Monitoring of patients treated by anti-TNF-α

Abstracts & Posters

Juin 2012
**Infliximab impact on the occurrence of cutaneous and articular paradoxical manifestations in IBD: prospective case control study**

Julie Chapuis, Keltoum Mamou, Edouard Chabrun, Anne Cadier, Valérie Labat-Debelleix, Patrick Blanco, Victor de Lédinghen, David Laharie.

**Introduction:** Among side effects of anti-TNF, dermatosis and arthralgia have been recently described. They are called paradoxical manifestations (PM) as anti-TNF are effective treatments of serious psoriasis and inflammatory rheumatisms. PM pathogenesis remains poorly known and could make appear high plasmatic tough concentration of anti-TNF. The aim of our study was to research an association between residual plasmatic levels of infliximab (IFX) and the occurrence of articular and cutaneous PM.

**Patients and Methods:** Between May 2010 and January 2011, all patients suffering from inflammatory bowel disease (IBD) and treated by IFX on maintenance therapy have been consecutively included in a monocentric study. At the inclusion (first IFX perfusion during the study period), the occurrence of a PM was identified on clinical and chronological criteria secondarily validated by an organ specialist (dermatologist or rheumatologist). Infliximab and anti-infliximab antibodies (anti-IFX Abs) tough concentration (LISA-TRAKER® kit, BiomedicaL Diagnostics BMD) was measured at the inclusion. Infliximab and anti-IFX Abs values were compared between patients that developed paradoxical cutaneous manifestations (PCM) or paradoxical articular manifestations (PAM) and the others, according to the Mann-Whitney Test, and a multivariate analysis of the predictive factors of occurrence of these paradoxical effects was performed.

**Results:** Among the 160 patients chosen during the period, 121 (69 W; mean age: 27.4 years old) were treated by IFX on maintenance therapy and were analysable. Nine (7%) had developed a PCM (8W), which were in 8 cases a psoriasis, and 10 (8%) a PAM (7W), such as disabling arthralgia. Median tough concentration of Infliximab were respectively of 5.87 (extremes: 0.52 – 19.53) µg/mL in case of PAM and of 5.57 (0.00 - 49.12) µg/mL in absence of PAM (P=0.560). Sixteen (13%) patients had detectable anti-IFX Abs (> 10g/mL), whose three had a PAM (P=0.128) and none had a cutaneous PM (P=0.605). In multivariate analysis, no predictive factor of occurrence of these paradoxical effects was identified, and only the factor associated to the PAM occurrence was a rate of anti-nuclear antibodies (ACAN) > 1/100e at the inclusion.

**Conclusion:** Tough concentration of IFX and anti-IFX Abs are not different among patients suffering from IBD and treated by IFX on maintenance therapy, that developed cutaneous or articular PM, and the others. The occurrence of PAM could be the consequence of an immune response induced, as evidenced by the presence of ACAN.

Translated from french.
INFLIXIMAB BUT NOT ADALIMUMAB SERUM TROUGH LEVEL IS USEFUL PREDICTOR OF DEEP REMISSION IN PATIENTS WITH CROHN’S DISEASE

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This abstract is Basic Science and should be considered for a Basic Science Session: No

INTRODUCTION: Deep remission (DR) defined as clinical remission and endoscopically proved mucosal healing is an important treatment goal in patients with Crohn’s disease. Biologic therapy including infliximab (IFX) and adalimumab (ADA) is associated with high chance of achieving DR, but optimization of such therapy is still necessary.

AIMS & METHODS: The aim of the current study was determination of the association between infliximab and adalimumab trough levels (ITL resp. ATL) and DR. A total of 100 Crohn’s disease (CD) patients were enrolled in to the analysis. The average age of 35 male and 65 female patients was 34.7±9.7 years. Median length of therapy was 19.5 months (2-76) and median dose interval was 8 weeks (4-8) in 52 infliximab patients and 2 weeks in 48 adalimumab patients. All but 2 patients received standard IFX dose of 5mg/kg and all patients in ADA group received 40 mg every 2 weeks. The groups did not differ in age or length of treatment. DR was achieved in 27 patients in INF group (51.9%) and in 24 patients in ADA group (50%). INF and ADA serum trough levels were analyzed using LISA TRACKER Premium Infiximab or Adalimumab EIA kit which allows simultaneous determination of TNF-α, IFX or ADA level, and anti-infliximab neutralizing antibodies (ATI) or anti-adalimumab antibodies. Degree of mucosal healing was regularly checked endoscopically.

