Serum Biomarkers For Verifying And Predicting Multiple Sclerosis Relapse

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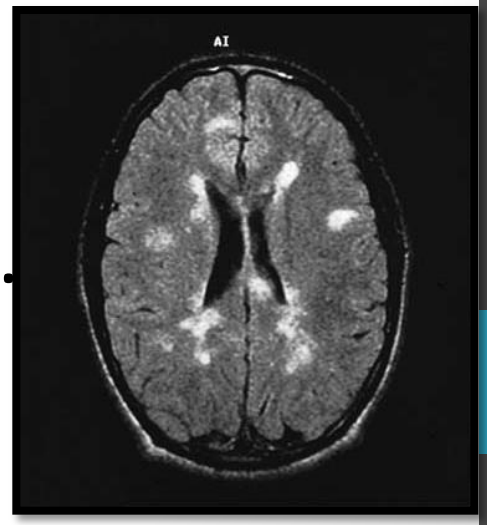
Multiple Sclerosis – Clinical Definitions

- Multiple Sclerosis - autoimmune inflammatory disease of the CNS.
- Relapsing Remitting (RRMS) - the most common disease course.
- Relapse - acute episode of new disease activity (CNS lesions).
  - It varies greatly between different patients.
  - Defined exclusively by clinical terms.
- How does it feel?

MS Relapse - Pathophysiology

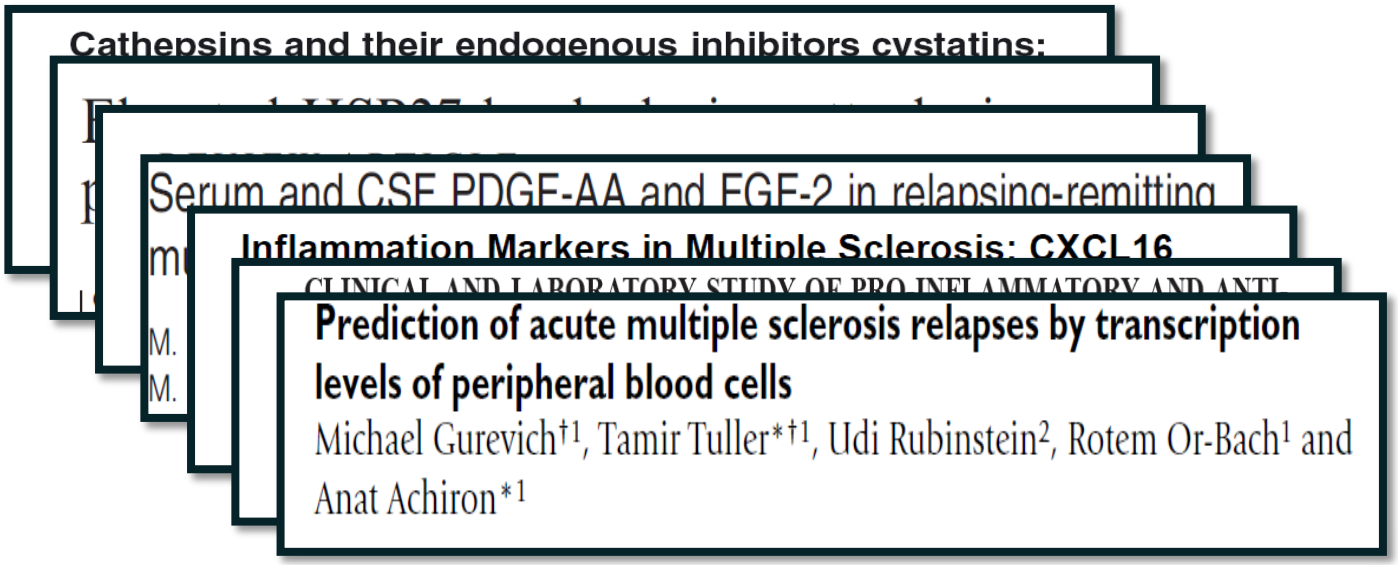
BBB breakdown → **Lymphocytes migration to the CNS** → **Attack on myelin sheaths and axons** → **Demyelinated lesions**

- Studies show:
  - **10 new lesions** is required for a clinical symptoms.
  - New lesions are formed **during remission.**
- The lesions accumulates.
- Does the inflammation accumulate?



