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Infeção e Imunomodulação: um novo paradigma de infeção

Infection and Immunomodulation: a new paradigm of infection

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Infection and Immunomodulation: a new paradigm of infection

Infeção e Imunomodulação: um novo paradigma de infeção

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List of Publications

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

Abreu C, Magro F, Santos-Antunes J, Pilão A, Rodrigues-Pinto E, Bernardes J, Bernardo A, Magina S, Vilas-Boas F, Lopes S, Macedo G, Sarmento A.

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1 - Second European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease

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Lectures

For post- graduate medical students, at invitation of the Course organizers:

2017

2º Curso Risco de Infeção na imunomodulação / Imunossupressão (CRINI) – O que precisamos saber? - Serviço de Infeciologia e Medicina Tropical. Centro Hospitalar de Lisboa Ocidental, Lisboa, 23th June – 14th July, 2017

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2015

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Tuberculose e anti-TNF.

2013

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2014
5th December, 2014

Vacinação antimicrobiana.

2013
13th November, 2013

Vacinação antimicrobiana.

Oral communications in congresses and other scientific meetings

2017

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Serial tuberculosis screening in IBD patients treated with infliximab in a cohort from an intermediate-incidence country

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Abreu C.

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Connecting for the Future II – Porto 24th May, 2014
Managing Infectious Diseases under Biologics: HBV Infection & Tuberculosis

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12th Medinterna International Meeting - Cutting Edge Autoimmunity, Porto
27th February– 01st March, 2014

Infection and Vaccination by Zoster Virus in Elderly and Autoimmune Diseases
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2013

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Tuberculosis in anti-TNFα treated Patients: Analysis of 25 patients matched with a control Population

Abreu C, Magro F, Santos-Antunes J, Pilão A, Rodrigues-Pinto E, Bernardes J, Bernardo A, Magina S, Vilas-Boas F, Lopes S, Macedo G, Sarmento A.

2012

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Disseminated Tuberculosis in a patient with Crohn's disease on adalimumab
Albuquerque A, Lopes S, **Abreu C**, Bettencourt H, Macedo G.

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Rodrigues S, Magro F, **Abreu C,** Cardoso S, Sarmento A, Macedo G.

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Abbreviations

Anti-TNFα - Anti tumor necrosis factor alfa

AS - Ankylosing arthritis

BCG - Bacillus Calmette–Guérin

BRC - B cell receptor modulation

BLys - B lymphocyte stimulator

CMV - Cytomegalovirus

DII - *Doença Inflamatória Intestinal*

DMARDs - Disease Modifying Anti Rheumatic Drugs

EBV- Epstein Barr Virus

Gedii - *Grupo de Estudo da Doença Inflamatória Intestinal*

HIV - Human Immunodeficiency Syndrome

HSV – Herpes Simples Virus

IBD - Inflammatory Bowel Diseases

IL - Interleukin

IRIS - Immune Reconstitution Inflammatory Syndrom**e**

IGRA - Interferon Gamma Release Assay's

mAbs - monoclonal Antibodies

NICE- National Institute for Health and Care Excellence

PML - Progressive Multifocal Leukoencephalopathy

PA - Psoriatic Arthritis

QFT-GIT - Quantiferon Gold-In-tube Test

RA - Rheumatoid Arthritis

TST - Tuberculin Skin Test

TB - Tuberculosis

Abstract

Introduction

The cross talk between infectious agents and the human host are far from being completely understood and from both sides (host and infectious agents) changes along times are to be expected. Biological therapies for inflammatory immune-mediated diseases, alone or in association with (or following) immunomodulators, changed the perspective of dealing with these pathologies and brought a new paradigm of its treatment. Opposite glucosteroids and the old immunomodulators, such as methotrexate and thiopurines inhibitors, biological therapies act in specific and very restrict points of the immune system, and unexpected immune answers may arise. The main concerns regarding these therapies are the potential induction of auto immunity, neoplastic diseases and infection. In the field of biologics, infection behaviour in different, and sometimes unexpected ways, compared with the non-immunosuppressed host. Also, as these therapies decrease or suppress the immune answer, the risk of infection rises. Tuberculosis (TB) associated with anti-tumor necrosis factor alfa (anti-TNFα) therapies and progressive multifocal leukoencephalopathy (PML) associated with the prescription of natalizumab, an humanized monoclonal antibody against the cell adhesion molecule α4-integrin, were milestones of the infectious risk of these new therapies. As there are still gaps in the knowledge we are in need of a deeper understanding of the infectious risk and the behaviour of infection under biological therapies to increase patient safety.

Among biological drugs, anti-TNFα enabled a large field of study, as they are prescribed for the treatment of several pathologies since 1998. Granulomatous and intracellular infections are of special concern in patients treated with anti-TNFα because the capacity of containing infection in the case of granulomatous infections is lost and the defence against intracellular agents weakened. Despite this knowledge, several questions need further research, such as: (i) how do TB behaviour in patients treated with anti-TNFα in terms of clinical manifestations, diagnosis and response to treatment and how different is from the non immunosuppressed population? (ii) is the TB incidence in a country such as Portugal, that has an intermediate risk of the disease, enough to justify a different management of latent TB screening before and during biologics from countries with a low and very low TB incidence? (iii) which are the best tests for the diagnosis of latent TB to avoid false negative results? (iv) should we retest for TB and how during anti-TNFα and other biological therapies that have the risk of reactivation of *Mycobacterium tuberculosis*? (v) how can the global infectious risk associated with these therapies be better mitigated?

Objectives and Methods

Considering the existing gaps of knowledge and clinical needs, the work carried out in the scope of the present thesis concerning infection in adults with immune-mediated inflammatory diseases, had four major objectives: the first one was to investigate TB - active disease and its prevention in patients treated with anti -TNFα; the second and third objectives were the study of other granulomatous and intracellular severe infections diagnosed in patients treated with anti-TNFα and immunomodulators, namely invasive listeriosis, nocardiosis and disseminated herpes virus infection; the fourth main objective was focused on infection prevention in patients under immunomodulatory and biological therapy, and ways to reduce the risk of infection under these therapies, contributing to increase drug safety and patient well-being. The cohort studied were adult patients suffering from inflammatory immune-mediated diseases and treated at Centro Hospitalar S. João, Porto, Portugal for the last 16 years. Retrospective, prospective and descriptive methods were applied in this study.
The criteria for latent TB diagnosis tests was based on the positivity of a TST or/and a specific interferon gamma release assay's (IGRA) test.

To attain the first objective, the burden of active TB in this cohort of Portuguese patients under anti-TNFα therapy and the safety of anti-TNFα reintroduction after TB treatment were assessed. Then the work was focused on latent TB and two prospective studies were done. In the first, the sensitivity and specificity of the TST compared with T-SPOT.TB and Quantiferon Gold in-Tube test (QFT-GIT) - before or in the switch of anti-TNFα therapy - was studied in patients with inflammatory bowel diseases (IBD). In the second study, a serial screening for latent TB in patients under anti-TNFα along 26 months was done.

Concerning the second objective, two cases of invasive listeriosis in the cohort of anti-TNFα and or immunomodulated IBD treated patients were reviewed, followed by a literature review. Also two cases of a puzzling and repeated stool isolation of *Nocardia nova,* in patients suffering from inflammatory bowel disease under immunomodulators were analysed and a literature review of the disease in immunosuppressed patients was done.

To attain the third objective a disseminated cutaneous herpes virus infection and a severe varicella in patients under immunomodulators plus, respectively, steroids and anti-TNFα therapy were presented and confronted with a literature review.

Finally, the fourth main objective was attained through the development of a practical approach to prevent infection in patients suffering from immune-mediated inflammatory diseases. This approach had a rational and was based on different recommendations from several authors that we adapted to the follow up of this cohort.

Results

Regarding the study that addressed active TB, 25 TB cases from 765 patients treated with anti-TNFα drugs among 2001-2012 were reviewed. The estimated incidence of TB per 100,000 patient-year of anti-TNFα prescription was 1337, 792 and 405 for those under infliximab, adalimumab and etanercept, respectively. Sixty per cent of the patients had extra-pulmonary TB. Seventeen patients from the 25 were screened for latent TB before anti-TNFα: 13 were negative and four positive. Three from these four patients were treated with isoniazid. Compared with a control group of community-acquired TB matched for sex, age and data of diagnosis, were more frequent in our cohort extra-pulmonary TB, fever on presentation and less positivity of direct microbiological exams; although the outcome of TB was not worse than in the general population.

Lately, concerning the restart of anti-TNFα after TB, from 28 patients with TB, eight restarted anti-TNFα therapy: five have done so after and three during TB treatment. With a follow-up period of more than 2.5 years no recurrence of TB was verified.

Facing TB prevention a prospective screening of TB in 250 patients with IBD about to start or switch anti-TNFα therapy was done. The diagnosis of latent TB was done in 29 % of them. We found TST had an overall higher sensitivity (81 vs. 35%) and a higher Negative Predictive Value (93% vs. 80%) when compared to QFT-GIT irrespective of the presence and kind of backbone immunosuppressive therapy. The concordance between both tests was weak.

Another study concerning the serial screening for TB in 46 IBD patients under infliximab (after a negative screening before anti-TNFα), was performed six times across 26 months, using T-SPOT.TB, TST and, for three screenings points, also QFT-GIT. Sixteen (29%) patients turned positive at least in one test (T.SPOT-TB, QFT-GIT or TST). The TST had the highest sensitivity, followed by T-SPOT.TB. Male patients and patients with long duration of IBD converted more frequently TB tests; test results were not concordant and positive IGRA's results were not reproduced in the following screenings and its results were close to the cut-off values. From the 16 patients 15 were treated with isoniazid; no cases of active TB were elicited.

Concerning other granulomatous infection under anti-TNFα, two cases of invasive listeriosis (meningitis) were diagnosed and treated in patients with ulcerative colitis under infliximab; one of them was infected with human immunodeficiency virus (HIV). Both had a good outcome without sequelae and one restarted infliximab.

From two IBD no symptomatic patients, treated with azathioprine and pulses of steroids, a pathogenic *Nocardia*, *Nocardia nova*, was isolated in several samples of stool; cutaneous and

invasive disease were excluded. One patient was treated with cotrimoxazole during the first three months of anti-TNFα introduction. The concern is that this colonization may disseminate and cause disease under deeper immunosuppression. This clinical situation was followed by a literature review of Nocardiosis in patients under immunomodulators and biologics.

In the setting of herpes virus infection, a severe disseminated cutaneous herpes simplex infection was diagnosed in a young patient suffering from Crohn's disease that was treated with steroids and azathioprine. She had a good outcome after acyclovir treatment.
In a young male with Crohn's disease treated with azathioprine and infliximab, a severe varicella that required inotropic support and mechanical invasive ventilation was described. The patient was discharged without remarkable sequelae and restarted anti-TNFα. In both situations a review of the literature concerning the disease in immunosuppressed patients was done.

Related to the fourth objective a practical protocol of screening, infection prevention (including immunizations) and counselling in patients about to star biologics or immunomodulators due to an auto- inflammatory immune-mediated disease was elaborated and tested in this cohort.

Conclusions

In the combination between anti-TNFα therapies (alone or in association with immunomodulators) and infection, in the studied cohort, TB was the biggest concern and extra-pulmonary and disseminated forms were more common than in the general population, although TB outcome was not worse than in the general population. Reintroduction of anti-TNFα therapies after or during TB treatment was safe. Concerning latent TB screening before anti-TNFα therapy, we found that TST remains important due to a higher sensitivity compared with IGRA's tests even in patients under immunosuppressive therapies. On the setting of TB screening during anti-TNF α prescription, subject to much more debate, our results showed also higher sensitivity of TST and conversion and reversion of IGRA's results that were closed to the cut-off values; thus, the suggestion is to screen regularly in the first years of prescription and in a tailor based way, according to perceived TB risk. Against the actual mainstream, probably would be advisable to maintain the TST before better tests for latent TB diagnosis become available.

There is also risk of other severe granulomatous and intracellular infections, as we have diagnosed in our cohort, especially on the association of steroids with immunomodulators or biologics, and the risk of infection may be even partially unknown for the most recent therapies, so surveillance of patients under these therapies is crucial.

Regarding the real risk of infection as we found in the treated cohort, counselling screening and prophylaxis, including immunization, before biologics and immunomodulators is appropriate to mitigate the risk of infection. From this study also resulted a practical protocol (with an introduction on its rational) for the follow-up of immune-mediated diseases treated patients whose propose is to avoid infections that can result in treatment interruptions and worsening of the medical condition.

