Ελληνική Πνευμολογική Εταιρεία

26ο Πανελλήνιο ΠΝΕΥΜΟΝΟΛΟΓΙΚΟ ΣΥΝΕΔΡΙΟ

9-12 Νοεμβρίου 2017

Athens Hilton Hotel, Αθήνα

Ανάγκη για νέους βιοδείκτες στη φυματίωση

Σταματούλα Τσικρικά

Πνευμονολόγος-Φυματιολόγος

Ιατρός Δημόσιας Υγείας

ΓΝΑ Ευαγγελισμός

Καμία σύγκρουση συμφερόντων

Τι καλούμε βιολογικό δείκτη?

Μετρήσιμες ουσίες σε ένα βιολογικό σύστημα που οι διαφορές στη συγκέντρωσή τους αντανακλούν διαταραχές στην φυσιολογική λειτουργία του συστήματος

Δίνουν απάντηση για τις ενδεχόμενες επιδράσεις ευεργετικές ή μη ενός φαρμάκου ή μιας θεραπευτικής αγωγής, σε συγκεκριμένους ασθενείς, ώστε οι γιατροί να γνωρίζουν εκ των προτέρων πριν προχωρήσουν σε χορήγηση της όποιας θεραπείας

Biomarkers working group, 2001

Ιδανικός β.δείκτης…

Ως εργαλείο…

- Εύκολα μετρήσιμος
- Υψηλή ειδικότητα
- Υψηλή ευαισθησία
- Άμεση συσχέτιση με την κλινική κατάσταση και την πρόγνωση του ασθενούς
- Χωρίς αυξημένο κόστος

McNerney et al.2012

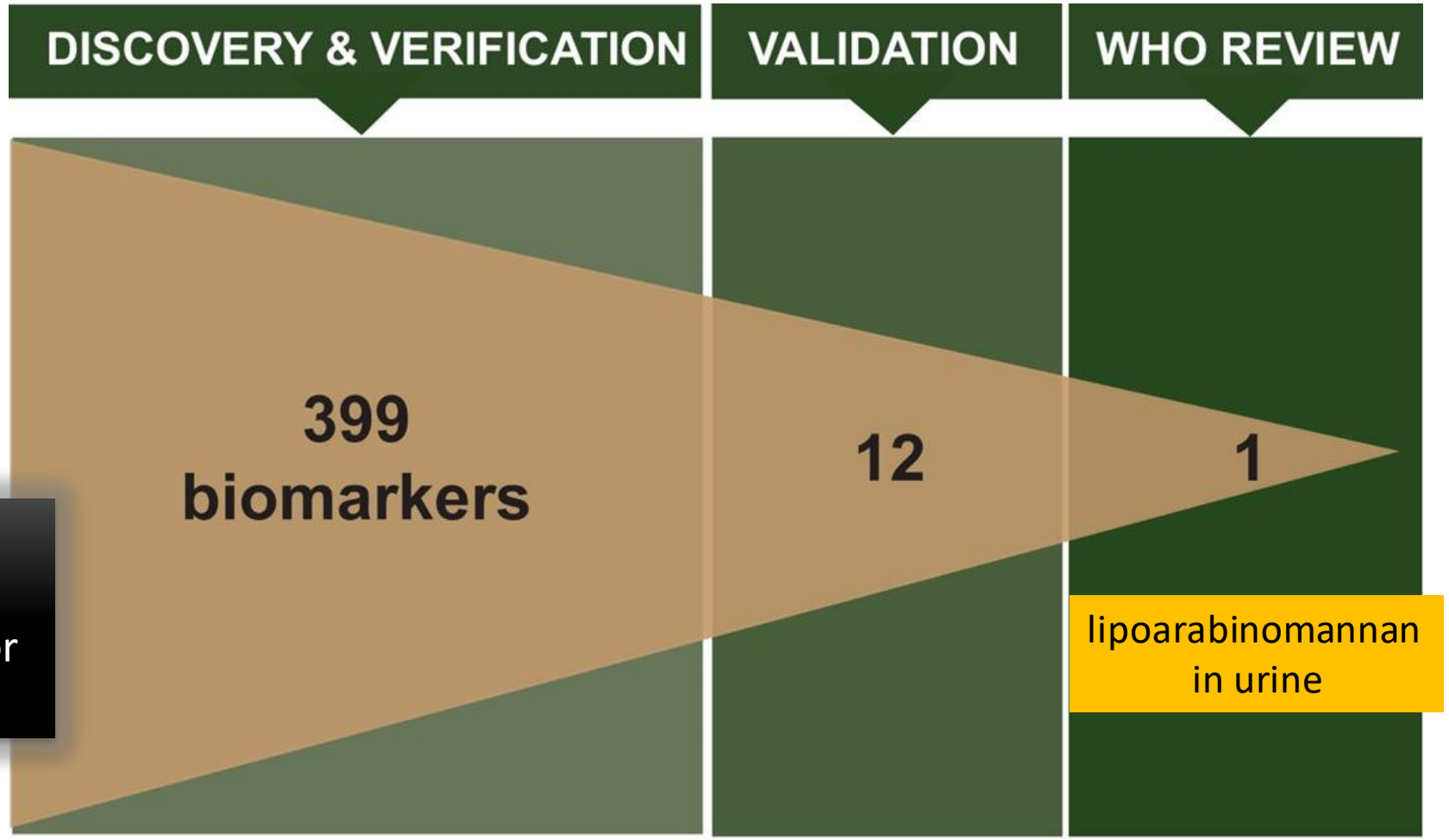
Σε σχέση με την ασθένεια…

- Ανίχνευση ασθένειας
- Δείκτης κλινικής παρακολούθησης
- Δείκτης εργαστηριακής παρακολούθησης
- Δείκτης σταδιοποίησης
- Δείκτης πρόγνωσης

Wallis et al., 2010

- 763 μελέτες
- 2010 – 2015

The Bill & Melinda Gates Foundation announced $7.7 million to identify biomarkers for diagnosing TB



Tuberculosis Biomarker Pipeline

International Journal of Infectious Diseases 2017

Β. ΔΕΙΚΤΗΣ/ ΦΥΜΑΤΙΩΣΗ

- Λανθάνουσα νόσο
- Ενεργό νόσο
- Σοβαρές μορφές νόσου
- Νόσος με υποτροπές - IRIS
- Ειδικούς πληθυσμούς (HIV, παιδιά)
- Ανάπτυξη ανθεκτικότητας
- Ανάπτυξη εμβολίου

Wallis et all. Lancet 2013

Βιολογικά υλικά και ΤΒ β.δείκτες

- Πτύελα και σίελο
- Ούρα
- Πλευριτικό, περικαρδιακό υγρό
- Ολικό αίμα, ορός/πλάσμα
- ΕΝΥ
- Αναπνοή

Biobank initiative	Biobank type	Objective	Sample type	Location
Grand challenge in global health of protective immunity against TB in the context of HIV/AIDS in Africa	Public–private partnership	Study correlates to immune protection for biomarkers discovery	Various	Uganda, The Gambia, Malawi, South Africa, Ethiopia
GSK TB Biobank	Public–private partnership	Predictive surrogate biomarkers	Blood, sputum	Africa and Europe
TB Immunology Research Tissue Bank	Public–private partnership	Collaborative research on TB	Fluid and tissue sample types	Africa and Europe
TBA	Public–private partnership	TB treatment predictive biomarkers	Various	South Africa and India
Catalysis Foundation for Health (TB Diagnostic Biomarker Research)	Nonprofit	TB treatment predictive biomarkers	Various	South Africa, Southeast Asia, USA
CTB2	Independent research	TB treatment predictive biomarkers	Various	South Africa, India, Southeast Asia, USA
WHO/TDR	Public	TB diagnostic biomarkers	Various	South Africa, Peru, Colombia, Vietnam, Kenya, Canada, The Gambia, Uganda, Brazil, Spain, Bangladesh, Zambia
TBRU	Public	Surrogate biomarkers of protective immune response: able to do early-stage clinical trials	Various	USA, Uganda, Brazil, Philippines, South Africa
TBTC	Public	Clinical trials	Human samples	USA, Brazil, Peru, Spain, South Africa, Uganda, Kenya, Vietnam, Hong Kong
National Reference Laboratories (eg, RIVM, the Netherlands; Swiss TPH; National TB Reference Laboratory, Borstel, Germany; Swedish National Institute of Public Health, Stockholm)	Public	Public health	*M. tuberculosis* strains, clinical isolates	South Africa-NHLS (CTB), Lesotho, Uganda
K-RITH	Independent research	Biomarkers, sequencing genomes of multidrug-resistant TB, characterizing the human immune response to TB	Various	South Africa (KwaZulu-Natal)
SUN-IRG	Independent research	Immunology of *M. tuberculosis* infection and host biomarkers	Various	South Africa (Cape Town)
SATVI	Independent research	Clinical trials	Various	South Africa (Cape Town)

WHO 2017
TB biomarkers

Table 1. Diagnostic markers of TB under research.

