Old and New biomarkers

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NORTHUMBRIA ORTHOPAEDIC INFECTION SERVICE

Northumbria Healthcare NHS
NHS Foundation Trust

Current Industry Disclosures

GRANTS

Academic Health Science Network - NENC and **Heraeus Medical** - Spreading the use of high dose antibiotic cement to prevent infection following surgery for hip fracture (lead applicant) -£84,452

Stryker - A randomised multicentre trial of 964 patients comparing the Thompsons stem with the Exeter/ unitrax for hemiarthroplasty (chief investigator) £313,003 with treatment costs

Heraeus Medical GMBH - Investigation of NucB anti-biofilm role in joints (co-applicant) £84,000

Zimmer Educational fellowship grant £45,923

Convatec Clinical audit: £30,000

SPEAKER FEES

Zimmer Biomet

Heraeus

The challenge

- **Is it infected or not?**
- **Which test do you use?**
- **MSIS?**
- **New biomarkers– cytokines, CD-64, Alpha defensin**
- **Who / which do you believe**

MSIS criteria

Clin Orthop Relat Res (2011) 469:2992–2994
DOI 10.1007/s11999-011-2102-9

Clinical Orthopaedics and Related Research®
A Publication of The Association of Bone and Joint Surgeons®

SYMPOSIUM: PAPERS PRESENTED AT THE 2010 MEETING OF THE MUSCULOSKELETAL INFECTION SOCIETY

New Definition for Periprosthetic Joint Infection

From the Workgroup of the Musculoskeletal Infection Society

Javad Parvizi MD, Benjamin Zmistowski BS, Elie F. Berbari MD, Thomas W. Bauer MD, PhD, Bryan D. Springer MD, Craig J. Della Valle MD, Kevin L. Garvin MD, Michael A. Mont MD, Montri D. Wongworawat MD, Charalampos G. Zalavras MD

Published online: 22 September 2011

Major criteria

One of the following:

1. Sinus communicating with the prosthesis
2. A pathogen is isolated from at least 2 separate tissue or fluid samples

Our previous gold standard

Minor Criteria

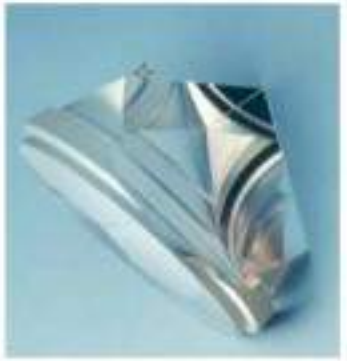
1. Three of the following six criteria exist:
   - Elevated ESR (>30)
   - CRP (>10 / 100)
   - Elevated synovial leukocyte count
   - Elevated synovial neutrophil percentage
   - Isolation of a microorganism in one culture of periprosthetic tissue or fluid, or
   - > 5 neutrophils per high-power field in five high power fields.

Our new gold standard?

Problems with MSIS

- **Not validated**
- Thresholds are controversial
- Technical issues around cell counts and differentials eg. Bloody tap and high viscosity
- Histology also technically challenging

Old Biomarkers?



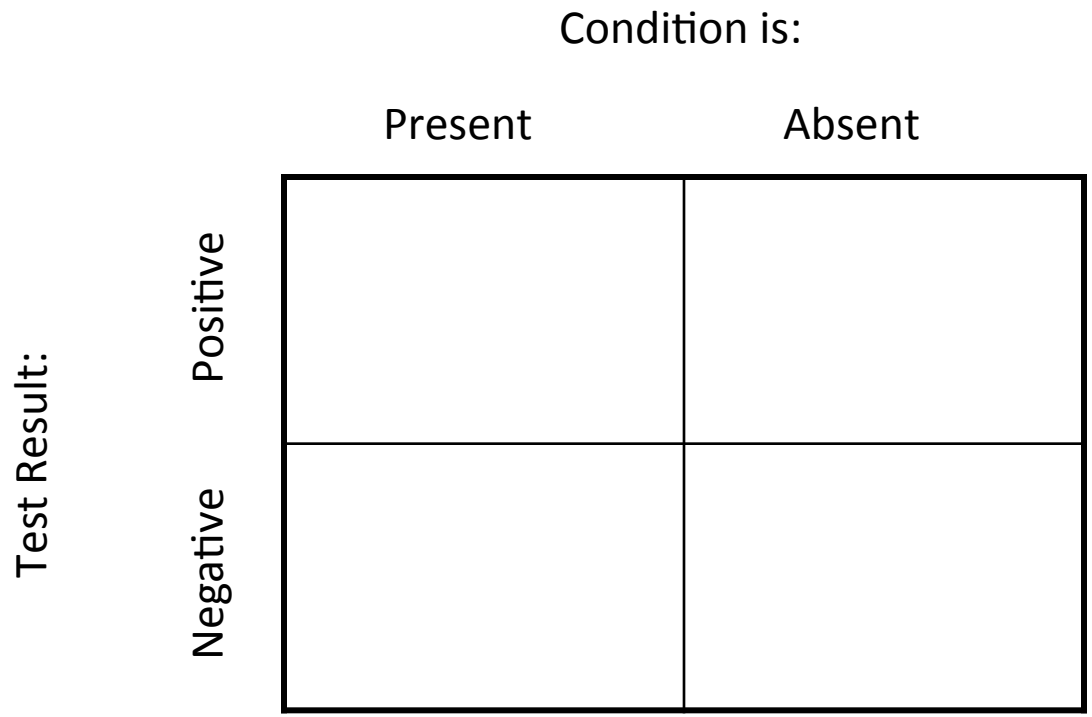
- First publication on “Biomarker” 1973
- What is a biomarker?
- “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention”

The ideal biomarker

- Quick
- Small volume of blood or saliva
- Accurate
- Stable

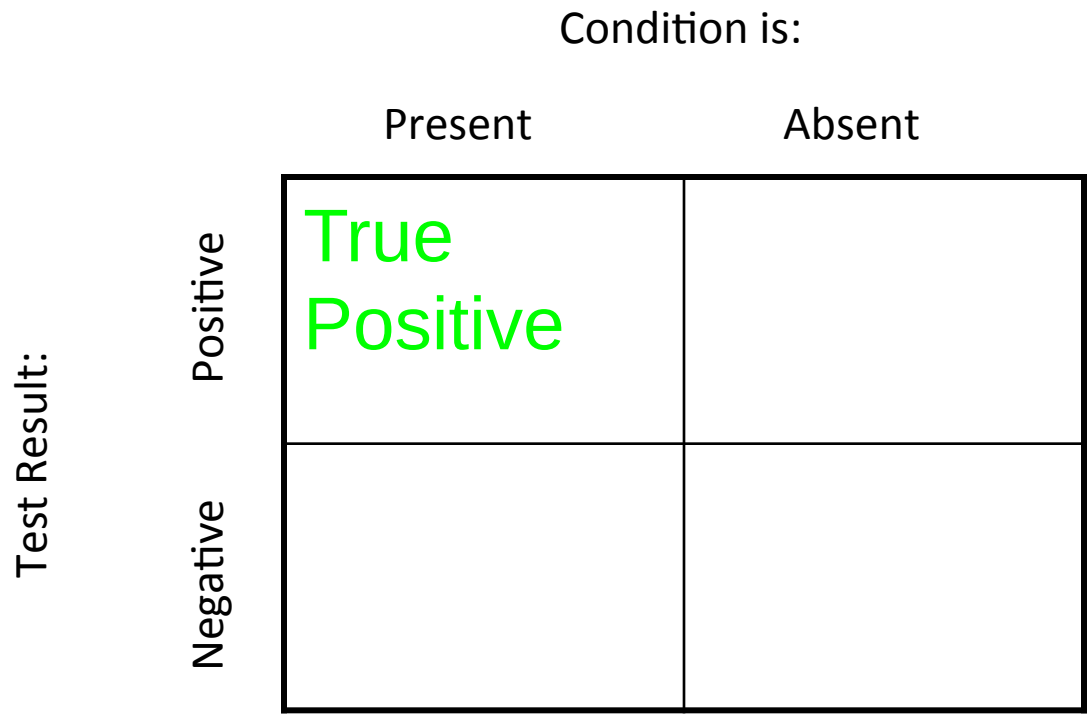
Sensitivity and Specificity

- Four possible situations:



