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UNIVERSITÀ DEGLI STUDI FIRENZE
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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

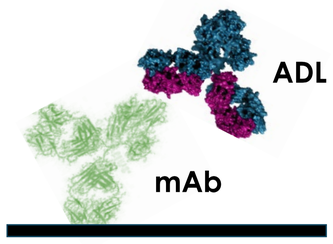
SPR-based biosensor

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

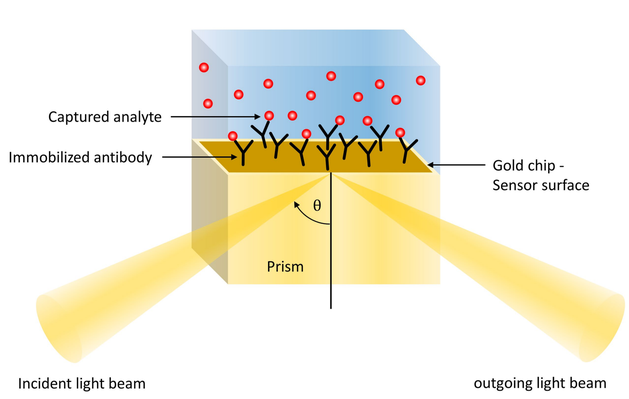
Patients and samples, materials and reagents, immobilization and analysis conditions, statistics



Adalimumab quantification

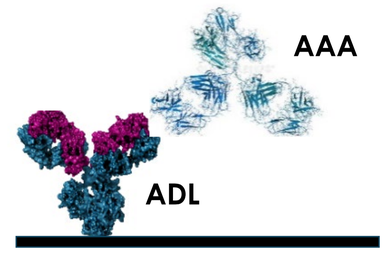
SPR signal correlated with increased concentration of ADL with a 4-parameters polynomial correlation

	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

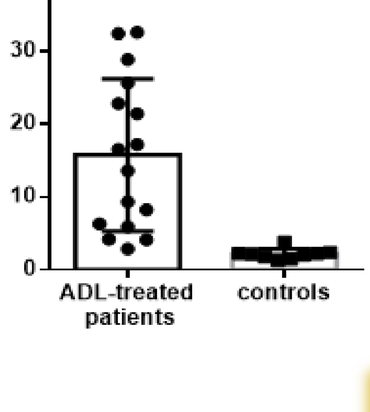
SPR signal linearly correlated with increased concentration of mAb ($r^2 > 0.998$)

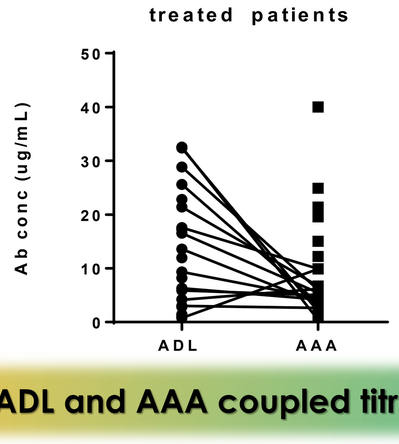


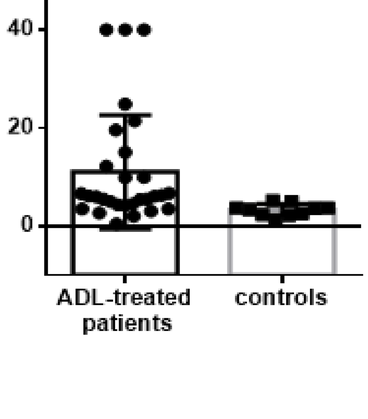
	C AAA (ng/mL)	n	Mean (ng/mL)	CV (%)	SD (ng/mL)	Accuracy (%)	Precision (%)
Intraday	100	3	102.0	7.8	8.0	102.0	92.2
	800	3	805.0	1.0	8.2	100.6	99.0
Interday	100	11	99.2	4.7	4.7	99.2	95.3
	800	11	791.2	0.8	6.3	98.9	99.2

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

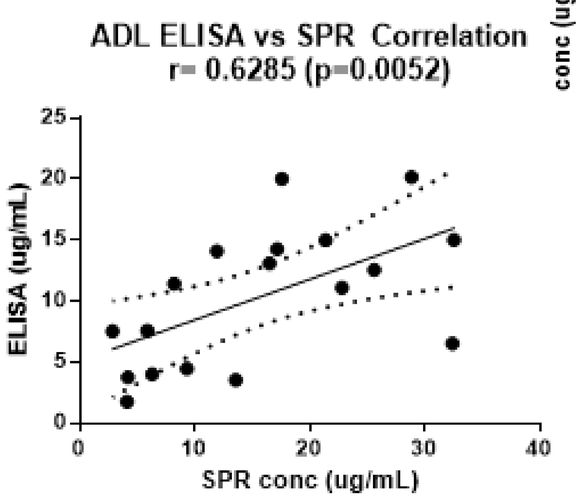
Data distribution of ADL serum titers measured. Cut-off was set at 5 µg/mL.





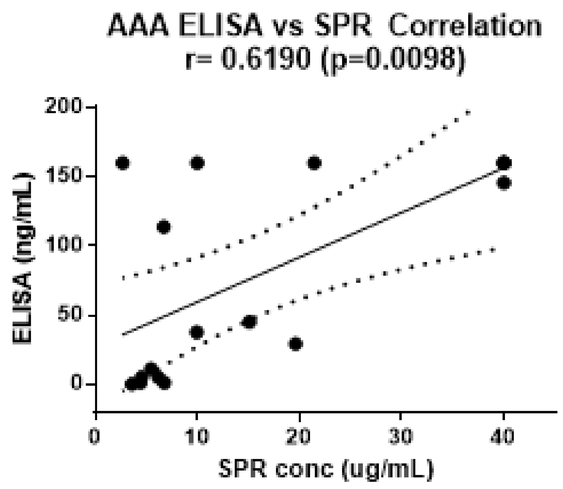


Data distribution of AAA serum titers measured. Cut-off was set at 7.5 µg/mL.



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

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