Marker	Sample and/or method	Associated outcome
Antigens for immunological assays		
HBHA protein	PBMCs, IFN-γ concentration	LTBI ↑
DosR proteins	PBMCs, IFN-γ concentration	LTBI ↑
RD peptides and proteins	PBMCs, IFN-γ concentration / frequency of IFN-γ-producing cells	Contact with *M. tuberculosis* ↑
Chemokines and cytokines		
IP-10	Plasma concentration	Contact with *M. tuberculosis* ↑
IL-4/IL-4δ2, IL-4/IFN-γ	mRNA from unstimulated PBMCs	Treatment response ↓ Infection reactivation ↑
Antigen detection		
LAM	Urine concentration	Active TB ↑/ treatment response ↓
Antigen 85	Serum/sputum concentration	Active TB ↑/ treatment response ↓
Rv1681	Urine concentration	Active TB ↑
Molecular methods		
Transrenal DNA	Urine	Active TB ↑
LAMP	Sputum	Active TB ↑
Non-specific markers		
Volatile organic compounds	Breath	Active PTB ↑
Neopterin	Serum concentration	Treatment response ↓/ relapse ↑
sICAM1	Serum concentration	Treatment response ↓
suPAR	Serum concentration	Risk of mortality ↑

EBioMedicine. 2017

A. Pathogen biomarkers

1. **Mycobacterial load**
   a. Mycobacterial count in sputum
   b. Early bactericidal activity decrease in colony forming units (CFU) in sputum during early phase of treatment
   c. Sputum culture: conversion after two months' therapy (time to detection)
   d. Sputum culture: conversion after two years' therapy
2. **Mycobacterial cell wall products**
   a. Lipoarabinamannan
   b. Phenolic glycolipid-1
   c. Dectin
3. **Mycobacterial antigens**
   a. Antigen 85
   b. Antigen 85B
   c. Antigen 85B RNA
4. **Mycobacterial nucleic acids**
   a. Mycobacterial DNA and its fragments (cannot distinguish live from dead bacilli)
   b. Mycobacterial mRNA and its fragments
5. **Mycobacterial volatile metabolites in breath**

B. Host biomarkers

1. **Clinical markers**
   a. Symptoms (nausea, anorexia, weight loss, cough, chest pain, sputum, haemoptysis, wasting, night sweats)
   b. Skin tests
   c. Chest X-ray (extent of disease, severity of disease, cavitary disease)
2. **Inflammatory markers**
   a. C-reactive protein
   b. Erythrocyte sedimentation rate
3. **Biochemical markers and liver function tests**
   a. Adenosine deaminase
   b. Liver enzymes (serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, alkaline phosphatase)
   c. Volatile metabolites (breath markers)
4. **Immune markers**
   a. B-cell markers – antibody/*M. tuberculosis* antigen studies
   b. T-cell markers
      i. associated with Th1 responses: LAG-3V3, splice variant of LAG.
      ii. associated with Th2 responses: sCD30, total IgE, CCL22
   c. Immune cytokines
      i. Type 1 (Th1) cytokines (IL-2, IL-7, IL-12, IFN-γ)
      ii. Type 2 (Th2) cytokines (IL-4, IL4δ2, IL-4/IL-4δ2 ratios, TNF-α, IL-1β, IL-6, IL-10)
      iii. Inflammatory cytokines (TNF-α, IL-1β,IL-6, IL-10)

Lancet Infect Dis 2007

	Biomarker	Neonates, children, adolescent or adult population	Location
Validated correlates of TB risk			
Commercial or traditional tests for LTBI diagnosis	RD1-specific immune response in IGRA, immune sensitisation to PPD in TST	Children and adults	Global
Molecular tests	mRNA expression signature of 16 IFN response genes	Adolescents	Africa
Cell activation markers	Increased HLA-DR-expressing $CD4^+$ T-cells	Infants	Africa
Blood cell counts	Elevated monocyte/lymphocyte ratio	Adults	Africa
Unvalidated correlates of TB risk			
Molecular tests	IL-13 and AIRE mRNA expression signature	Adults	Europe
	Elevated expression signatures of IFN response and T-cell genes	Infants with strong response to BCG vaccination	Africa
	Elevated expression signatures of inflammation, myeloid and glucose metabolism genes	Infants with weak response to BCG vaccination	Africa
Antigen-specific T-cells	Increased IFN-γ-expressing Ag85A-specific T-cells	Infants	Africa
	Increased Th1-cytokine-expressing BCG-specific $CD4^+$ T-cells	Infants with strong response to BCG vaccination	Africa
Cell differentiation markers	Downmodulation of CD27 in $CD4^+$ T-cells	Adults	Africa
Serum/plasma cytokine tests	Increased levels of IP-10	Adults	Africa
Antigen-specific antibodies	Elevated levels of anti-Ag85A-binding IgG	Infants	Africa
Responses to latency antigens	IFN-γ response to *in vitro* stimulation of PBMCs using HBHA	Adults	Europe
	IFN-γ response to *in vitro* stimulation of whole blood using Rv2628	Adults	Europe
$CD8^+$ T-cell response	IFN-γ response to *in vitro* RD1 stimulation of PBMCs	Children and adults	Africa Europe

ERJ 2016

	Associated outcome	Proposed level of certainty*
Month 2 culture status	Required duration of treatment[4] Relapse[5]	III III
Mycobacterium tuberculosis DNA (GeneXpert MTB/RIF assay)	Treatment effect[6] Extent of disease at start of treatment[7,8]	I I
M tuberculosis RNA	Treatment effect[9-11]	I
Liquid culture time to positive in automated liquid culture	Treatment failure[12] Treatment effect[13-16] Relapse[17]	I I I
Early bactericidal activity, 7–14 days	Inability to distinguish curative vs non-curative treatment[18] Inability to distinguish 6 vs 18 month regimens[19] Inability to detect curative effect of linezolid[20]	No role† No role† No role†
Serial colony counts, 1–2 months	Treatment effect Correlation with month 2 status[21,22]	I I
PET/CT imaging	Treatment effect[23]	I
Sputum Ag85	Treatment failure and relapse[16-18]	II
Antiphospholipid antibody	Treatment effect[24]	I
Urine lipoarabinomannan	Treatment effect[25]	I
Urine tuberculosis DNA	Treatment effect[26]	I
Antialanine dehydrogenase antibody	Treatment failure[27]	II
Volatile organic compounds	Active tuberculosis[28-30]	I
Interferon γ release assay (ELISPOT or whole blood)	Latent infection treatment (no effect)[31,32] Latent infection effect[33] Treatment failure[34] Subsequent active tuberculosis[35-37]	No role† I I III
Whole blood bactericidal activity	Treatment effect[38,39] Correlation with month 2 status[40]	II II
Neopterin	Treatment effect[41] Relapse[42]	II II
Soluble intercellular adhesion molecule-1	Treatment effect[43]	I
Soluble interleukin-2R	Treatment effect[44]	I
Soluble tumour necrosis factor receptor, granzyme B	Extent of disease at start of treatment[45]	I
Sputum interferon γ	Treatment effect[46]	I
C-reactive protein	Treatment effect, death[47-49]	I
Soluble urokinase plasminogen activator receptor	Death[50] Correlation with month 2 status[51]	II II

(Continues on next page)

	Associated outcome	Proposed level of certainty†
(Continued from prevous page)		
Natural killer T cells	Extent of disease at start of treatment[28]	I
Mycobacterial growth inhibition assays	Vaccine effect[52,53] Revaccination (no effect)[54] Correlation with other markers[52,53,55-59]	II II II
Interleukin 18	Subsequent active tuberculosis[60]	II
Natural killer and CD4 T cells	Subsequent active tuberculosis[60]	II
Bcl2	Subsequent active tuberculosis[60]	II
Whole blood interferon γ release assay	Subsequent active tuberculosis[35,61] Diagnosis of subclinical tuberculosis[36]	II II
Transcriptomics	Diagnosis of active tuberculosis vs latent infection[62,63] Treatment effect[62,87]	I I
Proteomics	Diagnosis of active tuberculosis vs other chronic inflammatory diseases[65]	I I
Metabolomics	Diagnosis of active tuberculosis vs latent disease[66]	I
microRNA	Diagnosis of active tuberculosis vs latent disease[67]	I