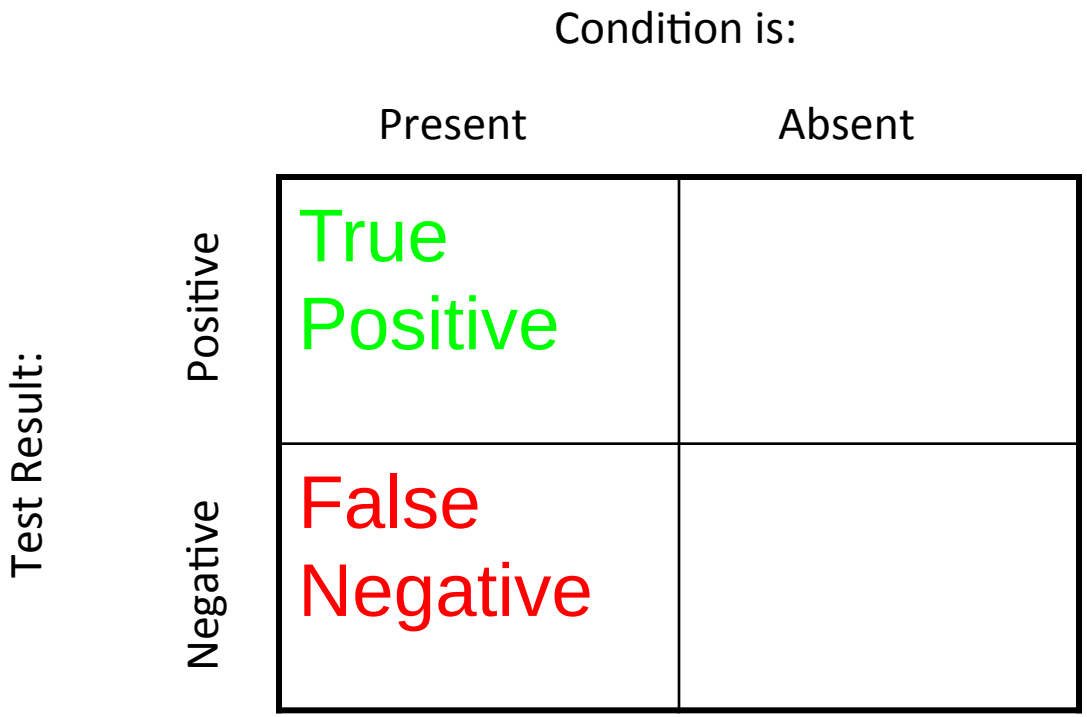
Sensitivity and Specificity

- Four possible situations:



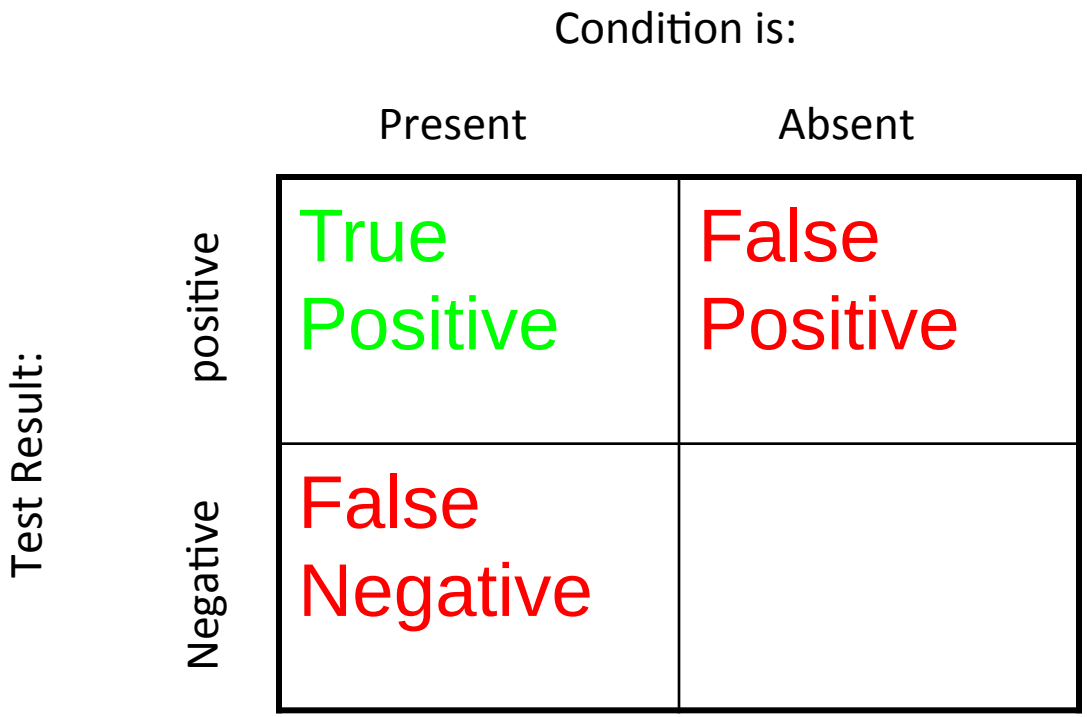
Sensitivity and Specificity

- Four possible situations:



Sensitivity and Specificity

- Four possible situations:



Sensitivity and Specificity

- Four possible situations:

Test Result:	Condition is: Present	Condition is: Absent
Positive	True Positive	False Positive
Negative	False Negative	True Negative

Sensitivity

- *Sensitivity* is the proportion of patients with the disease with a “positive” test result

$$Sensivity = \frac{\text{True Positives}}{\text{True Postives + False Negatives}}$$