MS Relapse - Biomarkers

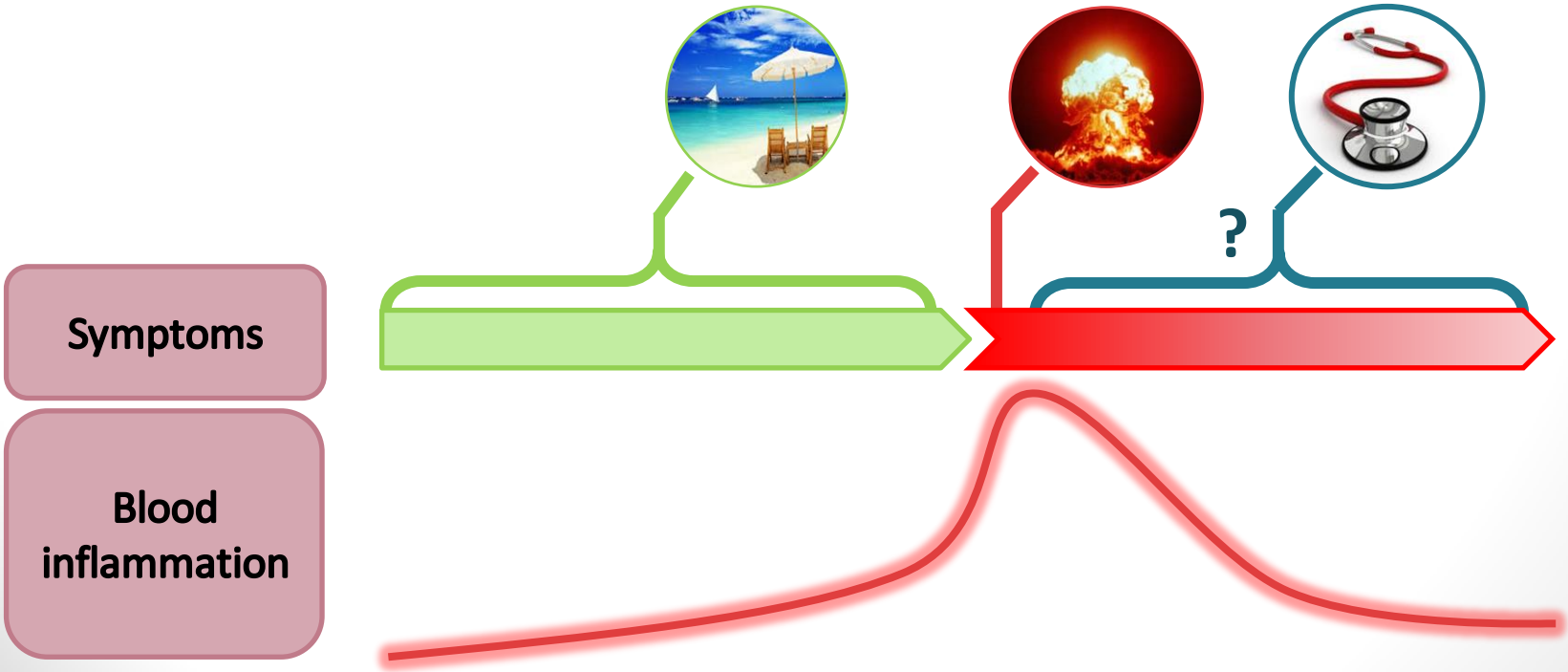
- Many biomarkers are suggested to be involved in relapse pathogenesis.
  - Cytokines, chemokines, vascular adhesion and permeability molecules, etc.



- However results were not reproducible.

MS Relapse - Biomarkers

- Suggestions for the inconstant results :
  - Variability in population between studies.
  - "The timeline" - did we miss the peak of the inflammatory activity?