RESULTS: IFX serum through level more than 2 µg/ml was associated with significantly higher chance of DR (19 patients out of 27) than ITL < 2 µg/ml (8 pt out of 27); (70.4 vs. 29.6%, p<0.001). ADA serum trough level more than 2 µg/ml was associated with DR in 21 of 24 patients (84%) but among patients without DR , ATL ≥ 2 µg/ml was found in 17 of 24 patients (70.8%, p-NS). In IFX group, ITL ≥ 2 µg/ml was found just in 5 patients without DR (20%). We did not find any significant association of drug antibodies or serum TNF-α level with DR.

CONCLUSION: Infliximab but not adalimumab serum trough level is good predictor of deep remission in Crohn’s disease patients. Measurement of ITL is helpful in clinical management of CD patients on IFX therapy.

I confirm having declared any potential Conflict of Interest for ALL authors listed on this abstract: Yes

E-mail Address: drastich@hotmail.com

Disclosure of Interest: None Declared

Keywords: adalimumab trough level, Crohn’s disease, deep remission, infliximab trough level
Simultaneous determination of anti-infliximab antibodies and residual infliximab levels to monitor anti-TNF therapy

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Introduction

Some patients with autoimmune inflammatory diseases and treated with infliximab (an anti-TNF therapy), are suspected of acquired therapeutic resistance and loss of response. Several therapeutic strategies are possible for those patients: increasing infliximab dosage or switching to another TNF-inhibitor or another therapy. However, most of the strategies are based on clinical data. The aim of this study was to evaluate the presence of anti-infliximab antibodies together with residual infliximab levels in such patients.

Patients

We studied 41 patients treated by infliximab and suspected of therapeutic loss response:
- Rheumatoid arthritis: RA (n=17)
- Mean DAS28 = 5.7 (range 3.9-7.9)
- Psoriasis (n=2)
- Active RA
- Rheumatoid arthritis: RA before switch to anti-TNF therapy

Patients treated by infliximab (an anti-TNF inhibitor) or another therapy.

Methods

Residual infliximab levels and anti-infliximab antibodies were determined by ELISA (LISA-TRACKER, BMD, France) with a threshold of 10 ng/ml for anti-infliximab antibodies and 0.1 µg/ml for infliximab.

In 22/25 sera (88%), an undetectable or low infliximab concentration was associated to the presence of anti-infliximab antibodies. However, the absence of anti-infliximab antibodies is more difficult to interpret in patients with low but detectable infliximab levels. For two patients, we found detectable anti-infliximab antibodies (156 and 23 ng/ml) although the sera were obtained more than one year after the last infliximab perfusion.

Results

<table>
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<tr>
<th>Infliximab (µg/ml)</th>
<th>Anti-Infliximab (ng/ml)</th>
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<tr>
<td>&lt;0.1</td>
<td>&gt;200</td>
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<td>&lt;0.1</td>
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<td>0.22</td>
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Conclusion

Our preliminary results suggest that in patients suspected of loss of anti-TNF response, the undetectable level of infliximab was mostly related to the presence of anti-infliximab antibodies confirming the necessity in those patients to switch to another TNF-inhibitor instead of increasing infliximab doses.

In 16 patients the loss of response to infliximab was not explained by weak infliximab levels. However therapeutic concentrations need to be defined.
Background/ Purpose: Infliximab is a chimaeric monoclonal antibody targeting tumour necrosis factor alpha (TNFα) indicated in ankylosing spondylitis (AS). We analyze association between blood level of TNFα, infliximab, or antibodies toward infliximab (ATI) with AS activity.

Methods: In a monocentric cross-sectional study, 30 AS patients treated with infliximab were collected. Detail of infliximab, prednisone and methotrexate (MTX) were stated since the last infusion. AS patients are the main characteristically of AS with 66% than, with a mean age of 44.4 years old (from 17 to 63) treated since 37.4 months (from 0.5 to 106.4). Only 2 patients received also methotrexate. AS activity was assessed by Ankylosing Spondylitis Disease Activity Score (ASDAS). TNFα, infliximab, and ATI serum concentrations were measured in the blood collected before the next infliximab infusion by enzyme-linked immunosorbent assay (LisaTracker, BMD, France). Data were analyzed using the Kruskall–Wallis one-way analysis of variance (ANOVA) test, and correlation between TNFα concentration and ATI was also observed using the Kruskal-Wallis one-way analysis of variance (ANOVA) test.