Resumo

Introdução

A interação entre agente infecioso e hospedeiro humano é complexa e está longe de estar completamente esclarecida, sendo expectável que ao longo do tempo venham a ser identificados novos aspetos. As terapêuticas biológicas prescritas no tratamento de doenças inflamatórias imuno-mediadas, isoladamente e/ou em associação ou na sequência de tratamento com imunomoduladores, mudaram a perspetiva de abordar estas patologias e trouxeram um novo paradigma ao seu tratamento. Em oposição aos fármacos mais antigos, os corticosteroides e os imunomoduladores, tais como metotrexato e inibidores das tiopurinas, as terapêuticas biológicas atuam em pontos específicos e restritos do sistema imune e, por isso, respostas imunológicas não esperadas podem surgir. As fontes de maior preocupação relativamente a estas novas terapêuticas são a possibilidade de indução de auto-imunidade, doenças neoplásicas e infeções. Nos doentes sob tratamento biológico, a infeção comporta-se de forma diferente relativamente a hospedeiros não imunossuprimidos, assumindo por vezes formas inesperadas. Além disso, dado que estas terapêuticas diminuem ou suprimem a resposta imune, o risco de infeção aumenta. O reconhecimento da associação entre as terapêuticas anti-fator de necrose tumoral alfa (anti-TNFα) e o desenvolvimento de tuberculose (TB), e entre a administração de natalizumab (um anticorpo monoclonal contra a adesão celular à molécula α4-integrina) e a ocorrência de leucoencefalopatia multifocal progressiva, constituíram marco importante na evidência do risco infecioso associado a estas novas terapêuticas. Contudo, continua a haver falhas nesse conhecimento sendo necessário aprofundá-lo e compreender melhor o comportamento da infeção no decurso da terapêutica biológica para aumentar a segurança dos doentes sob estas terapias.

Entre os fármacos biológicos, os anti-TNFα têm permitido um largo campo de estudo por serem prescritos desde 1998, no tratamento de diversas patologias imuno-mediadas. As doenças granulomatosas e intracelulares são uma preocupação particular no tratamento com estes fármacos pois a capacidade de conter as infeções granulomatosas perde-se e a defesa contra agentes infeciosos intracelulares fica enfraquecida. Apesar do conhecimento já reunido, diversos aspetos necessitam de mais investigação, tais como: (i) como é que a TB se comporta em doentes tratados com anti-TNFα em termos de manifestações clínicas, diagnóstico e resposta à terapêutica e quão diferente é esse comportamento do que ocorre em hospedeiros não imunodeprimidos? (ii) num país como Portugal com uma incidência intermédia de TB justifica-se fazer de forma diferente o seu rastreio nos doentes antes de iniciar a terapêutica e durante a terapêutica biológica comparativamente aos países com baixa ou muito baixa

incidência de TB? (iii) quais são os melhores testes para o diagnóstico de TB latente de modo a evitar resultados "falsos"negativos? (iv) deveremos, durante a terapêutica com anti-TNFα e outras terapêuticas biológicas que tenham risco acrescido de reativação da infeção por *Mycobacterium tuberculosis*, rastrear para TB e, em caso afirmativo, como e com quê? (v) como podemos diminuir eficazmente o risco infecioso associado a estas terapêuticas imunomoduladoras e biológicas?

Objetivos e Métodos

Considerando as lacunas de conhecimento e as necessidades clínicas, o trabalho da presente Tese teve quatro objetivos principais: o primeiro foi a investigação de TB nas vertentes da prevenção e doença ativa em doentes sob anti-TNFα; o segundo e o terceiro foram o estudo de outras doenças graves granulomatosas e de doenças causadas por agentes infeciosos intracelulares em doentes tratados com anti-TNFα e/ou imunomoduladores, nomeadamente listeriose invasiva, nocardiose e infeções herpéticas disseminadas. O quarto objetivo focou-se na prevenção de infeção em doentes tratados com imunomoduladores e biológicos e formas de reduzir o risco de infeção, tendo sido desenvolvido um protocolo prático para rastreio e imunização, baseado em recomendações de diferentes autores e adaptado a esta população de doentes.

A população estudada foi a dos doentes adultos com doença inflamatória imuno-mediada seguida no Centro Hospitalar S. João, Porto, Portugal nos últimos 16 anos. Métodos retrospetivos, prospetivos e descritivos foram utilizados neste estudo. O critério de diagnóstico no que concerne aos testes de diagnóstico de TB latente foi baseado na positividade do TST e/ou dum teste IGRA específico para *M. tuberculosis*.

Para atingir o primeiro objetivo foram avaliadas primeiro a TB ativa numa população de doentes portugueses com doença inflamatória imuno-mediada sob anti-TNFα e depois a segurança de reintrodução do anti-TNFα após tratamento de TB. Posteriormente o foco de estudo foi a TB latente e dois estudos prospetivos foram efetuados. No primeiro a sensibilidade e especificidade do TST comparada com com a dos testes IGRA´s (T-SPOT.TB e QFT-GIT) antes ou na troca de anti-TNFα foi estudada em doentes com doença inflamatória intestinal (DII). No outro estudo foi efetuado o rastreio seriado de TB latente em doentes sob anti-TNFα ao longo de 26 meses.

Relativamente ao segundo objetivo, na *cohort* de doentes com DII tratados com anti-TNFα e/ou imunomoduladores, foram revistos dois casos de listeriose invasiva, a que se seguiu revisão da literatura. Dois outros casos de um isolamento nas fezes, repetido e paradoxal, de *Nocardia nova* em doentes sob imunomoduladores com doença inflamatória intestinal foram

estudados e foi efetuada uma revisão da literatura sobre infeção por *Nocardia spp.* em doentes sob imunomoduladores e/ou biológicos.

Quanto ao terceiro objetivo, foram estudados um caso de infeção herpética cutânea e disseminada e um caso de varicela grave ambos em doentes sob imunomoduladores associados respetivamente a corticoide e a anti anti-TNFα, tendo sido feita uma revisão da literatura sobre estas doenças em doentes sob imunomoduladores e ou biológicos.

O quarto objetivo foi atingido através do desenvolvimento de normas práticas de prevenção de infeção em doentes com doença inflamatória imuno-mediada. Estas normas foram fundamentadas de acordo com recomendações de vários autores e adaptadas à população estudada.

Resultados

O estudo que focou a TB ativa reviu 25 casos de TB, diagnosticados de entre os 765 doentes tratados com anti-TNFα entre 2001-2012, o que reflete uma incidência estimada de TB por 100,000 doentes/ano de prescrição de anti-TNFα de 1337, 792 e 405 para os doentes tratados com infliximab, adalimumab e etanercept, respetivamente. Sessenta por cento desses 25 doentes apresentaram TB com envolvimento extra-pulmonar. Antes da prescrição de anti-TNFα, 17 dos 25 doentes fizeram rastreio de TB latente, tendo quatro rastreio positivo, três dos quais fizeram tratamento com isoniazida. Em relação a uma população controle da comunidade da região do Porto com diagnóstico de TB ativa, emparelhada por sexo, idade e data de diagnóstico, os doentes tratados com anti-TNFα estudados apresentaram maior incidência de tuberculose extra-pulmonar e de febre na apresentação, e uma menor frequência de resultados positivos nos exames microbiológicos diretos. No entanto, o resultado não foi pior do que o da população controlo.

Posteriormente e relativamente à TB associada a anti-TNFα, a retoma do fármaco após (ou durante) a terapêutica foi verificada em oito dos 28 doentes com diagnóstico de TB: cinco retomaram esta terapêutica depois de tratada a TB e três ainda durante o tratamento. Com uma mediana de seguimento de mais de 2,5 anos não se verificou em nenhum doente recorrência de TB.

Relativamente à prevenção de TB, o rastreio prospetivo efetuado em 250 doentes com DII antes do início ou em troca de anti-TNFα, permitiu o diagnóstico de TB latente em 29% deles. Genericamente, a sensibilidade da prova de tuberculina foi mais elevada (81% *versus* 35%) assim como o valor preditivo negativo (93% *versus* 80%, respetivamente) quando comparado com o teste QFT-GIT, independentemente da presença e do tipo de terapêutica imunossupressora. A concordância entre estes dois tipos de testes foi baixa.

Num outro estudo que englobou 46 doentes com DII sob infliximab, após rastreio negativo de TB prévio ao infliximab, foi efetuado o rastreio seriado de TB seis vezes ao longo dos 26 meses, tendo sido utilizados os testes T-SPOT.TB, tuberculina e em três dos rastreios também o QFT-GIT. Dezasseis (29%) dos doentes tiveram pelo menos um dos três testes positivo ao longo dos 26 meses. O TST teve a maior sensibilidade, seguido do T-SPOT.TB. Doentes do sexo masculino e com longa duração da DII converteram mais frequentemente estes testes, cujos resultados foram muito pouco concordantes entre eles; os testes IGRA positivos não se reproduziram nos rastreios subsequentes e tiveram valores muito próximo do limite da positividade. Dos 16 doentes com diagnóstico de TB latente, 15 foram tratados com isoniazida; não se verificou nenhum caso de doença tuberculosa.

Relativamente a outras doenças granulomatosas, diagnosticaram-se e trataram-se dois casos de listeriose invasiva (meningite) em doentes com o diagnóstico de colite ulcerosa tratados com infliximab, tendo um deles infeção pelo vírus da imunodeficiência humana. Ambos tiveram evolução favorável sem sequelas e um deles retomou infliximab.

Em dois doentes tratados com azatioprina e pulsos de corticosteroides por DII, uma *Nocardia* usualmente patogénica, *Nocardia nova*, foi isolada em subsequentes amostras de fezes, sem sintomatologia associada. Foi excluída doença cutânea e invasiva. Um destes doentes foi tratado com cotrimoxazol nos três primeiros meses após ter iniciado anti-TNFα. A preocupação foi a de que a colonização pudesse disseminar e causar doença sob terapêutica mais imunossupressora. A situação clínica foi seguida e foi efetuada uma revisão da literatura relativa a nocardiose em doentes sob imunomoduladores/biológicos.

Relativamente à infeção por vírus do grupo herpes sob imunomoduladores/biológicos, uma forma grave de *Herpes simplex* cutâneo disseminado foi diagnosticada numa jovem com doença de Crohn tratada com corticoide e azatioprina, cuja evolução clínica foi favorável sob tratamento com aciclovir endovenoso.

Uma forma grave de varicela num jovem com doença de Crohn tratado com infliximab e azatioprina foi também estudada, tendo sido necessária ventilação invasiva e suporte inotrópico. A evolução foi favorável não sendo detetadas sequelas e foi retomado o anti-TNFα com sucesso. Em ambas as situações foram efetuadas revisões da literatura destas doenças sob imunossupressão.

No que se refere ao quarto objetivo, foi desenvolvido um protocolo de fácil utilização relativo a rastreio, prevenção de infeção (incluindo imunizações) e aconselhamento em doentes que vão iniciar imunomoduladores ou biológicos devido a doença auto inflamatória, que foi testado nesta *cohort,*

Conclusões

Na interseção entre terapêutica anti-TNFα (só ou em associação com imunomoduladores) e infeção, a TB foi a principal preocupação na amostra de doentes adultos estudados, sendo as formas extrapulmonares e disseminadas muito mais comuns do que na população em geral, ainda que o resultado não tenha sido pior. A reintrodução de terapêutica com anti-TNFα depois da TB revelou-se segura. Relativamente ao rastreio de TB antes da medicação com anti-TNFα, a prova de tuberculina permanece importante já que mostrou maior sensibilidade quando comparada com os testes IGRA mesmo em doentes sob terapêuticas imunossupressoras. No rastreio de TB durante a prescrição de anti-TNFα, que tem sido alvo de muitas mais dúvidas, os nossos resultados mostraram também maior sensibilidade da prova de tuberculina do que os testes IGRA, e a frequente conversão e reversão dos resultados destes últimos, com valores próximos do limiar da positividade. Assim, sugere-se a manutenção da prova da tuberculina no rastreio da TB enquanto não existirem testes melhores e, no rastreio durante a prescrição de anti-TNFα, que o mesmo seja feito nos primeiros anos da prescrição e sempre de uma forma personalizada de acordo com o risco percetível de TB.

Conforme indicado pelos nossos resultados em concordância com o referido na literatura, existe também risco de outras infeções graves granulomatosas e intracelulares, associadas à prescrição de anti-TNFα e/ou imunomoduladores. Não estando ainda estabelecido o risco de infeção em doentes medicados com as terapêuticas biológicas mais recentes, a vigilância clínica nestes casos é de importância acrescida.

Atendendo ao risco real de infeção verificado também nesta *cohort,* o aconselhamento, rastreio e profilaxia, incluindo imunização preferencialmente antes dos imunomoduladores e biológicos são medidas importantes na redução do risco de infeção. Deste estudo resultou um protocolo prático cujo propósito é evitar infeções que possam resultar em interrupção da terapêutica biológica e imunomoduladora e agravamento da condição médica.