Specificity

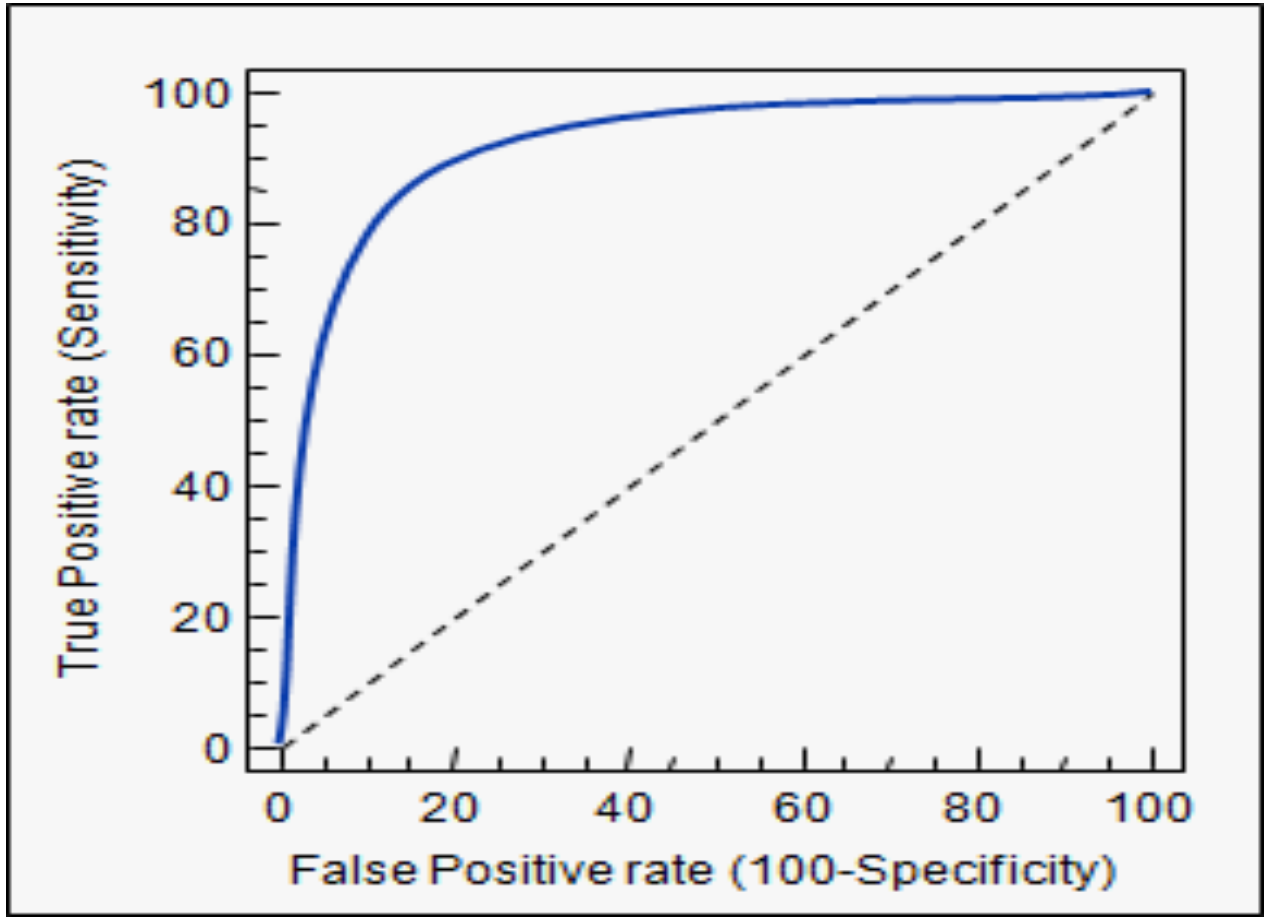
- Specificity is the proportion of patients **without** the disease with a “negative” result

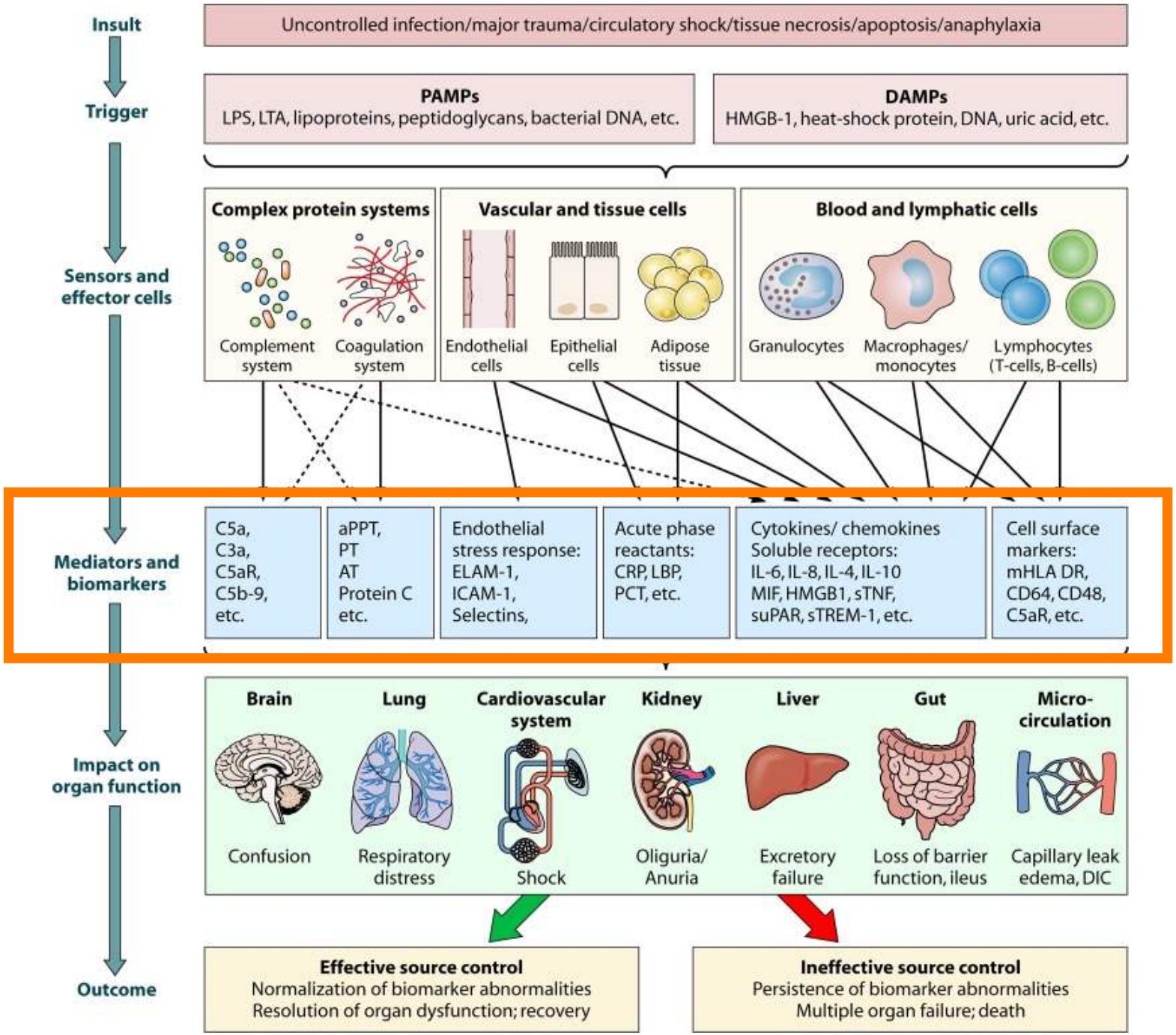
$$Specificity = \frac{\text{True Negative}}{\text{True Negative + False Positive}}$$

ROC curve

- Receiver operating characteristic curve
- Plot of true positive rate (sensitivity) against false positive rate (1-specificity)
- Diagnostic accuracy given by the area under the curve

ROC curve


True Positive rate (Sensitivity)
100
80
60
40
20
0
0
20
40
60
80
100
False Positive rate (100-Specificity)