Results: We observe heterogeneity in TNFα concentration according to AS activity (P < 0.001). A close TNFα effect in the blood was observed according to AS activity assessed by ASDAS. Similarly, infliximab concentration was heterogeneity in the 30 AS patients (P < 0.02). Except for inactive AS, we observed an opposite dose effect between infliximab concentration and ASDAS (P < 0.05). Significant level of ATI were observed in 4 AS patients. One had a moderate activity and 3 had a very active disease. In particular, we observed a positive correlation between TNFα concentration and ATI was also observed (Spearman correlation coefficient = -0.47, P < 0.01). Furthermore, a negative correlation between infliximab concentration and ATI was also observed (Spearman correlation coefficient = -0.47, P < 0.01).

Conclusion: Our study suggested that remaining circulating TNFα is associated with AS disease activity assessed by ASDAS. These confirm interest of a personal monitoring in artistic patients treated by TNFα blockers. Furthermore studies including interventional approaches need to confirm impact of monitoring in the daily practice.

INTRODUCTION: The management of AS treatment is a big issue due to the small number of effective drugs. Biologic markers of TNFα-blockers seems to be interesting to help us in the therapeutic decision.

RESULTS: We observed:
- Heterogeneity in serum TNFα and infliximab concentration according to AS activity (respectively P < 0.001 and P < 0.02, Figure 2 and 3).
- Significant level of ATI in 4 AS patients, 3 had a very active disease and 1 a moderate activity (P < 0.05, Figure 4). In this population, there is:
  - a negative correlation between infliximab concentration and ATI was also observed (Spearman correlation coefficient = -0.47, P < 0.01; Figure 5).
  - a positive correlation between TNFα concentration and ASDAS (Spearman correlation coefficient = 0.48, P < 0.01; Figure 6).

CONCLUSION: ATI could be usefull to manage AS treatment, but it should be proved in other studies including interventional approaches.
Impact of infliximab dosage and anti-infliximab antibodies in the therapeutic optimization of MICI patients that are non-responders to infliximab therapy

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(1) Saint Etienne; (2) Saint-Priest-en-Jarez

Introduction

Therapeutic failure to infliximab (IFX) is widely described in the literature during MICI. Our current recommendations propose to increase the IFX dosing or to reduce time between infusions before envisaging a therapeutic switch. In a previous study, we had identified a cut-off for trough concentration of IFX associated to the therapeutic response (2µg/ml) and a therapeutic cut-off related to resistance in case of anti-infliximab antibody (ATI) above 200ng/ml. The aim of our work was to appreciate the positioning of these cut-off values in case of therapeutic failure to IFX in order to guide our optimization.

Patients and Methods

MICI patients in therapeutic failure with an IFX dose of 5mg/kg were included in a prospective manner. All patients had to be primary responders to IFX. In case of concomitant immune-suppressor treatment (IS), the medication had to be pursued without any change in the posology. A therapeutic failure was defined by a CDAI >220 for Crohn disease (CD) with a high level of inflammatory marker (CRP or calprotectin) and for chronic ulcerative colitis (CUC) a score from mayo clinic > 7 with a sub score mayo > 1. All patients were tested for TNF, IFX and ATI (Lisa-Tracker, bmd) before infusion to IFX with double dosing (10mg/kg). These results were generated in a blind manner to the clinic and reported to 8 weeks. Patients were examined after 8 weeks.

Results

30 patients were included among which 12 CUC and 18 CD. The mean age of the population was 32 and the sex ratio M/W equal to one. 60% of patients were under IS associated treatment. 15 patients were in clinical remission 8 weeks after therapeutic adaptation. The mean trough concentration of IFX before optimization was significantly higher in patients that did not respond to the optimization (3.5 µg/ml vs 1.6µg/ml; p=0.005). The 4 patients that had ATI concentration above 200ng/ml did not show any response to the dose increase. Among the 15 patients with IFX trough concentration above 2µg/ml, only 4 of them did respond to the dose increase. The 11 patients showing a trough concentration of IFX < 2µg/ml with a titer of ATI < 200ng/ml would show clinical remission at week 8. Our cut-off levels would have prevented an ineffective increase of IFX dose in 50% of the cases but would also have led to inappropriate therapeutic change in 13% of the cases.

