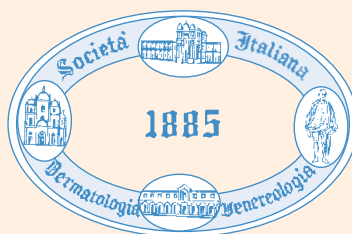


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E D I Z I O N I M I N E R V A M E D I C A

Detection of antibodies to anti-TNF agents in psoriatic patients: a preliminary study

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Aim. The efficacy of anti-TNF α agents in psoriasis (Pso) has been established in different clinical trials and in every day practice. However, a portion of patients (non-responder patients) persists with active disease or relapses even during current biological therapy. In addition to this, the use of biologic agents can give adverse drug reactions. All these factors may be due to the immunogenicity of these new biological drugs. All the biological proteins, including fully human proteins, have the potential to induce immunogenicity which leads to the development of specific antidrug antibodies (ADAs).

Methods. In a retrospective way, we studied ADAs, using ELISA test, in a group of 51 patients with moderate to severe Pso treated with anti TNF α drugs: 18 Etanercept (ETN), 15 infliximab (IFX) and 18 adalimumab (AdA).

Results. Anti-IFX antibodies were positive in 13.3%, anti AdA in 16.6% while all the patients treated with ETN did not present ADAs. Our opinion is that it is important to verify non-responder patients due to neutralizing ADAs so that we can change the drug before patients get worse. Moreover, it is important to detect ADAs since positive patients are more likely to develop acute hypersensitivity reactions. The development of antimono-clonal specific IgGs may also lead to an effector mechanism involving complement activation and production of anaphylotoxins which determine side effects that can be, in some cases, very severe. ADAs can be responsible of the poor response to the drugs and can impact the safety profile of the drugs.

Conclusion. We think that the detection of ADAs will be useful for the management of the drugs and the disease in all patients with psoriasis in treatment with anti biological drugs as a routine clinical practice.

KEY WORDS: Psoriasis - Antibodies - Biological agents.

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Biological therapy, in particular anti-TNF α drugs, has become a common treatment not only in Crohn's disease (CD) and rheumatoid arthritis (RA) but also in psoriasis (Pso) and psoriatic arthritis (PA). The efficacy of anti TNF agents has been established in different clinical trials and in every day practice. The endpoints of the therapy are the reduction of Psoriasis Area and Severity Index (PASI), of body surface area (BSA) or the reduction of Dermatologic Quality of Life Index (DQLI). However, a portion of patients persists with active disease (non-responder patients) or relapses even during current biological therapy. In addition to this, the use of biologic agents can give adverse drug reactions. All these factors may be due to the immunogenicity of these new biological drugs. It is known that many drug-related or patient-related factors contribute to the immunogenicity of biologics. All the biological proteins, including fully human proteins, have the potential to induce immunogenicity which leads to the development of specific antidrug antibodies (ADAs).¹ ADAs have been studied in different diseases such as RA and CD but they have not been detected very often in Pso and PsA patients.² We studied ADAs in a group of patients with Pso treated with anti-TNF α drugs: etanercept (ETN), infliximab (IFX) and adalimumab (AdA).

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Materials and methods

We conducted a retrospective study including a total of 51 patients (36 males and 15 females) aged between 28 and 85 (mean age 55 years) with moderate to severe plaque psoriasis. Patients with PsA, even in treatment with anti-TNF α drugs were excluded. Patients with Pso, eligible to a treatment with biologic drugs, having an active psoriasis with a PASI>10, or BSA>10% or DLQI>10, non responders or intolerant to at least 2 systemic, conventional drugs namely acitretin, methotrexate (MTX) and cyclosporine A, were included. Concomitant use of systemic drugs in particular MTX was not permitted. Only patients who had been using the same biological drug for 24 weeks were included in this study. Eighteen patients received ETN 50 mg BIW for 12 weeks and 50 mg OIW for at least 12 weeks; 18 received AdA 80 mg and 40 mg week 1 and 2 and then 40 mg every other week for at least 24 weeks; 15 patients received IFX 5 mg/kg/day week 0, 2 and 6 and then every 8 weeks at least for 24 weeks. ADAs were studied using ELISA test (Immun Diagnostik AG, Stubenwald-Allee 8a D-64625 Bensheim, Germany). All patients sera were collected before the next infusion or injection. The detection of ADAs was carried out according to the following steps: in a first incubation step, the free anti therapeutic antibodies from the sample were bounded to drugs (AdA or ETN or IFX) coated on the plate. To remove all unbound substances, a washing step was carried out. In a further incubation step, Peroxidase-labeled therapy antibody was added. After another washing step, to remove all unbound substances, the solid phase was incubated with a substrate (tetramethylbenzidine). An acid stop solution was then added. After the color converted to yellow, the absorbance of the color compound was determined photo-metrically at 450 nm. The intensity of the color is directly proportional to the amount of bound ADAs from the sample. The results were evaluated by a cut off value as specified by the kit (1 for AdA ELISA; 1,2 for ETN ELISA; 1,2 for IFX ELISA).