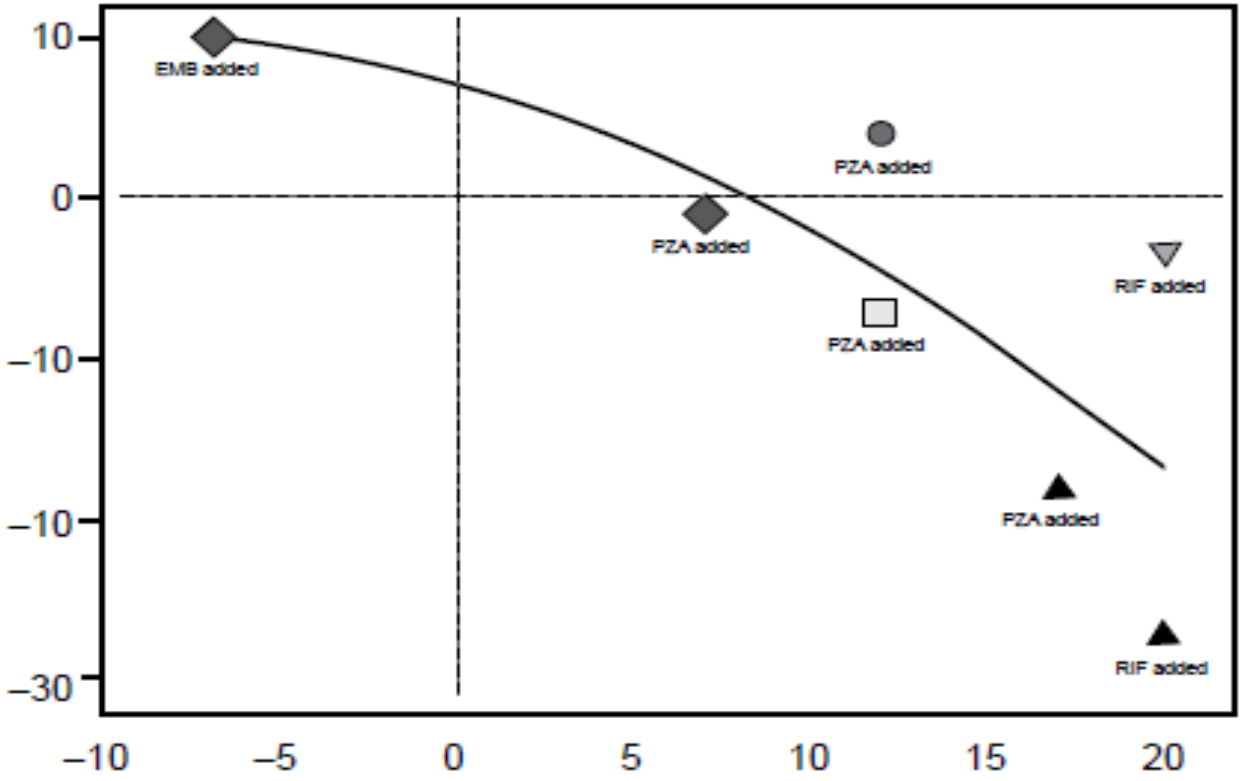
ELISPOT=enzyme-linked immunosorbent spot assay. *Data do not support a role as biomarker. †III=high certainty, capturing differences in clinical outcomes across treatment groups in prospective randomised trials; II=intermediate certainty, predicting differences in outcomes in patients in non-interventional studies; and I=low certainty, biological plausibility without association with clinical outcome.

Table: **Candidate biomarkers in tuberculosis**

Καλλιέργεια πτυέλων στους δύο μήνες
Οι υπόλοιποι δείκτες δεν διαπιστώθηκαν επαρκείς

Lancet 2013

Biomarkers M.TB



EMB, ethambutol; PZA, pyrazinamide; R, rifampicin; T, thiacetazone.

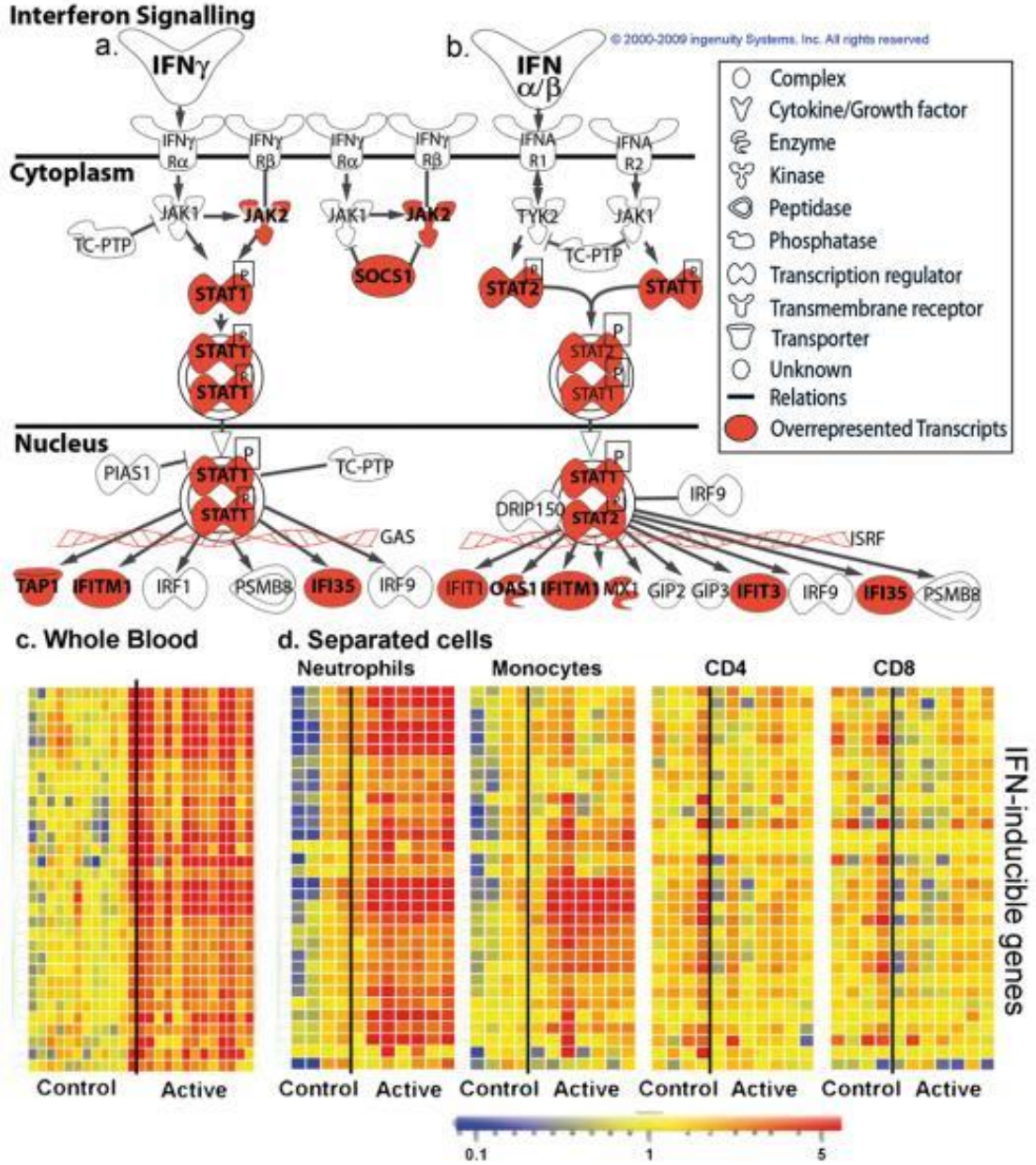
Each symbol represents the difference between experimental and control regimens within a single trial. A significant relationship was identified by Spearman rank correlation ($R = -0.76$ $P = 0.038$).