Conclusion

The achievement of IFX trough concentration and ATI dosage before therapeutic optimization in case of therapeutic failure within MICI under IFX prove to be very useful. By applying our cut-off previously defined, it has allowed the prognosis of the therapeutic response to the optimization in 87% of the cases.

Translated from French
Interest in Infliximab dosage and antibodies against Infliximab in the therapeutic response under Infliximab therapy in MICI

S. Paul (1), H. Marotte (1), E. Del Tedesco (1), M. Rinaudo-Gaujous (1), C. Jarlot (1), A. Moreau (1), Jm. Phelip (2), C. Genin (1), X. Roblin (2)

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Introduction

Within the family of anti-TNFα antibodies, Infliximab (IFX) is one of the major molecules for the therapeutic care of Crohn disease (CD) and chronic ulcerative colitis (CUC). In the literature, many contradictory studies have been reported describing in some cases an association between the trough concentration of infliximab, free TNFα, anti-infliximab antibodies (ATI) titers and the therapeutic response to the molecule. In this study, we have looked at a correlation between IFX, ATI titers and the therapeutic response during MICI and have tried to identify a predictive value in such case.

Patients and Methods

Longitudinal and monocentric study including all MICI patients under IFX treatment with an interview every 8 weeks. During inclusion and the day of the IFX infusion, a CDAI score for CD and Lichtiger for the CUC was calculated. Before infusion, a blood sample was taken for IFX, ATI and TNFα dosage which was conducted in a blind manner from clinical data (Lisa-Tracker, bmd). A remission was defined for CD with a CDAI <150 and a Lichtiger <3 for CUC.

Results

120 patients were included (mean age: 40 years old, sex ratio M/W: 1.3). 54% of patients had CD and 55% showed a clinical remission. No adverse effect to infusion was reported. Average IFX levels were significantly higher in CD where patients were in remission (2.8 µg/ml vs 0.8µg/ml, p<0.0001) and in CUC (1.9 vs 0.9; p=0.01). The average levels for ATI (ng/ml) were comparable in patients in clinical remission or not whatever the MICI disease classification (p=0.64). An ATI rate above 200ng/ml was strongly associated with a therapeutic failure (p: 0.032, Sensitivity: 15.7%, Specificity: 93.98%). No ATi was ever observed in presence of IFX. The TNFα dosage showed no correlation to clinical response for neither CD nor CUC. A cut-off value for IFX below (<) 2µg/ml was strongly associated to the absence of clinical remission with a sensitivity of 76% and a specificity of 82.3 % (AUROC=0.6).

Conclusion

A strong correlation between circulating IFX level and the clinical remission in MICI either CD or CUC was shown. A cut-off value of 2µg/ml for infliximab appears to be a better discriminator. Only ATI rates above 200ng/ml indicated a therapeutic failure with a strong specificity (94%).

Translated from French
Etanercept monitoring by 3 months allows predicting the therapeutic response at 6 months

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Context
Among patients suffering from Rheumatoid Arthritis (RA), it is not clearly defined whether the response to anti-tumor necrosis factor (TNF)alpha has to be measured between 3 and 6 months according to the recommendations. In daily practice the question is often to continue or not anti-TNF beyond 3 months in case of no response or partial response. Consequently it would be interesting to have bio-markers allowing predicting the interest to continue the treatment in case of incomplete response at 3 months.

Objective
To determine whether TNF alpha, etanercept (ETN) and anti-ETN antibodies concentrations at 3 months are predicting the clinical response to ETN at 6 months among patients suffering from RA. Second objective is to measure the optimal cut-off of these markers for the therapeutic decision.

Patients and Methods
Eighteen women affected by TNF alpha, responding to ACR 1987 standards and for which a treatment by etanercept has been decided, have been included. Mean age was 57 ± 12 years old; median RA duration was 5 (1-38) years, 71% had a positive rheumatoid factor and the DAS28 at the inclusion was of 4.8 ± 1.1. EULAR response was measured at 3 and 6 months. TNF alpha, ETN and anti-ETN antibodies concentrations were measured in the serum, using LISA TRACKER ® Premium etanercept kit (LTE001, Biomedical Diagnostics ®, France), at 3 and 6 months.

