Journal of Crohn's and Colitis (2013) 7, e486–e492

Available online at www.sciencedirect.com

ScienceDirect

Tuberculosis in anti-TNF-α treated patients remains a problem in countries with an intermediate incidence: Analysis of 25 patients matched with a control population

Cândida Abreu [a,b,1], Fernando Magro [c,d,e,*,1], João Santos-Antunes [c,f], Artur Pilão [g], Eduardo Rodrigues-Pinto [c], José Bernardes [h], Alexandra Bernardo [h], Sofia Magina [d,i], Filipe Vilas-Boas [c], Susana Lopes [c], Guilherme Macedo [c], António Sarmento [a,b]

[a] Department of Infectious Diseases, Faculty of Medicine, Centro Hospitalar S. João, Porto, Portugal
[b] Nephrology Research and Development Unit, University of Porto, Portugal
[c] Gastroenterology Department, Faculty of Medicine, Centro Hospitalar S. João, Porto, Portugal
[d] Institute of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, Portugal
[e] IBMC, Institute for Molecular and Cell Biology, University of Porto, Portugal
[f] Department of Biochemistry (U38-FCT), Faculty of Medicine, University of Porto, Portugal
[g] Center of Pulmonary Diseases, National Health System, Porto, Portugal
[h] Rheumatology Department, Faculty of Medicine, Centro Hospitalar S. João, Porto, Portugal
[i] Dermatology Department, Faculty of Medicine, Centro Hospitalar S. João, Porto, Portugal

Received 7 February 2013; received in revised form 5 March 2013; accepted 9 March 2013

KEYWORDS
Anti-TNF-α therapy;
Tuberculosis;
Latent tuberculosis screening

Abstract

Background and aims: An increased incidence of tuberculosis (TB) in patients under anti-TNF-α therapy has been reported, but outcome compared with TB in the general population are unknown.
Methods: Patients who had active tuberculosis while taking anti-TNF-α drugs were studied and compared with a control group of community-acquired TB matched for sex, age and data of TB.
Results: Twenty-five cases of TB were reported from a cohort of 765 patients under anti-TNF-α from 2001 to 2012. The incidence of TB per 100,000 patient-years was estimated to be 1337, 792 and 405 respectively for those on infliximab, adalimumab and etanercept. Twelve patients had inflammatory bowel disease, ten had rheumatologic diseases and three had psoriasis. From the 17 patients screened for latent TB before anti-TNF-α, three were treated with isoniazid. TB was diagnosed 1–108 months after starting anti-TNF-α, being the median time six, seven and 89 months respectively for those on infliximab, adalimumab and etanercept. Sixty per

* Corresponding author at: Institute of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, Al. Prof. HernâniMonteiro 4200 319 Porto, Portugal. Tel.: +351 22 551 3600; fax: +351 22 551 3601.
E-mail address: fm@med.up.pt (F. Magro).
[1] These authors contributed equally in the design, conception, analysis, and paper writing.

1873-9946/$ - see front matter
http://dx.doi.org/10.1016/j.crohns.2013.03.004

cent of the cases had extra-pulmonary TB. No deaths occurred in the case groups, while two died in control TB patients. Patients on anti-TNF-α drugs had more frequent extra-pulmonary TB, fever on presentation, higher mean C-reactive protein and lower positive rate of acid-fast bacilli.

Conclusions: TB may still occur in those with negative testing, some of them probably representing new infections instead of reactivations. Three out of 25 patients had TB in spite of previously treated LTB, although, the outcome of TB was not worse than in the general population.

1. Introduction

Tumor necrosis factor alpha (TNF-α) is essential for granuloma formation and maintenance, and plays an important role in host defense against diseases caused by intracellular pathogens like *Mycobacterium tuberculosis*, *Histoplasma capsulatum* and *Listeria monocytogenes*. The increased clinical use of TNF-α antagonists dramatically improved the management of immunomediated diseases, but has led to a higher incidence of infections with intracellular agents.[1–8] Keane et al. were the first to describe an increased incidence of tuberculosis (TB) in patients with rheumatoid arthritis (RA) treated with TNF-α blockers,[9] and a relatively large proportion of extra-pulmonary and disseminated forms has been diagnosed, despite the previous latent TB screening and treatment.[10,11]

There is no gold standard method to define latent TB, and its treatment before anti-TNF-α drugs institution may not be sufficient to protect from the disease. The clustering of TB reports, shortly after initiation of treatment with infliximab, is consistent with reactivation of latent infection, although in some cases, it occurs later and may represent new infections.