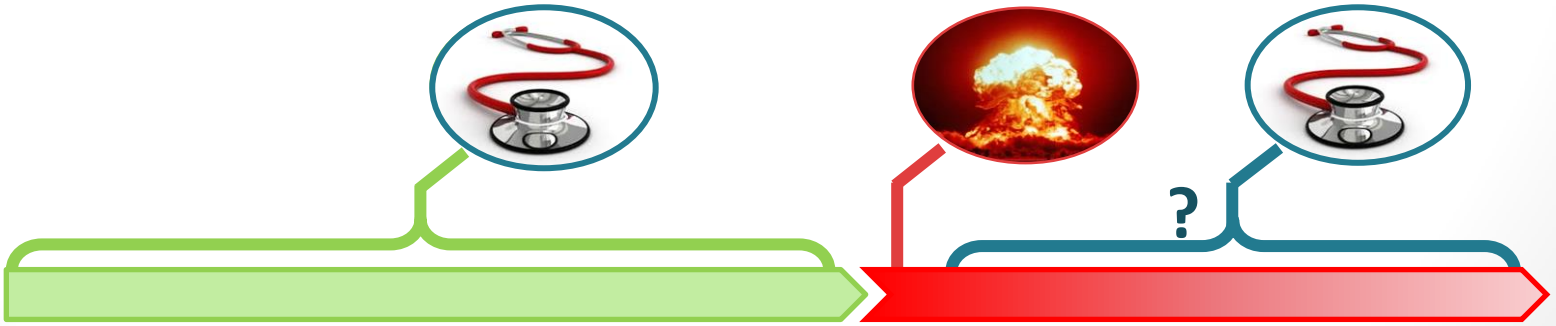
Motivation

- We refer to two major **needs** regarding relapse:
  - **Verification -** borderline relapses place a challenge for physicians.

  - **Prediction** - no tools for relapse prediction, leaving patients in a constant uncertainty.

Hypothesis

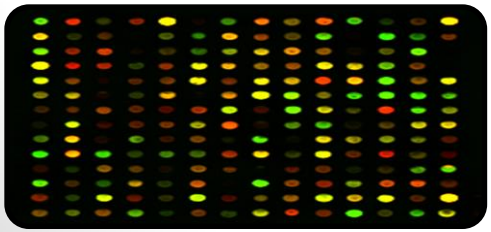
- Relapse is a **visible outcome** of subclinical inflammation during remission.
- Inflammation biomarkers profile during remission might reflect disease activity.
- Monitoring patients during remission can help **verify** clinical relapse and might **predict** it.

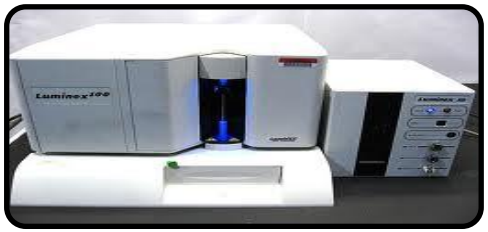


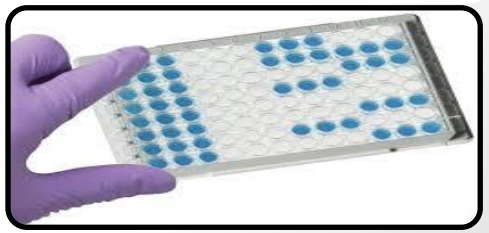
Goal and Experiment Designing

- **The Goal** - understand inflammation profile during relapse and remission in peripheral blood.
  - Observe inflammatory changes in patients with different time to upcoming relapse.
  - To find specific biomarkers which reflect disease activity.
- Experiment design:

Gene expression → **Protein verification** → **Towards clinical application**

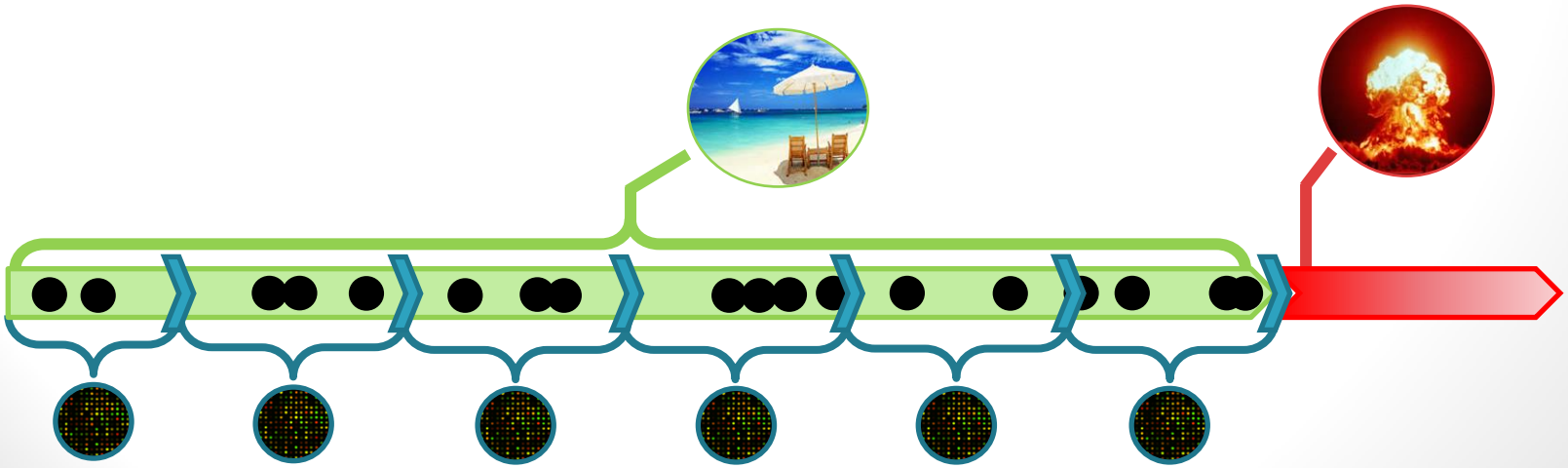




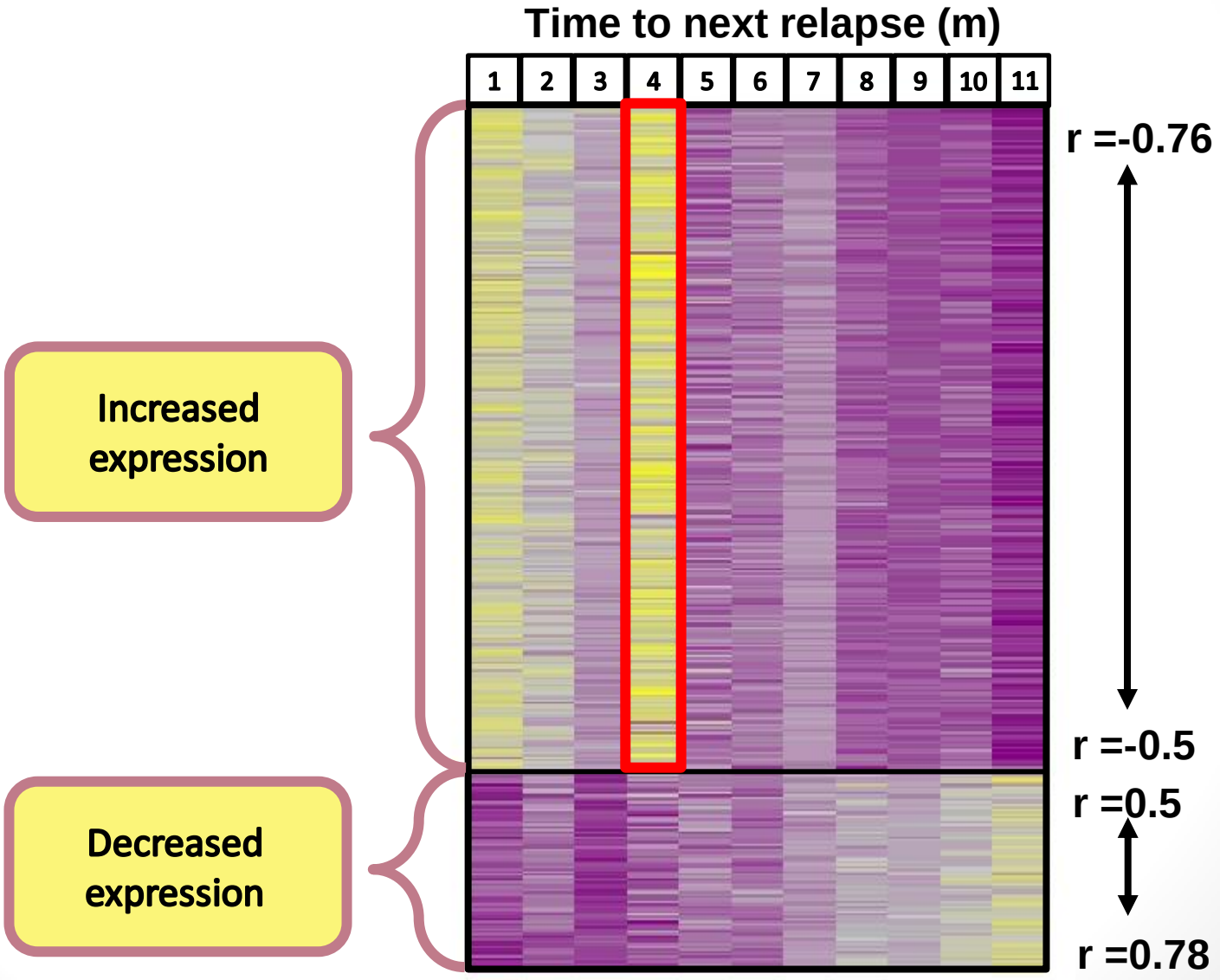


First step – Gene Expression

- Understand how blood **gene expression** changes throughout remission period.
- Data pool of gene expression from 70 MS patients.
