Biomarkers in and of Critical Illness:
What do they mean and are they useful?

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Definition of a Biomarker

- A biochemical feature or facet that can be used to diagnose and/or measure the progress of disease or the effects of treatment.
Content

- Cardiac biomarkers
  - Troponin (I, T, & C) / CKMB
  - B type Natriuretic Peptide (BNP)
- Sepsis
  - Procalcitonin
- AKI
  - creatinine, urea, new biomarkers
- Liver failure
  - Liver enzymes
- Brain failure
  - Neuron specific enolase
- PE - D dimer
- Clotting - APTT / INR
Cardiac Biomarkers

MI biomarkers - Troponin / CK, CKMB
Heart failure biomarkers - BNP
Biomarkers of Myocardial Infarction
Basis of Injury

- Severe acute ischaemia:
- Lowers the resting potential (makes it less negative)
- Shortens the duration of the action potential and changes the shape of the plateau (phase 2) - in the ischaemic area.
WHO definition of MI

- 2 of 3 of the following criteria
  - Typical symptoms
  - Typical ECG changes
  - Elevated cardiac biomarker
Case studies...
36 year old female

- 3 day history of shortness of breath
- Flu like symptoms
- Decline in exercise tolerance
- Generalised malaise
- Fevers
- Presents to the ED with chest pain
Sent to PCI facility

- Angiogram NAD
- Raised Troponin I

- Met all 3 of the WHO criteria for an MI
  - Chest pain
  - ST elevation
  - Raised troponin
84 year old female

- 4 day history productive cough
- Chest x-ray - lobular consolidation
- Treated with ceftriaxone - of course...
- Noted to be “between the flags”
  - Heart rate 93
  - Respiratory rate 22
  - WCC - low
  - Temp 35.8°C
- Raised troponin - diagnosed as non STEMI
ECG
Case 1 - 36 year old female
The trouble with biomarkers...
Other conditions can alter the ST segment / T wave

- Electrolyte abnormalities
- Post-cardiac surgical state
- Anemia
- Fever
- Acidosis or alkalosis
- Catecholamines
- Drugs
- Acute abdominal process
- Endocrine abnormalities
- Metabolic changes
- Cerebrovascular accidents
- Diseases such as myocarditis, pericarditis, cardiomyopathy, pulmonary emboli, infections, amyloidosis, systemic diseases, lung diseases

- Abnormal T waves and ST segments may also be seen in healthy individuals, including well trained athletes.
The trouble with biomarkers...

- Can’t rely on the ECG
- Can’t rely on troponin
The Ideal Biochemical Cardiac Marker

- **High Sensitivity** - High concentration of the marker in the myocardium
- **High specificity** - Absent concentrations in non-cardiac tissue and not detectable in blood from non-diseased subjects
- **Release** - rapid release for early diagnosis and long half life in the blood for early diagnosis
- **Analytical** - cost effective, short turn around time, precise, accurate
- **Clinical** - ability to influence therapy and so improve patient outcomes, validated by clinical studies.
Troponin
Troponin

- Specific to cardiac tissue
- 3 types of troponin
- Takes 6-12 hours to be excreted
- Stays in your blood for 10-14 days
WHO definition of MI

- 2 of 3 of the following criteria
  - Typical symptoms
  - Typical ECG changes
  - Elevated CKMB
- The addition of Troponin testing demonstrated that approximately half the number of patients did not meet the WHO criteria with a raised troponin
- More ACS patients with MI
Troponin T and Mortality

Graph showing cumulative probability of death over months for different troponin T levels.
Positive Troponins in the Critically Ill

- Commonly (always) raised in the ICU population
- The sensitivity of Troponin is high in patients presenting with signs of acute coronary syndrome
- The sensitivity of troponin in ICU has been quoted to be in the range of 56%.

????????????????
- Pericarditis & myocarditis - 30% have raised troponin / CKMB
- PCI - CKMB & Troponin raised post procedure, prognostic indicator.
- Heart failure - troponin elevated and is a prognostic indicator
- PE - troponin elevated. Amount correlates with degree of ventricular dysfunction and prognosis.
- Renal failure - troponin elevated, increased risk of death
- Sepsis - elevated. Not prognostic indicator
- Critical Illness - elevated. Not prognostic indicator
- Acute stroke - elevated predict mortality - but link with ACS.
The role of echocardiography in establishing:

- the diagnosis,
- location,
- extent of MI,
- in diagnosing mechanical complications of infarction,
- providing prognostic information that is important for risk stratification will be reviewed.
RWMA’s changes occur prior to the onset of electrocardiographic changes or the development of symptoms.