Results

Anti AdA antibodies were positive in 3 out of 18 patients (16.6%), anti-IFX were positive in 2 out of 15 patients (13.3%), while all the patients treated

TABLE I.—*Relationship between ADAs and clinical responses.*

Patients	Therapy	ADAs	Clinical response
1	AdA	NEG	Reduction PASI 75%
2	AdA	POS	Reduction PASI 75%
3	AdA	NEG	Reduction PASI 75%
4	AdA	NEG	Worsening
5	AdA	POS	Unchanged
6	AdA	NEG	Reduction PASI 75%
7	AdA	NEG	Unchanged
8	AdA	NEG	Worsening
9	AdA	NEG	Worsening
10	AdA	NEG	Reduction PASI 75%
11	AdA	NEG	Reduction PASI 75%
12	AdA	NEG	Reduction PASI 75%
13	AdA	NEG	Reduction PASI 75%
14	AdA	NEG	Reduction PASI 75%
15	AdA	NEG	Reduction PASI 75%
16	AdA	NEG	Worsening
17	AdA	POS	Reduction PASI 75%
18	AdA	NEG	Reduction PASI 75%
19	ETN	NEG	Reduction PASI 75%
20	ETN	NEG	Reduction PASI 75%
21	ETN	NEG	Reduction PASI 75%
22	ETN	NEG	Worsening
23	ETN	NEG	Unchanged
24	ETN	NEG	Reduction PASI 75%
25	ETN	NEG	Reduction PASI 75%
26	ETN	NEG	Reduction PASI 75%
27	ETN	NEG	Worsening
28	ETN	NEG	Reduction PASI 75%
29	ETN	NEG	Reduction PASI 75%
30	ETN	NEG	Reduction PASI 75%
31	ETN	NEG	Reduction PASI 75%
32	ETN	NEG	Worsening
33	ETN	NEG	Worsening
34	ETN	NEG	Reduction PASI 75%
35	ETN	NEG	Worsening
36	ETN	NEG	Reduction PASI 75%
37	IFX	NEG	Reduction PASI 75%
38	IFX	NEG	Reduction PASI 75%
39	IFX	NEG	Worsening
40	IFX	NEG	Reduction PASI 75%
41	IFX	NEG	Reduction PASI 75%
42	IFX	POS	Reduction PASI 75%
43	IFX	NEG	Reduction PASI 75%
44	IFX	NEG	Reduction PASI 75%
45	IFX	NEG	Reduction PASI 75%
46	IFX	NEG	Unchanged
47	IFX	NEG	Unchanged
48	IFX	NEG	Worsening
49	IFX	POS	Reduction PASI 75%
50	IFX	NEG	Reduction PASI 75%
51	IFX	NEG	Unchanged

ADAs: specific antidrug antibodies; AdA: Adalimumab; ETN: Etanercept; IFX: infliximab; PASI: Psoriasis Area and Severity Index

with ETN did not present ADAs Concerning the clinical responses at the time of the detection of ADAs, the patients positive for anti AdA antibodies 3 had a reduction of 75% of the PASI while 1 remained clinically unchanged. As regards the 2 ADAs-positive patients treated with IFX both had a reduction of 75% of the PASI. The results are summarized in Table I.

Discussion and conclusions

IFX and AdA are monoclonal antibodies (mAbs) while ETN is a soluble receptor for TNF. IFX is a chimeric mAb made from a variable region of a murine source and a constant region of a human immunoglobulin. For its specific conformation it is thought to have the potential to induce immunogenicity leading to the development of specific ADAs. However, the degree of immunization can also be determined by glycosylation or by other structural modifications of the drug as well as other non immunogenic factors that may influence immunogenicity.³ In our cases, ADAs were present in about 13% of IFX patients, in accordance with the literature. In fact, anti-IFX antibodies vary from 12 to 44% in RA and from 6 to 61% in patients with Crohn's disease.⁴⁻⁶ In an Italian series of patients with different immune-mediated diseases, the incidence of anti-IFX antibodies was about 14%.⁷ In our psoriatic patients treated with AdA the percentage of ADAs was slightly higher, 16.6%. AdA is a fully human antibody but it also has been described to induce marked immune-response. In fact, AdA has been described to induce ADAs in 2.8-3.7% of patients with Crohn's disease and up to 70% of RA patients.^{1, 3} This can be explained by the fact that human therapeutic monoclonal autoantibodies determine the production of anti idiotypic antibodies. A fusion protein such as ETN can also induce an antibody response in 0-3% of patients.⁸ In our study, no ETN patients was positive for ADAs. A higher percentage of anti-ETN antibodies (18%) was found by Tying *et al.*⁹ However, patients with Pso or PsA had non neutralizing ADAs. In fact, their presence had no apparent impact on the safety and activity profile of ETN.⁹ However, some of the patients positive for ADAs did not have a reduced activity of the drugs. On the contrary, patients without ADAs can be non-responders to one

