A SEQUENTIAL ALGORITHM FOR CSF-TESTING USING NEW AND CONVENTIONAL B CELL-RELATED MARKERS FOR THE DIAGNOSIS OF MULTIPLE SCLEROSIS: FIRST TUNISIAN PILOT PROJECT

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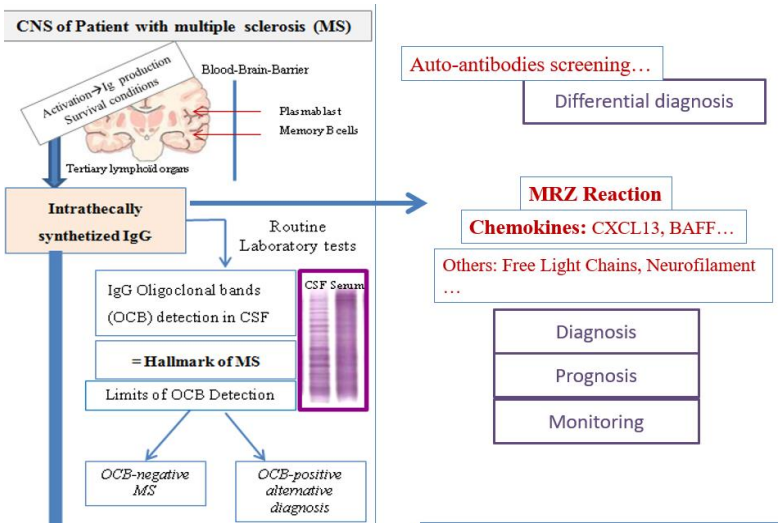
Introduction

In multiple sclerosis (MS) disease, the importance of the intrathecal B cell response classically revealed as IgG oligoclonal bands (OCB) in CSF was reaffirmed again in the **recently revised diagnostic criteria**.

Since there are several limitations related to OCB testing, measurement of other B cell-related molecules (Ig free light chains (FLC) and CXCL13 (*B-Cell Attracting chemokine1)* metrics) in CSF has been suggested as quantitative standardizable and simple alternative to detect this intrathecal synthesis (IS) of IgG.

After an experience of about 10 years of biological investigation for MS diagnosis (first Tunisian pilot project), **Our aim** was to optimize **the algorithm of routine CSF investigation during MS** by studying the performance of the new B cell-related markers and software for the detection of the intrathecal inflammation in MS context.



Material and Methods

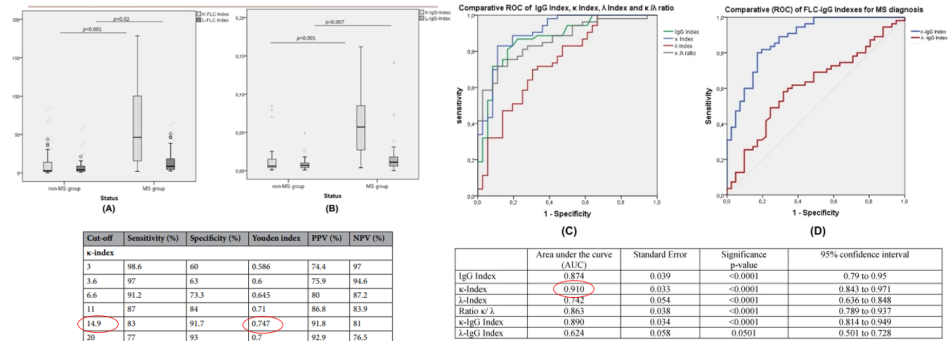
420 paired CSF-serum samples were collected from 210 patients :

with MS (n =110) and non-MS diseases (n =100) for:

- IgG Index calculation and OCB testing (CSF isofocusing test)
- Free Light Chains (FLC) metrics (κ/λ ratio, FLC Indexes, FLC IgG Indexes, κFLC intrathecal fraction (IF)) calculation using formulas and specific software.
- Levels of CXCL13 determination in CSF.
- MRZ-reaction (intathecal humoral response against-Measles (M), Rubella (R) and Varicella-Zoster (Z) viruses) testing.