- Patients were divided into groups by the time (months) to their next relapse.
- For every month, we calculated the **average change** in all the genes.



The Change in Gene Expression

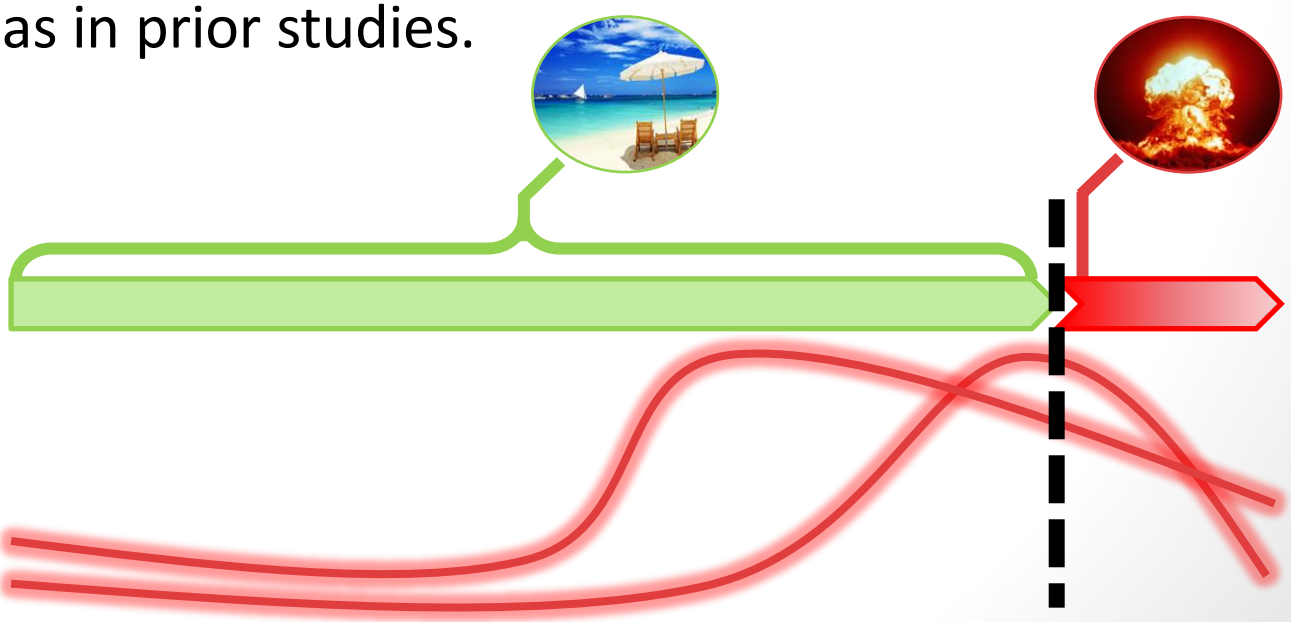


Gene Expression – Conclusions

- Peak in **inflammation gene expression** 4 month prior to visible clinical relapse.

