37th EPS European Peptide Symposium
14th IPS International Peptide Symposium
25 - 29 August 2024
Satellite Workshop 30 - 31 August 2024
Florence, Italy- 2024

UNIVERSITÀ DEGLI STUDI FIRENZE
NEUROFARBA
DIPARTIMENTO DI NEUROSCIENZE, PSICOLOGIA, AREA DEL FARMACO E SALUTE DEL BAMBINO

https://doi.org/10.17952/37EPS.2024.P2134

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ADALIMUMAB AND ANTI-ADALIMUMAB ANTIBODIES: A NOVEL METHOD OF DETECTION AND QUANTIFICATION IN HUMAN SERA BASED ON SURFACE PLASMON RESONANCE TECHNIQUE

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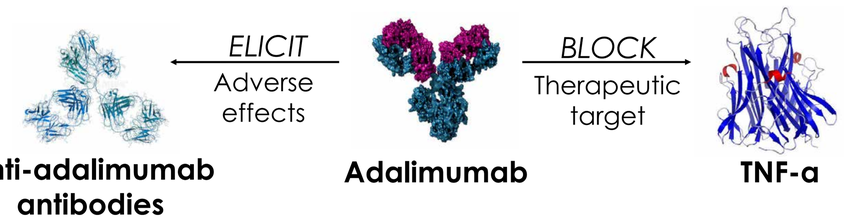
Adalimumab

Biologics targeting the tumor necrosis factor alpha (TNF-a) have revolutionized treatments in a wide variety of autoimmune diseases, such as rheumatoid arthritis and juvenile idiopathic arthritis. **Adalimumab (ADL)** is the most widely used drug in this field, but, despite being a fully human antibody, **anti-adalimumab antibodies (AAA)** are reported at a rate up of 8% after 8 weeks and 24% after 60 weeks of treatment.[1] Their presence is correlated to an inadequate response to initial treatment (primary failure), the loss of response over time (secondary failure), and the development of potentially therapy-limiting adverse events.[2]



SPR for detection of both ADA and ADAbs
Biacore™ X100 from Cytiva (Uppsala, Sweden)

- ❖ Immobilization of an anti-ADL mAb to detect ADL
- ❖ Immobilization of ADL o detect AAA
- ❖ Reference subtraction using an immobilized human pAb

Anti-adalimumab antibodies (AAA) monitoring

Problems caused by:[3,4]

- Proper secondary reagent to discriminate AAA and ADL
- Drug-antibody complexes and complexity of the matrix (serum)
- Heterogenicity of the assays and lack of a gold standard

Aim of the study

The purpose of this work is to overcome the problems of AAA monitoring, proposing a novel method able to detect and quantify AAA as well as the free drug ADL

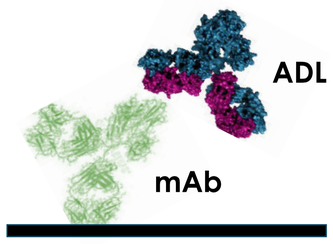
SPR-based biosensor

Surface plasmon resonance (SPR) allows the detection of mass changes on the chip surface in real-time, registering the binding between the immobilized ligand and the analyte in solution.

- ✓ No sample pre-treatment
- ✓ No need for secondary reagent
- ✓ Parallel measurement exploiting different channels
- ✓ Fully automatized
- ✓ Able to detect low-affinity antibodies

Materials and methods

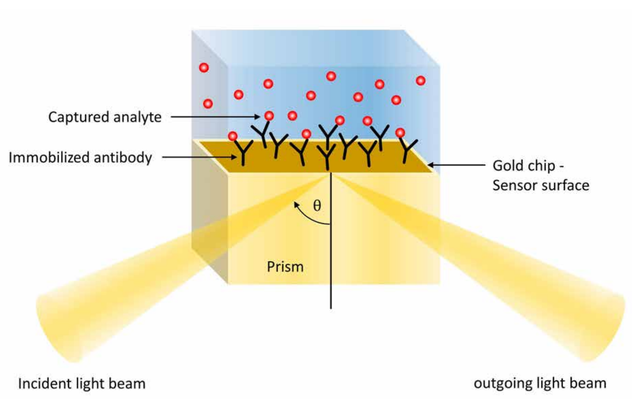
Patients and samples, materials and reagents, immobilization and analysis conditions, statistics



Adalimumab quantification

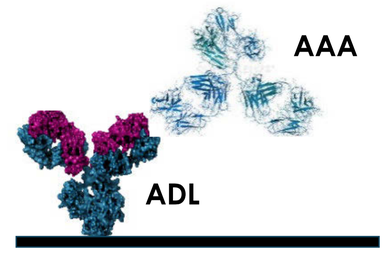
SPR signal correlated with increased concentration of ADL with a 4-parameters polynomial correlation

	C ADL (ng/mL)	n	Mean (ng/mL)	CV (%)	SD (ng/mL)	Accuracy (%)	Precision (%)
Intraday	100	3	103.5	3.2	3.3	103.5	96.8
	800	3	832.1	3.8	32.0	104.0	96.2
Interday	100	7	99.0	1.6	1.6	99.0	98.4
	800	7	800.3	0.1	0.8	100.0	99.9