1. Thesis Outline

1. Thesis outline

The present PhD thesis is based on several different studies, which are organized and detailed in nine chapters.

Chapter 2 presents an introduction on immunomodulation and risk of infection, concerning different therapies and risk evaluation.

Chapter 3 resumes the aim and specific objectives of this thesis.

Chapters 4, 5, 6 and 7 present the main results of the different studies developed to achieve the four main objectives of the thesis.

Chapter 4 presents original cohort studies related to TB: first the cases of active TB in patients under anti-TNFα because of several immune-mediated diseases, then the re-institution of anti-TNFα after TB. At last the prospective screening of latent TB: before anti-TNFα or on the switch of anti-TNFα and then during infliximab therapy.

Chapter 5 presents original case studies in patients with other granulomatous infection (invasive Listeriosis, Nocardiosis) and a literature review.

Chapter 6 presents original cases study of intracellular herpes virus infection (severe varicella and disseminated cutaneous herpes simplex infection) and a literature review.

Chapter 7 presents a proposed protocol for counselling, screening, and prophylaxis including immunization, before prescription of common immunomodulators and biological therapies.

Chapter 8 includes a brief general discussion of the work and some recommendations based on our results.

Chapter 9 depicts some final remarks.

2. Rationale

2. Rationale

2.1. Infection

Infection remains in humankind from ancestral times. However, just recently, due to advances in medical science and technology, namely due to the use of antimicrobials and vaccines, was possible to protect the human population from important infectious agents. Several potential severe infections like measles, poliomyelitis, tetanus, diphtheria, to speak only a few are now controlled in developed countries. The increase in life expectancy during the 20th century was largely due to reductions in infectious disease mortality in consequence of immunization and improvements in child survival[1]. However, infectious diseases remain a major cause of illness, disability and death. Remarkable, concerns about infection changed across times. Recently, globalization enabled the likelihood of infectious diseases to occur and spread rapidly across the planet.

In the eighties of the XX century, the human immunodeficiency virus (HIV) infection become a challenge in infectious diseases, and a model to understand the implications of a virus that attacks directly the immune system that is crucial for the protection against infection. Diagnosing and treating the opportunistic infections in HIV infected persons that occur in consequence of immune depression has been a singular and huge experience. The research and workup enabled what at first was just a mirage: the reconstitution of immunity by the suppression of the viral load and rise of TCD4 lymphocytes count. A new era in HIV history was accomplished. Yet, no cure is available despite the huge progress made.

Although other conditions are relevant in the field of infection - old and very old persons, immunosuppressed patients due to severe medical conditions and treatments, haematological or solid organ transplanted receptors - are on the rise and have an increased risk of infection[2-5]. The crosstalk between the human host and the infectious agent is far from being completely understood as it has several types of response and the immune pathways involved are complex. Furthermore, new challenges arrived with the use of biological therapy and immunomodulators for the treatment of inflammatory immune-mediated diseases.

2.2. Inflammatory Immuno-mediated Diseases

For several reasons, some of them unknown, the increase of inflammatory immune-mediated acquired disorders observed, particularly of inflammatory bowel diseases (IBD), in the newly industrialised countries is significant[6-9]. Genetic and environmental factors, and innate immunity are involved in the genesis of these diseases[10]. The "hygiene hypothesis" suggests that the decline in infectious diseases due to better hygiene may have contributed to the increase of immune-mediated diseases[11]. In patients with such diseases the capacity of self-tolerance is lost[12]. These diseases themselves increase the susceptibility to infection, especially among patients with the most active ongoing inflammatory disease activity[10].

The development of therapies to treat such disorders by inducing self-tolerance started 60 years ago, and has been particularly remarkable in the last decades. Since then, the therapeutic approach has been largely the treatment with steroids, immunomodulators and more recently also biological drugs that modulate the immune system answer. The goal of immunomodulation in the treatment of immune-mediated diseases is to produce self-tolerance, without a severe immunosuppression that may put the patient at increased risk of malignancies and infection[12]. The main current challenge of this therapeutic approach is that such pharmacological action often interferes with the immune system in varied and sometimes unexpected ways.

2.2.1. Therapies of inflammatory immunomediated diseases and risk of infection

Early therapeutic agents, like glucocorticoids and thiopurines, lack specificity[13]. Broad suppression of immune cell function and replication caused by these agents frequently also induce toxicity and other adverse effects, including infections. Glucocorticoids for instance decrease the number of circulating lymphocytes and eosinophils; macrophages and monocytes reduce the production of inflammatory cytokines when steroids are administered and adverse metabolic and endocrine effects are to be expected, especially in patients under prolonged and high dose treatment[13]. Glucocorticoids therapies are associated with infections caused by bacteria, micobacteria, virus and parasites[14,15].

Immunomodulators like anti proliferative agents such as methotrexate (T cell inhibitor of folate metabolism), leflunomide (inhibitor of T and B cell purine synthesis) and azathioprine (inhibitor of purine nucleic acid metabolism) have also anti-inflammatory actions with potential adverse effects and risk of infections, namely those dependent on T cell immunity as those caused by the herpes virus group and *Pneumocystis jiroveci*[16].

More recently, biologic therapeutic agents were developed. They are proteins with human or human/murine constructs that are directed against cells from the inflammatory process or against inflammatory cytokine activity[17]. They target particular receptors of the immune response and changed the paradigm of treatment, owing to its good efficacy and better safety profiles[10,13,18,19]. The targets of these biological therapies are cytokines, B cells, and co-stimulation molecules[18]. Anti-cytokines include anti-TNFα, anti-interleukin (IL) 1, anti-IL 6 anti-IL 12, anti-IL 17 and anti-IL 23 molecules. B-cell depletion drugs include anti-CD20 antibodies agents and B cell receptor (BCR) modulation by the B-lymphocyte stimulator (BLyS)[18]. More than 45 antibodies are now marketed for therapy (including in oncology) or imaging in western countries. Nowadays a focus on early treatment with biological therapies is recommended, although none is yet available as first-line medication for autoimmune diseases[18].

A sharp rise on the development of biological agents for the treatment of immune-mediated inflammatory diseases is expected for the next years and side effects, including the risk of infections, are partially unknown contrary to older immunomodulatory therapies. The challenge remains to selectively interfere with immune responses that causes autoimmunity while keeping an intact response to infectious agents, and this is difficult to reach[20].

Some of the biologic therapies have been useful in several immunomediated diseases while others are specific for a few or a single disease. Anti-TNFα monoclonal antibodies, for instance, are effective in the treatment of various inflammatory diseases, such as IBD, rheumatoid arthritis (RA), ankylosing spondylitis (AS) and psoriatic arthritis (PA). Infliximab, adalimumab, and golimumab are monoclonal antibodies directed against TNF-α, etanercept is a soluble receptor for TNF-α and certolizumab pegol is a pegylated fragment of a humanized anti-TNFα monoclonal antibody[19]. Several years of knowledge and prescription makes anti-TNFα a reference in biologic therapies. Infliximab was the first anti-TNF α to be approved (1998) for the treatment of IBD and RA, and a large clinical experience of its use already exists. Being TNFα fundamental for activation of macrophage and phagosome, differentiation of monocytes into macrophages and recruitment of neutrophils and macrophages, granuloma formation and its maintenance and clearance of intracellular pathogen, anti-TNF α drugs increase considerably the risk of granulomatous, namely TB, and intracellular infections in patients under these therapies[21]. TB was really the first infectious of high concern associated with biological therapy[22].

2.2.2. Tuberculosis

Tuberculosis was first associated to anti-TNFα therapy in 2001[22], about three years after infliximab approval for treating IBD and RA patients. In general, the TB occurring in patients under anti-TNFα therapy results mainly from reactivation, frequently is extra-pulmonary and disseminated, progresses more rapid than in other patients and has less granuloma formation[23,24]. The risk of TB occurrence is not the same for different anti-TNFα therapies[25] and is also dependent on the local TB incidence. The standard performance of TB screening and treatment before anti-TNF α (and also before leflonamide, teriflunomide, ustekinumab and alemtuzumab administration) is considered to reduce the risk of the disease by 80%[26]. Although, it should be taken into consideration that the most part of the studies concerning patients treated with anti-TNF α are from western countries with high income that have a low or very low incidence of TB. Thus, more studies are needed, especially in countries with intermediate TB incidence like Portugal, or higher TB incidence, as the risk may be higher. Considering the risk of TB associated with the therapy anti-TNF α and the intermediate or high TB incidence in such countries, several questions arise, such as: (i) how should the TB screening proceed under anti-TNF α therapy? (ii) should the rescreening be done only in patients at higher risk of TB? (iii) how frequently should the rescreening be done (e.g. 6 months, annual)? (iv) which are the most efficacious tests for both screening and rescreening (i.e., tuberculin or specific IGRA's tests)? (v) what about the cut-off for tests positivity? These last questions are pertinent especially because under immunosuppressive therapy, the TST may be less often positive and, due to the reduction of interferon gamma produced by the lymphocytes, QFT-GIT and T-SPOT.TB may be also less often positive[27,28]. In short we still miss better tests for a correct diagnosis of latent TB.

2.2.3. Other granulomatous and intracellular infections

In addition to TB, other intracellular and granulomatous infections, such as those caused by *Listeria monocytogenes, Legionella pneumophila* and *Nocardia,* several fungal infections (aspergilosis, candidosis, cryptosporidiosis) and the more rare endemic mycosis (histoplasmosis, coccidioidomycosis), are associated with anti-TNFα therapy[21,29].

Intracellular DNA virus infection and biological or immunomodulatory therapy reactivation of Herpes virus family agents, in particular *Herpes simplex*, are a frequent concern in patients under anti-TNFα treatment. In fact, a significantly increase of *Herpes simplex* reactivation in such patients with inflammatory rheumatic diseases was found by some authors[30,31]. Outside of anti-TNFα therapies, fingolimod that prevents lymphocyte egress from lymphoid tissues, and alemtuzumab, an anti CD52 drug, were both associated with herpes virus infections[32], as well as tofacitinib, an oral Janus Kinase (JAK) 1/3 inhibitor used for the treatment of rheumatoid arthritis[31,33], IBD and psoriasis[34]. Concerning alemtuzumab, for the treatment of multiple sclerosis, there is even the recommendation of treatment with acyclovir at least on the first month after infusion[35].

Considering that age is a relevant risk for the development of varicela zoster virus reactivation, for those older adults treated with biological therapies herpes zoster is a concern.

Another concern is the risk of hepatitis B virus reactivation under anti-TNFα therapies[36,37]. This reactivation may result in fulminant liver failure and death[38,39]. Although the greatest risk concerning this intracellular DNA virus reactivation is rituximab and other anti CD20 therapies that cause depletion of B-cells.

2.2.4. The complexity and hidden faces of this risk of infection

Age, the presence of comorbidities, such as diabetes mellitus, chronic kidney disease or impaired lung function and concomitant glucocorticoid prescription or a previous serious infection are considered factors that increase the risk of serious infection across many studies in patients treated with immunomodulators and or biologics[3,5]. The dose of the therapeutic biological agent, the disease to be treated, past immunosuppression or concomitant therapy are to be considered in risk evaluation[2,40,41]. For instance, in RA patients treated with tocilizumab (anti-IL6), when rituximab was previously prescribed a greater risk of mild and moderate infections was found[42]. Demography issues and asymmetries in risk of infectious diseases distribution are important concerning, for instance, TB and endemic mycosis[43,44]. Also, lymphopenia associated with immunomodulators or biologics, whenever is severe and prolonged, may constitute a risk for infection. For instance, alemtuzumab for the treatment of B cell chronic lymphocytic leukaemia may be associated with low CD4+ and CD8+ lymphocyte counts that may not return to baseline values for more than one year, being preconized monitoring cytomegalovirus (CMV) infection and prescribe prophylactic medication against *Pneumocystis jiroveci* and Herpes virus infection (http://www.campath.com/hcp/AboutCampath.html). Also lymphopenia associated with the prescription of dimethyl fumarate should be considered a risk for PML[45].

In real life, patients with inflammatory immunomodulated diseases get concomitant (or past) prescription of immunomodulators and glucocorticoids and thus the risk of biological monotherapy is hard to extrapolate. Concerning the risk of infection associated with these therapies, there is a need to know if any increase in risk of infection is constant over time, or whether there are times when the risk is higher or lower and define an at-risk window; that is, the period when adverse events should be attributed to a drug[46]. And of course in these studies our main focus was severe infections: those requiring hospitalization, if outpatient at onset, or those in the need of intravenous treatment.

