



Research progress in biomarkers for effective triage for viral and bacterial infections

Jianwei Wang/ Lili Ren

Christophe Mérieux Laboratory, Institute of Pathogen Biology, CAMS & PUMC-Fondation Mérieux

Why should we look for biomarkers?

- Infectious diseases are the major threat to global health
- Etiological diagnosis of some infections lack and are inaccessible in resource lack area or primary health settings, which may influence the patient management and delay the epidemic warning and lead to inappropriate use of antibiotics



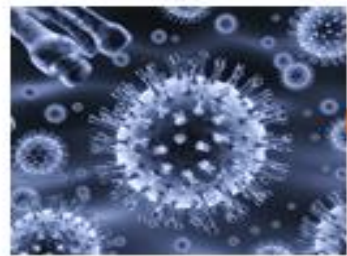
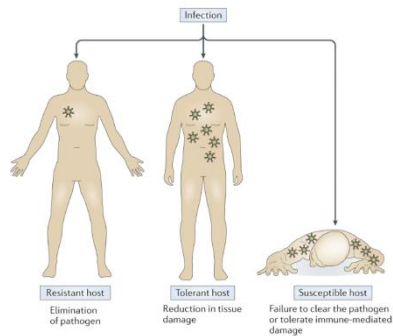
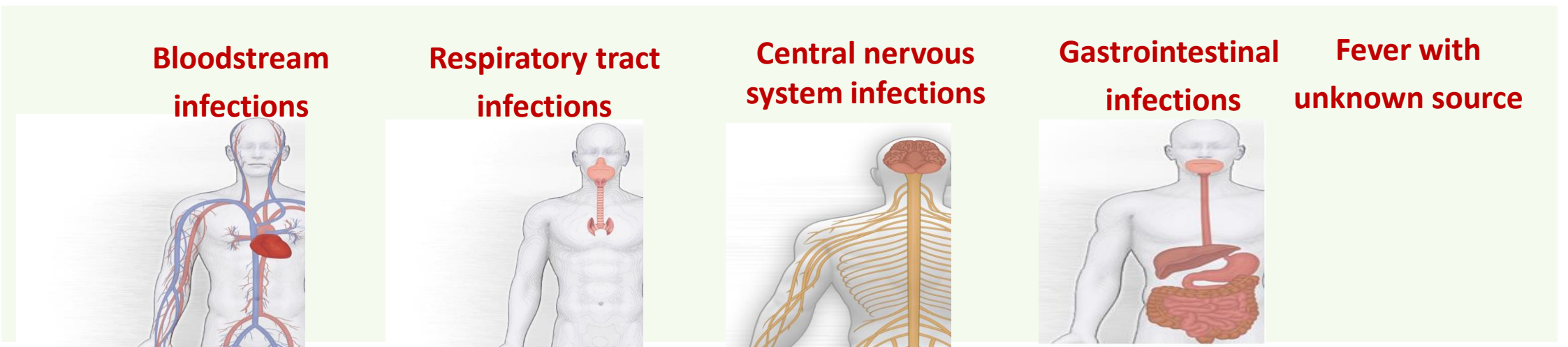
2019, WHO

10 threats to global health

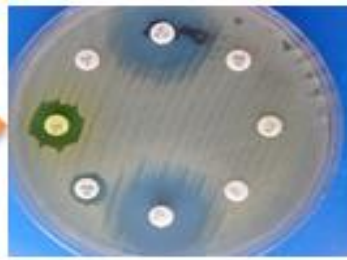
6 related to infectious disease

Etiological diagnoses in infectious diseases are complicated

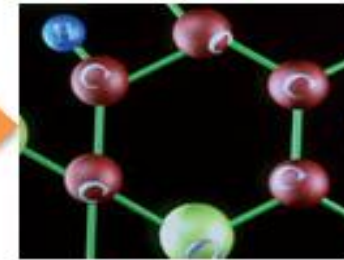
- Most infections are characterized fever with or without other symptoms
- Clinicians are perplexed by complex infections in case manner
- Clinic outcomes should be predicted involving infectious pathogens and host factors