Portugal has an intermediate incidence of TB[12] (22 cases/100,000 habitants, data from 2009) when compared to the rest of Western Europe; therefore, in patients under anti-TNF-α, the risk of TB infection is expected to be higher.

We report 25 cases of active TB in adults under anti-TNF-α drugs diagnosed in our center. The aim was to determine the features and outcome of TB in patients under anti-TNF-α drugs and compare them to a control population in a retrospective 1:3 matched case–control study.

2. Materials and methods

2.1. Case groups

Medical files of adult patients taking anti-TNF-α agents (namely infliximab, adalimumab, etanercept and golimumab) between January 2001 and August 2012 were retrospectively reviewed, and those who developed active TB while on treatment with these drugs were selected. Data was collected from the Gastroenterology, Infectious Diseases, Rheumatology and Dermatology departments of Centro Hospitalar São João in Porto, Portugal. All patients had been vaccinated with BCG vaccine at birth. Diagnosis of latent TB was based on tuberculin skin test (TST), performed according to standard rules of Mantoux method, and postero-anterior chest X-ray. A positive TST was considered when reached 5 mm or more of induration. This cutoff is recommended by the National Rheumatology and Gastroenterology Societies.[13] The aim is to achieve a very high sensitivity, even with a decrease in the specificity, in patients frequently under other immune suppressive therapies, namely steroids.

Whenever possible, TST was repeated one to two weeks after the first one if it yielded a negative result, to evaluate the booster effect, and considered positive if 5 mm or more of induration. A positive TST or chest radiograph consistent with prior TB was an indication for chemoprophylaxis with isoniazid 300 mg/day for nine months and anti-TNF-α was started at least four weeks after beginning isoniazid.

2.2. Control group

In order to obtain a ratio of three controls for each case, a group of outpatients with a diagnosis of active TB and without other significant co-morbidities (namely inflammatory diseases or neoplastic conditions) or immunosuppressive treatment were randomly collected from the general population, matched for age, sex and year of TB diagnosis. They were treated in a National Health System outpatient clinic, responsible for the management of TB in our city. Clinical presentation, microbiological data, therapy and outcome of the two groups were compared.

2.3. Diagnosis and clinical characterization of TB

TB was diagnosed by clinical, radiologic and microbiological findings. Mycobacteriological procedures and drug susceptibility tests were made according to standards.[14] The demographic and clinical characteristics of the patients such as age, sex, contact with TB infected cases, history of past TB treatment, type of TB (pulmonary versus extra-pulmonary or disseminated forms when two or more extra-pulmonary organs were involved) were recorded. From the 25 patients on anti-TNF-α drugs, type and duration of primary disease and concomitant immunosuppressive treatment were reviewed.

2.4. Statistical analysis

Categorical variables were described as absolute (n) and relative frequencies (%), and median was used for continuous variables when they were not normally distributed. Continuous variables were analyzed with Student's t-test or Mann–Whitney nonparametric tests according to their distribution. When testing a hypothesis about categorical variables, a chi-square test or Fisher's exact test was used, as appropriate. The significance level used was 0.05. Statistical analysis

was performed using the software Statistical Package for the Social Sciences (SPSS) v. 20.0.

In order to calculate the incidence of TB for 100,000 patients (expressed in patient-years), we divided the number of patients under a drug who developed tuberculosis for the sum of months of all patients exposed to that drug, multiplying the final for 12 (to obtain the incidence per year) and 100,000.

3. Results

3.1. Case groups

From a population of 765 persons with inflammatory diseases under anti-TNF-α, twenty-five Caucasian patients were diagnosed with active TB: 12 with inflammatory bowel disease, 10 with rheumatologic conditions (six with RA and four with ankylosing spondylitis) and three with psoriasis (two of them with arthritis). Sixteen (64%) patients were on infliximab, six (24%) on adalimumab and three on etanercept. In this cohort, the incidence of TB was 1337 for patients treated with infliximab, 792 for patients treated with adalimumab and 405 per 100,000 patient-years for patients treated with etanercept. Seventeen (68%) of them were male and of the eight females, six had rheumatologic diseases (one had concomitant Crohn's disease). The mean age at TB diagnosis was 48 ± 14 years.