Concerning the infection risk over time, there are studies where this risk was greatest during the initial 6 months of treatment and then decreased over time[47], suggesting positive correlation between infection and the systemic inflammatory burden of the underlying disease. Such correlation may explain why severe infections tended to be higher in patients with RA and IBD than in patients with psoriasis or AS[41].

Large national and international registers may reveal some of the patterns of rare adverse events that might otherwise be hidden. The overall incidence of severe infections in patients treated with anti-TNFα has been reported from large numbers of treated patients for several autoimmune diseases. For instance, concerning RA in European patients treated with anti-TNFα and anakinra, an anti-IL-1 therapy, the incidence of serious infection was 2.7-2.8 times higher in patients receiving biologics than in those treated with conventional disease-modifying anti rheumatic drugs (DMARDs)[48]. In another meta-analysis including 42,330 patients with RA from 106 randomized trials, a standard or higher dose of biological drugs (pooled analysis of etanercept, adalimumab, infliximab, golimumab, certolizumab pegol, anakinra, tocilizumab, abatacept, and rituximab) was associated with an increase of serious infections compared with the use of traditional DMARDs in patients with RA. Although, this association was not evident under low-dose biological drug treatment[40]. Moreover, in another meta-analysis of 44 randomized controlled trials involving 11,700 patients treated with anti-TNFα and 5,901 subjects receiving placebo or traditional DMARDs, patients with RA receiving anti-TNFα monoclonal antibodies (mAbs) other than etanercept (adalimumab, certolizumab pegol, and infliximab) had a higher risk of serious infection than those receiving placebo or traditional DMARDs[49].

Nevertheless other studies concerning the use of biologic agents didn't result in increased risk of infection[50-52]. The same was reported from four large US administrative databases (the Safety Assessment of Biologic Therapy project), where anti-TNFα use was not more commonly associated with hospitalization than use of DMARDs in patients with various autoimmune disease, such as RA, AS, IBD, and psoriasis[52].

Another issue is the risk of infection among different anti-TNFα treatments: - infliximab and adalimumab (mAbs) - compared with etanercept: from a Dutch[53] and Italian[54] registry mAbs were associated with a higher infection rate than etanercept, but not in a British registry[47].

2.2.5. Infectious risk mitigation strategies: counselling, prophylaxis and immunization

Due to the infectious risk associated to biologics, immunomodulators and steroids, mitigation strategies are welcome.

Granulomatous and intracellular infections are of concern, especially when anti-TNFα drugs are prescribed. TB is a key issue in prevention; concerning other granulomatous infections transmitted from food or water and spread in hot tubs, counselling according to the risk is important. The risk of herpes reactivation deserves prophylaxis in some situations. Prophylaxis for *Pneumocystis jiroveci* is needed in patients treated with some immunomodulators especially when in association.

Finally immunizations, indications and contraindications, have to be considered as strategies to mitigate the infectious risk and to keep safety on biological therapy.

3. Objectives

3. Objectives

This thesis had four main goals:

Goal 1:

Tuberculosis: from the disease to latent tuberculosis screening (Chapter 4)

The first aim of this thesis was to summarize the evidence regarding TB in Portuguese patients treated with anti-TNFα therapies. First, patients with active disease were reviewed, and then the safety of the reintroduction of anti-TNFα therapy after a TB diagnosis was evaluated. Concerning latent TB screening, the prospective screening before anti-TNFα or on the switch of anti-TNF therapy was done, as well as the screening in patients during infliximab therapy.

Goal 2:

Other severe granulomatous infections (Chapter 5)

The second aim of this thesis was to identify other severe granulomatous infections and to do a literature review on the subject.

Goal 3:

Severe intracellular DNA herpes virus infections (Chapter 6)

The third aim of this thesis was to summarize severe herpes virus infections and to do a literature review.

Goal 4:

Infectious prevention (Chapter 7)

The fourth aim of this thesis was to develop a practical approach to screening, monitoring and prophylaxis on patients about to start biologics, based on the risks identified on goal 1, 2 and 3.

4. Tuberculosis: from the disease to latent tuberculosis screening

4. Tuberculosis: from the disease to latent tuberculosis screening

As previously indicated, the first aim of this thesis was to summarize TB in Portuguese patients treated with anti-TNFα therapies. To reach this goal, first 25 cases of active disease from a population of 765 treated patients were reviewed. Then, the reintroduction of anti-TNF α therapy after TB diagnosis was followed.

The incidence of TB per 100,000 patient-years was estimated to be 1337, 792 and 405 for those on infliximab, adalimumab and etanercept therapy, respectively, and 60% of the cases were extra-pulmonary forms of TB.

From the 28 patients with TB associated with anti-TNF α drugs (25 published in 2013) eight of them restarted anti-TNF α therapy, three of them even during TB treatment, and no recurrence of TB was observed.

Two studies were conducted:

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

Journal of Crohn's & colitis 2013; 7:e 486-492.

Abreu C*, Magro F*, Santos-Antunes J, Pilão A, Rodrigues-Pinto E, Bernardes J, Bernardo A, Magina S, Vilas-Boas F, Lopes S, Macedo G, Sarmento A.

*These authors contributed equally in the design, conception, analysis and paper writing

4.2 Reintroduction of anti-TNF α therapy after (or even during) anti-TNF α associated tuberculosis in immune-mediated diseases

Journal of Crohn's & colitis 2016; 10:120-121.

Abreu C, Sarmento A, Magro F.

The following work was about latent TB in IBD patients: in the first study a prospective screening of latent TB (being the diagnosis based on a positive TST or/and QFT-GIT test in the absence of active disease) in 250 patients about to start or switch from anti-TNF α therapy was done. In the second study a prospective screening involved 46 patients treated with infliximab along 26 months. The diagnosis of latent TB was based on positivity of TST, TB.SPOT.TB and/or QFT-GIT tests that were applied six times across the study.

In the first study, 72 (29%) patients had a diagnosis of latent TB. When compared the performance of TST and QFT-GIT tests, TST had an overall higher sensitivity (81% versus 35%) and a higher Negative Predictive Value (93% versus 80%) irrespective of the presence and kind of backbone immunosuppressive therapy.

On the second study 16 from 46 patients convert at least one TB test during the follow-up of 26 months and again the TST had a higher sensitivity than the IGRA's tests; 15 of them were treated with isoniazid and none had a diagnosis of active TB.

Two additional studies were conducted:

4.3 The tuberculin skin test still matters for the screening of latent tuberculosis infections among inflammatory bowel disease patients.

Digestive and Liver Diseases 2016; 48: 1438-1443

Abreu C, Almeida F, Ferraz R, Dias CC, Sarmento A, Magro F.

4.4 Serial tuberculosis screening in inflammatory bowel disease patients under anti-TNF alpha therapy

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Abreu C, Afonso J, Dias CC, Ruas R, Sarmento A, Magro F.

4. Tuberculosis: from the disease to the screening of latent tuberculosis

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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

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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

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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

4. Tuberculosis: from the disease to the screening of latent tuberculosis

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.

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4.2 Reintroduction of anti-TNF α therapy after (or even during) anti-TNF α associated tuberculosis in immune-mediated diseases

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Letter to the Editor

Letter to the Editor

Reintroduction of Anti-TNFα Therapy After (or even During) Anti-TNFα-associated Tuberculosis in Immune-mediated Diseases

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Dear Editor

There is no consensus on whether it is safe to undertake retreatment with anti-tumour necrosis factor α (TNFα) drugs in patients in whom active tuberculosis (TB) has been associated with this therapy. Only a few cases of readministration of TNFα inhibitor after TB have been reported so far, and there are still no reports concerning other, more recent biological drugs, such as ustekinumab. The American College of Rheumatology guidelines state that TNFα inhibitors could be resumed for rheumatoid arthritis management after completion of anti-TB treatment if clinically relevant, though with a low level of evidence (level C). More recently, in Italy, SAFEBIO (safe biological therapy) consensus statements concerning rheumatology and dermatology patients requiring anti-TNFα treatment stated that, in the case of high disease activity, low-risk biologicals may be restarted after 2 months of TB induction therapy.[1] The rules for restarting anti-TNFα in inflammatory bowel disease patients are not clearly defined by the 2014 ECCO guidelines, although reintroduction of immunomodulators once TB responds to treatment has been considered.[2] The decision to restart anti-TNFα drugs depends on the potential risk of TB recurrence and the severity of the immune-mediated disease. Recently, in a Crohn's disease patient, the recurrence of TB was reported after its successful initial treatment, following the readministration of anti-TNFα therapy during TB treatment, resurrecting the need to answer this unsolved question.[3] Yet in patients with rheumatoid arthritis there are some data concerning the resumption of adalimumab/infliximab treatment to improve the paradoxical response to anti-TB therapy.[4]

In our cohort of 28 patients with TB associated with anti-TNFα drugs (25 published in 2013),[5] all patients discontinued TNF inhibitors when treatment of TB was started. We prospectively evaluated safety and outcome in 8 of these patients (6 with disseminated TB) who restarted anti-TNFα therapy: 5 after and 3 during TB treatment (Table 1). With a median follow-up period of more than 2.5 years (1–65 months), no recurrence of TB was verified. We noted a case of meningitis 1 month after the reintroduction of adalimumab. The work-up showed clear cerebrospinal fluid; a positive China ink test result was obtained but cryptococcal antigen and culture tests were negative. Adalimumab was stopped and the patient was successfully treated with antifungal therapy and ongoing TB treatment. Pneumococcal pneumonia and chronic myeloid leukaemia were diagnosed after the reintroduction of anti-TNFα in 2 patients, which dictated the change of therapy to ustekinumab and imatinib. In conclusion, the restarting of anti-TNFα drugs seems to be safe regarding tuberculosis risk, but prospective studies with large sample sizes and for a longer time are warranted to strengthen our results. Further studies are also required to establish the ideal time for the reintroduction of anti-TNFα therapy once TB treatment is established, and to find out whether secondary severe infections are more common after anti-TNFα reintroduction.

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Table 1. Restarting anti-TNF after or during TB treatment in patients with inflammatory diseases.

	Patient							
	1	2	3	4	5	6	7	8
Disease	AP	AP	CD	CD	CD	UC	AS	CD
Age at TB diagnosis (y)	53	65	57	34	32	25	45	24
Date of TB diagnosis	8/2007	4/2010	2/2011	12/2012	4/2012	3/2014	7/2009	5/2013
Ongoing therapy at TB diagnosis	INFX + CYCLP	ETNR + MTX	ADA	INFX	ADA	ADA	ADA	AZA + ADA
Clinical form of TB	Diss (G, U)	P	Diss (P, U, G)	P + G	Diss (P, PL, G, S)	Diss (P, S, G)	DIss (P, G, I)	Diss (P, Pl, I)
TB treatment (months)	9	9	12	9	12	9	8	9
Months without anti-TNF	17	11	10	12	20	5	8	7
Anti-TNF reintroduced	ADA	ETNR	ADA	INFX	INFX (+ AZA)	ADA	ADA	ADA
Time of follow-up (mo)	65 (stopped)	31 (stopped)	43	34	8	1 (stopped)	65	21
Health issues after reintroduction of anti-TNF	Pneumococcal pneumonia: stopped ADA, started ustekinumab	Chronic myeloid leukaemia: stopped ETNR, started imatinib				Meningitis (China ink positive): stopped ADA, started amphotericin + cytosine		

AP, psoriatic arthritis; CD, Crohn's disease; UC, ulcerative colitis; AS, ankylosing spondylitis; INFX, infliximab; ADA, adalimumab; CICLP, cyclosporin; ETNR, etanercept; MTX, methotrexate; AZA, azathioprine; Diss, disseminated; G, ganglionic; U, urinary; P, pulmonary; PL, pleural; S, splenic; I, intestinal.

4. Tuberculosis: from the disease to the screening of latent tuberculosis

4.3 T**he tuberculin skin test still matters for the screening of latent tuberculosis infections among inflammatory bowel disease patients.**

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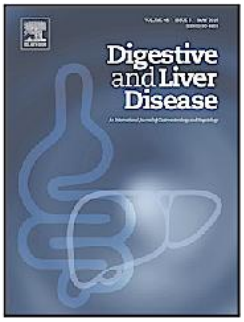

Title: The tuberculin skin test still matters for the screening of latent Tuberculosis infections among Inflammatory Bowel Disease patients

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4. Tuberculosis: from the disease to the screening of latent tuberculosis

Title: The tuberculin skin test still matters for the screening of latent Tuberculosis infections among Inflammatory Bowel Disease patients

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4. Tuberculosis: from the disease to the screening of latent tuberculosis

Abstract

Background and Aims

There is a high risk of Tuberculosis among patients medicated with anti-tumour necrosis factor α (anti-TNFα) that can be mitigated by treating latent Tuberculosis infections (LTI). This study aimed to evaluate the performance of Tuberculin Skin test (TST) and Quantiferon-TB Gold in Tube (QFT-GIT) in a population of patients suffering from Inflammatory Bowel Diseases.

