  - Inhibit expression of adhesion molecules

*Anti-inflammatory*

*Circulation.* 2001; 103:993-999.
C- Reactive Protein ...Implications for prevention...cont.

- The first data to link the CRP and statin therapy was from the Cholesterol and Recurrent Events (CARE) trial.
CRP levels measured at baseline and at 5 years in 472 patients in the CARE trial.

- Showed that:
  - Statins lowered CRP levels
  - Statins anti-inflammatory effect

C- Reactive Protein ...Implications for prevention...cont.

- The Paravastatin Inflammation CRP Evaluation (PRINCE), 2884 patients received paravastatin and placebo

- CRP and LDL levels were lowered by paravastatin in unrelated manner

⭐Statins anti-inflammatory effect
C- Reactive Protein ... Implications for prevention... cont.

- Statins may have anti-inflammatory effects in addition to lipid-lowering

- Statins may play important role in primary prevention of cardiovascular disease in HEALTHY individuals with high CRP and normal lipid profile (50%)
Conclusion

- Our understanding of atherosclerosis has evolved beyond the view that the lesion consist of lipid debris.

- Current evidence support a central role for inflammation that can be used to predict future cardiovascular risk.
Conclusion...cont.

- Clinical studies correlate the circulating inflammatory markers with prevention, prognosis and therapy of cardiovascular diseases.

- Future studies will prove the utility of inflammatory markers as a guide to monitor the Statin therapy in primary prevention of cardiovascular diseases.
Thank You
Interaction between cholesterol & C-reactive protein (CRP) in determination of your risk for heart attacks & strokes. From Sci. Amer. May 2002.
The End
Conclusion
Event Rates and Number Needed to Treat Among Those Allocated to Lovastatin or Placebo in the AFCAPS/TexCAPS Trial, According to Baseline Levels of LDL Cholesterol and CRP

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Event Rates/5 Years</th>
<th>NNT*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low LDL, low CRP</td>
<td>0.20</td>
<td>0.22</td>
</tr>
<tr>
<td>Low LDL, high CRP</td>
<td>0.09</td>
<td>0.01</td>
</tr>
<tr>
<td>High LDL, low CRP</td>
<td>0.70</td>
<td>0.00</td>
</tr>
</tbody>
</table>

The NNT can not be calculated for those in the low LDL, low CRP strata because there was no evidence of efficacy of lovastatin in this subgroup.

Adapted from Ridker et al.
Cholesterol plaque contains many of the inflammatory elements that are designed to destroy infectious germs such as *Chlamydia pneumoniae* - the Heart Attack Germ - and repair damaged tissue. These elements are drawn to injured and infected arteries as part of the healing process.
there's a new test you can take that measures the levels of chronic inflammation in your bloodstream. This test - called hs-CRP or high-sensitivity C-reactive protein - is a more accurate predictor of heart attack than cholesterol levels, even in people who are currently healthy.
Scientific Basis of Inflammation in Atherogenesis...cont.

**Leukocyte Binding**

1. ↑ expression of adhesion molecules (VCAM-1)
2. ↓ local production of endothelium-derived NO
3. Augment the production of certain leukocyte adhesion molecules (intracellular adhesion molecule molecule-1 [ICAM-1])
4. Promote production by arterial smooth muscle cells (SMCs) of proteoglycans
The latest research has proven a link between inflammation and atherosclerosis. In fact, it's now known that inflammation is responsible for the birth and growth of atherosclerosis.
C-Reactive Protein and risk of Cardiovascular Events...cont.

Fig. 3. In patients with unstable angina, CRP values greater than 0.3 mg/dL predict increased likelihood of future heart attacks (Tomassi, et al. 1999).
Scientific Basis of Inflammation in Atherogenesis...cont.

**Leukocyte Binding**

[A: C S Versican Perlecan Biglycan Decorin]

[B: Medium Cells ~KD C S]

Versican 420
Perlecan 500
Biglycan 400
Decorin 52

C- Reactive Protein and risk of Cardiovascular Events...cont.

**Fig. 2.** Relative risk of cardiovascular events among apparently healthy postmenopausal women. When CRP levels are low and cholesterol levels are *high*, the risk is significantly *less* than when cholesterol levels are *low* and CRP levels are *high*. Naturally, it is best to have both low cholesterol and low CRP (Sparrow, et al, 1986).
The Anatomy of Atherosclerotic Plaque

- Intima
- Fibrous cap
- Lipid core
- Media
- T lymphocyte
- Macrophage
- Foam cell (tissue factor)
- "Activated" intimal SMC (HLA-DR)
- Normal medial SMC

Triggers for Inflammation in Atherogenesis...cont.

Nutrition, Age, Race, Genes, Fetal Programming

Atherosclerosis

Cytokines

Liver

Adipose Tissue

Beta Cell

Glucose Intolerance

Insulin Resistance

Acute-Phase Response

Fibrinogen

VLDL

Serum Amyloid A

HDL ↓

Leptin

Brain

ACTH

Cortisol

Central Obesity

Hypertension

Insulin Resistance

Pickup JA, Crook MA
Diabetologia 1998; 41:1241-48
Pro-Inflammatory Risk Factors (oxidized LDL, infectious agents, etc)

vascular and extravascular sources

Primary Pro-Inflammatory Cytokines (e.g., IL-1, TNF-α)

- IL-6
  - “Messenger” Cytokine
  - ICAM-1
  - Selectins, HSPs, etc.
  - CRP
  - SAA
  - Liver

Endothelium & other cells

Circulation