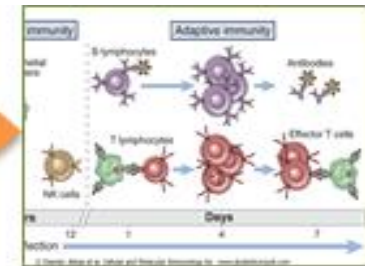
Pathogens identification



Virulence factor



Host RNA signature



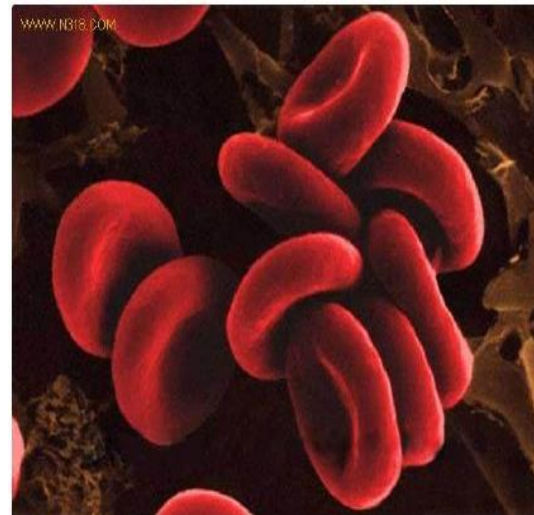
Host protein factors

Bacterial detection

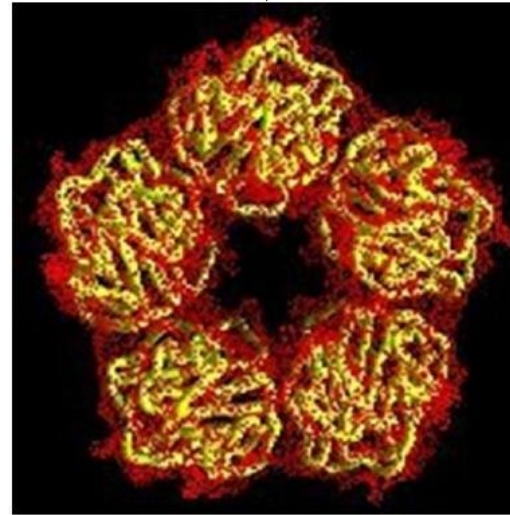
Gold standard



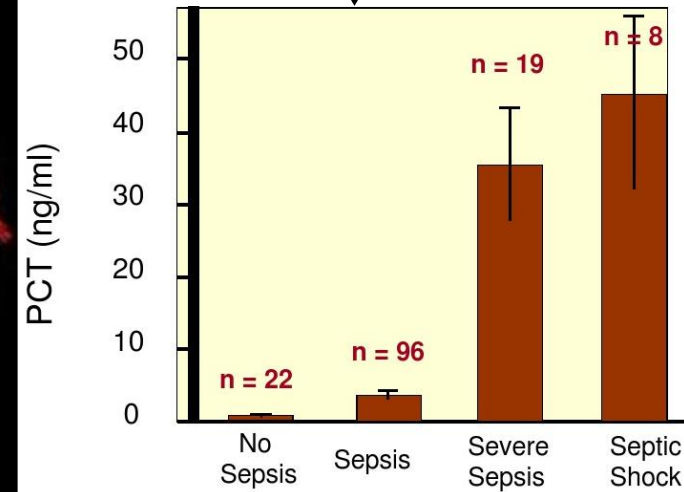
General indicators



ESR

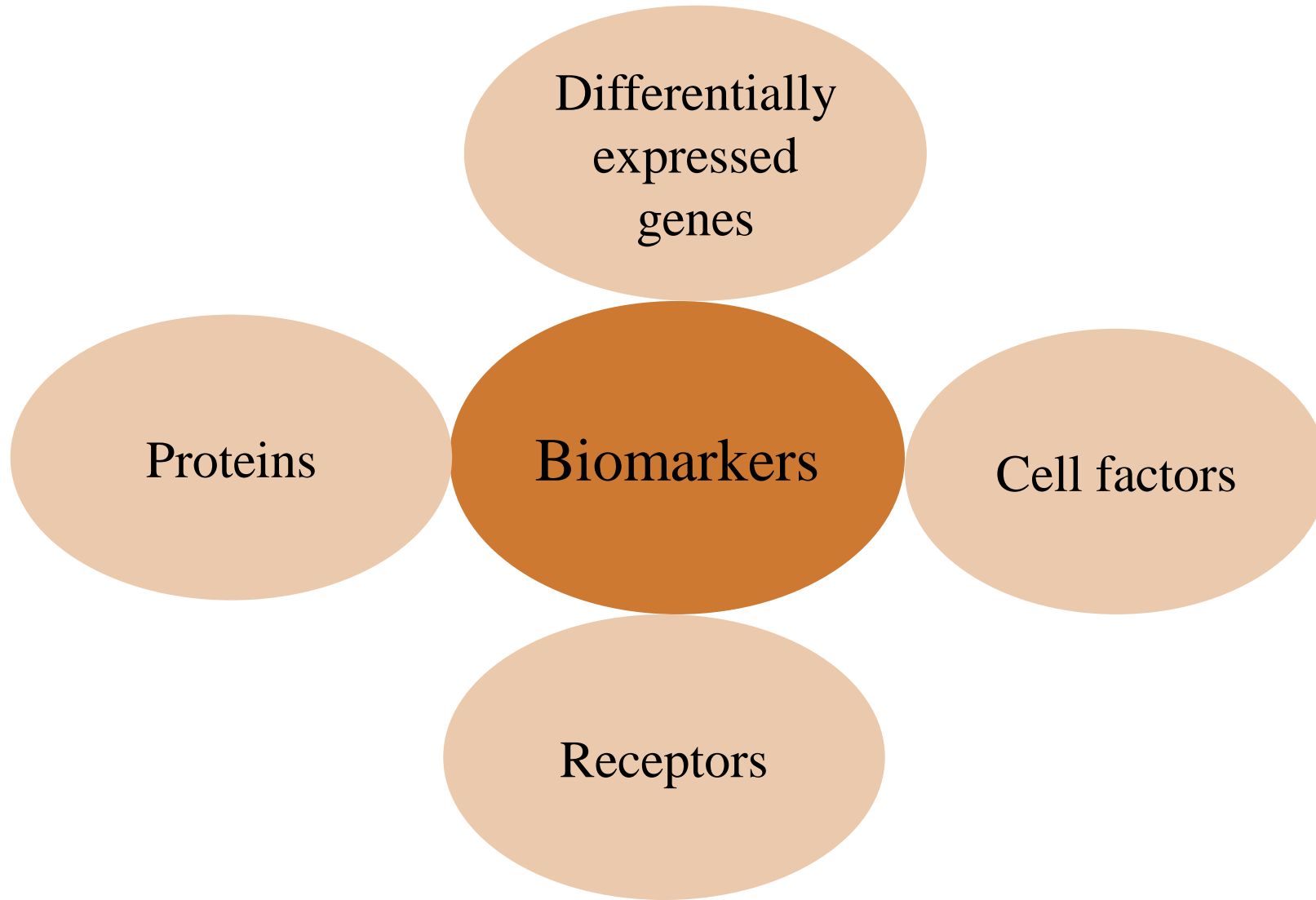


CRP

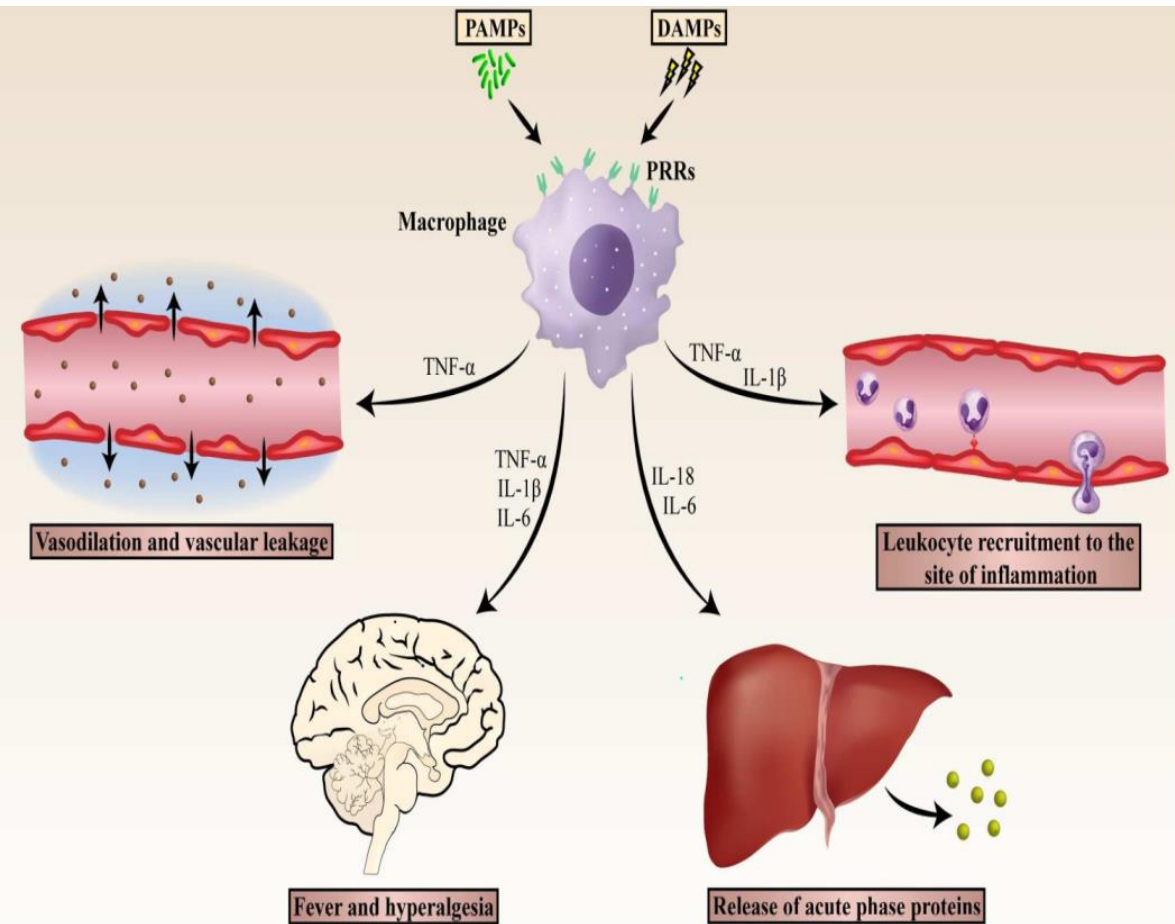


PCT

Literature Analysis



Protein markers



Plasma cytokine concentrations	Bacterial Infection	Viral Infection
IL-1 α	Low/ N.D.	N.D.
IL-1 β	Increased	Increased
IL-1R α	Increased	Normal/N.D.
IL-2	Increased	Increased
IL-6	Increased	Low / ND
IL-18	Increased	Increased
IFN- α	N.D.	Increased
IFN- γ	Increased	Increased
TNF- α	Increased	Normal/Increased

N.D., not detected

Slaats J, et al. PLoS Pathog 2016

Acute phase proteins	Viral and bacterial Infection
C-reaction protein	Stimulated by both viral and bacterial infections, but reaches higher values during bacterial infections
Serum amyloid A	
Procalcitonin	
Ferritin	Elevated in viral infections
Retinol	Decreased during infections
Haptoglobin	Not significantly different between neonates with and without an infection
α 1-antitrypsin	
LPS binding protein	Elevated in bacterial infections as compared to viral infections
sTREM-1	
Neutrophil lipocalin	More elevated in bacterial infections as compared to viral infections

CRP testing on antibiotic prescription

- CRP, C-reactive protein, an acute-phase biomarker of inflammation.
- Highly sensitive (92% for 20 mg/L and 86% for 40 mg/L), moderately specific in identifying bacterial infections.