Methods

The cohort analysed in this study consisted of 250 patients, of whom 15% were therapy-naïve and 85% were medicated: 70% under immunosuppressive therapy and 30% on anti-TNFα. A LTBI was diagnosed following a positive result in either of the tests and their performance and concordance were evaluated.

Results

Fifty-eight and 24 patients had a positive TST and QFT-GIT, respectively. In 72 (29%) patients LTBI was diagnosed, of whom 8 (21%) were therapy-naïve. TST had an overall higher sensitivity (81% *vs.* 35%) and a higher Negative Predictive Value (93% *vs.* 80%) when compared to QFT-GIT test; this superiority was consistently maintained irrespective of the presence and kind of backbone immunosuppressive therapies. The concordance between both tests was weak

Conclusions

Our results underscore the need to maintain the TST on LTBI diagnosis in patients about to start or switch anti-TNFα therapy in an intermediate Tuberculosis incidence context.

Keywords (max. 3): anti-TNFα; QuantiFERON-TB Gold In-Tube;

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Introduction

Inflammatory Bowel Disease (IBD) patients are frequently treated by suppressing their immune system activity, either through the intake of classical drugs – azathioprine (AZA), methotrexate (MTX) or steroids, among others – or biological anti-tumour necrosis factor α (anti-TNFα) medication, as for instance Infliximab (IFX) and Adalimumab (ADA). The latter ones are a well-known risk factor for the reactivation of latent Tuberculosis infection (LTBI). Portugal still presents an intermediate Tuberculosis (TB) burden, and therefore the possible reactivation of LTBI in anti-TNFα-treated patients poses a serious problem. An accurate screening of LTBI, followed by the prophylactic TB treatment should an infection be revealed, significantly reduces the risk of active disease. LTBI screening is currently performed using the classical Tuberculin Skin Test (TST) and/or the more recent and specific Interferon-ϒ release assays (IGRAs) tests, delivered in the form of commercial kits such as Quantiferon–TB Gold in-Tube (QFT-GIT) (Cellestis, Carnegie, Australia®) and T-SPOT.TB (Oxford Immunotec, Oxford, United Kingdom®). However, neither of these tests is considered to be a gold standard. Whereas both detect immunological evidence of host sensitization to *Mycobacterium tuberculosis* antigens, neither of them can differentiate between LTBI and active TB disease, nor distinguish a reactivation from a reinfection[1]. Besides, both tests have a low predictive value for the progression from LTBI to active TB[1], and neither of them is able to assess the progression of TB disease[2].

Tendentiously, the classical TSTs are being replaced by IGRA tests, in accordance to different recommendations concerning the LTBI diagnosis[2-5] and with the justification that the IGRA tests may improve the diagnostic accuracy[3,4]. The QFT-GIT detects an immune response against three mycobacterial proteins - early secretory antigenic target-6 (ESAT-6), culture filtrate protein-10 (CFP-10) and TB-7.7 (p4) - which are expressed by all *M. tuberculosis* and pathogenic *Mycobacterium bovis* strains, but not by the Bacille Calmette–Guerin (BCG) nor by the majority of non-tuberculous mycobacteria (with the exception of *Mycobacterium kansasii,*

4. Tuberculosis: from the disease to the screening of latent tuberculosis

Mycobacterium szulgai, *Mycobacterium flavescens* and *Mycobacterium marinum)*[4,6]. QFT-GIT tests quantify the antigen-specific IFN-ϒ secretion by the circulating T cells by performing an *in vitro* Enzyme-Linked Immunosorbent Assay (ELISA) in a whole-blood sample. In comparison, TST is considered to have a low sensitivity, particularly in patients under immunomodulatory therapy, as well as a low specificity for pathogenic *M. tuberculosis* complex strains, given the known cross-reactivity with the *Mycobacterium bovis* (used in the TB vaccination with BCG) and several environmental mycobacteria[5].

However, the sensitivity and performance of both TST and IGRA tests are dependent on a number of factors, such as the, different types of immunosuppressive/immunomodulatory therapies being followed by the patients at the time of the screening, and the TB incidence in the country or region where the patient lives. The World Health Organization (WHO) has recently published guidelines on the management of LTBI, where it is stated that either TST or IGRA can be used for LTBI screening in high-income and upper middle-income countries with an estimated TB incidence below 100 per 100 000[2].

This study aimed to evaluate the performance of TST (one or two-steps method) and QFT-GIT tests in the detection of LTBI among a cohort of 250 IBD Portuguese patients whom were either therapy-naïve or were under different immunosuppressive or immunomodulatory therapies.

Methods

Patients and therapies

The cohort analysed in this study was prospectively enrolled between 2012 and 2015 and consisted of 250 IBD adult patients (older than 18 years), whom were candidates to start or switch anti-TNFα therapy. According to the National Plan of Vaccination, all patients received the BCG vaccination at birth, and some of them were prescribed with one or more additional doses during childhood. All patients were asymptomatic for active TB and had a normal pulmonary X-ray. Demographic, clinical, and microbiological individual information was taken into account for all the patients, including active TB contact trace. Patients with a clinical history of treated LTBI or active TB were excluded from the study, as were HIV-infected patients. This study was approved by the local ethics committee.

A patient was considered to be under an immunosuppressive therapy if any of the following criteria were fulfilled: intake of steroids for a period equal of superior to two weeks; intake of thiopurines, MTX or cyclosporine for a period equal or superior to 2 months; prescription of anti-TNFα; intake of any two or three immunosuppressors associated with or in the absence of concomitant anti-TNFα prescription.

The study get the approval of the Ethical Committee of Centro Hospitalar São João.

Tests and LTBI diagnosis

The TST was performed according to the Mantoux procedure: 0.1 mL of tuberculin PPD (0.04 micrograms of Tuberculin PPD RT 23 – Statens Serum Institute, Copenhagen, Denmark, 2 T.U.) was injected in an intradermal fashion over the forearm. The transverse induration diameter was measured 48 to 72 h later. The TST result was considered to be positive if the induration was equal or superior to 5 mm. Patients that were under immunosuppressive therapy and that had an initial TST negative result were boosted by a second test two to three weeks later (2-steps TST), using the same positivity criteria as for the initial TST.

The QFT-GIT test was performed following the manufacturers' recommendations. In brief, a whole-blood sample was collected and divided among the three Quantiferon-TB Gold collection tubes: one of them contained heparin (the nil tube, or the negative control); another one contained the T cell mitogen phytohemagglutinin (the mitogen tube, or the positive control); and the third one contained the *M. tuberculosis*-specific antigens ESAT-6, CFP-10, and TB7.7 (the TB antigen tube). The tubes were shaken to ensure that the entire inner surface of the tube was appropriately coated with blood, and after a period not superior to 12 hours were incubated at 37ºC for 16 to 24 hours. The quantification of the interferon-gamma released was performed by testing the collected plasma samples with an enzyme-linked immunoassay. The final result was determined following the guidelines proposed by the Centre for Disease Control and Prevention and using the QFT-GIT analysis software. The result was considered positive if the nil was ≤8.0 and TB antigen minus nil was ≥ 0.35 UI/mL and ≥25% of nil, with any mitogen response; negative if the nil was ≤8.0 and TB antigen minus nil was <0.35 IU/mL, or <25% of nil with mitogen response <0.5; and indeterminate if nil ≤8.0 and TB antigen minus nil was <0.35 IU/mL or <25% of nil or if nil antigen was >8 IU/mL[4].

Positive TST or QFT-GIT tests were, alone or in combination, considered criteria for LTBI diagnosis. Both tests were performed at the same time point whenever possible, or the TST followed the QFT-GIT separated by a maximum period of 10 days.

Statistical analyses

Categorical variables were described through absolute and relative frequencies and continuous variables were described through the mean and standard deviation, or median and interquartile (IQR) range, whenever appropriate. The discriminative power of each test was assessed through the sensitivity and negative predictive value (NPV) regarding final diagnosis. The concordance between the two tests and between each test and the diagnosis were evaluated through the kappa value (with 95% confidence intervals-CI) and the global accuracy.

All the reported p values were two-sided, and p values of <0.05 were considered statistically significant. All data were arranged, processed and analyzed with SPSS ® v.23.0 data (Statistical Package for Social Sciences).

Results

Characterization of the cohort

The cohort under study, which was prospectively recruited, consisted of 250 Caucasian IBD patients of whom 81% were diagnosed with Crohn's disease (CD) and 19% were diagnosed with Ulcerative Colitis (UC) (Table 1). The median (IQR) time elapsed from diagnosis was 5 (1-10) years. The majority of patients were female (56%), and their median (IQR) age was 36 (27-46). The index event was the LTBI screening, elicited by the need to start or switch anti-TNFα therapy. At the beginning of the study, 38 (15%) patients were naïve to therapy and 85% were medicated: 70% were under immunosuppressive therapy (*i.e.*, azathioprine, methotrexate (MTX), steroids, cyclosporine or different combinations of these drugs) and 30% were on biologicals (*i.e.,* anti-TNFα, either alone or in combination with immunosuppressors).

LTBI screening

The LTBI screening of the 250 patients enrolled in this study resulted in 72 (29%) TST and/or QFT-GIT positive test results. Overall, the QFT-GIT was positive for 24 (10%) of the patients (6 had an indeterminate result), and the TST was positive for 58 (23%) of the patients (Table 2). TST had ten or more millimetres of induration in 41 (72%) of the patients (data not shown). Ten patients (4%) had a double positive result and 175 (70%) had a double negative result. From the six patients with an indeterminate QFT-GIT test result (three were medicated with azathioprine and one with steroids), three were TST positive and three were TST negative. Sixty-six patients were boosted during the TST screening, out of which four (6.1%) turned out to be positive (Table S-1).

The distribution of TST and QFT-GIT positive test results upon stratifying patients according to their therapeutic regimen reveals that a LTBI diagnosis was made for 21% of the therapy-naïve patients, 31% of the patients medicated with a single drug and 26% of the

patients medicated with two drugs (Table S-1). Concerning the specific therapeutic regimens, the highest rate of LTBI diagnosis was made among AZA-medicated patients (40%), followed by patients medicated with AZA and steroids (29%). The other high diagnosis rates correspond to therapies involving a small number of patients, and may therefore not be indicative. The percentage of patients with a positive TST test result was consistently higher than that of patients that tested positive for QFT-GIT across all different drug regimens.

Performance of TST and QFF-GIT tests

For the sake of this study, a LTBI diagnosis was made for all patients that had either a TST or a QFT-GIT positive test result, and the sensitivity and negative predictive values (NPV) of both tests were computed assuming this premise (Table 3). Overall, TST had an 81% sensitivity, whereas that of QFT-GIT was 35%. Considering only therapy-naïve patients, sensitivity values were 75% and 50% for TST and QFT-GIT, respectively. These values varied widely upon stratifying patients according to their therapeutic regimen. In fact, TST sensitivity ranged from 50% (eight patients under a low dosage of steroids) to 100% (patients receiving steroids irrespective of the dosage), whereas QFT-GIT sensitivity ranged from 0% (patients receiving steroids irrespective of the dosage) to 50% (eight patients under a low dosage of steroids). With the exception of the patients medicated with a low dosage of steroids, the TST sensitivity was always superior to that of QFT-GIT. Accordingly, so were the rates of true negative results (NPV), the accuracy, and the Kappa measurement of concordance with diagnosis (Table 3). Interestingly, the 95% CIs of the accuracy and Kappa (to the diagnostic) for each therapeutic regimen were always overlapping those of therapeutic-naïve patients, which suggest that the therapies being followed by the patients in this cohort do not affect the performance of TST and QFT-GIT.

A comparison of TST and QFT-GIT results resulted in an overall slight agreement (Kappa: 0.135 [0.002-0.268], Table 3), according to the interpretation of kappa values defined

by Landis and Koch[7]. This agreement was fair for therapy-naïve patients (Kappa= 0.313 [0.102-0.729]). However, there were many therapeutic regimens for which no agreement could be found (Table 3)

Increasing the TST threshold positivity to 10 or more millimetres of induration, the accuracy for diagnosis is 88%, and the agreement between the two tests increased: a Kappa of 0.216 [0.057-0.374] was the result for the overall cohort, whereas a Kappa of 0.371 [0.068-0.810]) and 0.453 [0.218-0.688] were the results for the therapy-naïve patients and those medicated with a single immunosuppressor, respectively (data not shown).