Insult
Uncontrolled infection/major trauma/circulatory shock/tissue necrosis/apoptosis/anaphylaxia
Trigger
PAMPs
LPS, LTA, lipoproteins, peptidoglycans, bacterial DNA, etc.
DAMPs
HMGB-1, heat-shock protein, DNA, uric acid, etc.
Sensors and effector cells
Complex protein systems
Complement system
Coagulation system
Vascular and tissue cells
Endothelial cells
Epithelial cells
Adipose tissue
Blood and lymphatic cells
Granulocytes
Macrophages/ monocytes
Lymphocytes (T-cells, B-cells)
Mediators and biomarkers
C5a, C3a, C5aR, C5b-9, etc.
aPPT, PT AT Protein C etc.
Endothelial stress response: ELAM-1, ICAM-1, Selectins,
Acute phase reactants: CRP, LBP, PCT, etc.
Cytokines/ chemokines Soluble receptors: IL-6, IL-8, IL-4, IL-10 MIF, HMGB1, sTNF, suPAR, sTREM-1, etc.
Cell surface markers: mHLA DR, CD64, CD48, C5aR, etc.
Impact on organ function
Brain
Confusion
Lung
Respiratory distress
Cardiovascular system
Shock
Kidney
Oliguria/ Anuria
Liver
Excretory failure
Gut
Loss of barrier function, ileus
Micro-circulation
Capillary leak edema, DIC
Outcome
Effective source control
Normalization of biomarker abnormalities
Resolution of organ dysfunction; recovery
Ineffective source control
Persistence of biomarker abnormalities
Multiple organ failure; death

New biomarkers – personalised medicine?

The literature

No shortage…

Caution

Issues with PJI biomarker literature

- Small series
- Acute and/or chronic in same series
- Different diagnostic criteria

Peripheral blood tests

- CRP and ESR
- Alternatives – IL-6 , Procalcitonin,

2010

Inflammatory Blood Laboratory Levels as Markers of Prosthetic Joint Infection

A Systematic Review and Meta-Analysis

By Elie Berbari, MD, Tad Mabry, MD, Geoffrey Tsaras, MD, Mark Spangehl, MD, Pat J. Erwin, MLS, Mohammad Hassan Murad, MD, James Steckelberg, MD, and Douglas Osmon, MD

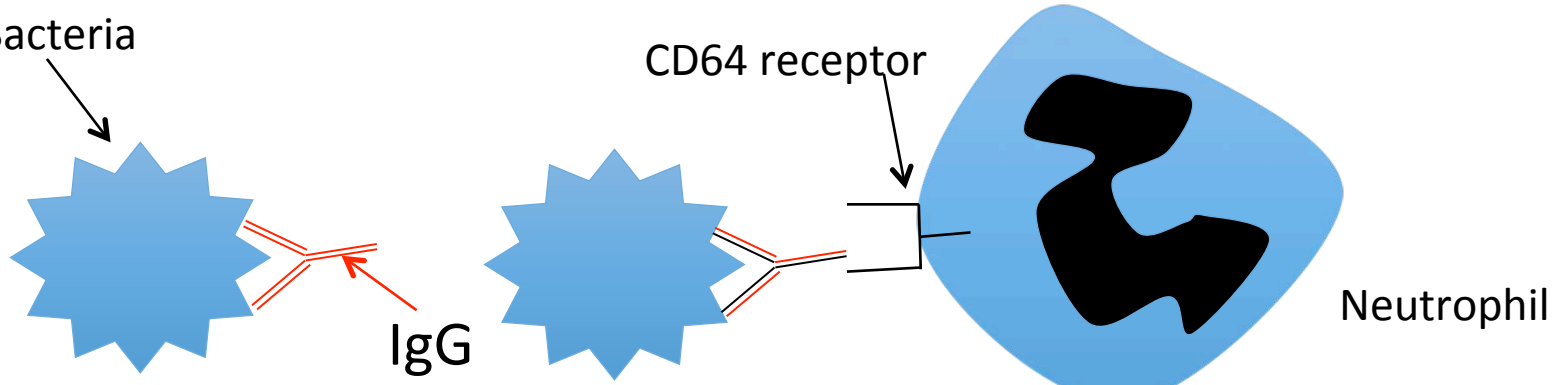
Investigation performed at Mayo Clinic College of Medicine, Rochester, Minnesota

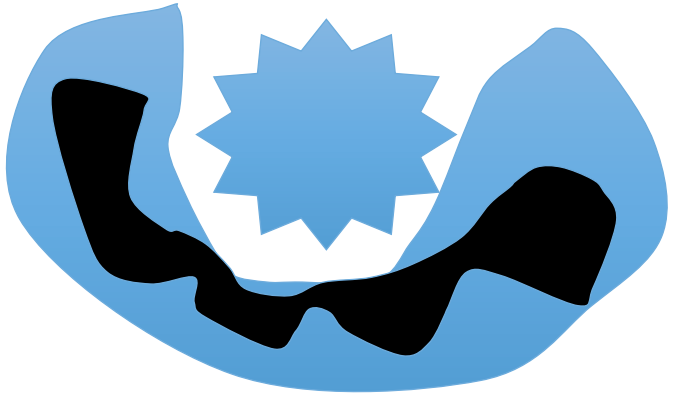
- **IL-6 superior to CRP**

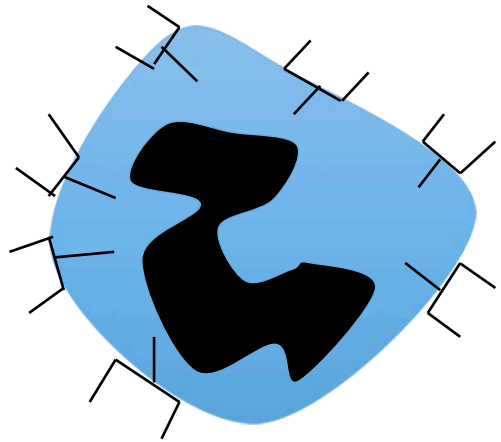
What about CD64?

- CD64 shows short lived response to surgery
  - normal by day 7 vs CRP 21 days to normalise
- Validated infection marker in active rheumatoid arthritis