Μυκοβακτηριδιακό φορτίο στα πτύελα

- Κ/α πτυέλων **δυο μήνες** μετά τη χορήγηση αντιΤΒ αγωγής
- Στατιστική σημαντικά σχέση κ/α πτυέλων στους 2 μήνες και υποτροπής (R=-0.753, p=0.001)
- Περιοδικότητα των δειγμάτων από ένα δείγμα τουλάχιστον δύο ανά εβδομάδα (ανά μήνα)
- Μέτρηση CFU εβδομαδιαίως από τη 2η μέρα αγωγής
- Μη αρνητικοποίηση των πτυέλων στους 2 μήνες αποδεικνύει υποτροπή
- Η προσθήκη EMB δεν επηρέασε το χρονικό διάστημα δυο μηνών ή την πιθανότητα υποτροπής

Benator et al. 2002
Wallis & Johnson, 2006

An Interferon-Inducible Neutrophil-Driven Blood Transcriptional Signature in Human Tuberculosis. Nature 2010

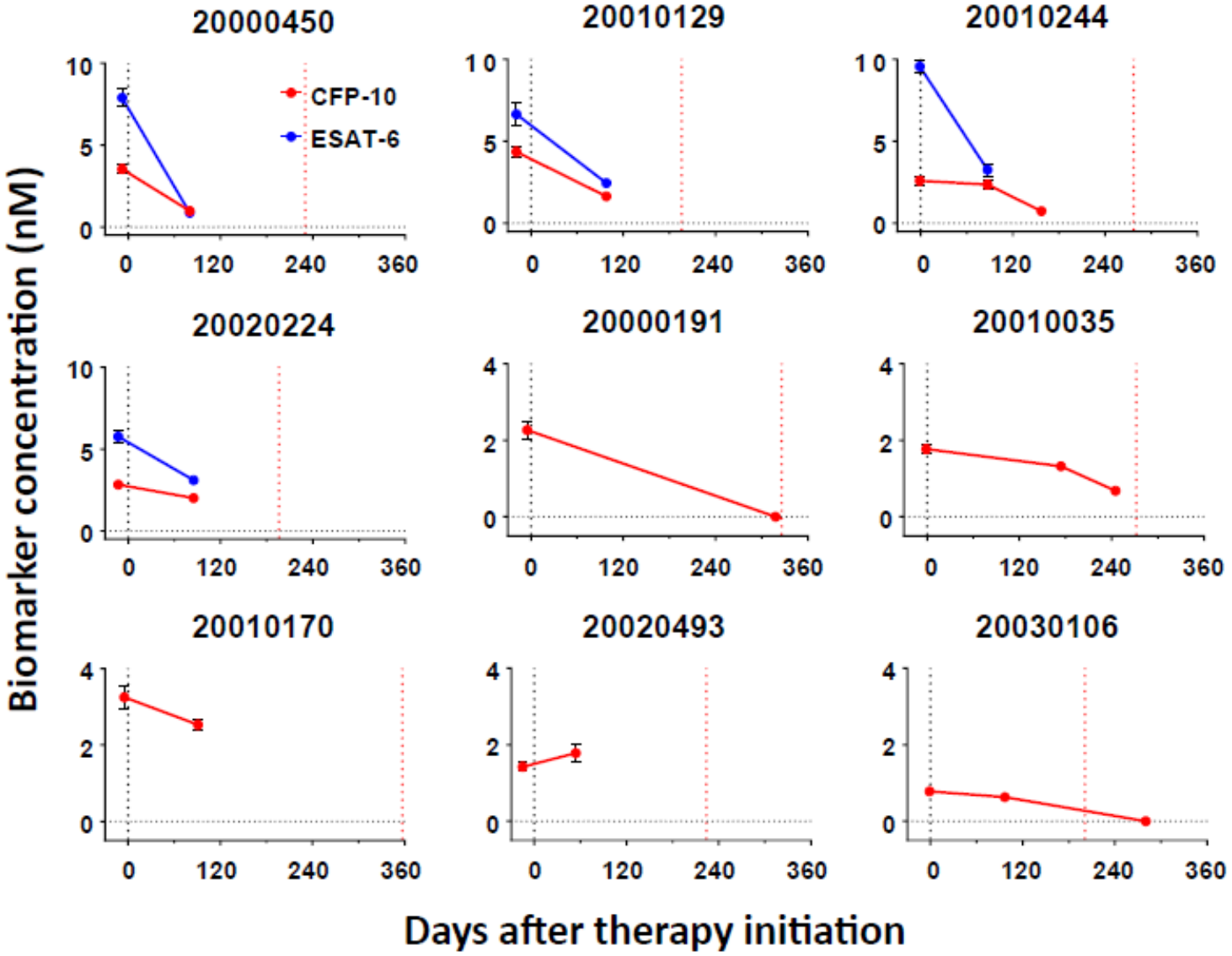


- 393 transcript signature for active TB in intermediate and high burden settings, correlating with radiological extent of disease and reverting
- UK Test Set & South African Validation Set
- 2-12 mo after therapy
- IFN-γ and Type I IFN α/β receptor signalling were significantly over-represented in blood from active TB patients
- Neutrophils are the predominant cell type infected with rapidly replicating M.Tb

Quantification of circulating *Mycobacterium tuberculosis* antigen peptides allows rapid diagnosis of active disease and treatment monitoring

Chang Liu[a,b,c], Zhen Zhao[d], Jia Fan[a,c], Christopher J. Lyon[a,c], Hung-Jen Wu[a,e], Dobrin Nedelkov[f], Adrian M. Zelazny[d], Kenneth N. Olivier[g], Lisa H. Cazares[h], Steven M. Holland[i], Edward A. Graviss[j], and Ye Hu[a,b,c,1]

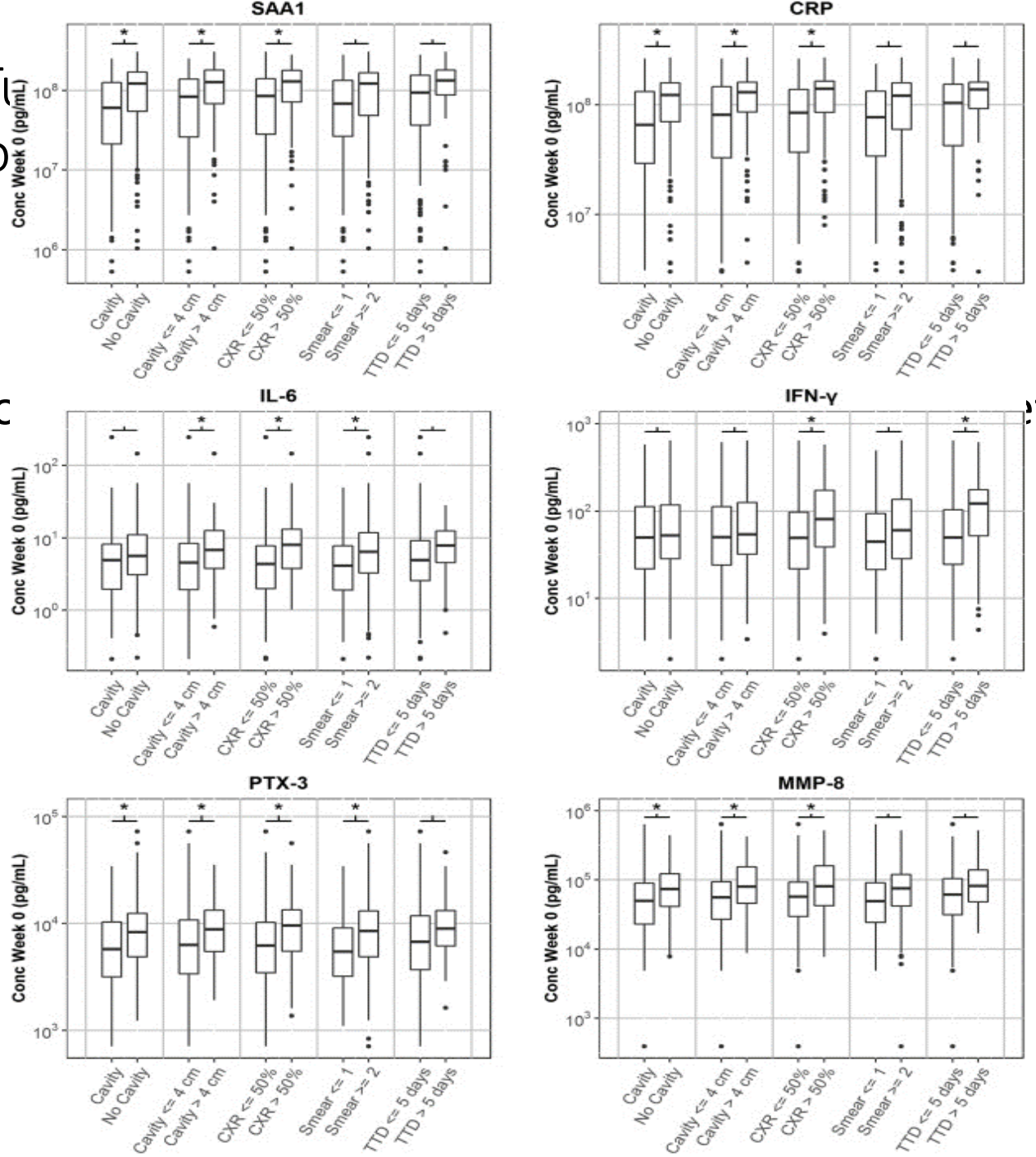
- Mtb-specific peptide fragments
- HIV-negative TB patients
- TB therapy (2–11 mo)
- Cutoff values of CFP-10 (200 pM)
- Cutoff values of ESAT-6 (650 pM)