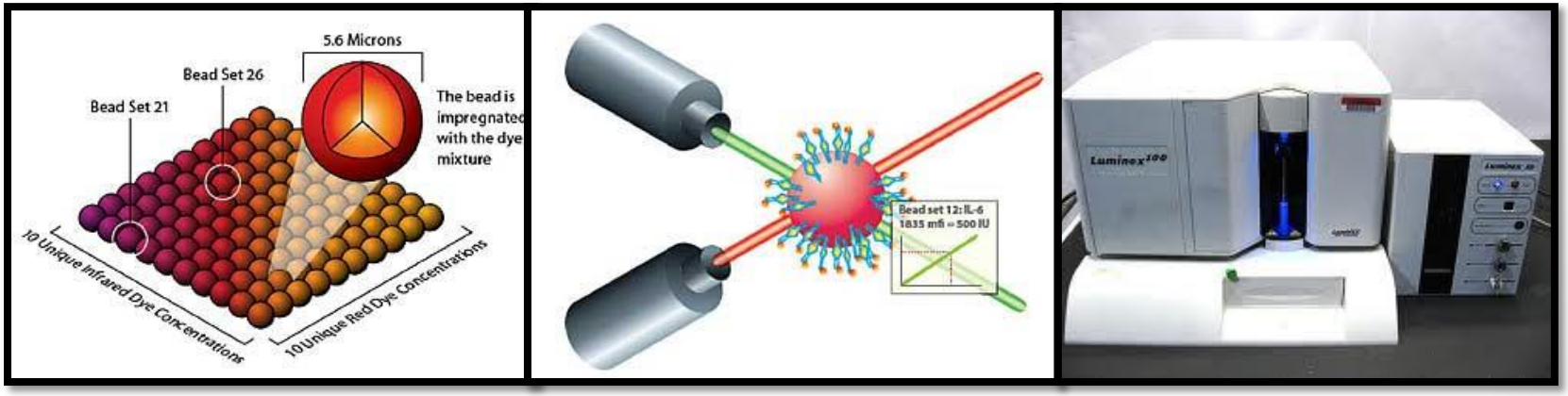
Intermediate Conclusions

- Relapse process begins **long time** before clinical symptoms.
- Different **"cutoff"** between relapse and remission – a possible bias in prior studies.



Second Step – Protein Level

- Verify results using a **different mechanism.**
- The **Luminex® system** can measure the level of 50 proteins in up to 96 patients simultaneously.