Sixteen (64%) patients were on combined immunosuppressive therapy. Seven patients were on azathioprine, four on methotrexate plus steroids, four on steroids and one on rituximab plus steroids.

Besides anti-TNF-α and the other immunosuppressive therapies, no other risk factors for TB were identified, namely active TB infection among relatives. Diagnosis of latent TB was elicited in 17 (68%) patients, and in six this diagnosis was made before national guidelines regarding latent TB screening on patients under anti-TNF-α. From those 17 patients tested for latent TB, 13 had negative tuberculin test (two of them boosted tuberculin) and negative pulmonary X-ray, nine of them on immunosuppressive therapy: seven on azathioprine and two under steroids in low doses (in one associated to methotrexate). One patient had a positive tuberculin test (10 mm induration) but a negative IGRA test, and latent TB treatment was also not done; disseminated TB developed 21 months after the beginning of IFX. In the remaining three patients, the Mantoux test was positive and they were prescribed isoniazid for nine months; in this group the diagnosis of active TB was made 8, 12 and 24 months after isoniazid treatment.

TB was diagnosed 1.5 to 108 months after starting anti-TNF-α drug (median 28 ± 34 months); for those on infliximab the median time was six months, for patients on adalimumab was seven months and for those on etanercept was 89 months. Pulmonary TB was diagnosed in ten (40%) patients and extra-pulmonary TB in 15 (60%), being nine of them disseminated forms (Table 1). Extra-pulmonary forms of TB were more common from 2001 to 2006, the first years of treatment: among these five cases of TB, all were extra-pulmonary forms, being four of them disseminated TB. When analyzed by age, gender, inflammatory disease and anti-TNF-α agents, no significant risk factors for extra-pulmonary TB were identifiable. Regarding clinical presentation (fever and other constitutional symptoms) and serum biomarkers no differences were found between pulmonary and extra-pulmonary forms.

3.2. TB diagnosis

Considering the case groups, TB diagnosis was supported by mycobacteriological or histological results in 23 (92%) patients; in 21 (84%) patients the microbiology workup was positive (Table 2). *M. tuberculosis* was isolated in 20 patients, at least in one sample. One of them had genetic resistance to rifampicin, not detected in in vitro sensibility tests; another respiratory sample had in vitro resistance to isoniazid and streptomycin. Ziehl–Neelsen was positive in 11 (44%) patients. Ten of the 11 histological exams were positive for TB: three at lymph nodes, three at bronchial biopsy, and one each at hepatic biopsy, pleura, sciatic nerve and synovial tissue from the wrist. One patient had epithelioid granulomas on sciatic nerve (histological exam) and another one had necrotizing granuloma on lung tissue.

3.3. Treatment (case groups)

All patients but two started therapy with four drugs (isoniazid, rifampicin, ethambutol and pyrazinamide); the remaining were kept on isoniazid, rifampicin and ethambutol. Four of the 23 patients on quadruple therapy were sequentially treated with aminoglycosides and levofloxacin: two due to hepatic toxicity to isoniazid and rifampicin, one due to detection of genetic resistance (though not detected on posterior in vitro susceptibility test) and one due to in vitro resistance to isoniazid. Otherwise, treatment was well tolerated on clinical and analytical follow-up.

3.4. Outcome

Direct and cultural exams became negative except for the patient with genetic resistance to rifampicin that still had positive smears seventy days after starting therapy and positive cultures for *M. tuberculosis* after the second month of therapy. Four patients are still on TB treatment, and the others were successfully treated from six to 24 months (the last case in a patient with cerebral tuberculoma).

During TB treatment and after becoming asymptomatic three patients developed symptoms of immune reconstitution inflammatory syndrome (IRIS): in one of them the same therapeutic schedule was maintained, with gradual improvement; in the other two steroids were prescribed (prednisolone 1 mg/kg/day) and resolution was reached on the 10th day; the third patient also became febrile and prostrated, one month after starting TB therapy but he gradually improved without other intervention.

Sequela was elicited in three patients: lung cavitated lesions persisted in two patients after therapy and the patient with sciatic nerve TB remains with motor disability of the left leg. Two died for non-related conditions, several years after TB diagnosis. Anti-TNF-α (adalimumab and infliximab) was resumed in four. In two patients with Crohn's disease infliximab was resumed after tuberculosis treatment with a follow-up of 5 and 12 months without intercurrences. One patient on etanercept resumed anti-TNF-α therapy five

Table 1 Extra-pulmonary tuberculosis: characteristics at tuberculosis diagnosis.