Biomarkers of Tu A Directed Screen of 70 Ho

EBioMedicine. 2017

- CDC-sponsored
- daily regimen cc ethambutol
- smear-positive,
- 70 biomarkers
- 319 patients

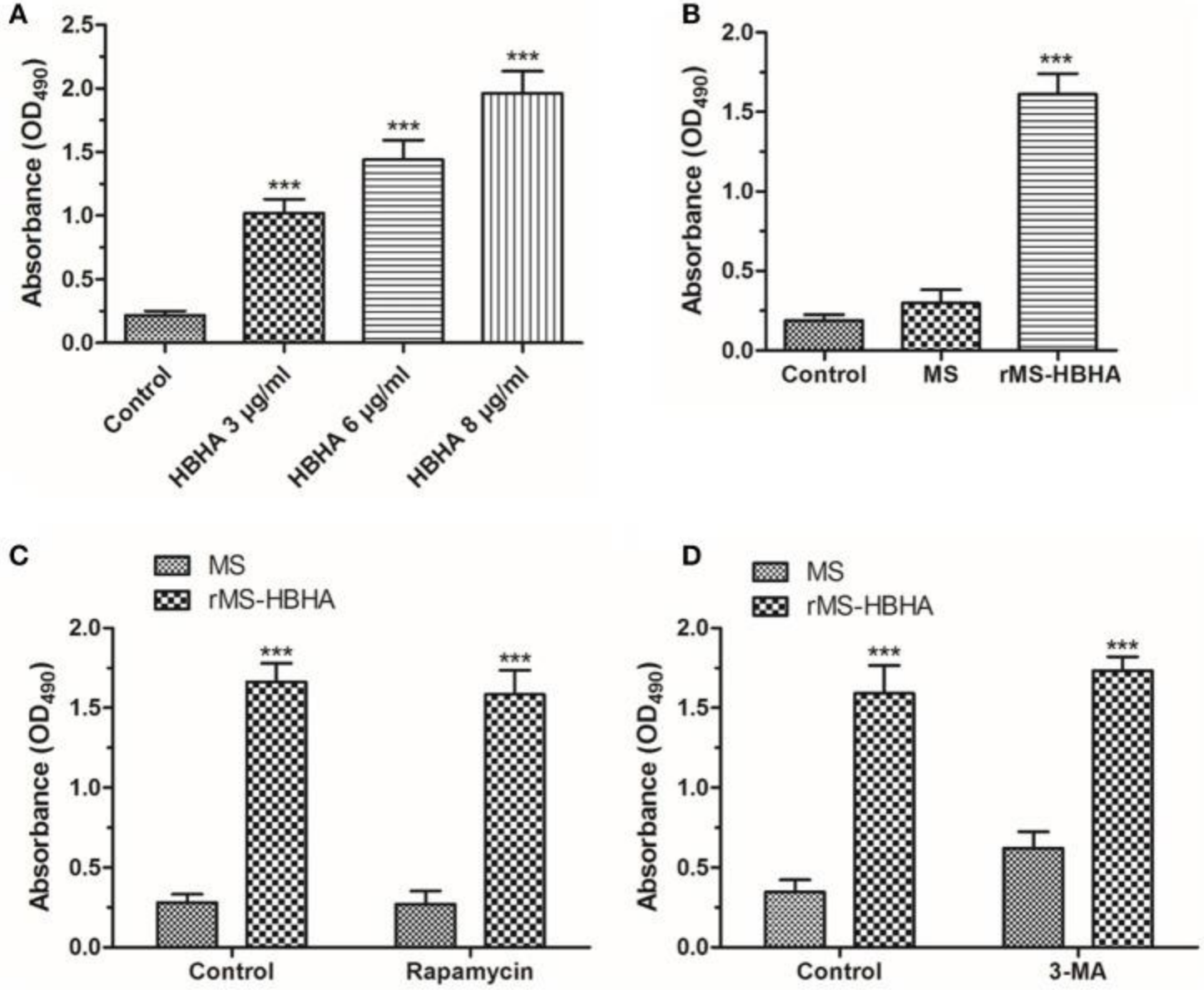


SAA1
CRP
IL-6
IFN-γ
PTX-3
MMP-8

Heparin-binding Hemagglutinin (HBHA) of *Mycobacterium tuberculosis*

Front Cell Infect M

- Attachment l role in the dissemi y infection
- M.Tb may u ate infection
- The role of in host airway epith
- HBHA coulc S intracellular



Diagnostic Potential of Novel Salivary Host Biomarkers as Candidates for the Immunological Diagnosis of Tuberculosis Disease and Monitoring of Tuberculosis Treatment Response. Plos one 2016

Table 2. Median levels and interquartile ranges (in parenthesis) of host markers detected in baseline saliva samples from the TB patients (n = 18) and individuals with ORD (n = 33) and their diagnostic accuracies for TB disease.

Marker	ORD	TB Disease	P value	AUC	Cut off value	Sensitivity %(95% CI)	Specificity %(95% CI)
A2M	1966(1546–3503)	1390(1169–1926)	0.013	0.67(0.5–0.84)	< 1351	50(26–74)	88 (72–97)
ECM-1[a]	14.6±14.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	8 ((31–66)
GDF-15	0.02(0.02–0.1)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	6 (58–89)
Granzyme B[a]	0.02±0.05	0.001±0.003	0.036	0.55	< 0.0150	100 (81–100)	27 (13–46)
IL-1β	36.4(15.9–183.4)	16.9(4.3–39.4)	0.027	0.69(0.52–0.86)	< 7.285	39(17–64)	97 (84–100)
IL-16	56.1(22.0–144.3)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	5 (68–95)
IL-17A	6.1(2.2–10)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	7 (48–82)
IL-23	0(0–0)	0.3(0–2.0)	0.0023	0.73(0.59–0.88)	> 0.1550	61(36–83)	85 (68–95)
Myoglobulin	0.4(0.2–0.7)	0.6(0.3–0.8)	0.097	0.64(0.49–0.80)	>0.2500	100(81–100)	27 (13–46)
SAP	61.0(5.5–61.0)	5.5(5.5–5.5)	0.031	0.67(0.51–0.82)	<33.26	83(59–96)	52 (34–69)

IL-1β, IL-23, ECM-1, HCC1 and fibrinogen, which diagnosed TB disease with a sensitivity of 88.9% and specificity of 89.7%, regardless of HIV infection status

the concentrations of 11 of the markers changed during treatment

Κυτταροκίνες

- IL-2, IL-6, MIP-1β may improve diagnostic sensitivity for M.Tb compared with assessment of IFN-γ

  Cooper et al., 2011

- IP-10, MCP-1, MCP-2, MCP-3,IL-1RA in supernatants from whole blood stimulated with M.Tb antigens was also reported to be a marker of tuberculosis infection

  Nemeth et al., 2011

- IP-10 may be a better alternative marker for latent TB infection diagnosis among immunocompromised individuals and children

  Khader et al., 2009

- IL-2, IFN-γ, TNF-α expression profiles of CD4+ T cells promise in detecting active TB

  Mihret A et al., 2013

- TNF, IL-12(p40), IL-17 are increased in TB cases and can discriminate active TB disease /LTBI