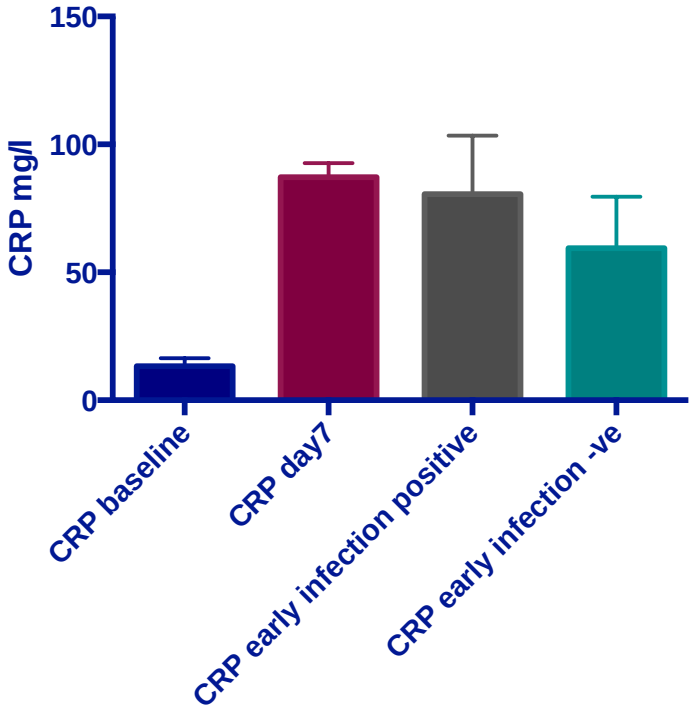
CD64 mechanism

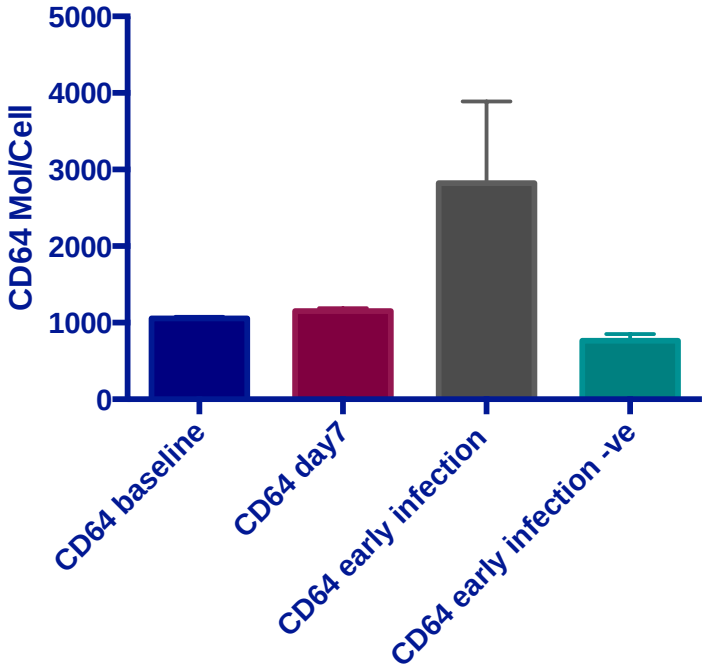






Some data ..


150
100
50
0
CRP mg/l
CRP baseline
CRP day7
CRP early infection positive
CRP early infection -ve


5000
4000
3000
2000
1000
0
CD64 Mol/Cell
CD64 baseline
CD64 day7
CD64 early infection
CD64 early infection -ve

Synovial fluid tests

- Closed compartment
- Higher concentrations of markers

BUT

- Invasive
- Expensive
- ? dangerous

Synovial fluid biomarkers – measure the host response to infection

- Acute phase – CRP
- Pro-inflammatory cytokines – eg. IL-6
- Antimicrobial peptides – eg. Alpha Defensins, Beta defensins,
- Enzymes – Leukocyte Esterase
- OMICS approaches

The search for the perfect biomarker

2011 - Leukocyte Esterase

Diagnosis of Periprosthetic Joint Infection: The Utility of a Simple Yet Unappreciated Enzyme

Javad Parvizi, MD, FRCS, Christina Jacovides, BS, Valentin Antoci, MD, PhD, and Elie Ghanem, MD

Investigation performed at the Rothman Institute of Orthopedics at Thomas Jefferson University Hospital, Philadelphia, Pennsylvania

108 revision knees
30 infections
Sensitivity 80.6% specificity 100%
But issues with bloody taps – can we do better?

Clin Orthop Relat Res (2014) 472:3254–3262
DOI 10.1007/s11999-014-3543-8

Clinical Orthopaedics and Related Research®
A Publication of The Association of Bone and Joint Surgeons®

SYMPOSIUM: 2013 MUSCULOSKELETAL INFECTION SOCIETY

Diagnosing Periprosthetic Joint Infection

Has the Era of the Biomarker Arrived?

Carl Deirmengian MD, Keith Kardos PhD,
Patrick Kilmartin, Alexander Cameron, Kevin Schiller,
Javad Parvizi MD

95 revisions
Hips and knees
29 infections – only 2 hips
6 culture negative
Mean CRP in infection 122 (72 – 184)

5 “perfect biomarkers”

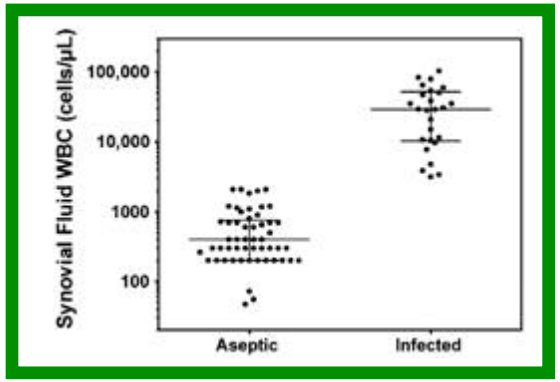
Table 4. Diagnostic characteristics of synovial fluid biomarkers

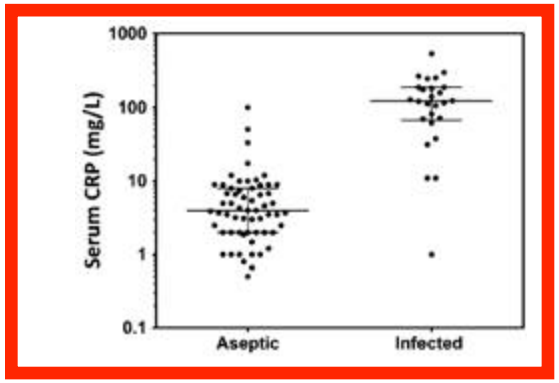
Biomarker	AUC	Cutoff	Specificity (%)	95% CI (%)	Sensitivity (%)	95% CI (%)
α-Defensin	1.000	4.8 μg/mL	100	95–100	100	88–100
ELA-2	1.000	2.0 μg/mL	100	95–100	100	88–100
BPI	1.000	2.2 μg/mL	100	95–100	100	88–100
NGAL	1.000	2.2 μg/mL	100	95–100	100	88–100
Lactoferrin	1.000	7.5 μg/mL	100	95–100	100	88–100
IL-8	0.992	6.5 ng/mL	95	87–99	100	87–100
SF CRP	0.987	12.2 mg/L	97	90–100	90	73–98
Resistin	0.983	340 ng/mL	100	95–100	97	82–99
Thrombospondin	0.974	1061 ng/mL	97	90–100	90	73–98
IL-1β	0.966	3.1 pg/mL	95	87–99	96	82–100
IL-6	0.950	2.3 ng/mL	97	89–100	89	71–98
IL-10	0.930	32.0 pg/mL	89	79–96	89	72–98
IL-1α	0.922	4.0 pg/mL	91	81–97	82	63–94
IL-17	0.892	3.1 pg/mL	99	92–100	82	63–94
G-CSF	0.859	15.4 pg/mL	92.	82–97	82	62–94
VEGF	0.850	2.3 ng/mL	77	65–87	75	55–89

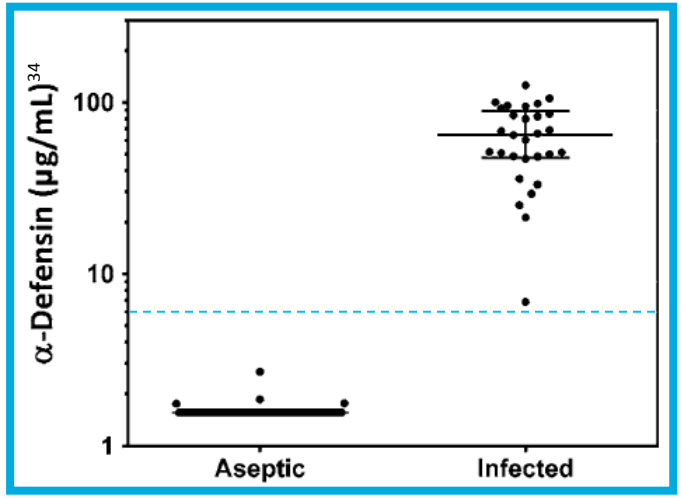
AUC = area under the curve; α-defensin = human α-defensin 1-3; ELA-2 = neutrophil elastase 2; BPI = bactericidal/permeability-increasing protein; NGAL = neutrophil gelatinase-associated lipocalin; SF = synovial fluid; CRP = C-reactive protein; G-CSF = granulocyte colony-stimulating factor; VEGF = vascular endothelial growth factor.