Age	Gender	IMD	Anti-TNF	Months with anti-TNF	Other IS	Ziehl–Neelsen/ Lowenstein	Histology	Tuberculosis form	Fever	Constitutional syndrome	Screening latent TB	Outcome
81	M	CD	IFX	1.5	Steroids	Urine; liver/ blood	Yes/hepatic	Disseminated	Yes	No	No	Favorable
47	F	CD + AS	IFX	5	Steroids	LN/SP	No	Disseminated	Yes	No	No	Neurologic sequels
57	M	CD	ADA	2	–	LN/LN, SP, urine, GF, BL, blood	No	Disseminated	Yes	No	Yes/negative	IRIS; favorable
48	M	CD	IFX	1.5	AZA	SP, LN/SP, LN	Yes/LN	Lymphatic + pulmonary	Yes	Yes	Yes/negative	Favorable
32	M	CD	ADA	6	AZA	–/SP, GF	Yes/pleura	Disseminated	Yes	No	Yes/negative	IRIS; still in treatment
50	M	CD	IFX	1.5	AZA + steroids	–/urine, GF	Yes/wrist	Disseminated	No	No	Yes/negative	Favorable
47	M	CD	IFX	20	–	SP, feces/SP	–	Pulmonary, intestinal	Yes	Yes	Yes/positive IST negative IGRA; not treated	Still in treatment
21	F	CD	IFX	8	AZA	–/BL	Yes	Disseminated	Yes	Yes	Yes	Still in treatment
63	M	PS + AS	IFX	1.5	–	LN/urine	Yes/LN	Disseminated	Yes	Yes	Yes/negative	Favorable
40	M	PS	ADA	2	–	BL/GF, BL	Yes/bronchial mucosa	Disseminated	Yes	Yes	Yes/negative	Favorable
47	F	RA	ET	89	Steroids	–	Yes/sciatic nerve	Sciatic nerve	No	Yes	Yes/negative	Motor sequela
53	F	RA	ADA	8	–	–/BL, urine, GF	No	Renal + pulmonary	Yes	Yes	Yes/negative	Favorable
64	M	RA	IFX	43	MTX + steroids	–/pleural fluid	No	Pericardic	Yes	Yes	No	Still in treatment
45	M	AS	ADA	31	–	SP/SP, feces	No	Disseminated	Yes	Yes	Yes/positive	Favorable
25	M	AS	IFX	3	–	–/GF	Yes/LN	Disseminated	Yes	Yes	No	Favorable

IMD — immunomodulatory disease; IS — immunosuppressors. LN — lymph node; GF — gastric fluid; BL — bronchial lavage; SP — sputum. IFX — infliximab; ADA — adalimumab; ET — etanercept; AZA — azathioprine. TB — tuberculosis.

Table 2 Comparison of tuberculosis characteristics between cases and controls.

		Cases (n = 25) n (%)	Controls (n = 73) n (%)	p
Clinical	Pulmonary	10 (40)	55 (75)	0.001
	Extra-pulmonary	15 (60)	18 (25)	
	Disseminated	9	0	
	Fever	19 (76)	35 (48)	0.015
	Constitutional syndrome	17 (68)	51 (70)	0.862
Laboratory	Mean C-reactive protein (mg/L)	77	29	0.007
	Positive direct exam (Ziehl–Neelsen)	11 (44)	47 (64)	0.027
	Positive culture (Lowenstein-J.)	17 (68)	53 (73)	0.605
	Positivity on hystological exam	10 (40)	18 (25)	0.099
Treatment	Four drugs (1st line)	21 (84)	66 (90)	1.000
	Three drugs	2 (8)	7 (10)	
	Others	2 (8)	0	
	On treatment	4 (16)	13 (18)	
Outcome	Patients with completed treatment	21 (84) [a]	60 (82)	0.122
	With sequelae	3 (12)	22 (30)	
	Dead	0	2 (3)	

[a] Two patients died after treatment from conditions not associated with tuberculosis.

months after tuberculosis treatment and is well at the 11th month after TNF-α; another one, with psoriasis, started adalimumab 22 months after tuberculosis treatment and is well with a follow-up of 23 months (all data from February 2013).

One secondary case of TB was reported in a child of a patient with a pulmonary form.