Febrile patients, primary care
Thailand and Myanmar

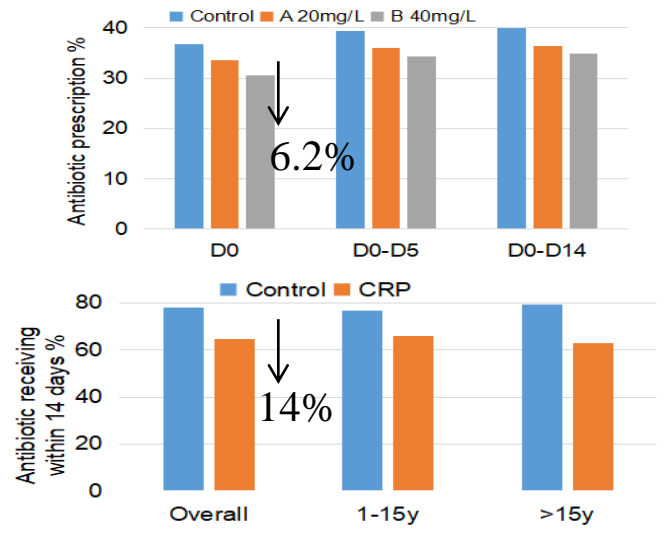
Thresholds
>1y, 40 mg/L

Acute respiratory tract infection
Northern Vietnam

1-5 yrs, 10 mg/L
6-65yrs, 20 mg/L

LRTI or rhinosinusitis
Netherlands

>18yrs, median 18mg/L

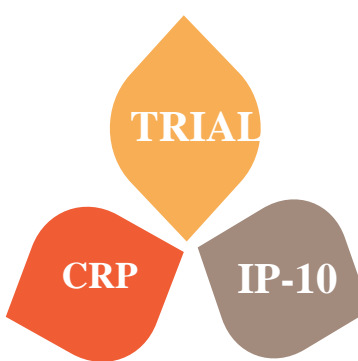


Reduction of 12% (65% vs 53%)

Do NT, et al. Lancet Glob Health 2016
Althaus T, et al. Lancet Glob Health 2019
Cals JW, et al. Ann Fam Med 2010

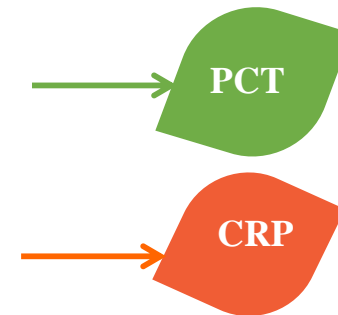
Diagnostic classification with multiple protein markers

- **CRP, TRAIL, and IP-10** improved diagnostic classification for viral infections than PCT or CRP
- Double-blinded prospective validation study
- 577 preschool children, 2 and 60 months
- Fever with RTs or unknown source



	Majority cohort	Unanimous cohort
CE-IVD cutoff		
Number of patients in subgroup	443	354
Sensitivity	86.7 (75.8-93.1)	87.8 (74.5-94.7)
Specificity	91.1 (87.9-93.6)	93.0 (89.6-95.3)
Positive predictive value	60.5 (49.9-70.1)	62.1 (49.2-73.4)
Negative predictive value	97.8 (95.6-98.9)	98.3 (96.1-99.3)

cutoff , CRP40 mg/mL, PCT, 0.5 ng/mL



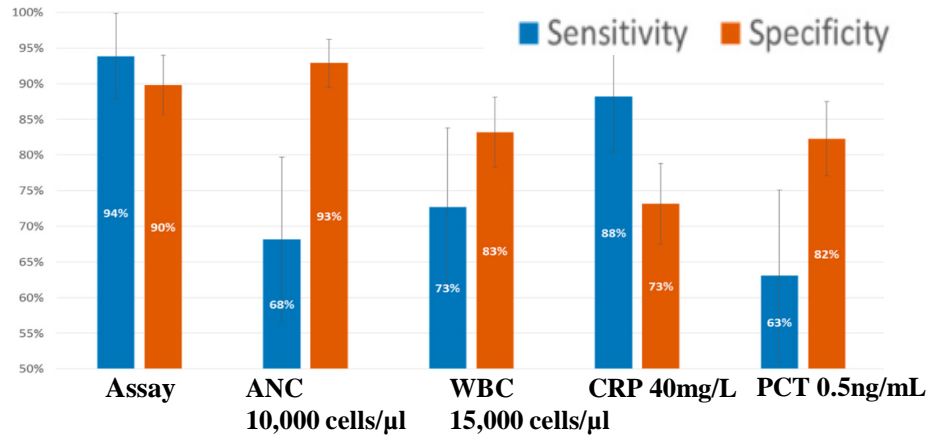
Improved reclassification of infections

Virus (%)	Bacteria(%)
6.3	5.4
P=0.02	
8.6	5.0
p<0.0001	

*TRAIL, Tumour necrosis factor-related apoptosis-inducing ligand
IP-10, interferon gamma induced protein-10*

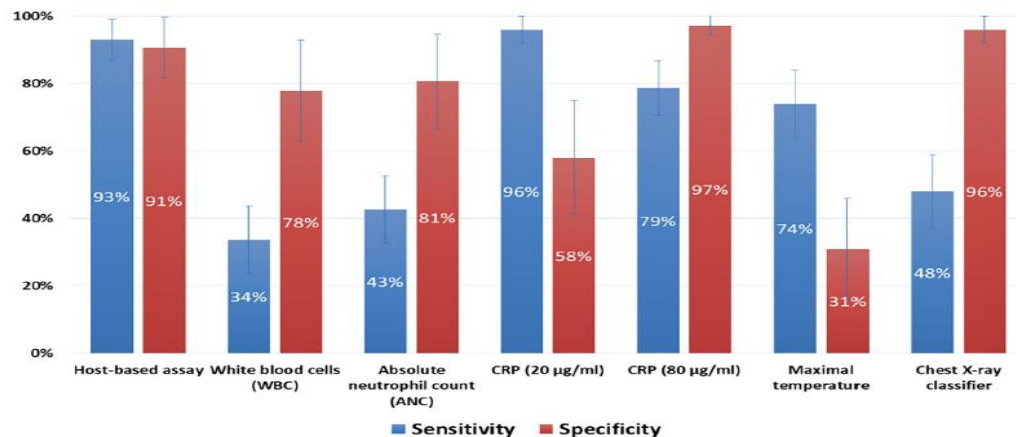
- Two independent research confirmed the higher accuracy of the host signature assay, **CRP, TRAIL, and IP-10**

- 597 acute infection patients, 3 months - 18 years



	Sensitivity (%)	Specificity (%)
Assay	93.8	89.8
CRP	88.2	73.2
PCT	63.1	82.3

- 124 LRTIs pediatric (54) and adult (70) patients



	Sensitivity (%)	Specificity (%)
Assay	93	91
CRP20	96	58
CRP80	79	97
WBC	34	78
ANC	43	81