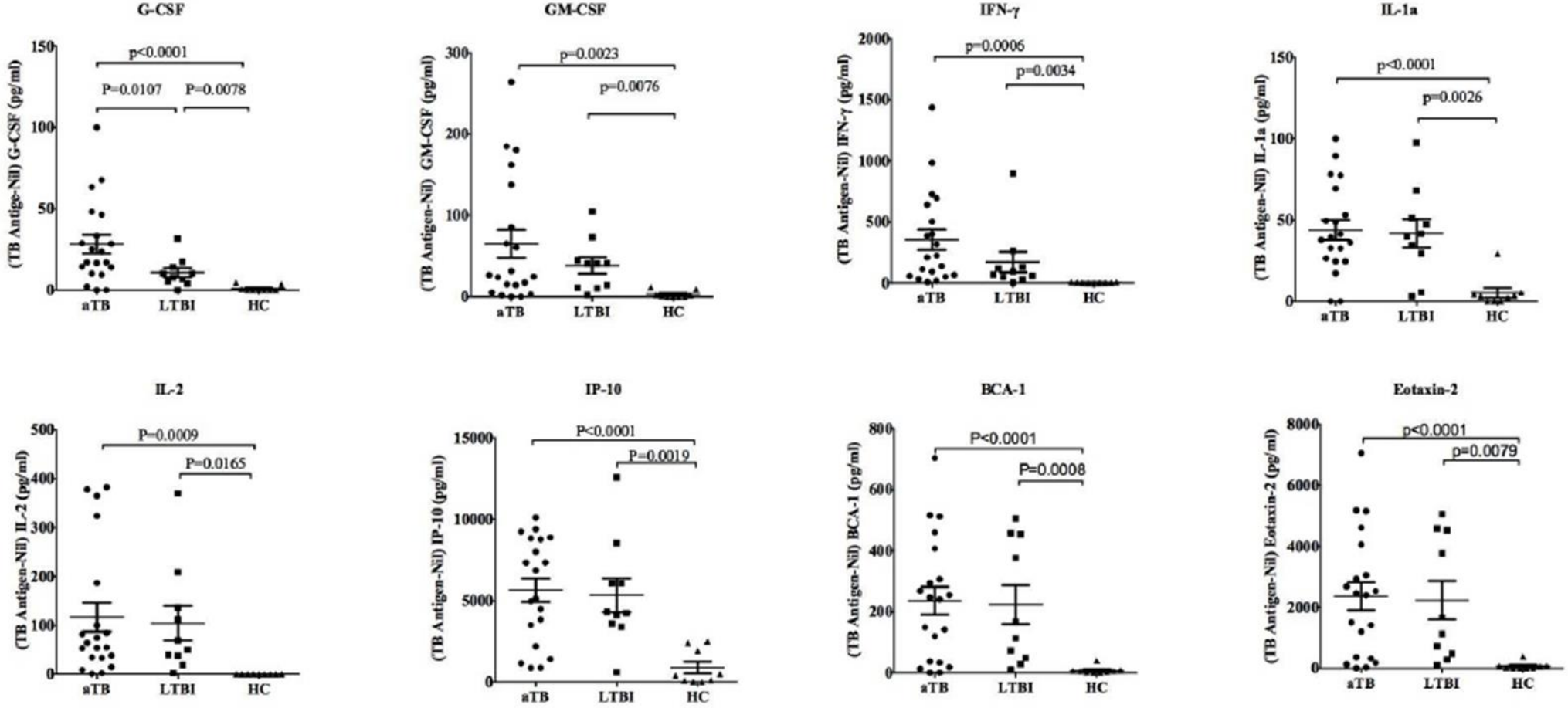
  Basile et al., 2011

- IFN-γ (but not IP-10, MCP-2 and IL-2) response to RD1 selected peptides is associated with active TB with a higher specificity than QFT / TST

  Scriba et al., 2008

Multiplex analysis of plasma cytokines/chemokines showing different immune responses in active TB patients, latent TB infection and healthy participants.

Tuberculosis 2017

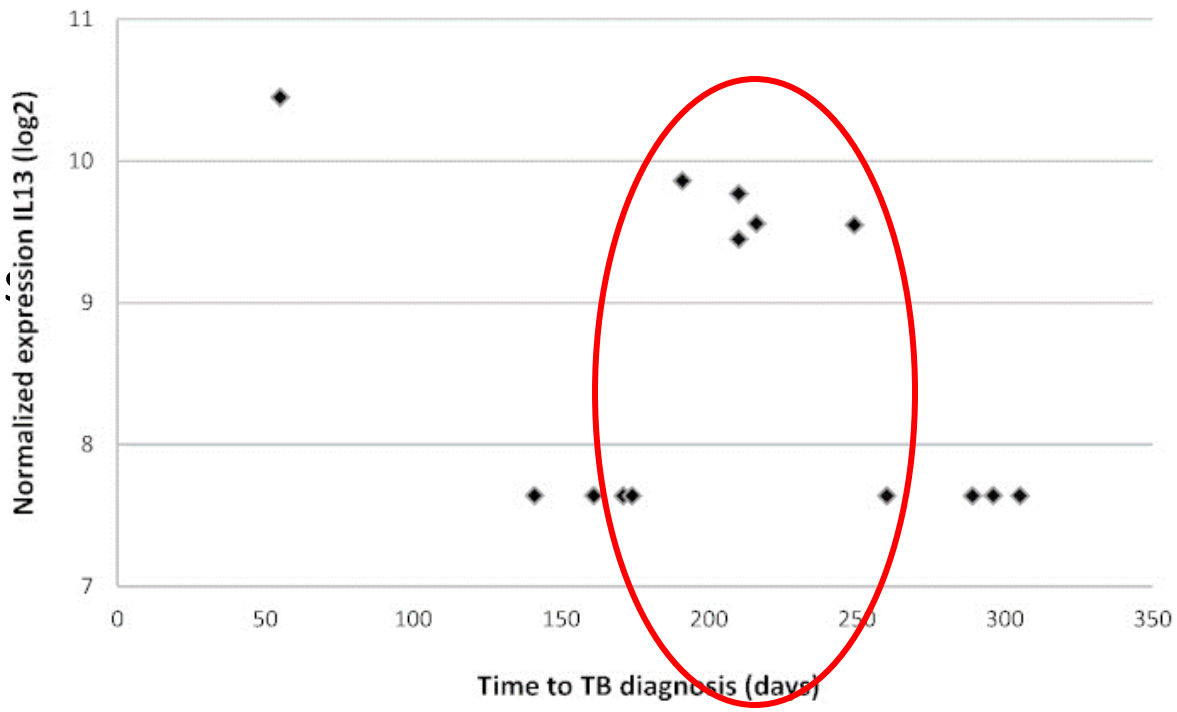


Biomarkers Can Identify Pulmonary Tuberculosis in HIV-infected Drug Users Months Prior to Clinical Diagnosis

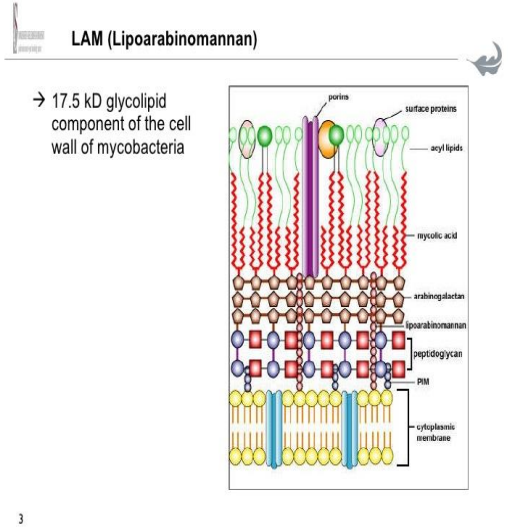
EBioMedicine. 2014

- 14 HIV-infected drug users obtained before TB diagnosis
- 15 controls
- 8 months prior to clinical diagnosis
- 141 genes
- AIRE: with increased expression in controls
- IL13: with increased expression in cases
- IL13 had been obtained

190–250 days prior to TB diagnosis



Biomarkers M.TB στα ούρα



- Εύκολη απόκτηση δείγματος (παιδιά, μη ομιλούντες γλώσσα)

- LAM (Lipoarabinomannan) is the main component of M.Tb cell wall actively secreted from infected alveolar macrophage

Wood R et al. BMC Infect Dis. 2012

- urinary LAM concentration after 2 months treatment of anti TB in TB patients

Shah M et al. J Clin Microbiol. 2010

- Ανίχευση M.Tb 156110DNA στα ούρα σε ασθενείς με πνευμονική φυματίωση και μείωση των επιπέδων δυο μήνες μετά την αγωγή

Cannas et al. Clint J Tuberc Lung Dis. 2008

The Diagnostic Value of Urine Lipoarabinomannan (LAM) Antigen in Childhood Tuberculosis.