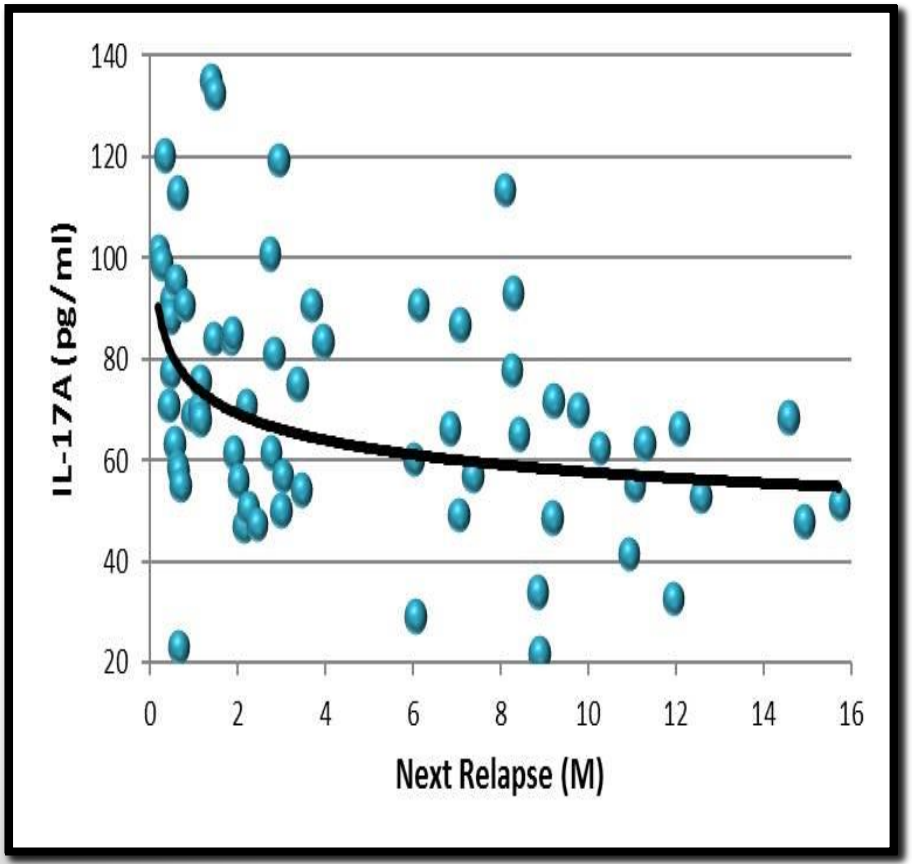


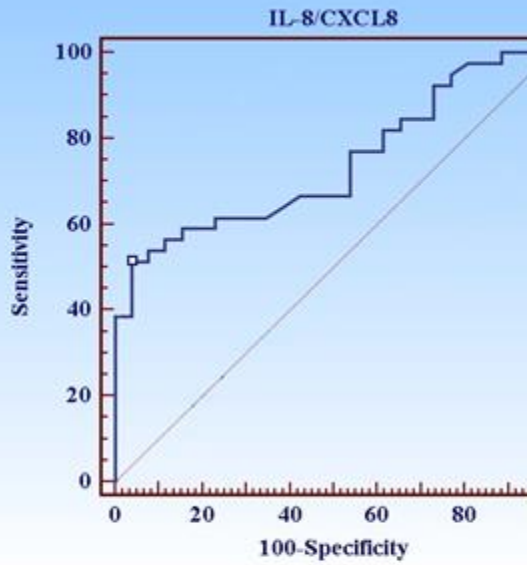
- We conducted a measurement of 50 highly expressed inflammatory proteins in 78 patients.

Protein Level Results

- 4 cytokines showed significant results:
  - **IL-8**, **IL-12**, **IL-13**, **IL-17A** (Th1, Th2, Th17 and B cell differentiation)

IL-8 | IL-12 | IL-13 | IL-17A





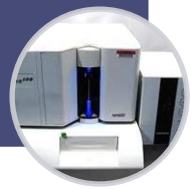
Significance (p-value)		0.001
IL-8 level	Sensitivity	Specificity
8.07	64.1	61.54

Third step – Clinical Application

- Next step - creating a clinical diagnostic tool.

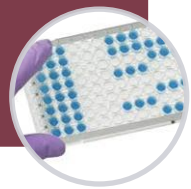
Luminex

- Expensive
- Rare
- Good for Screening



ELISA

- Cheap
- Common
- Good for individual diagnosis

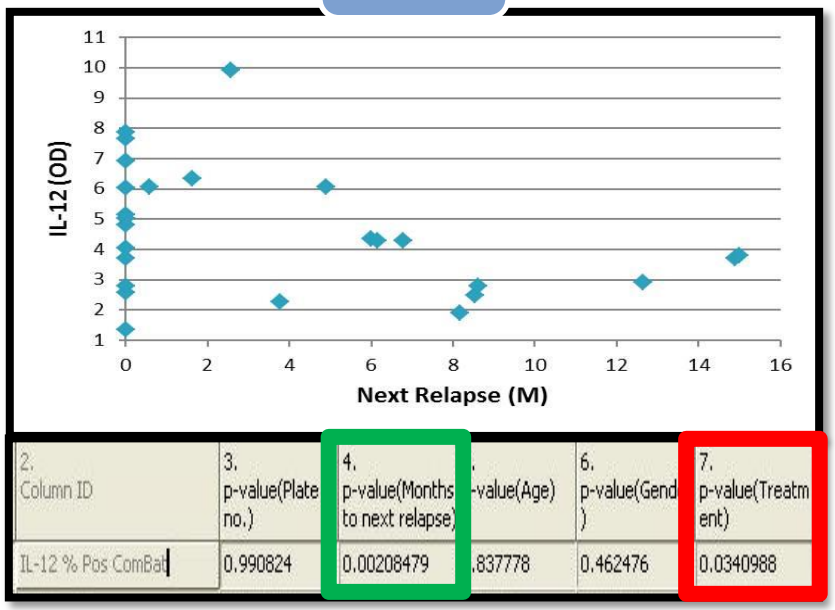


- We measured the 4 cytokines in 93 patients using ELISA test plates.