New marker improves the CRP capacity to predict inflammation

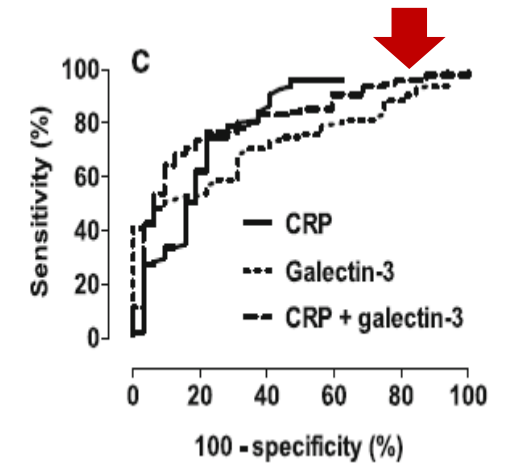
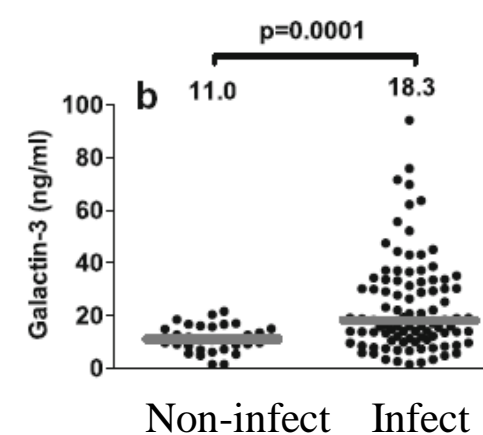
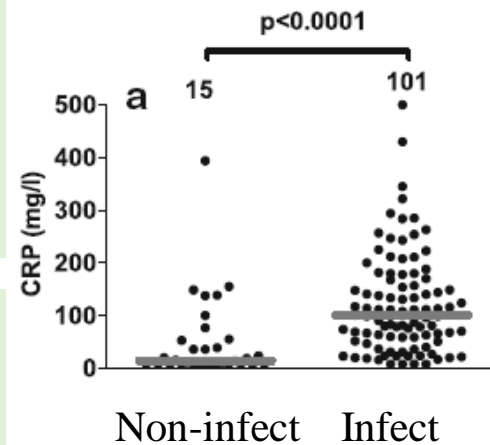
- Plasma circulating Galectin-3
- Galectin-3 (>20.6 ng/ml) with CRP (>156 mg/l) showed superior to CRP alone in distinguishing infectious diseases from non-infectious inflammation

Non-infectious inflammatory disorders

- Gout
- Auto-inflammatory syndrome
- Pancreatitis

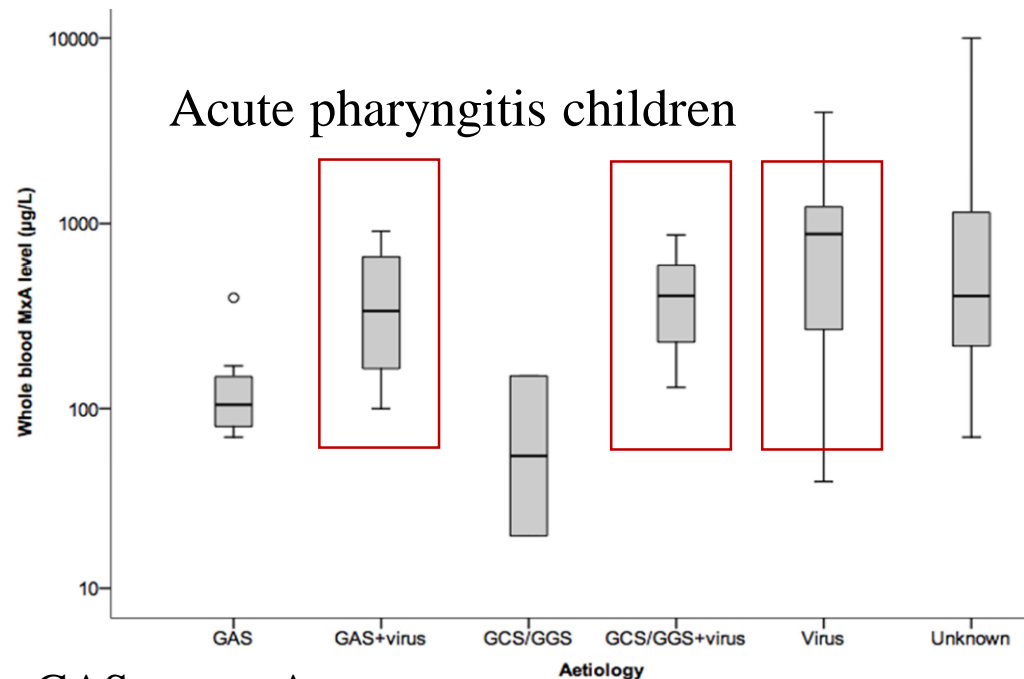
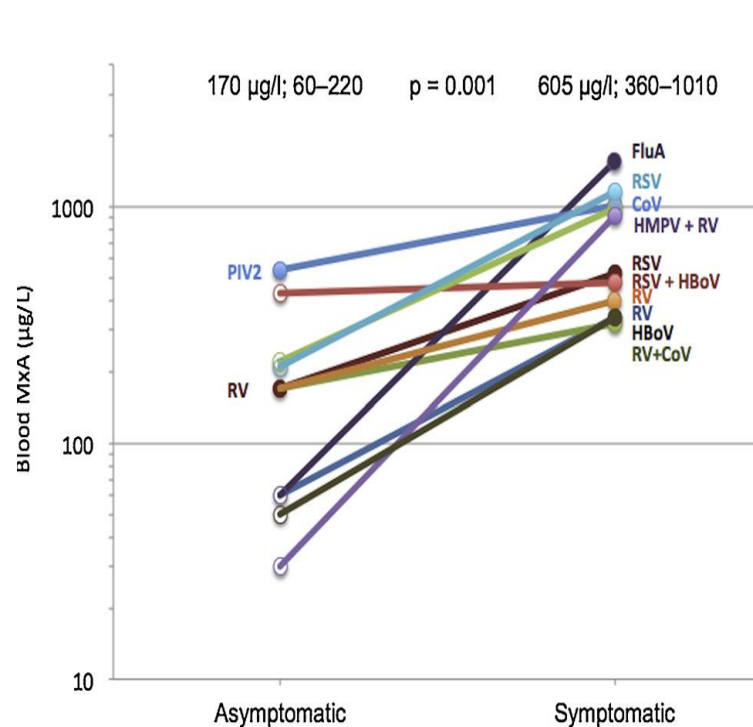
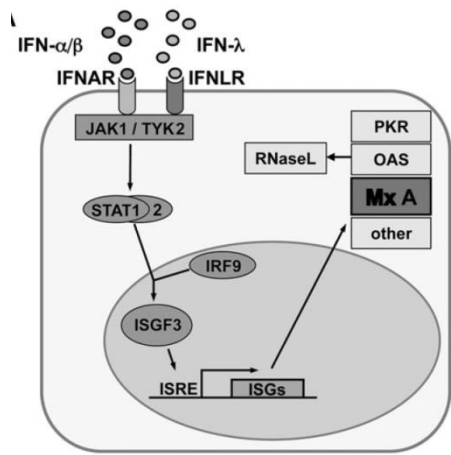
Infections

- Viral LRTIs
- Bacterial sepsis
- Candidaemia.