or another drug. In our study, the immunogenicity of anti-TNF α drugs is similar to the cases described in literature, particularly in Crohn's disease.³ RA patients present a higher percentage of ADAs that seems to depend on the disease itself. Rheumatoid factor (RF) and other non specific antibodies are produced in RA, this could mean that RA may be prone to immunogenicity.¹⁰ For this reason we have decided to select only patients with Pso. In fact, in Pso patients usually there is no production of autoantibodies of any type. In our work ADAs antibodies were detected using ELISA test which is said less specific than radioimmunoassay (RIA).¹¹ However RIA is more sensitive and specific than ELISA, its main drawback is its technical complexity due to the use of radioisotopes.¹² Is it really important to detect ADAs? We think that it is important to verify non-responder patients due to neutralizing ADAs so that we can change the drug before patients get worse. In this work we did not detect the plasma concentration of drugs which would have been low since we collected sera before the next infusion (IFX) or injections (AdA and ETN), particularly in patients with ADA positivity. Moreover, it is important to detect ADAs since positive patients are more likely to develop acute hypersensitivity reactions.⁷ The development of anti monoclonal specific IgGs may also lead to an effector mechanism involving complement activation and production of anaphylotoxins which determine side effects that can be, in some cases, very severe. In addition, although ADAs are mostly represented by IgG isotype, a proportion of antibodies may belong to IgE isotype. This retrospective small study wants to underline that Pso patients can develop ADAs. ADAs can be responsible of the poor response to the drugs and can impact the safety profile of the drugs in particular we detected ADAs of IgG isotype. Patients with positive ADAs should not be exposed to an intermittent therapy because it could increase the risk of reactions.⁷ However, we think that further studies should be carried out to evaluate: 1) if ADAs are neutralizing or not; 2) if non-response is correlated to a low plasma concentration of drug; 3) if drugs are stable; 4) if and for how long they herald a relapse of the disease; 5) if the plasmatic concentration of ADAs can decrease with the use of MTX as described in the literature. In accordance with Vincent *et al.*,¹⁰ we think that the detection

of ADAs would be useful for the management of the drugs and the disease, and an algorithm defining the presence of ADAs antibodies and identifying the therapeutic range of concentration for each anti-TNF agent should be suggested in all patients with psoriasis in treatment with anti biological drugs as a routine clinical practice.

Riassunto

Rilevamento di anticorpi agli agenti anti-TNF in pazienti psoriasici: studio preliminare

L'efficacia delle molecole anti-TNF α è stata riconosciuta in diversi studi clinici e nella pratica medica quotidiana. Tuttavia in una certa percentuale di pazienti la malattia rimane attiva (pazienti non responsivi) o presenta esacerbazioni anche nel corso di terapia biologica. Inoltre l'utilizzo di farmaci biologici può indurre reazioni avverse dovute alla immunogenicità di questi nuovi farmaci. Tutte le molecole biologiche, incluse quelle umane, sono in grado di indurre una immunogenicità che porta allo sviluppo di anticorpi specifici anti farmaco. In uno studio retrospettivo, abbiamo valutato mediante metodica ELISA, un gruppo di 51 pazienti affetti da psoriasi moderata lieve trattata con farmaci anti TNF α : 18 Etanercept (ETN), 15 Infliximab (IFX) e 18 Adalimumab (AdA). Gli anticorpi anti IFX erano positivi nel 13,3% dei pazienti in terapia con IFX, anti AdA nel 16,6% nei pazienti in terapia con AdA mentre nessuno dei pazienti trattati con ETN presentava anticorpi anti farmaco. Noi pensiamo che sia importante ricercare gli anticorpi specifici anti farmaco in maniera da poter sostituire il farmaco prima che questo perda di efficacia. Inoltre la determinazione di questi anticorpi è importante in quanto i pazienti con positività per gli anticorpi sembrano sviluppare maggiormente reazioni acute da ipersensibilità. Le IgGs monoclonali potrebbero indurre un meccanismo effettore, attivando il complemento e la produzione di anafilattossine, in grado di determinare effetti avversi anche gravi. Gli anticorpi specifici anti farmaco, essendo responsabili della ridotta risposta terapeutica e interagendo sul profilo di sicurezza del farmaco, devono essere ricercati nella pratica clinica quotidiana in tutti i pazienti con psoriasi in terapia con farmaci biologici.

PAROLE CHIAVE: Psoriasi - Anticorpi - Agenti biologici.

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Conflicts of interest.—The authors declare no conflicts of interest.

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