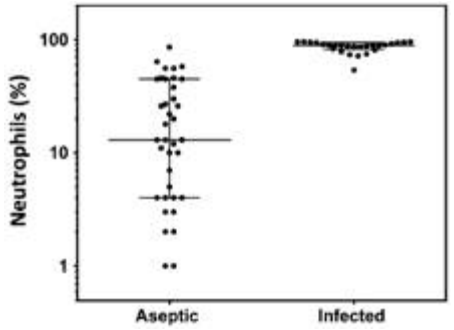
2013

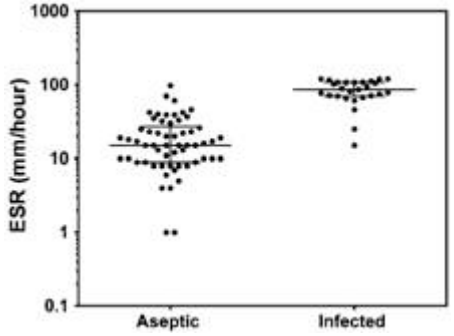
α-defensin, "our natural antibiotic"…











ANTIBIOTICS and α-defensin

The Effect of Antibiotics Treatment on Tests for PJI[21]						
Condition	Alpha-defensin	Serum CRP	ESR	Synovial fluid cell count	% Neutrophils	Culture Positive rate
PJI Group						
Patients receiving antibiotics	7.6	101.1	74.2	26,128	85.7	50%
Patients not receiving antibiotics	6.4	182.9	86.5	50,031	88.7	70%

Serum CRP was 45% lower in this population on antibiotics ($p = 0.039$),

Alpha-defensin level was unchanged.

Alpha defensin probably normalises when infection cleared

- Current evidence suggests that **alpha defensin normalizes after 3 months from stage 1 procedure** (range: 48-221 days) *– further studies needed.*

- Paper n.182 presented at AAOS 2015 –
- **Deirmengian** et al - Synovial Fluid Alpha-Defensin Levels Return to Normal After Treatment of a PJI with a Cement Spacer – AAOS 2015

Alpha defensin Vs Leucocyte Esterase

Clin Orthop Relat Res. 2015 Jan;473(1):
198-203.

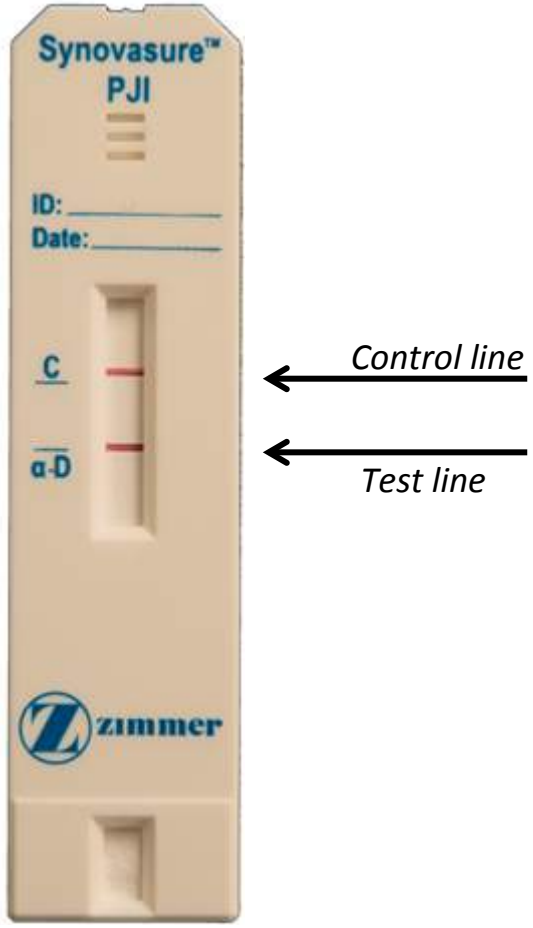
The alpha-defensin test for periprosthetic joint infection outperforms the leukocyte esterase test strip.

Deirmengian C1, Kardos K, Kilmartin P, Cameron A, Schiller K, Booth RE Jr, Parvizi J.

2015

Quick & easy

How to evaluate the result



How it will look	Result
Control line + Test line	+

57 lateral flows performed in Northumbria

Joint Type	Number of tests
Primary hip	22
Primary Knee	16
Hemiarthroplasty	5
Revision hip	5
Hip Spacer	4
Knee Spacer	2
Ankle	1
Periprosthetic fracture	1
Shoulder	1

Thanks to:
Allan Marriott
Ramsay Refaie

Indications for lateral flow

Indication for Synovasure	
Routine pre-revision workup / chronic infection	24
Acute infection	19
Between Stages	6
Dislocation	6
?contamination on 1st asp	1
Fracture	1

Positive lateral flows

- 17 positive tests
- 12 positive cultures on test fluid
- 5 negative cultures on test fluid – all patients on abx with previous known infections (4 primary hips post debridement and 1 hip spacer)

Negative lateral flows

- 38 negative tests
- 35 culture negative
- 2 contaminants – both negative on repeat aspiration
- **1 false negative – candida**

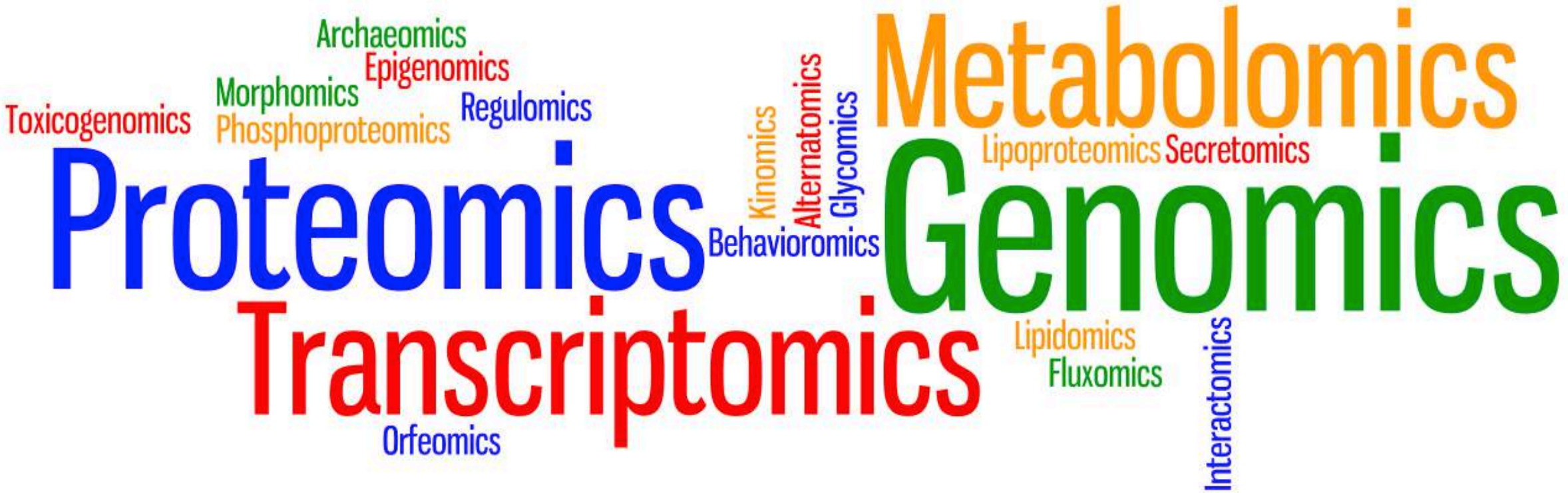
- 1 failed test
- (one test repeated after filtering the blood)