Host anti-viral protein as potential marker

- Blood concentration of myxovirus resistance protein A (MxA) , promising biomarker of virus infection
- MxA, cytoplasmic GTPase, against a wide range of viruses, induced by I, III-IFN, half-life of 2–4 days in blood leukocytes



GAS, group A streptococcus

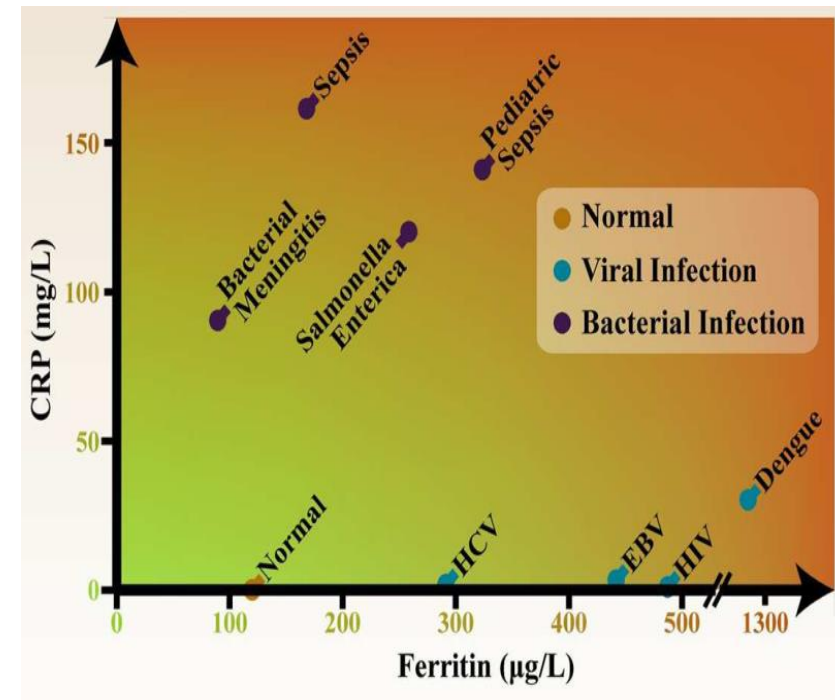
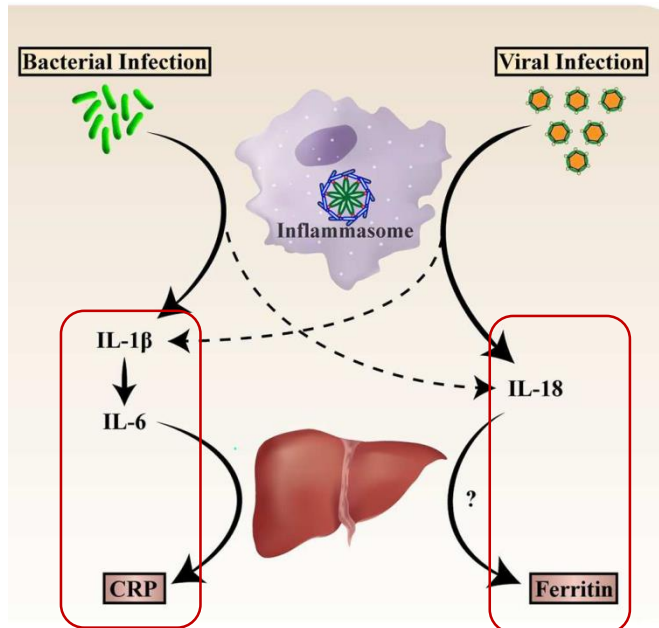
Ivaska L, et al. J Infect. 2017

Toivonen L, et al. J Clin Virol. 2015

Haller O, et al. J Interferon Cytokine Res 2011

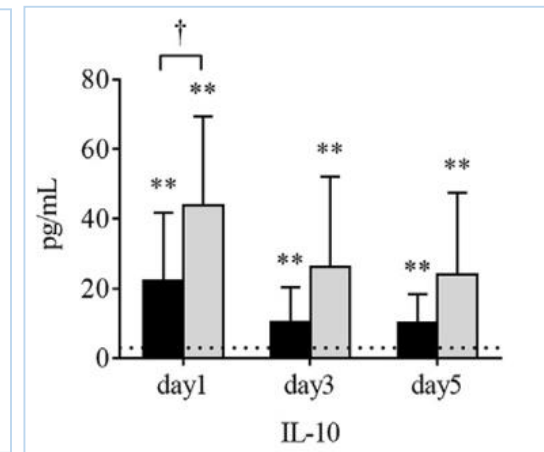
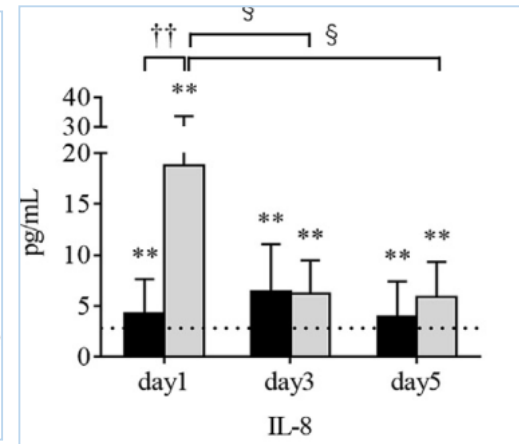
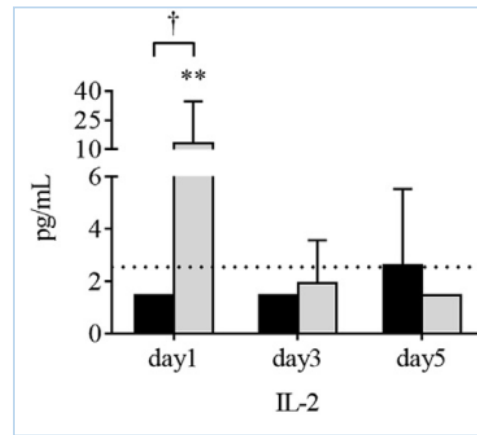
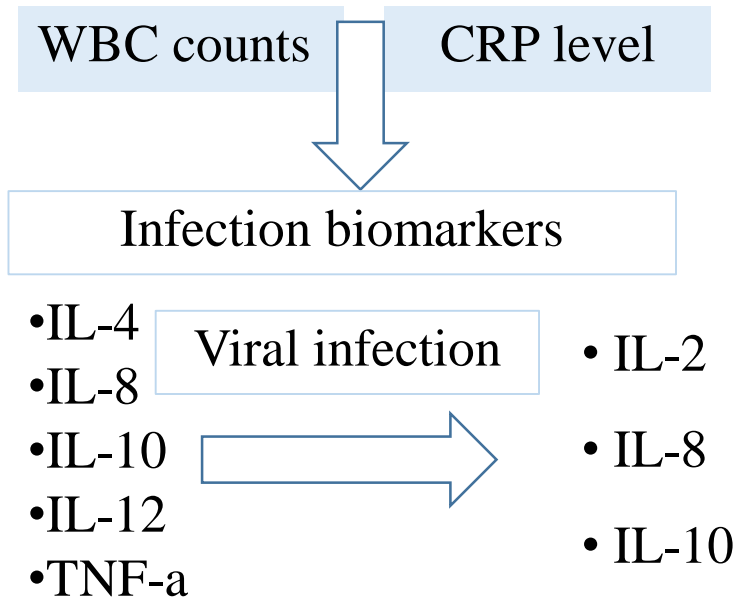
Inflammatory plasma markers as integral part of the diagnostic armamentarium

- Viral infections are characterized by high plasma ferritin with concomitant low circulating CRP
- Whether the types of inflammatory reactions (IL-1/IL-6/CRP versus IL-18/ferritin) fully reflect the bacterial \pm viral infection dichotomy need to be intensively investigated