3.5. Comparison between patients and controls

Seventy-three outpatients from the general population with active TB and without other significant co-morbidities were selected for comparison (Table 2). Patients on anti-TNF-α had more extra-pulmonary TB and no disseminated TB cases were reported among the control group. Fever was more common in immunosuppressed patients but the presence of other symptoms (constitutional syndrome) was identical in both groups. Concerning microbiological diagnosis, the rate of *M. tuberculosis* in culture and DNA was similar, but acid-fast bacilli were more identified in the control group.

Treatment approaches were similar, with most patients being treated with standard quadruple regimen. The mortality rate in controls was 3% (2/73). Two patients died of disease progression on the second month of therapy for pulmonary TB. Two secondary cases of pulmonary TB were identified in two close contacts of control patients with pulmonary TB.

4. Discussion

Herein, we report 25 active TB cases associated with anti-TNF-α drugs (infliximab, adalimumab and etanercept) in patients with autoimmune inflammatory diseases, namely inflammatory bowel disease, RA, ankylosing spondylitis from 2001 to 2012, followed at a Portuguese center. We stress a good clinical quality of our records because all patients on anti-TNF-α have been prospectively captured in a hospital database. The incidence of TB in this cohort of 765 patients was the highest of any other study published before. Juan et al. published in 2003 from a cohort of 5.198 rheumatological patients on anti-TNF-α therapy 17 cases of active TB, depicting a TB rate of 172 per 100,000 patient-years.[15]

In this study, as has been published in the literature, the anti-TNF-α that is more frequently associated with TB was infliximab (64% of cases). This increased risk of TB after infliximab therapy was initially noticed in 2001, using post-marketing surveillance data from the FDA Adverse Events Reporting System (AERS).[16] For AERS, the median monthly reactivation rate of latent TB for infliximab was estimated to be twelve times that of etanercept ($p < 0.001$), probably due to a more and prolonged TNF-α block. Furthermore, TB was also reported at an earlier stage after infliximab than etanercept.[1] Two from the three patients with TB under etanercept had RA. Several studies showed a two to ten fold increase in TB risk among RA patients naive to anti-TNF-α drugs compared to the background population.[17–19] So, a higher incidence of tuberculosis among RA patients may contribute to these incidences, in patients under etanercept. Countries with intermediate and high incidences of TB must be aware of latent TB diagnosis and reinfection. Latent TB infection screening before anti-TNF-α drugs is considered essential given the risk for progression to active TB and increased susceptibility to more severe forms of TB.[20] There is no gold-standard test for latent TB infection. TST and the newer IGRA tests, QuantiFERON-TB ® Gold in Tube (QTF-G-IT), and T-SPOT ® TB have false negative and false positive results, especially in immunosuppressed patients.[21] For instance, one of our patients was QTF-G-IT negative and TST positive and developed clinical TB under anti-TNF-α. For a more accurate diagnosis of latent TB, an IGRA test plus boosted tuberculin is probably the best strategy. We can argue that all patients negative for one latent TB test, namely TST or IGRA test, should also be tested sequentially for the other, and only

those with both tests negative, may be considered negative for latent TB.

In addition, the intermediate incidence of TB in Portugal raises the possibility of new infections. Indeed, seven (28%) cases of TB in this cohort were diagnosed more than two years after beginning anti-TNF-α therapy: three of them had had a negative TB screening before starting anti-TNF-α, and two had had clinical TB treated five and ten years before. As treatment of latent TB infection has been shown to be effective in reducing the risk of developing active disease,[22] in our cohort three patients developed TB eight months, one and two years after isoniazid, stressing again the hypothesis that they may represent new TB infection.

The Portuguese national statistics reported a male/female ratio for TB of 1.9/1.[12] However, in our twelve patients with inflammatory bowel disease, only three were female with a male/female ratio of 4/1. TB was more common in patients under two or more immunosuppressors, namely steroids, thiopurines or methotrexate.[23] TB diagnosis was supported by microbacteriological or histological results in 92% of patients and in 90% of controls. In both populations *M. tuberculosis* culture was more frequently positive than smears, and acid-fast bacilli were best identified in the control group than in anti-TNF-α patients. One possible explanation may be the more frequent pulmonary TB, being sputum smears for mycobacteria more frequently positive and easier to perform. Whenever done, histological exams were positive in all patients but one, in the patient group. Two patients had concomitant *Mycobacterium avium–intracellulare* and *Mycobacterium gordonae* isolation, raising the possibility that non-TB mycobacteria may be prevalent in patients under anti-TNF-α drugs.[24]