Discussion

The diagnosis of LTBI is extremely important for patients suffering from autoimmune disorders and elected for anti-TNFα therapy, as this treatment is known to be a risk factor for TB development. The detection of a LTBI is particularly challenging as there are no gold standard test for the diagnosis and the incidence of tuberculosis is not low, such is the case of Portugal. This study assessed the performance of two tests – TST and QFT-GIT – for the LTBI diagnosis in a cohort consisting of 250 IBD patients, of which 85% were already under immunosuppressed or immunomodulatory therapies. The criteria for LTBI diagnosis was the "either test positive strategy", as recommended by several orientations including ECCO (European Crohn and Colitis Organization) and NICE (National Institute for Health and Clinical Excellence guidelines) [8,9]. Following this approach, 79 (29%) patients were diagnosed with LTBI, of which 59 patients tested positive for a single test. This is a rather high prevalence when compared with other studies[10,11]. However, the incidence of TB in the city of Porto, where the care center that recruited the cohort was located, was of 46.3 cases per 1000000 inhabitants (data from 2014[12]), a value that is considerably higher than the national TB incidence (21.8 cases per 100000 habitants). Remarkably, LTBI diagnosis did not seem to be affected by the concomitant immunosuppressive or immunomodulatory therapies, as the 95% CIs for the tests accuracy and kappa regarding therapeutic-naïve patients overlapped those of patients following the different therapies. These results are supported by the literature, namely by the work of Kurti *et. al,* whom reported the rates of LTBI diagnosis in a cohort of Hungary adults that were vaccinated with BCG at birth, 6 and 14 years-old[13]. Their results were similar to ours, and suggested that immunosuppression does not have a significant impact on either IGRA or TST positivity rates, a conclusion also present in the results from Mantzaris et. al[14]. Notwithstanding, this issue is still debatable in the literature: several studies can be found reporting the anergy of IBD patients receiving immunosuppressive or anti-TNFα in response to TST[15], and TST, *Candida* and tetanus antigens[16]. Moreover, in a population with a high

incidence of TB, patients with rheumatoid arthritis medicated with modifying anti rheumatic drugs (DMARD's) or steroids were shown to have significantly less induration after a TST when compared to controls and more negative results on TST[17].

Overall, TST had a higher sensitivity for the detection of LTBI compared to that of QFT-GIT (23% cases detected by the former *vs.* only 10% by the latter), irrespective of the presence of immunosuppressive therapy, a result that is supported by other studies with similar conclusions[18,19]. Even if we choose a cut-off for TST of ten or more millimetres of induration TST had a higher sensitivity compared to that of QFT-GIT (17% cases detected by TST and 10% by QFT-GIT). The BCG vaccination at birth may elicit a positive TST result in the absence of infection, and could therefore be responsible for a number of positive TST results. However, and for most patients included in this cohort, more than 20 years have elapsed since the childhood vaccination, and a relevant effect of BCG on TST positivity after such a period of time is unlikely[20]. Accordingly, Wang *et. al* concluded that even if BCG inoculation took place after infancy, a positive TST result more than 15 years after vaccination is infrequent (relative risk 0.8; 95% CI: 0.74 to 0.85)[21]. Anyway, it is known that tuberculin reactions wane more slowly in subjects vaccinated with BCG after the first year of life[22,23], and a persistent tuberculin reaction may be still observed several years after. On the other hand, a positive TST result due to sensitivity to antigens of non-tuberculosis mycobacteria appears to be rare, especially outside warm climates[20,22,23] and, except for populations with a high prevalence of these atypical mycobacteria sensitisation and a very low prevalence of TB infection, is not an relevant cause of positive TST[23].

Finally, the boosting of TST was performed in 66 patients, and 4 (6,1%) of them had a positive result. This proportion is similar to the results of other series. In a cohort of 143 patients with rheumatoid arthritis[24], 84 patients repeated TST after an initial negative test, which was positive in 9 (6,3%). In another cohort of 106 patients with rheumatoid arthritis[25], two-step "boosted" TST was performed in 81 and gave a positive result in 9 (6,9%).

Although IGRA tests are considered to be more specific in what comes to LTBI diagnosis than TST, being unaffected by BCG vaccination, they are nevertheless unstable and present a considerable degree of variability, as it has been shown in several studies[26]. Moreover, IGRA results can also be negatively influenced by immunosuppression[8,11,27]. In our cohort, six (2.4%) patients had an indeterminate QFT-GIT test result. The use of steroids might be a possible explanation for this, as a high risk for indeterminate QFT-GIT results is associated with mitogen anergy[28,29]. Moreover, the literature refers that the rate of indeterminate responses is usually above 10% in patients medicated with biologics[30]. Others situations that may yield indeterminate results are: incorrect filling/mixing of the mitogen tube, lymphopenia, reduced lymphocyte activity due to inadequate specimen handling, or incapacity of the patient's lymphocytes to produce IFN-γ[18]. As the repetition of an indeterminate QFT-GIT test one month later is likely to yield a second indeterminate response (in 83.3% of the cases)[31], we have chosen not to repeat the QFT-GIT test in these situations.

The concordance between TST and QFT-GIT results was globally poor (Kappa lower than 0.2). Although it was slightly better for therapy-naïve patients (fair agreement, Kappa=0.313), there were many therapeutic-defined sub-groups with a null agreement between the tests, given the absence of QFT-GIT positive results. This agreement can be increased by lowering the TST sensitivity, *i.e.* considering a positive result only for indurations equal or above 10 millimetres.

A crucial aspect of our analyses is the definition of an "either test positive strategy" to diagnose LTBI. In fact, if each test was to be considered independently, the TST would diagnose LTBI in at least twice the number of patients as would the QFT-GIT test. Basing the LTBI screening on the IGRA test alone, as is currently suggested by several orientations[5] and recent WHO guidelines, would result in a LTBI diagnosis in only 24 patients, leaving 48 possibly-infected patients without the respective prophylactic TB treatment. Whether at least a few of them were actually infected and would suffer a reactivation during anti-TNFα therapy is a key

aspect to be considered. In our experience[32], TB development remains an unsolved problem in patients treated with immunosuppression therapies, namely those of a biological nature. For that reason, we believe that the safer approach is to favour a higher sensitivity, using the TST and an IGRA test and the "either test positive strategy" to diagnose LTBI, even at the expense of treating a number of uninfected patients. Such a consequence would be less serious than leaving a few infected patients untreated, potentiating TB reactivations – a possible scenario we may have to face should the TST becomes unavailable or exclusively replaced by an IGRA test.

This study had a few strengths that we hereafter highlight: the cohort was uniform in what comes to demographic issues - namely ethnicity, age, BCG vaccination and IBD diagnosis - therefore eliminating some potentially confounder factors. However, there were a number of limitations that should also be acknowledged: the low number of therapy-naïve patients precluded more assertive conclusions on the effect of immunosuppression on both tests' positivity; and the inexistence of a gold standard reference test to diagnose LTBI, which would enable the identification of false positives and false negatives results.

Given the high risk of TB reactivation in immunosuppressed patients, namely those medicated with anti-TNFα drugs, we believe that in this particular context a higher sensitivity should be favoured at the expense of a lower specificity in the detection of a LTBI. From our results, we may conclude that the utilization of the TST alone or in combination with an IGRA test is the best strategy to screen LTBI in patients suffering from IBD and candidates for a biological treatment. Whereas the need to develop better LTBI screening methods – insensible to past exposures and BGC vaccination – is a undeniable need in the field, the present context suggests that the elimination of the classical TST would be precocious and likely to have a negative impact in the screening of immunodepressed patients. In other contexts, where a higher specificity is the goal, IGRA tests alone would be useful by avoiding false positive results and the unnecessary treatment of uninfected patients. We believe our results are valid in

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countries with an intermediate TB prevalence and where the BCG inoculation is mandatory according to the National Vaccination Plan.

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Specific author contributions:

The authors made the following contributions to the development of this article:

Conception and design of the study – Cândida Abreu; Fernando Magro

Acquision and interpretation of Data –Francisco Almeida; Rita Ferraz; C. Camila Dias; Cândida Abreu

Drafting the article – Cândida Abreu; Fernando Magro

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Conflict of interests:

All authors have no competing interests to declare.

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1 **Table 1.** Cohort characterization

	n (%)
Female	141 (56%)
Male	109 (44%)
Age (years) – median (IQR)	36 (27-46)
Time elapsed from IBD diagnosis - median (IQR)	5 (1-10)
CD patients	203 (81%)
UC patients	47 (19%)
therapy-naïve patients – n (%)	38 (15%)
Patients receiving immunossupressors – n (%)	148 (59%)
AZA	75 (30%)
Steroids	25 (10%)
AZA+ Steroids	45 (18%)
MTX	1 (0.4%)
AZA + steroids + MTX or cyclosporine	2 (0.8%)
Patients receiving biologicals – n (%)	64 (26%)
Anti- TNFα	20 (8%)
Anti- TNFα + 1 or 2 immunossupressors	44 (18%)

2 IQR – interquartile range; CD- Crohn Disease; UC – ulcerative disease;
3 AZA – azathioprine; MTX - methotrexate

Table 2. TST and QTF-GIT tests results across the patients (244)[a]

	QFT-GIT		
TST	**Positive**	**Negative**	**Total** - n (%)
Positive	10	45	55 (23%)[b]
Negative	14	175	189 (77%)[c]
Total - n (%)	24 (10%)	220 (90%)	244

[a]six patients were excluded for having an indeterminate QFT- GIT.

[b]adding 3 patients with indeterminate QFT-GIT: 58 (23%) TST positive patients

[c]adding 3 patients with indeterminate QFT-GIT: 192 (77%) TST negative patients

Table 3. TST and QFT-GIT tests' performance stratified by therapeutic regimen.

Therapeutic regimen (n)	Tests	Sens (%)	NPV (%)	Accuracy (%)	Kappa (%) (CI 95%)	Kappa between tests (%) (CI 95%)
all (250)	TST	81	93	94% [91%-96%]	0.855 [0.780; 0.923]	0.135 [0.002-0.268]
	QFT-GIT (n=244)	35	80	81% [76%-85%]	0.433 [0.311; 0.555]	
therapy-naive (38)	TST	75	94	95% [88%-100%]	0.826 [0.594; 1.00]	0.313 [0.102-0.729]
	QFT-GIT	50	88	89% [79%-99%]	0.612 [0.281; 0.943]	
AZA (alone or in association) (159)	TST	82	91	93% [89%-97%]	0.855 [0.769; 0.941]	0.114 [0.0-0.265]
	QFT-GIT (n=157)	33	74	77% [70%-84%]	0.396 [0.259; 0.533]	
AZA + steroids (45)	TST	69	89	91% [83%-99%]	0.762 [0.546; 0.978]	0 [-]
	QFT-GIT	31	78	80% [68%-92%]	0.387 [0.105; 0.670]	
AZA + steroids (>10mg/day of prednisolone) (41)	TST	77	90	93% [85%-100%]	0.820 [0.628; 1.000]	0 [-]
	QFT-GIT	23	74	75% [62%-88%]	0.291 [0.022; 0.559]	
steroids (25)	TST	100	100	100% [-]	1.000 [-]	0 [-]
	QFT-GIT (n=21)	0	91	91% [79%-100%]	0 [-]	
steroids (≥10mg/day of prednisolone) alone or in association (66)	TST	78	92	94% [88%-99%]	0.836 [0.685;0.987]	0 [-]
	QFT-GIT	24	78	79%	0.310	

	(n=63)			[69%-89%]	[0.069; 0.551]	
steroids (<10mg/day of prednisolone) alone or in association (8)	TST	50	67	75% [45%-100%]	0.500 [0.019; 1.000]	0 [-]
	QFT-GIT	50	67	75% [45%-100%]	0.500 [0.019; 1.000]	
AZA or steroids or MTX (101)	TST	91	96	97% [94%-100%]	0,932 [0,855;1,000]	0.293 [0.093-0.493]
	QFT-GIT (n=95)	36	76	79% [73%-85%]	0,426 [0,243;0,608]	
anti-TNFα (20)	TST	67	94	95% [85%-100%]	0.773 [0.309; 1.000]	0 [-]
	QFT-GIT	33	90	90% [77%-100%]	0.459 [0.121; 0.609]	
Anti-TNFα (alone or in association) (64)	TST	75	92	93% [87%-99%]	0,818 [0,647;0,988]	0 [-]
	QFT-GIT	25	80	81% [71%-91%]	0,333 [0,080;0,586]	
anti-TNFα + AZA or steroids or MTX (35)	TST	88	96	97% [91%-100%]	0.915 [0.750; 1.000]	0 [-]
	QFT-GIT	13	79	80% [67%-93%]	0.179 [0.126; 0.484]	

Sens – sensitivity; NVP – negative predictive value; AZA - azathioprine; MTX – methotrexate;

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4.4 Serial tuberculosis screening in inflammatory bowel disease patients under anti-TNF alpha therapy

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Serial Tuberculosis screening in inflammatory bowel disease patients under anti-TNFα therapy

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Short title: Serial TB screening during anti-TNFα therapy

Abbreviations:

anti-TNFα: tumour necrosis factor-α monoclonal antibodies

AZA: azathioprine

CD: Crohn's disease

IBD: inflammatory bowel diseases

IFN-ϒ: interferon-gamma

IGRA: interferon-ϒ release assays

LTBI: latent tuberculosis infection

MTX: methotrexate

NICE: National Institute for Care and Excellence

QFT-GIT: Quantiferon gold in-tube

SFCs: spot-forming cells

TB: tuberculosis

TST: tuberculin skin test

UC: ulcerative colitis

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Summary(230 words)

Background and aims

One of the adverse effects of the tumour necrosis factor-α monoclonal antibodies for the treatment of immune-mediated inflammatory diseases is a higher propensity for tuberculosis development. The aim of this study was to explore the utility and sensitivity of serial tuberculosis screening during anti-TNFα treatment.