Results
Anti-TNF alpha concentrations increased significantly among all patients at 3 and 6 months (p<0,001). Anti-TNF alpha and ETN concentrations were significantly correlated at 6 months (correlation coefficient r = 0.60, p = 0.01). Anti-ETN antibodies have been found in only one patient, at 6 months. Anti-TNF alpha and ETN concentrations at 3 months were significantly lower among the nonresponders at 6 months compared with the responders (103 [0-481] vs 237,5 [146-565]pg/ml, p = 0,005 and 1,75 [0 to 3,6] vs 3,70 [1.0 -6,7] µg / ml, p = 0,03, respectively). In addition TNF alpha and ETN concentrations at 3 months were significantly correlated to the DAS 28 diminution between the inclusion and 6 months (correlation coefficient r = -0,52 p = 0,025 and r = - 0,62, p = 0,006, respectively). The cut-off of 3,1µg / ml for ETN at 3 months was associated to a sensitivity of 87% and to a specificity of 67% to predict the response at 6 months.
Conclusion

TNF alpha and ETN concentrations at 3 months allow predicting the response to the treatment by ETN at 6 months. Low ETN concentrations could explain the non response to the treatment. These results suggest either to adjust doses in some patients or to stop sooner anti-TNF in patients who have low ETN concentrations.

Translated from French
Anti-TNFα monitoring

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Anti-TNFα therapies such as Infliximab, Adalimumab, and Etanercept represent an important progress in therapy for inflammatory diseases such as rheumatoid arthritis, Crohn’s disease, ankylosing spondylitis, etc…However, there remain a proportion of patients who do not recover despite therapy. These drugs are expensive and have the potential of serious toxicity. Therefore, it would be ideal to predict the patients who will respond, so that the use of these drugs can be well targeted.

bmd has developed immunoassays, using ELISA technique, in order to detect TNFα antagonists (ADA, anti-drug antibodies: anti-Infliximab, anti-Adalimumab, anti-Etanercept) before the physical effect are observed.

These quantitative assays have been designed in order to help clinicians to monitor anti-TNFα therapy and predict a failure in the treatment. These fast (<4h) and easy-to-use immunoassays are sensible (ng/mL), specific (interferences not detected) and precise (CV<15%).

As preliminary data, we have found 18 samples that were, ADA « positive » (14 « positive » anti-infliximab and 4 « positive » anti-Adalimumab) out of 115 serum-samples from patients treated with anti-TNFα (Infliximab or Adalimumab). « Positive » samples (ADA+) from Infliximab or Adalimumab treated patients were confirmed « positive » when tested in an inhibition assay. No « positive » anti-Etanercept sample was found.

A prospective study based on more than 300 samples of patients (before and during the anti-TNFα treatment) will highlight the interest of anti-TNFα monitoring as useful information in the evaluation of treatment efficiency. Data will be presented in the poster.

ADA, anti-drug antibodies
Development of immunoassays (ELISA) to detect antibodies directed against anti-TNFα: Infliximab, Adalimumab and Etanercept.

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Introduction: Therapeutic agents like REMICADE®, HUMIRA® and ENBREL® are widely used to cure inflammatory diseases such as rheumatoid arthritis, Crohn’s disease, ankylosing spondylitis, etc. These drugs are able to block TNFα action which is responsible for the inflammatory state. But, during the treatment, patients may develop antibodies directed against the drug (ADA, anti-drug antibodies: anti-Infliximab, anti-Adalimumab, anti-Etanercept). Consequently, the treatment becomes less efficient. Bmd has developed immunoassays, using ELISA technique, in order to detect these antibodies.