Regional myocardial dysfunction, which can be detected as regional wall motion abnormalities on echocardiography, occurs before ECG changes or anginal chest pain.

Thus, echocardiography for an acute coronary syndrome has a **high sensitivity** but a relatively **lower specificity**.

- **Study of 180 patients** with chest pain in the emergency department; the following findings were noted:
  - RWMAs were present in **27 of 29** patients with an acute MI (sensitivity 93 %) (only 9 had ST elevation and 8 developed q waves)
  - RWMAs were indicative of acute MI in **only 31 percent** of 87 patients
Role of Troponin in the Critically Ill

- Useful in diagnosing MI in patients presenting with signs of ACS when you can use the WHO criteria
- Limited role in the critically ill
- Flags patients who may require an echo
BNP in Adult Cardiac Disease

- Widely used as a biomarker in the distinction between dyspnea caused by congestive heart failure and that caused by non cardiac aetiologies.  

BNP

- In 1988 Sudoh & colleagues described a novel natriuretic peptide in porcine brain\(^1\) (Brain Natriuretic Peptide)
- Subsequently found to be most abundant in the heart - renamed “B-type Natriuretic Peptide” (BNP)
- Release is triggered in large part by myocyte stretch
- BNP levels are easily quantified by several commercial assays.


a) An agent that promotes the excretion of sodium in the urine
b) Cardiac muscle cell.
**Primary actions**

- Vascular smooth muscle relaxation and anti-mitogenesis

- Mediated by cGMP, diuresis caused by a shift of fluid into the interstium and

- Natuiriuresis, caused by antagonism of renin and aldosterone release.

1. Mitogenesis = the initiation of the process of cell division, or mitosis.
NP Pathway

Pre-Pro BNP

Pro BNP

NT pro BNP

BNP

NT-ProBNP is excreted by the kidneys – thus renal function has a greater influence on NT-proBNP levels than BNP levels.

BNP may be a better dynamic measure as the half life is shorter (20 min vs 60-120 mins).
In addition to volume and pressure loading:
- Acute myocardial ischaemia
- Alpha agonist stimulation
- Endothelin-1 and inflammatory mediators, such as:
  - Tumor necrosis factor (TNF) alpha and interleukin (IL) beta

Result in a rapid ventricular expression of BNP
Role of BNP in the Critically Ill

Procalcitonin
Figure 1: (Adapted from DeGaudio 2009:710)

Gram Negative Sepsis

lipopolysaccharide

MD2 CD14 TL4 LPB

Cell Surface

Gram Positive Sepsis

teichoic acid & lipoteichoic acid

CD14 TL2

NF-κβ

Proinflammatory Cytokines
Cytokines

Pro inflammatory
- Tubular Necrosis Factor – α (TNF α)
- Interleukin 1 (IL1)
- Interleukin 1β (IL β)
- Interleukin 2 (IL2)
- Interleukin 6 (IL6)

Anti inflammatory
- Interleukin 4 (IL4)
- Interleukin 10 (IL10)
- Interleukin 13 (IL 13)
Sepsis and Septic Shock

- Early signs of sepsis (fever, leukocytosis) are non-specific.
- More specific signs (lactate, hypotension) are late and associated with a high mortality.
- Variety of biomarkers have been proposed:
  - Cytokines (IL6 & IL8)
  - Acute phase proteins (such as CRP)
  - Procalcitonin
Procalcitonin (PCT)

- Procalcitonin is a peptide of 114 amino acids, lacking the N-terminal dipeptide alanine-proline.
- Pivotal roles in the metabolic and inflammatory host response to microbial infections.
- Role in calcium homeostasis.
- Levels elevated in the setting of bacterial sepsis.
High PCT may exert harmful effects influencing mortality and morbidity:

- PCT injected into healthy hamsters causes minor changes in phosphate, calcium and glucose. ¹
- PCT injected into septic hamsters – increases mortality to almost 100%. ¹
- Immunoneutralisation ameliorates symptomatology and improves outcome. ¹

Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: A systematic review and meta-analysis

Bernard Uzzan, MD; Régis Cohen, MD, PhD; Patrick Nicolas, PharmD, PhD; Michel Cucherat, MD; Gérard-Yves Perret, MD, PhD

Objective: To quantify the accuracy of serum procalcitonin as a diagnostic test for sepsis, severe sepsis, or septic shock in adults in intensive care units or after surgery or trauma, alone and compared with C-reactive protein. To draw and compare the summary receiver operating characteristics curves for procalcitonin and C-reactive protein from the literature.

Data Source: MEDLINE (keywords: procalcitonin, intensive care, sepsis, postoperative sepsis, trauma); screening of the literature.