Methods

A cohort of 46 inflammatory bowel disease patients on infliximab was prospectively recruited and followed for 26 months. During this period of time, a tuberculosis skin test and two different interferon Ɣ release assays (QFT-GIT and T-SPOT.TB) were applied with four to six months of interval.

Results

Overall, 16 patients were diagnosed with latent tuberculosis infection after having at least one test conversion: 12 patients had a positive tuberculosis skin test, seven patients had a positive T-SPOT.TB and two patients had a positive QFT-GIT. Active tuberculosis was excluded in all; fifteen were treated with isoniazid. A comparison between tests showed a moderate accuracy (72% to 85%) but low kappa values (0.063 to 0.377). Concerning association to demographic and clinical characteristics, test conversion was more common among the male gender and those with a longer disease duration.

Conclusions

Tuberculosis tests conversions were common in inflammatory bowel disease patients treated with infliximab, alone or in association with immunomodulators. In these

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immunosuppressed individuals the classical tuberculosis skin test seems to have a higher sensitivity than the modern tests based on the release of interferonϒ.

Keywords: Tuberculin skin test; interferonϒ release assay; tuberculosis; infliximab; inflammatory bowel diseases.

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Introduction

The introduction of tumour necrosis factor-α monoclonal antibodies (anti-TNFα) in the therapeutic armamentarium against immune-mediated inflammatory diseases has improved the prognosis and health-related quality of life of patients afflicted with these diseases. However, anti-TNFα therapy is not exempt of risks, one of which is an increased propensity towards tuberculosis (TB) development. Among the different anti-TNFα currently approved for the treatment of immune-mediated inflammatory diseases, infliximab and adalimumab pose the highest risk for TB development[1,2]. Previous TB screening and adequate treatment upon evidence of a latent TB infection (LTBI) are crucial steps to prevent a TB reactivation during anti-TNFα therapy[3] and are now the standard of care. These actions are known to reduce the TB incidence by up to 85% in persons treated with anti-TNFα[4,5].Nevertheless, TB cases still occur in anti-TNFα-treated patients[2]. These cases might be explained by either failures in the previous LTBI diagnosis or treatment, or by incidental new infections. In what concerns LTBI diagnosis failures, it is important to stress that LTBI tests are far from perfect: both the classical tuberculin skin test (TST) and the more specific *Mycobacterium tuberculosis* interferon-γ release assays (IGRAs) are known to have a suboptimal performance particularly among immunosuppressed individuals[6-8]. Moreover, LTBI treatment adherence may be poor, and new infections can occur, particularly in countries with intermediate or high TB incidence rates[5,9].

Repeating the TB screening during the anti-TNFα therapy seems like a rational approach to minimize this issue. However, its utility and the best screening method (considering that the patients involved have been tested before) remain controversial[5,9,10]. The number of longitudinal studies assessing the performance of TB rescreening in patients receiving anti-TNFα therapies is rather limited[8,11], and both the interpretation of IGRA results and the criteria for LTBI treatment among individuals on anti-TNFα is yet to be established[12].

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This study was designed to evaluate the usefulness of TB serial rescreening – using TST and two different IGRA tests - among a population of inflammatory bowel disease (IBD) patients on infliximab therapy. The main goals were: a) to detect the frequency of IGRA conversions and reversions and evaluate its agreement with the TST results; and b) to evaluate if and how TB re-screening may contribute to a better and more accurate TB diagnosis and control. To do so, a population of inflammatory bowel disease (IBD) patients on infliximab was prospectively evaluated at defined time points to test the presence of LTBI using three different methods - two IGRA tests (T-SPOT.TB and Quantiferon gold in-tube [QFT-GIT]) and the TST – for a follow-up time of 26 months.

Material and Methods

Cohort and study design

This study involved IBD patients being treated with infliximab and prospectively and consecutively recruited from the outpatient department of a community hospital in the northern region of Portugal during July-August 2013. As inclusion criteria, patients were required to be over 18 years old and to have a negative test for TB before starting the anti-TNFα therapy (*i.e.*, a negative TST [boosted or no boosted] and chest-radiograph and, for patients starting anti-TNFα after 2011, a negative QFT-GIT test result). Patients with a previous TB positive screening and/or TB therapy and with a known contact with active tuberculosis patients were excluded from this analysis, as were women that were pregnant at recruitment. A few patients declined to participate as they were working abroad.

The patients' baseline assessment included a clinical interview, a chest radiograph and two TB screening tests: a TST and an IGRA test, the T-SPOT.TB. These two tests were repeated after 4, 8, 14, 20 and 26 months. A second IGRA test, the QFT-GIT, was performed at the 4th,8th and 20th month of follow-up.

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This study was approved by the Hospital Ethics Committee and all patients gave a written informed consent prior to their participation.

Tests application and interpretation

TST (Tuberculin PPD RT 23SSI, Statens Serum Institute) was performed according to the Mantoux method and an induration ≥5 mm was considered to be positive. A TST conversion was described as an induration of ≥5mm after an initial negative TST.IGRA tests (QFT-GIT [Cellestis Limited, Carnegie, Victoria, Australia] and T-SPOT.TB [Oxford Immunotec, UK]) were performed and interpreted according to the manufacturer's instructions by trained technicians that were blinded to the TST results. Briefly, and concerning the QFT-GIT test, the IFN-γ cut-off was 0.35 IU/mL, and each sample result was calculated by subtracting the negative control from the TB antigen-stimulated sample only and if the negative control was below 8.0 IU/mL. Whenever the IFN-γ response in the TB antigen-stimulated sample was below 25% of the negative control, the result was considered to be negative irrespective of its relation to the cut-off. Test results with a negative control over 8.0 IU/mL or a positive control less than 0.5 IU/mL were considered to be indeterminate. Concerning the T-SPOT.TB tests, the results were interpreted by subtracting the spot-forming cells (SFCs) count in the negative control from that of the sample stimulated by the ESAT-6 and CFP10 antigens. Samples were considered to be positive when ESAT-6 or CFP-10-stimulated wells had at least six spots over the number of spots found in the negative control, whenever the negative control had 10 spots or fewer. If the negative control well had more than 10 spots and/or there were less than 20 spots in the mitogen positive control wells, the result was considered to be indeterminate, according to the criteria explicited by the manufacturers version1 (TG-TB-UK-V1).

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Diagnosis criteria

LTBI was diagnosed upon the conversion of at least one of the TB tests in the absence of clinical, radiological and microbiological evidence of disease. Patients diagnosed with LTBI were treated with isoniazid, according to the usual proceedings. Moreover, and to rule out the hypothesis of active TB, these patients were subjected to a clinical interview, a chest–radiograph, a thorax CT scan and, whenever possible, sputum was collected and examined to assess the presence of *Mycobacterium spp*.

Statistical Analysis

Categorical variables were described through absolute and relative frequencies and continuous variables were described as mean and standard deviation, median, percentiles, minimum and maximum. The concordance between the three tests was analysed using the Cohen's kappa coefficient - kappa values >0.75 were considered to represent excellent agreement beyond chance, values 0.40–0.75 were considered to represent fair to good agreement beyond chance, and values <0.40 were considered to represent poor agreement beyond chance – and the global accuracy. All the reported p values were two-sided, and p values of <0.05 were considered statistically significant. All data were arranged, processed and analyzed with SPSS ® v.23.0 data (Statistical Package for Social Sciences).

Results

Cohort characterization

Forty-eight Portuguese patients with a good health condition and from a similar place of living were initially enrolled in the study, but two were later excluded: one due to lost to follow-up after the second screening, and the other because he was found to have been treated for LTBI before. From the 46 patients that composed the final cohort (Table 1), 61% were female, 85% had Crohn's disease (CD), 15% had ulcerative colitis (UC), and 72% were on

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immunosuppressive drugs other than infliximab (steroids, azathioprine [AZA], methotrexate [MTX], or steroids combined with AZA or MTX). Patients median age was 38 years, and they had been diagnosed in median 8.5 years before their enrolment in this study. Fifteen patients were on infliximab for less than a year, 16 were on infliximab for a period between one and three years, and 15 had more than three years of infliximab therapy. In accordance with the Portuguese National Vaccination Plan, all patients were BCG vaccinated at birth; for a few of them, this vaccine was repeated during childhood.

Test results

Overall, 649 TB screening tests were performed: 276 were T-SPOT.TB, 138 were QFT-GIT and 235 were TST. Of those, 32 (4.9%) had a positive result: 14 (6.0%) TSTs, 15 (5.4%) T-SPOT.TB and three (2.2%) QFT-GITs (Table 2). From a patients' perspective, 16 (34.5 %) had at least one positive test result along the 26 months of the follow-up, being therefore diagnosed with LTBI. Twelve (75%) patients were LTBI-diagnosed after a positive TST; seven (43.8%) patients were LTBI-diagnosed after a positive T.SPOT.TB; and only two (12.5%) patients were LTBI-diagnosed after a positive QFT-GIT test result (Table 3). In 15 (out of the 16) patients the test conversion occurred up to the 8thmonth of the follow-up (Tables 2 and 3). A comparison between the three tests was carried out to determine their LTBI diagnosis accuracy, and the results are depicted on Table 4: TST and T-SPOT.TB had the highest kappa (0.377) and an intermediate accuracy (78%), whereas T.SPOT.TB and QFT-GIT had the highest accuracy (85%) with a kappa of 0.166. The pair TST and QTF-GIT had the lowest kappa (0.063) and accuracy (72%).

Concerning the evolution of IGRA test results, two of the patients who tested positive with T.SPOT.TB maintained a positive (or borderline) response to this test in the following screenings. Regarding QFT-GIT, the patient that tested positive at the 4th month had another positive result at the 20th month, although a negative response was reported at the 8th month.

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TSTs were not repeated after displaying an induration of 10 mm or higher in accordance to the tuberculin guidelines administration[13].

Table 1 shows a comparison of the patients diagnosed with LTBI with those whom TB screening tests remained negative along the entire follow-up: the former had a significantly higher percentage of male patients as well as a longer IBD evolution (10.6 *vs.* 6 years).

Clinical evaluation and follow-up of LTBI-diagnosed patients

The 16 patients diagnosed with LTBI after a TB screening test conversion were immediately evaluated: all of them were asymptomatic and, to their knowledge, had no contact with an active TB patient. The chest X-ray and thorax CT scan did not reveal lesions. As so, all these patients were maintained on infliximab. Concerning LTBI therapy, 15 patients were started on isoniazid and pyridoxine for a predicted period of nine months[14,15]. Patient number 2 (Table 2) had a single positive test result (6 SFCs in the T-SPOT.TB) and was asymptomatic, being excluded from the LTBI treatment. Fourteen patients completed the nine months on isoniazid without any remarkable clinical or analytical intolerance; a single patient interrupted the prophylactic therapy after four months due to gastrointestinal intolerance. No case of active TB was reported among the 46 included patients during the entire follow-up period.

Discussion

Anti-TNFα-associated TB cannot be fully prevented with TB screening before treatment[16] .At the time being, the best procedure to tackle this issue remains unknown. In this study, 46 adult IBD patients on infliximab were followed for 26 months, during which the classical TST and two different IGRAs were serially applied. The first three screening points were done with a 4 months-interval, whereas the latter three were done with a 6 months-interval.