Anti-adalimumab antibodies quantification

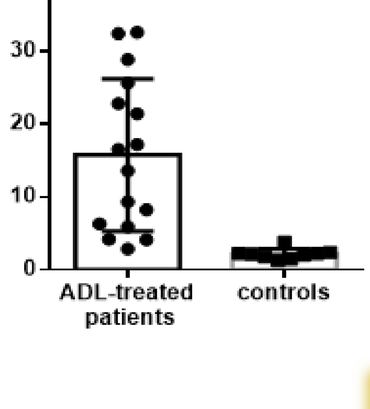
SPR signal linearly correlated with increased concentration of mAb ($r^2 > 0.998$)

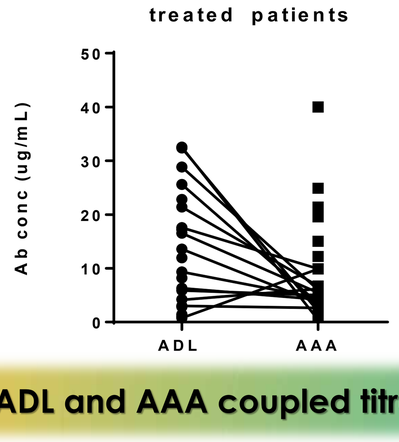


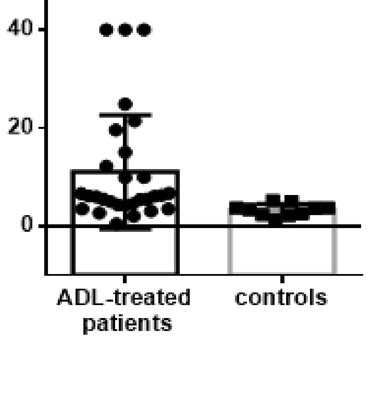
	C AAA (ng/mL)	n	Mean (ng/mL)	CV (%)	SD (ng/mL)	Accuracy (%)	Precision (%)
Intraday	100	3	102.0	7.8	8.0	102.0	92.2
	800	3	805.0	1.0	8.2	100.6	99.0
Interday	100	11	99.2	4.7	4.7	99.2	95.3
	800	11	791.2	0.8	6.3	98.9	99.2

Analysis of human sera samples
ADL-treated patients (n = 47) and controls (n = 13)

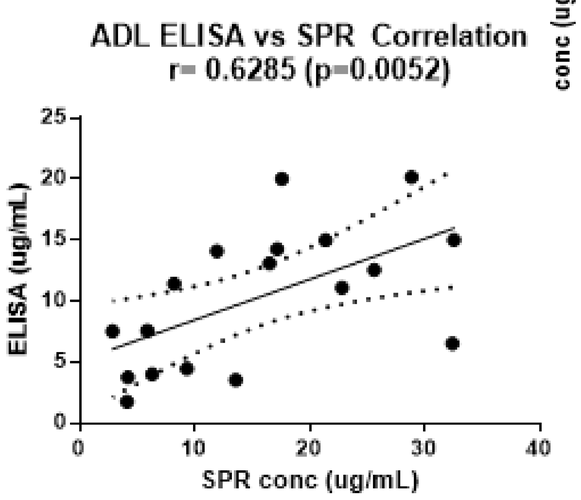
Data distribution of ADL serum titers measured. Cut-off was set at 5 µg/mL.





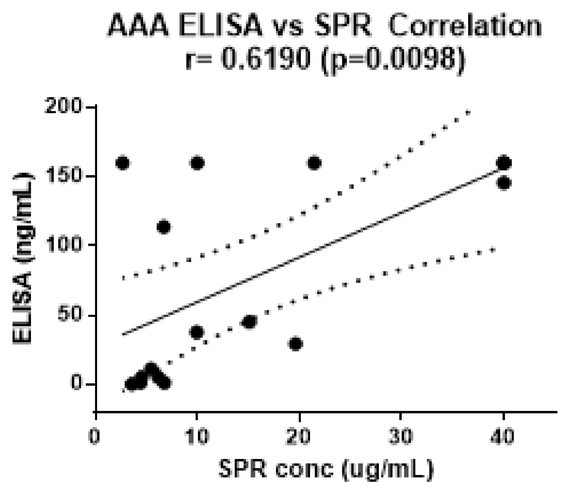


Data distribution of AAA serum titers measured. Cut-off was set at 7.5 µg/mL.



Comparison with commercially available ELISA kit

ADL and AAA coupled titres



Comparison with commercially available ELISA kit

Conclusions

- Methods developed
- Samples presenting AAA titres revealed lower traces of ADL
- ADL overall agreement with ELISA: 77%
- AAA overall agreement with ELISA: 79%

Acknowledgments

Thanks to MiUR for Andrea Di Santo PhD scholarship

Thanks to European Peptide Society for Andrea Di Santo awarded registration

Thanks to THE Tuscany Health Ecosystem for the grant ECS_00000017 and the Azienda USL Toscana Centro for the financial support to the project "Progetto ADA" (No. 1043/16-09-2022)

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