J Clin Diagn Res. 2017

Characteristics	Non-TB (n = 12)	TB (n = 49)
Urinary LAM (mg/l)	0.46±0.30	1.80±1.02

Urinary LAM	Diagnosis	
	TB	Non TB
≥ 0.98 mg/L	42	2
< 0.98 mg/L	7	10
Total	49	12

Sensitivity 83.3%
Specificity 83.7%

Urinary LAM	Microbiology examination	
	Positive	Negative
≥ 1.69 mg/L	7	16
< 1.69 mg/L	14	24
Total	21	40

Sensitivity 33%
Specificity 60%

Diagnostic accuracy of a low-cost, urine antigen, point-of-care screening assay for HIV-associated pulmonary tuberculosis before antiretroviral therapy: a descriptive study.
Lancet Infect Dis. 2012

	TB prevalence	Determine TB-LAM		Determine TB-LAM and sputum AFB*	
		Positive predictive value	Negative predictive value	Positive predictive value	Negative predictive value
All patients (n=516)	16·5% (13·4–20·0)	80·0% (61·4–92·3)	87·4% (84·2–90·3)	84·1% (69·9–93·4)	89·8% (86·7–92·4)
CD4 cell count (cells per μL)†					
<50	28·1% (17·6–40·8)	92·3% (64·0–99·8)	90·2% (78·6–96·7)	92·9% (66·1–99·8)	90·0% (78·2–96·7)
<100	22·7% (15·7–30·9)	93·8% (69·8–99·8)	87·5% (79·9–93·0)	95·0% (75·1–99·9)	90·7% (83·6–95·5)
<150	20·5% (15·4–26·4)	91·3% (72·0–98·9)	87·6% (82·2–91·8)	93·1% (77·2–99·2)	90·3% (85·2–94·0)
<200	18·2% (14·1–22·8)	88·5% (69·8–97·6)	88·0% (83·7–91·4)	88·6% (73·3–96·8)	90·3% (86·3–93·5)
≥200	13·2% (8·7–18·9)	66·7% (9·4–99·2)	87·1% (81·4–91·6)	75·0% (34·9–96·8)	89·5% (84·1–93·6)
WHO stage					
Stage 1 or 2	13·6% (10·2–17·7)	63·6% (30·8–89·1)	88·0% (84·1–91·3)	78·9% (54·4–93·9)	90·2% (86·4–93·2)
Stage 3 or 4	22·4% (16·3–29·4)	94·4% (72·7–99·9)	86·8% (80·3–91·7)	91·7% (73·0–99·0)	89·7% (83·5–94·1)
WHO symptom screen					
Positive	19·7% (15·7–24·2)	84·6% (65·1–95·6)	85·4% (81·1–89·0)	87·2% (72·6–95·7)	88·6% (84·6–91·9)
Negative	9·4% (5·3–15·0)	66·7% (9·4–99·2)	92·3% (86·9–96·0)	75·0% (19·4–99·4)	93·5% (88·4–96·8)
Any chest-radiograph abnormality‡	26·4% (20·9–32·5)	85·0% (62·1–96·8)	79·4% (73·4–84·6)	90·3% (74·2–98·0)	83·7% (77·9–88·5)

Αντιγόνο 85/85B

- Biomarker στα πτύελα
- Μέτρηση αντιγόνου 85/85B στα πτύελα με ELISA
- Μείωση τιμών ακόμα και από την πρώτη εβδομάδα
- Αυξημένα επίπεδα δηλώνουν πιθανή υποτροπή και μη ανταπόκριση στην αγωγή
- INH ευαίσθητο
- Για το αντιγόνο 85B RNA τα επίπεδα του στα πτύελα μειώνονται πολύ γρήγορα

Berry M.P et al, Nature. 2010

Biomarkers M.TB στην αναπνοή

- Ανίχνευση πτητικών ουσιών στην αναπνοή

  υπεροξείδιο του υδρογόνου

  επτάνιο, ναφθαλίνιο, εξάνιο, βενζόλιο
- Μη επεμβατικός βιολογικός δείκτης ανίχνευσης προϊόντων M.Tb κατά τη διάρκεια της αγωγής και σε πιθανή υποτροπή νόσου
- Αεριο-στερεο-χρωματογραφία
- Μη ικανοποιητικά αποτελέσματα

Kwiatkowska S et al. Respir Med 1999

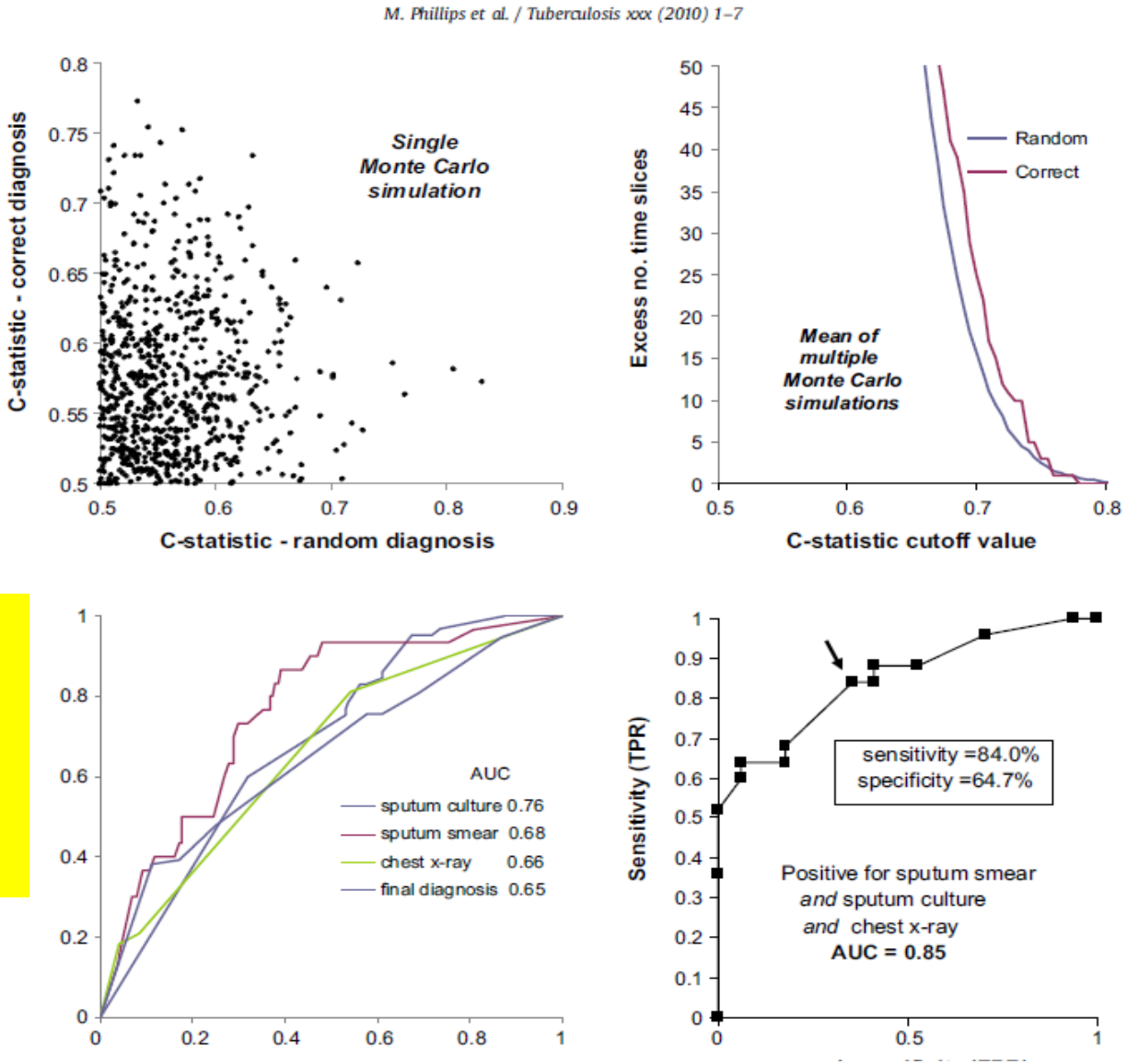
Breath biomarkers of active pulmonary tuberculosis

Tuberculosis 2010

Breath VOC biomarkers of active pulmonary TB.

Oxetane, 3-(1-methylethyl)-
Dodecane, 4-methyl-
Cyclohexane, hexyl-
Bis-(3,5,5-trimethylhexyl) phthalate
Benzene, 1,3,5-trimethyl-
Decane, 3,7-dimethyl-
Tridecane
1-Nonene, 4,6,8-trimethyl-
Heptane, 5-ethyl-2-methyl-
1-Hexene, 4-methyl-

Employing triple criterion of disease, breath biomarkers identified active TB with 85% accuracy, sensitivity 84%, specificity 64.7%



Improving tuberculosis diagnostics with biomarkers. Current Biomarker Findings 2015

5% of all TB forms
40%–63% of TPE
+

Pleural IFN-γ
IP-10
ADA
sFasL is released from T lymp and exerts cytotoxic effects on M.Tb hidden macrophages

s
sis

R3,
, FasL
PCT

- Sputum biomarkers, eg, urease
- Urine biomarkers, eg, LAM

TPE

- Pleural biomarker, eg, IFN-γ, ADA, DcR3, FasL, PCT
- Serum biomarkers, eg, PCT

PCT is lower in patients with TPE

INF-γ >75 pg/Ml
ADA >40 IU/mL
DcR3 >9.3 ng/mL
TNF-sR1 >3.2 ng/mL
independent factors associated with TPE