Cell factors

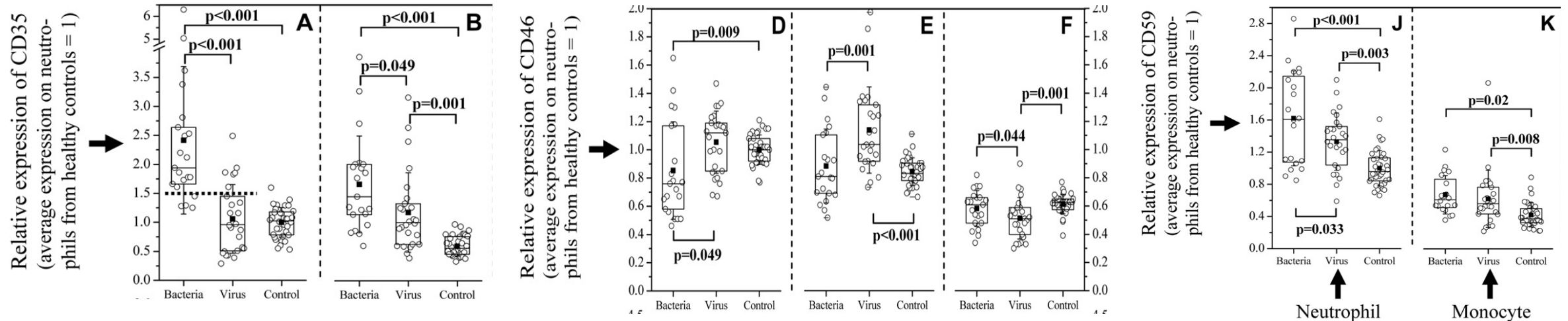
- IL-4, IL-8, IL-10, IL-12 and TNF-a, the potential biomarkers for infections
- IL-2, IL-8 or IL-10, potential distinguish markers between bacterial and viral infections.



Improve of classification effects

- Membrane-bound complement regulators----- CD 35、CD 46、CD 55, CD59

Increased levels	Viral infection	Bacterial infection	General infection
Neutrophils and monocytes	CD46	CD35, CD55	CD59



Cell factors have no improving classification effects

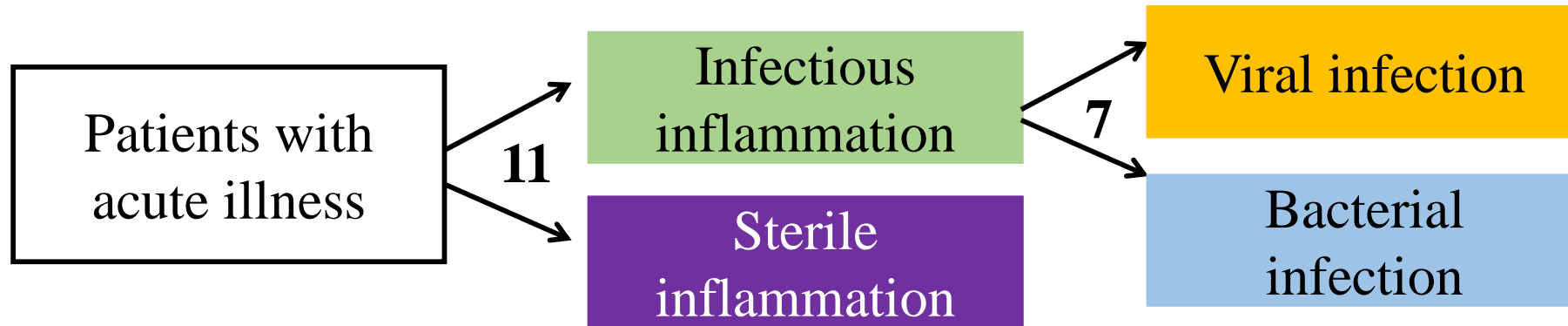
- Combining CRP with LBP, PCT, IL-6, IL-18, or sTREM-1 does not improve differentiation between bacterial or viral LRTI compared with CRP alone.

Marker	AUC	(95%CI)	P-value
CRP	0.64	(0.45–0.84)	
CRP + LBP	0.68	(0.50–0.86)	0.9
CRP + IL-6	0.70	(0.53–0.88)	0.8
CRP + PCT	0.69	(0.50–0.88)	0.7
CRP + sTREM-1	0.64	(0.46–0.83)	1.0
CRP + IL-18	0.62	(0.43–0.81)	0.7

- sTREM-1, soluble triggering receptor expressed on myeloid cells-1
- LBP, lipopolysaccharide binding protein

Host RNA signature

- Time-matched multicohort analysis
- Identification of 11 and 7 differentially expressed genes