Results

Free light chains (FLC) in Tunisian population

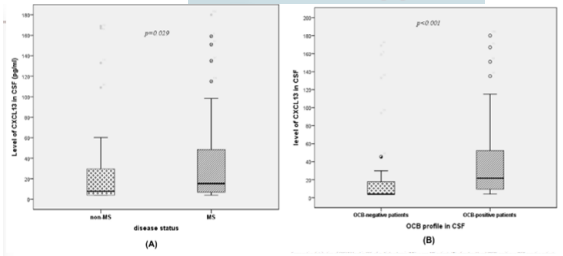


Cut-off	Sensitivity (%)	Specificity (%)	Youden index	PPV (%)	NPV (%)
κ-index					
3	98.6	60	0.586	74.4	97
3.6	97	63	0.6	75.9	94.6
6.6	91.2	73.3	0.645	80	87.2
11	87	84	0.71	86.8	83.9
14.9	83	91.7	0.747	91.8	81
20	77	93	0.7	92.9	76.5

	Area under the curve (AUC)	Standard Error	Significance p-value	95% confidence interval
IgG Index	0.874	0.039	<0.0001	0.79 to 0.95
κ-Index	0.910	0.033	<0.0001	0.843 to 0.971
λ-Index	0.742	0.054	<0.0001	0.636 to 0.848
Ratio κ/ λ	0.863	0.038	<0.0001	0.789 to 0.937
κ-IgG Index	0.890	0.034	<0.0001	0.814 to 0.949
λ-IgG Index	0.624	0.058	0.0501	0.501 to 0.728

k FLC optimal Cut-off for Tunisian population: 14,9

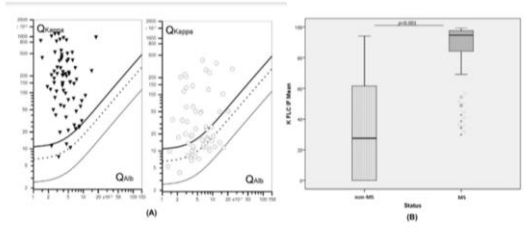
CXCL13 in Tunisian population



CSF level of CXCL13 Chemokine:

- is significantly higher in MS
- is correlated with the intrathecal humoral immune response

"FLC-κ statistics and graphic program":



(A) κ-FLC Reibergrams showing the relation between the CSF/serum κ-FLC quotient (Q kappa) and the CSF/serum albumin quotient (Qalb) for each patient.

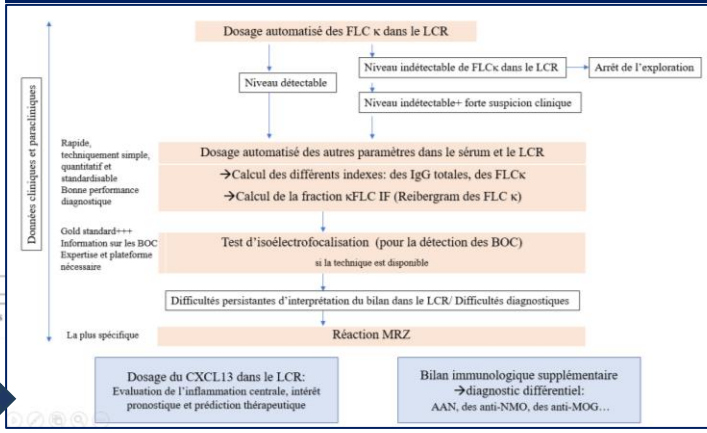
(B) Comparative distribution of κFLC intrathecal fraction (IF) in MS and non-MS groups using U test of Mann–Whitney. Horizontal solid lines indicate medians.

	Area under the curve (AUC)	Standard Error	Significance p-value	95% confidence interval
FLC IF	0.926	0.023	<0.0001	0.881 to 0.970

Figure 6. ROC curve for the κFLC IF performance to discriminate multiple sclerosis (MS) from non-MS patients. *ROC* receiver operating characteristic, *FLC IF* free light chain intrathecal fraction.

The quantitative, standardisable and simple FLCκ-metrics seems to be reliable in MS diagnosis.
CXCL13 is an effective parameter reflecting the intrathecal inflammation.
MRZ-reaction remains the most specific test described so far.

Conclusion



Optimized Algorithm for biological investigation of MS: Habib Bourguiba University Hospital