Biomarkers…… still loading

- LAG-3V3 (a soluble splice variant of lymphocyte activation gene) σχετιζόμενο με τη Th1
- IL-4/IL-4δ_2 ratio
- IL-4 αυξημένο σε TB
- IL-4δ_2 αυξημένο σε TB και LTBI

mRNA encoding IL-4δ_2

- IL-4/IL-4δ2 ratio στατιστικά αρνητική σχέση με τη σοβαρή νόσο (6mo)

European Union (EU)-funded University College London -VACSEL/VACSIS 2000
Scott & Rook, 2000
Dheda et al., 2005
Walzl et al., 2008

Αντισώματα ως β.δείκτες

- Αντισώματα έναντι των πρωτεϊνικών ενζύμων του M.tb

  alanine dehydrogenase

  malate synthetase

- Τα επίπεδα συνδέονται με την πιθανή αποτυχία στην αντιΤΒ αγωγή

Υψηλά επίπεδα αντισωμάτων alanine dehydrogenase συνδέονται με επιβαρυμένη νόσο και με χαμηλά ποσοστά επιτυχίας της θεραπείας και αυξημένη θνητότητα

Daley P, et al. Int Tuberc Lung Dis 2009

Μη ειδικοί β.δείκτες M.TB

Νεοπτερίνη

- Παράγεται από ενεργοποιημένα μονοκύτταρα/μακροφάγα
- Τα μονοκύτταρα παράγουν νεοπτερίνη όταν ενεργοποιούνται κυρίως από την γ-ιντερφερόνη
- Αυξημένα επίπεδα ανευρίσκονται σε διέγερση της κυτταρικής ανοσίας
- HIV-TB

Διάγνωση TB

Σε επιτυχή θεραπεία

Immannuel et al. 2001
Turgut et al., 2006
Wallis et al., 1996

Μη ειδικοί β.βιοδείκτες M.TB

CRP

- Οξεία φάση
- Υψηλά επίπεδα στη TB
- Αύξηση σε σοβαρή & εκτεταμένη νόσο
- Μείωση επιπέδων κατά τη διάρκεια της αγωγής
- Συνήθως 1mo μετά

Bajaj, Rattan & Ahmad, 1989
Lee & Chang, 2003

suPAR

- Υψηλά επίπεδα στην αρχική φάση TB
- Συσχέτιση επιπέδων στα πτύελα με κακή πρόγνωση και θνητότητα
- Μείωση επιπέδων κατά τη διάρκεια της αγωγής
- Διατηρούνται υψηλά ακόμη και 8 mo μετά

Eugen-Olsen et al., 2002

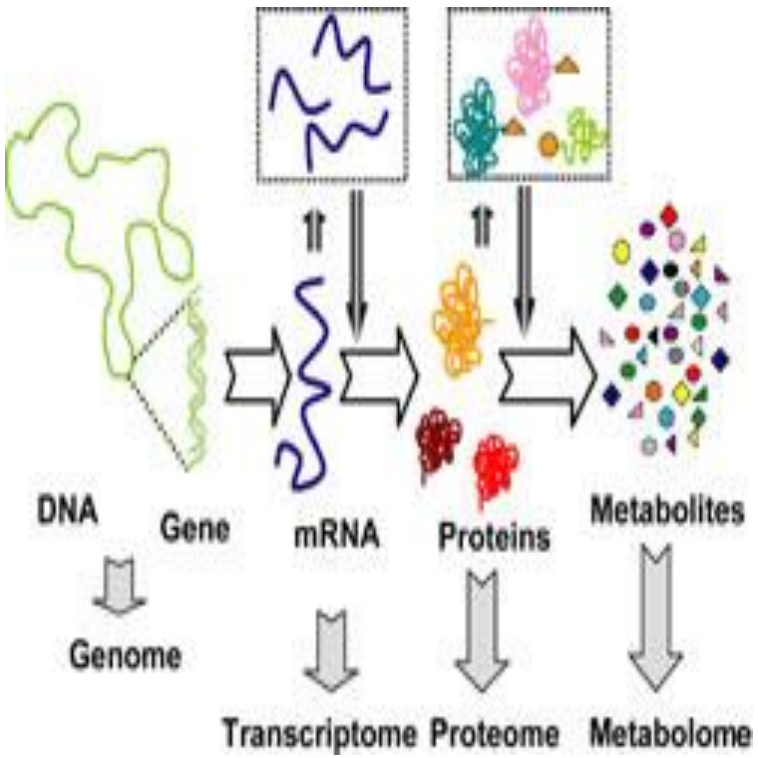
Advanced technological platforms for biomarker discovery

Transcriptomics Differentially expressed genes that distinguish latent from active TB	**RNA** (metabolites <1500 Da)	**100 000 transcripts**
Proteomics Differentially expressed proteins that distinguish latent from active TB	**Proteins**	**1 000 000 proteins**
Metabolomics Metabolomics exploits the identification of small metabolites	**Biochemical analytes**	**2400 compounds**
Combinations of above three platforms		

Courtesy of S. Parida

Biomarkers

Transcriptomics ***Μεταγράφωμα***	***Proteomics*** ***Πρωτεομική***	***Metabolomics*** ***Μεταβολωμικη***
• CD64 • Rab33A • Rab interactor 3 • LY6G6D • C14orf2 • TEX264 • ATP5G1 • NOLA3	• rRV3369 • rRV3874 • PstS1 protein • heat shock protein X • MPT64 • TrxC	• small molecules <1500 Da • serum or urine by mass spectrometry



Group	Sample	miRNA	References
Active TB and HCS	PBMC	miR-155, miR-144*	Wu et al. [17], Liu et al. [9]
Active TB and HCS	Serum	miR-29a, miR-93*	Fu et al. [18]
Active TB and HCS	Sputum	miR-29a	Fu et al. [18]
Active TB and HCS	Sputum	miR-19b-2*, miR-3179, miR-147	Yi et al. [19]
Active TB and HCS	Serum	miR-378, miR-483-5p, miR-22, miR-29c, miR-101 hsa-miR-320b	Zhang et al. [20]
Active TB and HCS	Serum	miR-361-5p miR-889 miR-576-3p	Qi et al. [26]
Active TB and HCS	Serum	miR-182 miR-197	Abd-El et al. [27]
Active TB and HCS	PBMC	miR-424 and miR-365	Wang et al. [4]
Active TB and HCS	PBMC & PFMC	miR-146a	Spinelli et al. [28]
Active TB and HCS	Serum	miR-148a miR-16 miR-192 miR-193a-5p miR-25 miR-365 miR-451 miR-532-5p miR-590-5p miR-660 miR-885-5p let-7e miR-146	Miotto et al. [29]
Active TB and HCS	PBMC	miR-150 miR-146a miR-125b miR-31 miR-10a miR-1 miR-155 miR-29	Zhou et al. [21]
PTB and other viral infections	Serum	miR-361-5p, miR-889, miR-576-3p	Qi et al. [26]
Active TB and LTBI	Macrophages	hsa-miR-16, miR-95, miR-101, miR-137, miR-193	Zheng et al. [30]
Active TB and LTBI	Serum	hsa-miR-196b and hsa-miR-376c	Zhang et al. [31]
Active TB and LTBI	PBMC	has-miR-21*	Xu et al. [32]
Active TB and LTBI	PBMC	miR-130b*, miR-223, miR-424, miR-302a, miR-21*	Wang et al. [4]
Active TB and LTBI	PBMC	miR-146a, miR-150, miR-16 miR-221	Wu [33]
Active TB and LTBI	PBMC	miR-155 and miR-155*	Stern et al. [23]
Active TB and controls	Plasma	hsa-let-7b and hsa-miR-30b	Xin et al. [25]

Biomarker and Therapeutic Targets for Tuberculosis: Role of Micro RNAs

Journal of Investigative Genomics 2016

Δυσκολίες

- Δύσκολα και ερευνητικά πρωτόκολλα
- Ακριβά λογαριθμικά πρωτόκολλα
- Μελέτες να συμπεριλαμβάνουν μεγάλο αριθμό ασθενών
- Χώρες με υψηλά ποσοστά υποτροπών και επαναλοιμώξεων από TB είναι οικονομικά πολύ ασταθείς και δεν προάγουν ανάλογες μελέτες
- HIV-TB

Ηθική δεοντολογία

Διαφορετικές πολιτιστικές κουλτούρες και θρησκευτικές πεποιθήσεις επιφέρουν τροποποιήσεις και προσαρμογές στα πρωτόκολλα συμμετοχής των ασθενών

ATHENS MARATHON. THE AUTHENTIC - 12 NOVEMBER 2017
12 November 2017
ATHENS MARATHON THE AUTHENTIC
opap
SEGAS
HELLENIC ATHLETICS FEDERATION

SAURABH
RANE
MARATHON RUNNER
(DR TUBERCULOSIS)