<table>
<thead>
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<th>Drug</th>
<th>Active principle</th>
<th>Type</th>
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<tr>
<td>REMICADE®</td>
<td>Infliximab</td>
<td>Chimeric IgG₁</td>
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<tr>
<td>HUMIRA®</td>
<td>Adalimumab</td>
<td>Fully humanized IgG₁</td>
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<tr>
<td>ENBREL®</td>
<td>Etanercept</td>
<td>Fusion protein consisting of 2 extracellular domains of the human p75 TNF receptor, linked to the Fc portion of human IgG₁</td>
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Test principle: We use a “bridging” immunoassay. First of all, the wells of a 96 polystyrene microplate (microplate) are coated with the drug. Then, a blocking solution is added to block all the uncoated surface. Serum samples, diluted into a buffer solution are added to the wells: if ADA are present, they can bind the coated drug. After a wash step, biotinylated-drug (drug-biot) is added: biotinylated drug and the coated drug are linked in a “bridging” assay format with the aid of ADA. After a new wash step, streptavidin (SA) conjugated to peroxidase (HRP) is added. A new wash step is conducted, then, peroxidase « substrate solution » is added to obtain a colored solution. Once the « stop solution » is added, measurement of optical density (OD) can be done on a spectrophotometer. The color intensity (reactivity) is proportional to the amount of ADA detected.

Cut-point determination: A sample is considered “positive” if the OD is above the cut-point. To determine the assay cut-point, we tested 144 serum-samples from healthy individual donors. We also tested a “Pool” sample composed of 10 healthy individual donors. We chose the 95th percentile (7 samples giving an OD above the assay cut-point) to determine the cut-point. OD can drift according to the day of the test, so we calculated a normalization factor (NF) which is defined as: OD from “Pool” x NF = OD cut-point. The NF are 1.37; 1.26 and 1.05 for anti-Infliximab, anti-Adalimumab and anti-Etanercept respectively. The figures below show the value of the 95th percentile (dotted green line) and the “Pool” value, for each immunoassay.
Limit of detection: The limit of detection is defined as the amount of ADA required to obtain an OD equal to the cut-point. Calibration curves were made with monoclonal antibodies directed against Infliximab, Adalimumab and Etanercept. Back-calculating the limit of detection from the curves resulted in a concentration of 9.6 ng/ml, 1.5 ng/ml and 2.8 ng/ml for anti-Infliximab, anti-Adalimumab, and anti-Etanercept respectively.

Test conducted on a population treated with anti-TNFα: 18 samples gave « positive » results (14 « positive » anti-Infliximab and 4 « positive » anti-Adalimumab) out of 115 serum-samples from patients treated with anti-TNF (REMICADE® or HUMIRA®). The 14 « positive » anti-Infliximab samples were confirmed « positive » with a kit already on the market. To confirm our results, we tested the 18 « positive » samples in a second test : inhibition assay. It consists in adding an increasing amount of drug into serum-samples to block ADA reactivity: if ADA are present, the reactivity is inhibited. But if ADA are not present, the level of reactivity is about the same as in the first test. The 18 samples tested showed a drop of reactivity when the drug was added. So, these samples were confirmed « positive » (ADA+). The figure below shows inhibition profiles for one « positive » anti-Infliximab sample (E1), for one « positive » anti-Adalimumab sample (E2), and for a « negative » ADA sample (E3), tested in the first test.

Other performances. Specificity: samples known as interferences agents were tested : rheumatoid factors, cryoglobulins, hypergammaglobulinemia, C1q protein, bilirubin, lipemic aspect, etc. All samples were « negative ». Serum samples from patients suffering from an auto-immune disease (SLE, Sjögren syndrom, CREST, systemic sclerodermy, polymyositis, mixt connectivite, etc.) were also tested. All samples were « negative ».

Precision: 4 samples (anti-Infliximab and anti-Adalimumab) and standards from the calibration curves were tested. Each sample and standard were tested four times in one assay. We performed four assays on four different days. The coefficient of variation (CV) was below 10% intra-assay and below 15% inter-assays.

Rugdeness: Time (+/-15min) and temperature (from 15°C to 30°C) of incubation steps didn’t affect the results.

Stability: reagents stability was not affected by temperature conditions which means the performances should remain stable in time.

Conclusion: Bmd has developed 3 immunoassays to detect antibodies directed against Infliximab, Adalimumab, and Etanercept. These semi-quantitative assays should help clinicians to monitor the state of their patients. These fast (<4h) and easy-to-use immunoassays are sensible (ng/ml), specific (interferences not detected) and precise (CV<15%). « Positive » samples (ADA+) from patients treated with REMICADE® were confirmed « positive » with a kit already on the market. « Positive » samples from patients treated with REMICADE® or HUMIRA® were confirmed « positive » when tested in an inhibition assay from Bmd. No « positive » anti-Etanercept sample was found.

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