Study Selection: Meta-analysis of all 49 published studies in medical, surgical, or polyvalent intensive care units or postoperative wards. Children, medical patients, and immunocompromised patients were excluded.

Data Extraction: Thirty-three studies fulfilled inclusion criteria (3,943 patients, 1,828 males, 922 females; mean age: 56.1 yrs; 1,825 patients with sepsis, severe sepsis, or septic shock; 1,545 with only systemic inflammatory response syndrome); eight studies could not be analyzed statistically. Global mortality rate was 29.3%.

Data Synthesis: Global odds ratio for diagnosis of infection complicated by systemic inflammation were 15.7 for the 25 studies (2,966 patients) using procalcitonin (95% confidence interval, 9.1–27.1) and 5.4 for the 15 studies (1,322 patients) using C-reactive protein (95% confidence interval, 3.2–9.2). The summary receiver operating characteristics curve for procalcitonin was better than for C-reactive protein. In the 15 studies using both markers, the Q* value (intersection of summary receiver operating characteristics curve with the diagonal line where sensitivity equals specificity) was significantly higher for procalcitonin than for C-reactive protein (0.78 vs. 0.71, p = .02), the former test showing better accuracy.

Conclusions: Procalcitonin represents a good biological diagnostic marker for sepsis, severe sepsis, or septic shock, difficult diagnoses in critically ill patients. Procalcitonin is superior to C-reactive protein. Procalcitonin should be included in diagnostic guidelines for sepsis and in clinical practice in intensive care units. (Crit Care Med 2006; 34:

Key Words: procalcitonin; biological marker; sepsis; septic shock; intensive care unit; postoperative complications; trauma; diagnostic test; meta-analysis
Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis

Benjamin M.P. Tong, Guy D. Eslick, Jonathan C. Craig, Anthony S. McLean

Procalcitonin is widely reported as a useful biochemical marker to differentiate sepsis from other non-infectious causes of systemic inflammatory response syndrome. In this systematic review, we estimated the diagnostic accuracy of procalcitonin in sepsis diagnosis in critically ill patients. 18 studies were included in the review. Overall, the diagnostic performance of procalcitonin was low, with mean values of both sensitivity and specificity being 71% (95% CI 67–76) and an area under the summary receiver operator characteristic curve of 0.78 (95% CI 0.73–0.83). Studies were grouped into phase 2 studies (n=14) and phase 3 studies (n=4) by use of Sackett and Haynes' classification. Phase 2 studies had a low pooled diagnostic odds ratio of 7.79 (95% CI 5.86–10.35). Phase 3 studies showed significant heterogeneity because of variability in sample size (meta-regression coefficient -0.592, p=0.017), with diagnostic performance upwardly biased in smaller studies, but moving towards a null effect in larger studies. Procalcitonin cannot reliably differentiate sepsis from other non-infectious causes of systemic inflammatory response syndrome in critically ill adult patients. The findings from this study do not lend support to the widespread use of the procalcitonin test in critical care settings.

Introduction
Sepsis is the leading cause of mortality in critically ill patients. Delay in diagnosis and treatment often results in rapid progression to circulatory collapse, multiple organ failure, and eventually death. Therefore, accurate and timely diagnosis will limit morbidity, reduce costs, and improve diagnostic accuracy of procalcitonin for sepsis. The search strategy used medical subject heading terms and text words, including the following: “procalcitonin”; “sepsis”, “sepsis syndrome”, “septicemia”, “infection”, “systemic inflammatory response syndrome”, and “SIRS”; and “sensitivity”, “specificity”, “predictive value”, and “likelihood ratio”.

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<table>
<thead>
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<th>Principal causes of hyperprocalcitonemia</th>
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<tr>
<td>A. Neuroendocrine tumors</td>
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<tr>
<td>Medullary thyroid cancer</td>
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<tr>
<td>Small cell lung cancer</td>
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<td>Carcinoid syndrome</td>
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<td>B. Noninfectious systemic inflammation</td>
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<td>Inhalational injury</td>
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<td>Pulmonary aspiration</td>
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<td>Pancreatitis</td>
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<td>Heat stroke</td>
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<td>Mesenteric infarction</td>
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<td>C. Severe infection</td>
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<td>Bacterial</td>
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<td>Viral</td>
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<td>Parasitic</td>
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<td>D. Sepsis</td>
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<td>E. Trauma</td>
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<td>Mechanical injury</td>
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Future
The Use of Gene-Expression Profiling to Identify Candidate Genes in Human Sepsis

Benjamin M. P. Tang1, Anthony S. McLean1, Ian W. Dawes2, Stephen J. Huang1, and Ruby C. Y. Lin2

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Rationale: Our understanding of the pathophysiology of sepsis remains incomplete. Genomewide study offers an unbiased, system biology approach to examine the expression patterns of circulating leukocytes and may reveal novel insights into the host response to sepsis.