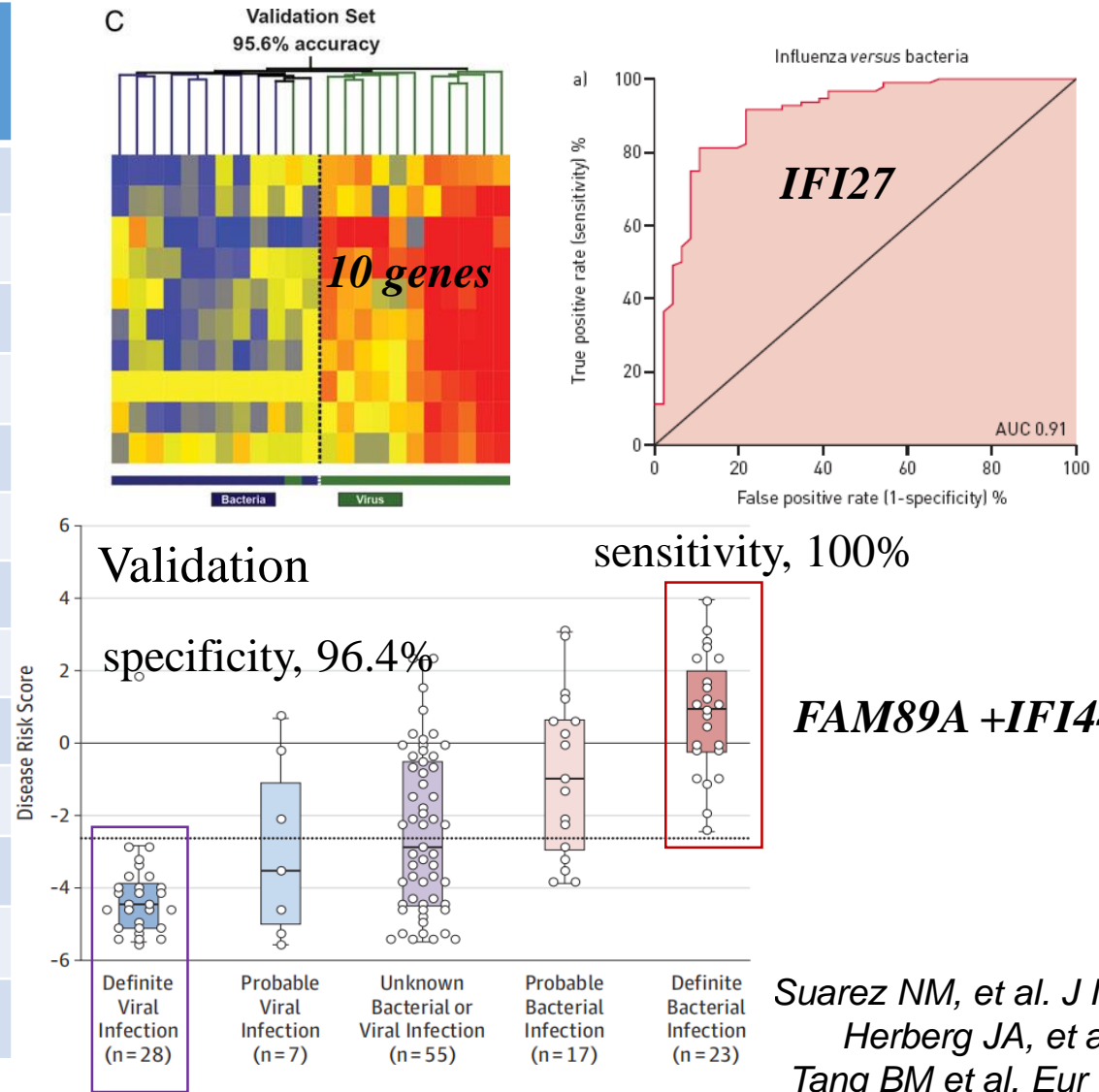


Overexpressed in sepsis	Underexpressed
<i>CEACAM1</i>	<i>KIAA1370</i>
<i>ZDHHC19</i>	<i>TGFBI</i>
<i>C9orf95</i>	<i>MTCH1</i>
<i>GNA15</i>	<i>RPGRIP1</i>
<i>BATF</i>	<i>HLA-DPB1</i>
<i>C3AR1</i>	

Higher in viral infections	Higher in bacterial infections
<i>IFI27</i>	<i>HK3</i>
<i>JUP</i>	<i>TNIP1</i>
<i>LAX1</i>	<i>GPAA1</i>
	<i>CTSB</i>

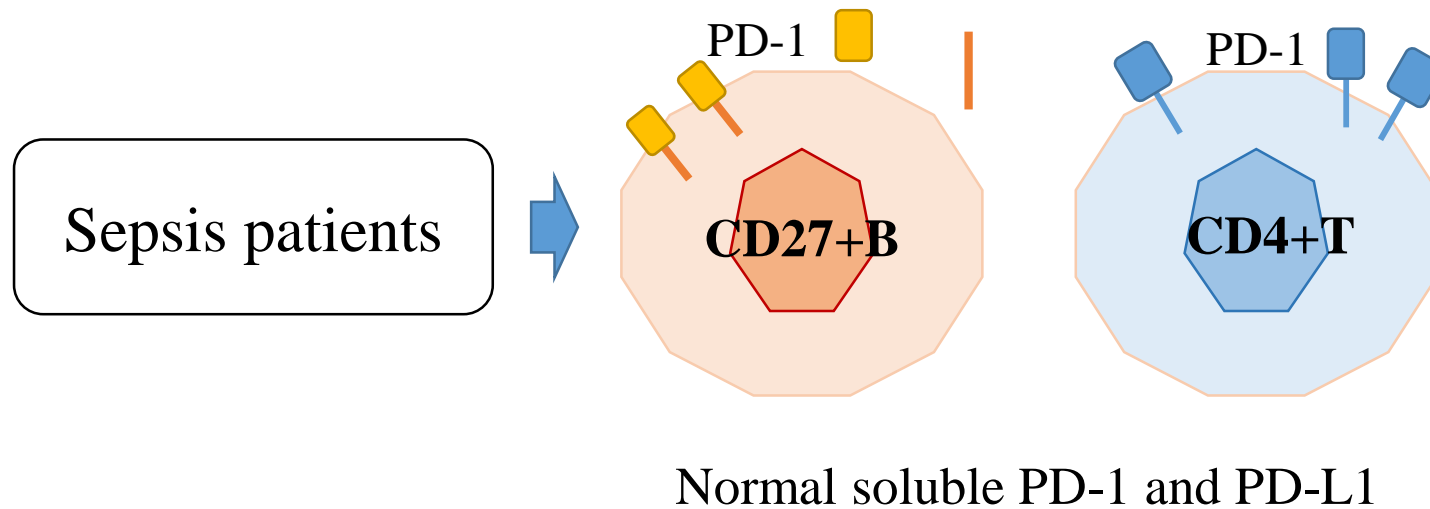
Host bacterial/viral classifier obtained from different cohorts

Cohorts	Gene	Bacterial infection	Viral infection
LRTI patients	<i>BTN3A3</i>		↑
	<i>IFI27</i>		↑
	<i>RSAD2</i>		↑
	<i>KIAA1618</i>		↑
	<i>OAS2</i>		↑
	<i>IFIT3</i>		↑
	<i>IFI44</i>		↑
	<i>OASL</i>		↑
	<i>IFIT2</i>		↑
	<i>PARP9</i>		↑
Febrile children	<i>FAM89A</i>	↑	
	<i>IFI44L</i>		↑
IFV patients	<i>IFI27</i>		↑ (IFV)



Receptors

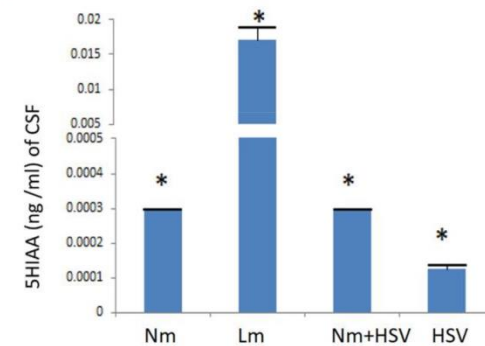
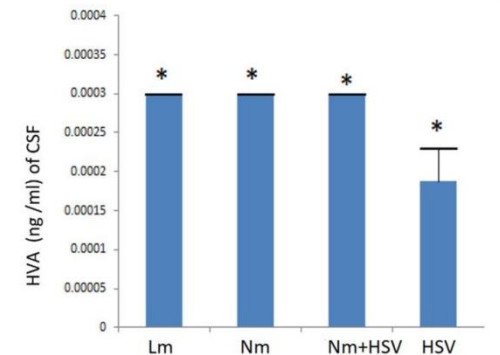
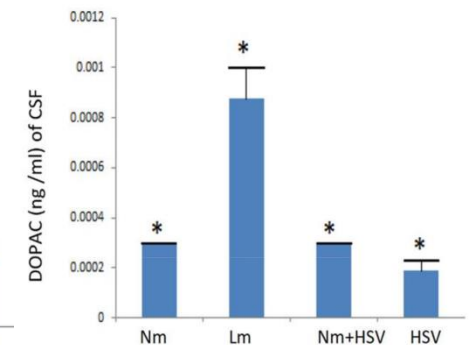
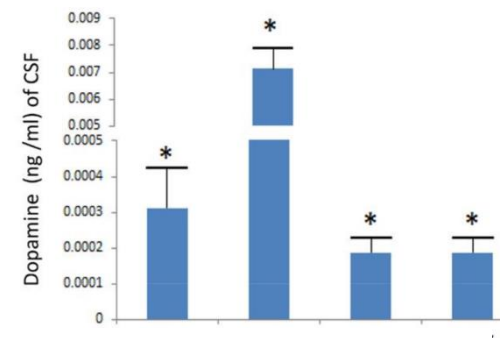
- sTREM-1
- PD1/PDL-1



Biomarkers in central nervous system (CNS) infections

- Biogenic amines — dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5HIAA)
- Low CSF lactate, ANC, serum ESR, and CRP rule out bacterial meningitis

Biomarker combinations	AUC
CRP& ESR& CSF-ANC& CSF-Lactate	0.994
CSF-Lactate & ESR & CRP	0.994
CSF-Lactate & ESR & CSF-ANC	0.984
ESR & CSF-Lactate	0.984
CRP & CSF-ANC & ESR	0.962
CSF-Lactate & CRP & CSF-ANC	0.958
CRP & ESR	0.942
CRP & CSF-Lactate	0.940
CSF-ANC & CSF-Lactate	0.923
CRP & CSF-ANC	0.917