Northumbria lateral Flow Synovasure

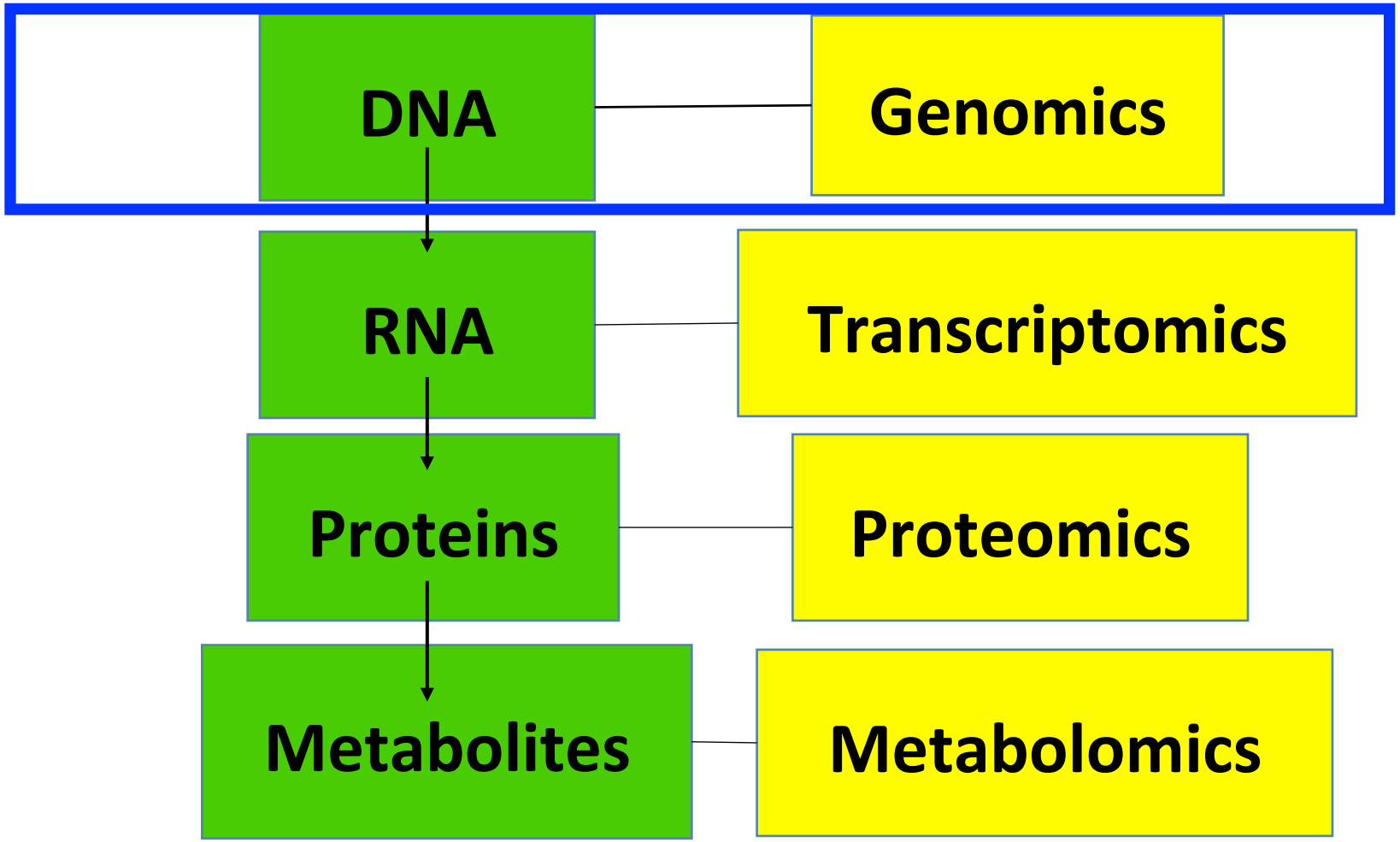
- If we assume the culture negative positive tests are false positives then:
- **Sensitivity is 92%, and specificity is 88%.**

- If we assume the culture negative positive tests are true positives then:
- **Sensitivity is 94%, and specificity is 100%.**

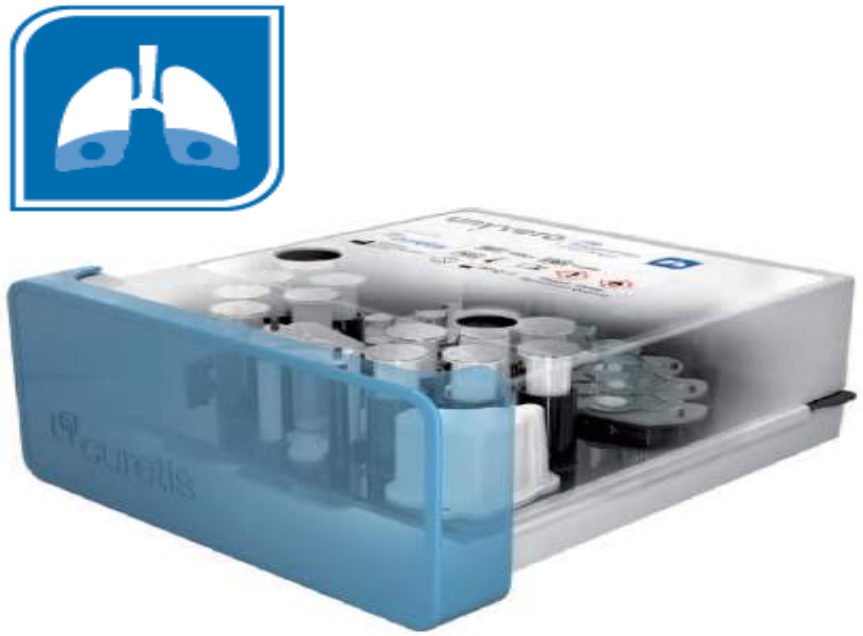
PJI biomarkers – Endless supply from OMICS?



PJI biomarkers – Endless supply from OMICS?