ELISA Results

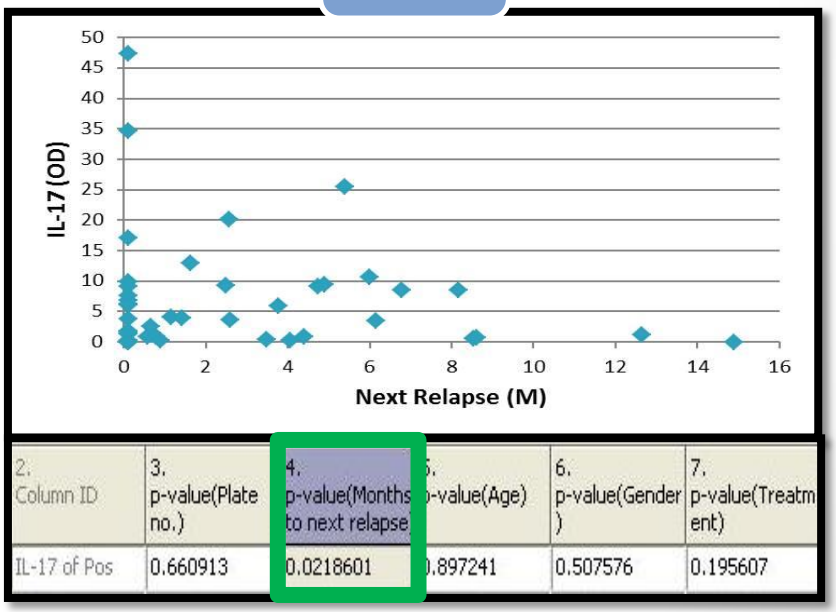
- The ELISA experiment showed some tendency, mainly regarding IL-12 and IL-17.

IL-12



2. Column ID	3. p-value(Plate no.)	4. p-value(Months to next relapse)	-value(Age)	6. p-value(Gend)	7. p-value(Treatm ent)
IL-12 % Pos ComBat	0.990824	0.00208479	.837778	0.462476	0.0340988

IL-17A



2. Column ID	3. p-value(Plate no.)	4. p-value(Months to next relapse	-value(Age)	6. p-value(Gender)	7. p-value(Treatm ent)
IL-17 of Pos	0.660913	0.0218601	.897241	0.507576	0.195607

- Technical challenges affected the quality of the results.

Outcome and Restrictions

- What can we do with that information?
  - **Clinical - monitoring** patients throughout the remission period, will allow us to **verify and predict relapse.**
  - **Research -** better understanding relapse mechanism.

- **Restrictions**
  - Previous relapses and unrelated inflammation can affect the protein and gene level.
  - Confounding variables - treatment, age, sex, disease state (EDSS), etc.

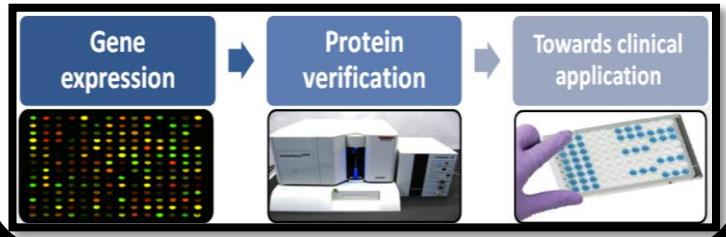
Summary

Needs

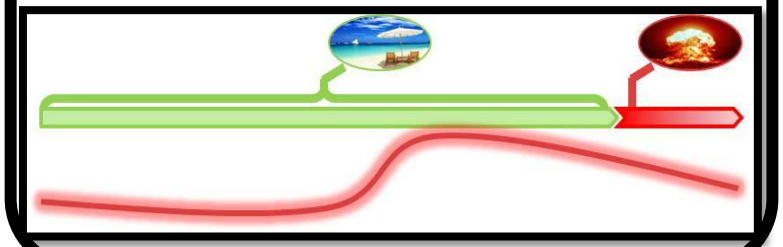
Verify

Predict

The Study



Findings



Biomarkers

IL-8

IL-12

IL-13

IL-17A

Further study is required to ratify the results before clinical application.


ANY
QUESTIONS
?