We found a higher incidence of extra-pulmonary disease (60%) in anti-TNF-α treated patients than in control group (28%)[2,24] (national Portuguese TB data report a percentage of 33%).[12] Furthermore, three cases experienced symptoms suggestive of IRIS. The occurrence of IRIS in patients who developed TB during treatment with-TNF-α antagonists has previously been reported.[25] In all our cases, anti-TNF-α treatment was stopped when TB was diagnosed. Anti-TNF-α drug withdrawal may precipitate a paradoxal response in patients for whom therapy is discontinued after a TB diagnosis.[26] Clinical experience with adjunctive TNF-α blockade in TB is limited,[27] and prednisone can be effective, however the necessary dose and duration of treatment remain unclear.[28]

TB in patients on anti-TNF-α therapy remains a huge concern in our country. The TB incidence in this cohort is so high that studies including more patients and for longer periods are needed. TB surveillance seems important during anti-TNF-α therapy because even with TB screening before anti-TNF-α institution and latent TB treatment whenever appropriate, active disease can occur, and it remains to be known if IGRA would improve latent TB diagnosis accuracy. Microbiologic and histological exams yield positive results in more than 90% of the cases and extra-pulmonary TB was more common than in the general population, although, the outcome was not worse. Despite of more severe forms of tuberculosis and much more frequent extra-pulmonary involvement when compared with the general population, no deaths were elicited. This is in contrast with the first report[26]; however, they included patients between 1998 and 2001[29], and nowadays we are particularly aware of tuberculosis risk in patients under anti-TNF-α.

References

1. Tubach F, Salmon D, Ravaud P, Allanore Y, Goupille P, Bréban M, et al. Risk of tuberculosis is higher with anti-tumor necrosis factor monoclonal antibody therapy than with soluble tumor necrosis factor receptor therapy: the three-year prospective French Research Axed on Tolerance of Biotherapies registry. *Arthritis Rheum* 2009;**60**:1884–18894.
2. US Food and Drug administration. Manufacturers of TNF blocker drugs must highlight risk of fungal infection. Silver Spring, Maryland: U.S. F.D.A.; 2008 [. http://www.fda.gov/bbs/topics/NEWS/2008/NEWS01879.html, accessed in October 25th 2012].
3. Bongartz T, Sutton AJ, Sweeting MJ, Buchan I, Matteson EL, Montori V. Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA* 2006;**295**:2275–85.
4. Dixon WG, Watson K, Lunt M, Hyrich KL, Silman AJ, Symmons DP. Rates of serious infection, including site-specific and bacterial intracellular infection, in rheumatoid arthritis patients receiving anti-tumor necrosis factor therapy: results from the British Society for Rheumatology Biologics Register. *Arthritis Rheum* 2006;**54**:2368–76.
5. Kaur N, Mahl TC. *Pneumocystis jiroveci* (*carinii*) pneumonia after infliximab therapy: a review of 84 cases. *Dig Dis Sci* 2007;**52**: 1481–4.
6. Netea MG, Radstake T, Joosten LA, van der Meer JWM, Kullberg BJ. Salmonella septicemia in rheumatoid arthritis patients receiving anti-tumor necrosis factor therapy: association with decreased interferon-gamma production and Toll-like receptor 4 expression. *Arthritis Rheum* 2003;**48**:1853–7.
7. Abreu C, Magro F, Vilas-Boas F, Lopes S, Macedo G, Sarmento A. *Listeria* infection in patients on anti-TNF treatment: report of two cases and review of the literature. *J Crohns Colitis* May 22 2012 [Epub ahead of print].
8. Ellerin T, Rubin RH, Weinblatt ME. Infections and anti tumor necrosis factor alpha therapy. *Arthritis Rheum* 2003;**48**:3013–22.
9. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001;**345**:1098–104.
10. Wallis RS, Broder MS, Wong JY, Hanson ME, Beenhouwer DO. Granulomatous infectious diseases associated with tumor necrosis factor antagonists. *Clin Infect Dis* 2004;**38**:1261–5.
11. Nacci F, Matucci-Cerinic M. Tuberculosis and other infections in the anti-tumour necrosis factor-alpha (anti-TNF-α) era. *Best Pract Res Clin Rheumatol* 2011;**25**:375–88.
12. Fonseca Antunes A. Relatorio para o dia Mundial da Tuberculose. Direcção Geral de Saúde; Março 2011 [In Portuguese].
13. Fonseca JE, Lucas H, Canhão H, Duarte R, Santos MJ, Villar M, et al. Recomendações para diagnóstico e tratamento da tuberculose latente e activa nas doenças inflamatórias articulares candidatas a tratamento com fármacos inibidores do factor de necrose alfa. *Acta Reumatol Port* 2006;**31**:237–45 [In Portuguese].
14. David H, Levy-Frebault V, Thorel MF. Méthodes de laboratoire pour mycobactériologie clinique. Unite de la Tuberculose et des mycobactéries. Paris: Institut Pasteur; 1989 [In French].
15. Gomez-Reino JJ, Carmona L, Rodríguez-Valverde V, Martín- Mola E, Montero MD. Treatment of rheumatoid arthritis with tumor necrosis factor inhibitors may predispose to significant increase in tuberculosis risk. *Arthritis Rheum* 2003;**48**:2122–7.
16. Culver EL, Travis SP. How to manage the infectious risk under anti-TNF in inflammatory bowel disease. *Curr Drug Targets* Feb 2010;**11**(2):198–218.