INITIAL APPLICATIONS



Pneumonia

Implant and soft tissue infection

qPCR

PATHOGENS

GROUP	PATHOGEN
Gram-positive bacteria	*Staphylococcus aureus*
	Coagulase negative staphylococci [1]
	Streptococcus agalactiae
	Streptococcus pyogenes [2]
	Enterococcus faecalis
	Enterococcus spp. [3]
Nutritionally variant Streptococci	*Granulicatella adiacens*
	Abiotrophia defectiva
Corynebacteriaceae	*Corynebacterium spp.* [4]

GROUP	PATHOGEN
Enterobacteriaceae	*Escherichia coli*
	Enterobacter cloacae complex
	Enterobacter aerogenes
	Proteus spp. [5]
	Klebsiella oxytoca
	Klebsiella pneumoniae [6]
Non-fermenting bacteria	*Acinetobacter baumannii complex*
	Pseudomonas aeruginosa
Anaerobic bacteria	*Propionibacterium acnes*
	Propionibacterium avidum/granulosum
	Finegoldia magna
	Bacteroides fragilis group [7]
Fungi	*Candida parapsilosis*
	Candida albicans

RESISTANCE MARKERS

RESISTANCES	RESISTANCE AGAINST
mecA	Oxacillin/ Methicillin
mecC (LGA251)	Oxacillin/ Methicillin
aac(6')aph(2")	Carbapenem
ermA	Macrolide
ermC	Macrolide
van A	Vancomycin
vanB	Vancomycin
rpoB	Rifampin *(S.aureus)*

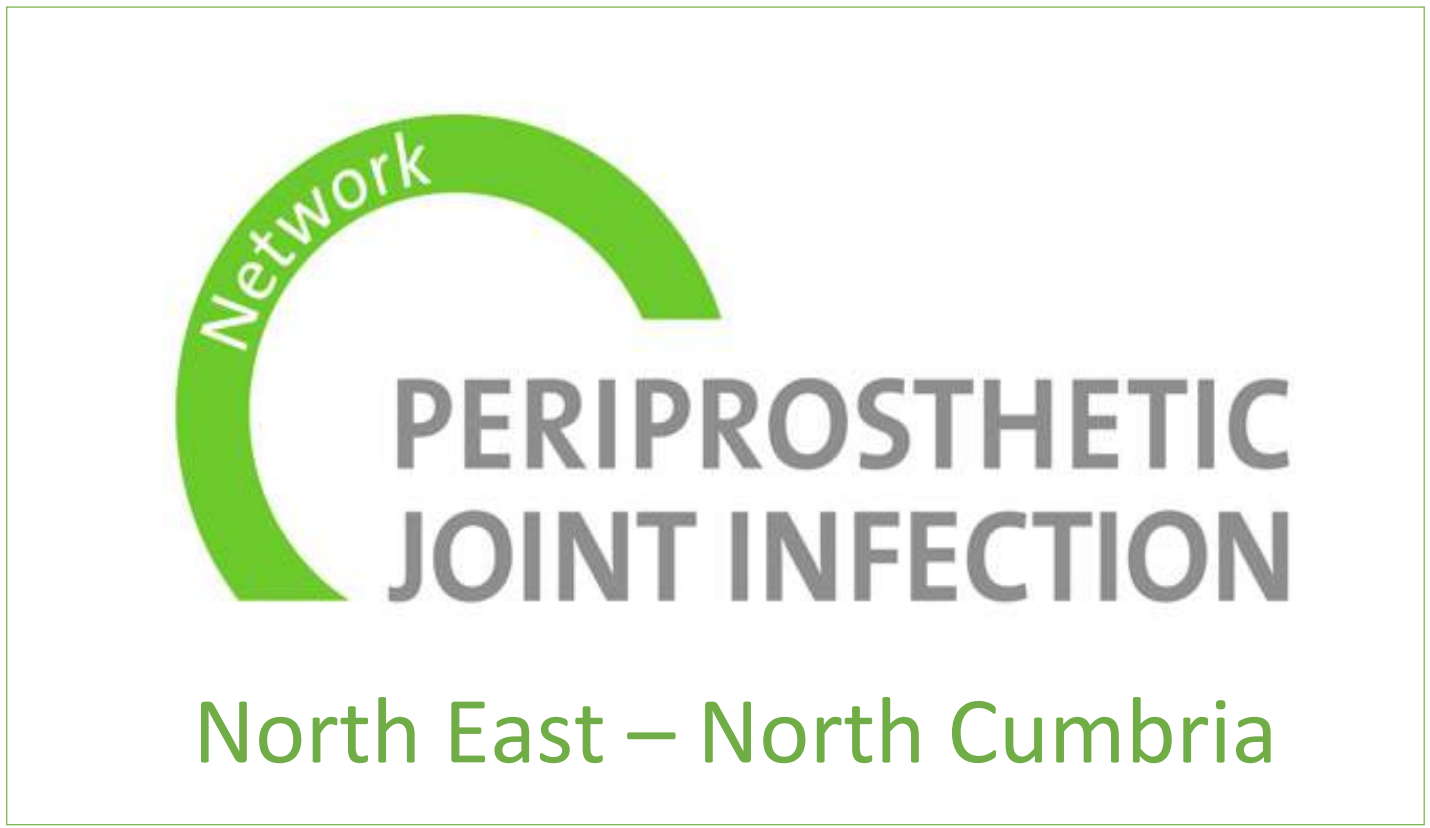
RESISTANCES	RESISTANCE AGAINST
ctx-M	3rd generation Cephalosporins
vim	Carbapenem
imp	Carbapenem
kpc	Carbapenem
ndm	Carbapenem
aacA4	Aminoglycoside
gyrA	Quinolones
oxa-23	Carbapenem
oxa-24	Carbapenem
oxa-48	Carbapenem
oxa-58	Carbapenem

mcr1 polymyxin

Old and New biomarkers?

- Need for large collaborative studies
- Need to distinguish markers for low grade indolent infection from acute infection markers

- Wider validation of markers required


Network
PERIPROSTHETIC
JOINT INFECTION
North East – North Cumbria

Systemic bloods tests

- CRP & ESR combined have excellent sensitivity & specificity; 90-95% but do not exclude low grade infection and need to be interpreted carefully in inflammatory arthropathy.

- Blood cultures should be taken in the setting of acute infection.

-

Microbiology

- The gold standard for PJI diagnosis is fluid or tissue taken from the joint.
- Swabs from superficial sinuses are unhelpful.
- All painful arthroplasties with any suspicion of infection should be aspirated with a strict aseptic protocol. All joints have some fluid; A ‘dry tap’ means the fluid has been missed.
- Patients with suspected chronic infection should not have had antibiotics in the preceding 2 weeks.

Aspirated fluids

Network PERIPROSTHETIC JOINT INFECTION North East – North Cumbria

  - Blood culture bottles (aerobic and anaerobic)
  - EDTA bottle for total WBC count and neutrophil count
  - Universal sterile container for C&S.
- >15 ml is the ideal volume.
- Each unit must be able to provide a white cell count and a differential for aspirated joint fluid. At this point this does not need to be a 24 hour service. Methodology used is to be standardised across the region.

Biopsies

- Intra-operative biopsies – minimum of 5 specimens (tissue or fluid, NOT swabs) using a bespoke sampling tray.
- There should be at least 5 tissue samples.
- We should aim to have the tissue at 1cm cubed size.
- Each unit should be performing extended incubation for at least 7 days. In fact, national guidance is for 14 days.
- Biopsies should not be obtained arthroscopically.
- Antibiotics and debridement should not be delayed pending aspiration in acute infection, where life threatening systemic sepsis may develop.

Synovasure

- There is an emerging role for a defensin (*synovasure*) yet to be defined
- High levels of specificity and sensitivity approaching 98%.
- It remains at the evaluation stage and is expensive and we cannot yet recommend its use.

Thank you.

mike.reed@nhs.net