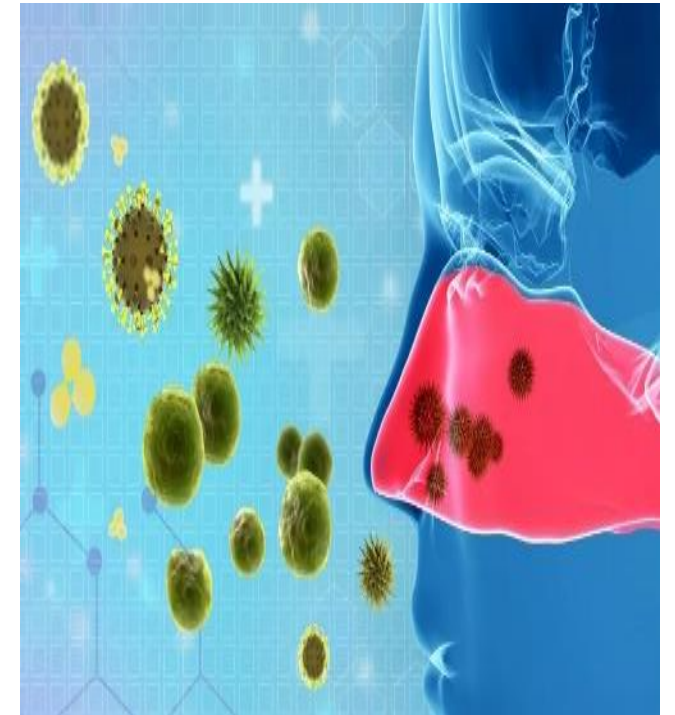
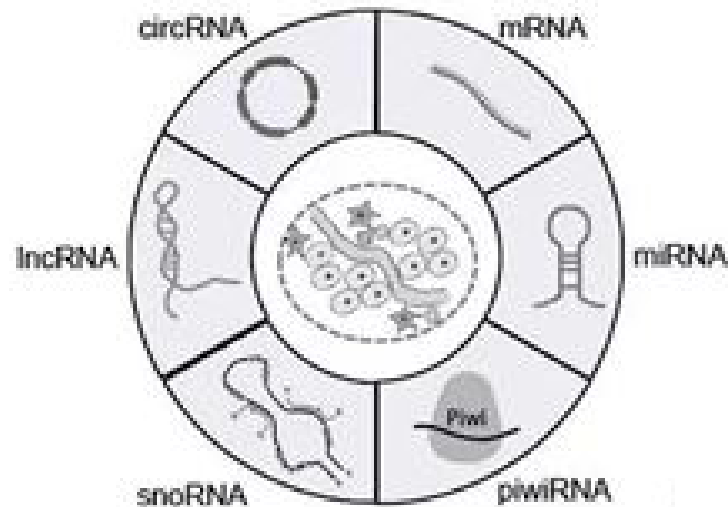


How to apply it to the clinic?

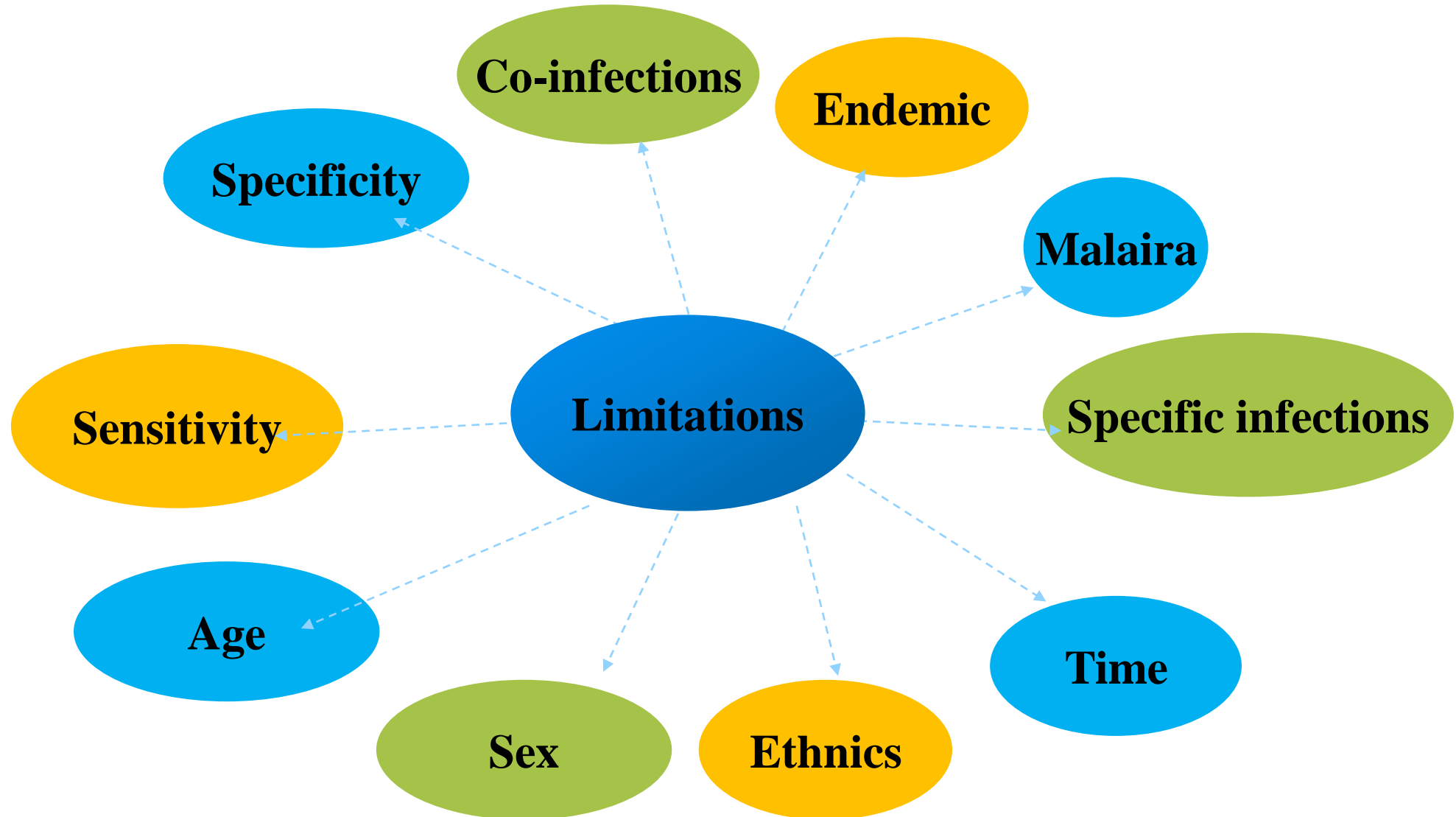
- Protein markers: Rapid and economical.
- RNA markers: Maintain RNA stability when collecting samples.
- Viral detections = viral infections?



Typical types of RNAs used as biomarker



Limitations of the existing markers



Prospective of biomarkers

- Combinations but not single marker
- RNA *vs* protein
- Sample size
- Multicohort, double-blind, multicenter research

Considerations

- Take advantage of the current network and biobank
- Specific objective: alternative diagnosis to acute infections
- Narrow down syndrome and subjects: acute fever with special symptoms (discussed?)
- Public health but not personnel medicine
- Subject recruitment: “acute fever with or without symptoms” , but sometimes challenged by complicated syndrome

Suggested topics

- Etiology of acute fever: multiple detection; known(PCR)+unknown (NGS)
- Evaluation of existing markers under different context: standardization and normalization; factors affected efficacy
- Evaluation of published markers
- Identification of novel markers or combination
- Development of rapid assays for biomarkers
- Development of new algorithms and AI tools

Thank you for your attention