17. Carmona L, Hernandez-Garcia C, Vadillo C, Pato E, Balsa A, González-Alvaro I, et al. Increased risk of tuberculosis in patients with rheumatoid arthritis. *J Rheumatol* 2003;**30**:1436–9.
18. Yamada T, Nakajima A, Inoue E, Tanaka E, Hara M, Tomatsu T, et al. Increased risk of tuberculosis in patients with rheumatoid arthritis in Japan. *Ann Rheum Dis* 2006;**65**:1661–3.
19. Sichletidis L, Settas L, Spyratos D, Chloros D, Patakas D. Tuberculosis in patients receiving anti-TNF agents despite chemoprophylaxis. *Int J Tuberc Lung Dis* 2006;**10**:1127–32.
20. Gardam MA, Keystone EC, Menzies R, Manners S, Skamene E, Long R, et al. Anti-tumor necrosis factor agents and tuberculosis risk: mechanisms of action and clinical management. *Lancet Infect Dis* 2003;**3**:148–55.
21. Elizabeth CH, Schluger N, Cush J, Chaisson RE, Matteson EL, Xu S, et al. Interferon-gamma release assay versus tuberculin skin test across RA, PsA, and AS patients prior to treatment with golimumab, a human anti-TNF antibody. *Arthritis Rheum* 2012;**64**(7):2068–77.
22. Centers for Disease Control and Prevention. Targeted tuberculin testing and treatment of latent tuberculosis infection. *MMWR* 2000;**49** [No. RR-6].
23. Klein NC, Go CH, Cunha BA. Infections associated with steroids use. *Infect Dis Clin North Am* 2001;**15**(2):423–32.
24. Kevin L, Winthrop S, Yamashita S, Beekmann E, Polgreen PM. On behalf of the Infectious Diseases Society of America Emerging Infections Network. Mycobacterial and other serious infections in patients receiving anti-tumor necrosis factor and other newly approved biologic therapies: case finding through the emerging infections network. *Clin Infect Dis* 2008;**46**:1738–40.
25. Garcia VC, Rodriguez FS, Martinez LJ, Salavert M, Vidal R, Carballeira MR, et al. Paradoxical response to antituberculous therapy in infliximab-treated patients with disseminated tuberculosis. *Clin Infect Dis* 2005;**40**:756–9.
26. Belknap R, Reves R, Burman W. Immune reconstitution to *Mycobacterium tuberculosis* after discontinuing infliximab. *Int J Tuberc Lung Dis* 2005;**9**:1057–8.
27. Wallis RS, Kyambadde P, Johnson JL, Horter L, Kittle R, Pohle M, et al. A study of the safety, immunology, virology, and microbiology of adjunctive etanercept in HIV-1-associated tuberculosis. *AIDS* 2004;**18**:257–64.
28. Arend SM, Leyten EMS, Franken WPJ, Huisman EM, Dissel JT. A patient with de novo tuberculosis during anti-tumor necrosis factor-α therapy illustrating diagnostic pitfalls and paradoxical response to treatment. *Clin Infect Dis* 2007;**45**:1470–5.
29. Keane J, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* Oct 11 2001;**345**(15):1098–104.