Objectives: We examined whether gene-expression profiling of neutrophils could identify signature genes and important pathways in the clinical syndrome of sepsis.

Methods: Gene-expression profiling was performed using oligonucleotide microarrays on peripheral blood samples of 94 critically ill patients (71 septic and 23 nonseptic). Using a supervised learning algorithm based on support vector machine, a molecular signature of sepsis was generated from a training set of 44 samples and validated in an independent set of 50 samples. The diagnostic performance of the signature genes was assessed against a reference standard based on the International Sepsis Forum Consensus Conference definition of infection.

Measurements and Main Results: A set of 50 signature genes correctly identified sepsis with a prediction accuracy of 91 and 88% in the training and validation sets, respectively. The diagnostic performance remained high regardless of patient’s age, comorbidities, or prior antibiotic treatment. Compared with controls, genes involved in immune modulation and inflammatory response had reduced expression in patients with sepsis. In particular, the activation of nuclear factor-κB pathway was reduced, whereas its inhibitor gene, NFKBIA, was significantly up-regulated.

AT A GLANCE COMMENTARY
Scientific Knowledge on the Subject
Current methods to diagnose sepsis in critically ill patients lack sensitivity and specificity.

What This Study Adds to the Field
Gene-expression profiling represents a novel approach to diagnose sepsis. It also provides important biological insights into the host response to sepsis.

Although clinical evaluation has been the mainstay of sepsis diagnosis, the physical signs of sepsis can be nonspecific. For example, fever and new chest radiograph infiltrates can be found not only in pneumonia but also in pulmonary embolus or postoperative atelectasis. Traditional markers of infection, such as leukocytosis or C-reactive protein, are often unhelpful because they are frequently elevated in critically ill patients. In addition, patients are commonly sedated and ventilated and may have multiple comorbidities, adding further difficulties to differentiating sepsis from other serious illnesses.
Role of Procalcitonin in the ICU

- Variable results from reviews and metanalysis
- May assist with diagnosis
- Other conditions apart from bacterial sepsis and septic shock may cause an elevated PCT
- Serial measurements may assist in prognosis, monitoring course of disease, and antibiotic description
Acute Kidney Injury
Bowman’s Capsule
- Creatinine
- Urea
- NGAL
RIFLE Criteria

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<th>GFR Criteria*</th>
<th>Urine Output Criteria</th>
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- Increased creatinine x1.5 or GFR decrease > 25%
- Increased creatinine x2 or GFR decrease > 50%
- Increase creatinine x3 or GFR dec >75% or creatinine ≥4mg/dl (Acute rise of ≥0.5 mg/dl)

- UO < .5ml/kg/h x 6 hr
- UO < .5ml/kg/h x 12 hr
- UO < .3ml/kg/h x 24 hr or Anuria x 12 hrs
- Persistent ARF** = complete loss of renal function > 4 weeks
- End Stage Renal Disease

Figure 1. RIFLE criteria for acute kidney injury. GFR, glomerular filtration rate; UO, urine output; doc, decrease; ARF, acute renal failure; ESRD, end-stage renal disease. Used with permission from Bellomo et al (20). *GFR changes are shown for general reference only. The criteria fulfilled by changes in serum creatinine relative to baseline.
Liver Function Tests
1) Enzyme tests

- Serum aminotransferases
  - alanine aminotransferase (ALT), and
  - aspartate aminotransferase (AST).
- alkaline phosphatase, and
- gamma glutamyl transpeptidase
Function of the Liver

- Storage, manufacturing and cleansing of blood
- Storage of many nutrients including:
  - vitamins A, D, E, K and B_{12}
  - trace minerals such as iron and copper
- Manufactures bile, albumin and clotting factors
- Cleansing the blood of toxins and bacteria
Complications of Liver Failure

Hepatic encephalopathy
Variceal bleeding
infection
2) tests of synthetic function

- principally the serum albumin concentration and prothrombin time
3) liver's ability to detoxify metabolites and transport organic anions into bile

- Bilirubin
4) Blood flow & hepatic function

- Indocyanine green dye clearance
Brain Damage
Neuron specific enolase
Pulmonary Emboli
D Dimer
Clotting
INR

APTT
Biomarkers in the critically ill

- No current clinical biomarker is perfect
- Troponin has a low sensitivity in the critically ill.
- Role of BNP limited to the ED - differentiating cause of respiratory failure
- PCT? Best available biomarker for sepsis but do not inject PCT into septic hamsters...
- Creatinine / Urea best available
- Liver
- +ve D dimer not useful
- APTT - INR