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Remarkably, 16 (34.5%) of these patients had at least one positive TB screening test result during the 26 months of the follow-up. It should be noticed that, besides infliximab, most patients (72%) were on concomitant therapies (steroids and immunomodulatory drugs), known to affect the TB screening tests' performance[8]. In most cases (12), the initial test conversion included a positive TST, whereas only 7 and 2 were positive for TSPOT.TB and QFT-GIT, respectively. There results were similar to the ones reported by Hatzara *et al.*, whom described 29% of TB tests' conversions in a population of 70 patients with rheumatic diseases after 12 months of anti-TNFα therapy [17]. Moreover, these authors also reported that positive results were more frequently found among TST than among IGRA tests. In this study, the differences between the test results are perceptible in the low kappa values and moderate accuracies found upon comparing them.

Patients diagnosed with LTBI were more frequently male and had IBD for a longer time than those whom TB screening tests remained negative throughout the follow-up. We wonder if IBD stability after a long medication period could somehow rescue part of the immune function, contributing to LTBI test conversion[18].The reasons for the higher conversion ratio among men are also not entirely clear. However, the results reported here are similar to those published by Cuomo *et al*, who found that the male gender was associated to a higher risk of TB conversion in an Italian cohort of patients with rheumatoid arthritis[19]. Interestingly, women are known to be prone to a deeper immunossupression - related to physiological aspects of pregnancy - and anergy to delayed hypersensitivity reactions is more commonly observed among women than men[20].

The classical TST has been pointed out as limited in some aspects One of them is the so-called boosting effect potentially elicited by repeated TSTs: according to this effect, the number of positive TSTs should increase with the number of repetitions, and therefore over time (in this study). However, 11 out of the 12 TST conversions occurred in the initial eight

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months of the study, and a single conversion occurred in the following 15 months. It should also be noticed that patient number 7 had a six mm induration at the 4thmonth, being therefore a good candidate for a "boosted" effect. However, all his following TSTs were negative. Accordingly, several studies agree that a boosting effect is uncommon when more than two months have elapsed between tests[21], and this study involved TST repetitions with four to six months of interval between them.

Another potential limitation of the TST is the fact that false positives may occur among patients whom have been vaccinated with BGC at birth, such as the patients enrolled in this cohort. However, it should be noticed that all patients had at least one negative TST before being placed on anti-TNFα therapy. Moreover, it has been argued that the effect of the BCG vaccination on the positivity of TST in adults aged more than 30 years is negligible[22], and eight of the 12 patients that had a positive TST in this study were indeed over 30. In fact, the 2016 National Institute for Care and Excellence (NICE) tuberculosis guidelines stated that, according to 14 different studies exploring the association between test positivity and BCG there was no strong evidence of an association between BCG vaccination history and TST positivity, indicating that the impact BGC vaccination on test positivity could be similar for IGRA and TST[23]. Finally, TST is not as specific as IGRA, and may have positive results following infections with atypical *Mycobacteria*. Still, this effect seems to be irrelevant in non-tropical areas[13,24], which is the case of the study reported here.

One should also consider the possibility that TST, which includes TB antigens that are present on the IGRA tests, leads to false positive results in the latter due to yet another boosting effect. This question was addressed in a previous systematic review that suggests the boosted effect seen when the QFT-GIT was performed over three months after TST administration was waned, although further studies are required to validate this conclusion[25].

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In what concerns T-SPOT.TB, a large study published in 2006 reported the absence of a boosting effect in up to two years after TST testing[26].

IGRA tests are more recent and have a few advantages over the TST - namely its specificity - for the LTBI diagnosis in the general population. However, and when considering immunosuppressed individuals, the picture is not as clear[27].In fact, anti -TNF therapies reduce the production of inflammatory cytokines like interferon-gamma(IFN-γ), interleukin-1, and TNF-α from T lymphocytes[28]. As the IGRA tests are precisely based on the quantification of the IFN-γ released after ESAT-6, CFP-10 and TB7.7 stimulation, the inhibition of IFN-γ production in anti-TNFα treated individuals may yield false negative and indeterminate results. In fact, a meta-analysis published by Wong *et al* has shown a markedly negative impact of steroids, oral immunomodulatory drugs and biological therapies on IGRA results[29]. This effect was apparently more relevant for the QFT-GIT than for the T-SPOT.TB. In this study, the number of positive results obtained with the latter was three-fold higher than that obtained with the former (7*vs.* 2). Still, QFT-GIT was performed in only three out of the six screening time points, which may have introduced some bias in the results. Nevertheless, the low number of positives obtained with the IGRA tests when compared to the TSTs, together with the borderline and indeterminate results, supports the idea that IGRA tests alone may not be the best choice to monitor TB in immunosuppressed individuals. It remains to be determined whereas the utilization of different cut-offs could improve these tests' performance in this specific population.

The analysis of the evolution of the IGRA test results after starting isoniazid therapy was limited by the small number of positive tests. Whereas positivity was maintained in a few patients, others reverted to negative test results, and no clear pattern could be established. Interestingly, in another recent study, the authors have failed to find the expected decrease in IFN production upon LTBI antibiotic therapy[30].

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One important limitation of this study is the fact that one cannot compare its results to the general population, as there are no similar studies performed in the latter. In fact the only healthy population group on whom serial testing of tuberculosis is performed are the healthcare workers (HCWs), who have a justifiable high risk of tuberculosis. One manuscript published in 2011 with data from HCWs working in our hospital found a conversion rate of 30. 7% for a second TST (increase of >10mm) and 11% for a second QFT test in a cohort of 670 HCWs (data was collected during 2007 to 2009)[31] Moreover, a third QFT test presented a conversion rate of 6.6%. However, the authors state that, at that time, the annual incidence of active tuberculosis in HCWs (192 per 100 000) was about six times higher than that in the general population in Portugal, so one can hardly extrapolate these results and compare the risk of HCWs with the risk of anti-TNF prescription.

Briefly, this study revealed a remarkably high number of positive TB results among a population of IBD patients being treated with infliximab with or without concomitant immunomodulatory drugs and steroids. TST had the highest ratio of positive results, followed by T-SPOT.TB and QFT-GIT: overall, test results were non-concordant between them. Male patients and those that had a longer disease evolution were more prone to test conversions. This suggests that re-testing should be tailored and focused on those more likely to develop TB. Still, the optimal timing and sensitivity of these re-testing procedures, as well as the most suitable IGRA test and respective cut-off for immunosuppressed patients and how to valorise the reversions and conversions of the test results are to be determined: larger, prospective and long-term studies are needed in order to do so. Meanwhile, and given the low ratio of positive results obtained from IGRA tests in the setting of retesting for TB, TST should likely be maintained as it offers a more high-sensitivity strategy, that minimises false negatives.

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Conflict of interests:

FM served as speaker and received honoraria from Merck Sharp & Dohme, Abbvie, Vifor, Falk, Laboratorios Vitoria, Ferring, Hospira and Biogen.

CA served as speaker and received honoraria from Pfizer, Janssen and Biogen

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Table 1 – Patients characteristics of converters and non-converters of TST and IGRA tests (T-SPOT.TB and QFT-GIT) along 2 years of the study

Characteristics	Total (n=46[#])	Converters (n=16)	Non Converters (n=30)	P value
Gender – female/ male	28/18	6/10	22/8	0.018
*Age** (years), mean ±SD	36.7±12.0	38.1±10.0	36.1±13.0	0.406
median	38	41	32.5	
With smoking habits	13	6	7	0.310
IBD type				
Crohn disease	39	14	25	0.674
Ulcerative colitis	7	2	5	
Disease duration (years)				
Mean ±SD	8.5±6.4	11.5±6.2	7.3±6.0	0.008
median	8.5	10.5	6	
Concomitant therapy (%)	34 (74)	13 (81)	21(70)	0.498
Steroids	1	1	---	
Azathioprine (AZA) or MTX	28=26+2	9=8+1	19=18+1	
Steroids +AZA or MTX	5	3	2	
Time of infliximab therapy				
< 1 year	15	6	9	
1 - <3 years	16	3	13	0.231
3 years and plus	15	7	8	

*Age at study enrolment

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Table 2- Follow-up of positive result tests along the study

Screening (months)	baseline		4th			8th			14th		20th			26th		
Patient Number	TST (mm)	T-Spot (SFC)	TST (mm)	T-Spot (SFC)	QFT-GIT (IU/mL)	TST (mm)	T-Spot (SFC)	QFT-GIT (IU/mL)	TST (mm)	T-Spot (SFC)	TST (mm)	T-Spot (SFC)	QFT-GIT (IU/mL)	TST (mm)	T-Spot (SFC)	INH
1	13	P (18)	--	Brd	N	--	Brd	N	--	P (14)	--	P (8)	N	--	P (10)	Yes
2	0	P (6)	0	N	N	0	N	N	0	N	0	N	N	0	N	No
3			20	P (14)	P (0.72)	--	P (16)	N	--	P (14)	--	P (14)	P (0.36)	--	P (10)	Yes
4			10	N	N	--	N	N	--	N	--	N	N	--	N	Yes
5			0	P (8)	N	0	N	N	0	I	0	N	N	0	I	Yes
6			10	P (14)	N	--	N	N	--	I	--	N	N	--	I	Yes
7			6	P (10)	N	0	N	N	0	N	0	N	N	0	N	Yes
8			6	N	N	8	N	N	0	N	0	N	N	9	N	Yes
9			0	P (8)	N	0	N	N	0	I	0	N	N	0	I	Yes
10			10	N	N	0	N	N	0	N	0	N	N	0	I	Yes
11			12	N	N	0	N	N	0	N	0	N	N	0	N	Yes
12						11	N	N	--	P (11)	--	N	N	--	N	Yes
13						10	N	N	--	N	--	N	N	--	I	Yes
14						13	N	N	--	N	--	N	N	--	I	Yes
15						0	N	P (0.37)	0	N	0	I	N	0	N	Yes
16											18	N	N	--	N	Yes

P- positive N – negative; I- indeterminate; Brd- borderline (panel B); SFC –spot forming colonies

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Table 3 – Characteristics of patients and time of first conversion test results (n=16)

Patient Number	Sex	Age	Diagnosis	Steroids*	IMM**	POSITIVE TST	POSITIVE T-SPOT.TB TEST	POSITIVE QFT-GIT	Positive in Screening (month of study)
1	F	54	UC	No	Yes	+	+	ND	0
2	M	48	CD	No	Yes		+	ND	0
3	M	28	CD	No	Yes	+	+	+	4
4	M	26	CD	Yes	Yes	+			4
5	M	35	CD	Yes	Yes		+		4
6	F	49	CD	No	No	+	+		4
7	F	45	UC	No	Yes	+	+		4
8	F	33	CD	No	Yes	+			4
9	F	43	CD	No	No		+		4
10	M	20	CD	No	Yes	+			4
11	M	39	CD	No	No	+			4
12	M	44	CD	No	Yes	+			8
13	M	21	CD	No	No	+			8
14	M	35	CD	No	Yes	+			8
15	F	44	CD	Yes	Yes			+	8
16	M	46	CD	No	Yes	+			18

**> 10mg/day prednisolone; * IMM- immunomodulators: azathioprine, methotrexate*

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Table 4- Concordance among test results (n=46)

	TST			
	Negative	Positive	Kappa	Accuracy
T-SPOT.TB				
Negative	31	8	0.377 (0.152)	78%
Positive	2	5		
QFT-GIT				
Negative	32	12	0.063 (0.106)	72%
Positive	1	1		
	QFT-GIT			
	Negative	Positive		
T-SPOT.TB				
Negative	38	6	0.166 (0.181)	85%
Positive	1	1		

5. Other severe granulomatous infections

5. Other severe granulomatous infections

The second aim of this thesis was to identify other granulomatous infections in patients treated with biologics, its behaviour and the outcome, and to perform a literature review on the topic.

We herein present two cases of invasive listeriosis, with meningitis, in two adults (51 and 69 years-old) suffering from ulcerative colitis and treated with infliximab plus steroids. One was HIV 1 infected. Both presented as an acute disease and were empirically treated since the admission, being the outcome good. The HIV infected patient reassumed infliximab and he is doing well with a follow-up period of six years. A total of 43 cases of invasive listeriosis associated with anti-TNFα were reported in the literature: 23 had rheumatoid arthritis, 14 Crohn's disease, three psoriatic arthritis, one ulcerative colitis and one Still's disease; 15 died.

Isolation of a pathogenic Nocardia, *Nocardia nova*, in stool was not, to our knowledge, documented before in patients with immune-mediated diseases. We found two patients with a persistent isolation of the bacteria, without invasive disease. Concerns due to the risk of dissemination under biological therapies drive the article that follows about Nocardiosis among immunomodulated inflammatory bowel diseases patients. Ten cases of invasive nocardiosis among IBD patients are published, seven of them were doing anti-TNFα.

Three studies were conducted:

5.1 Listeria infection in patients on anti-TNF treatment: report of two cases and review of the literature.

Journal of Crohn's & colitis 2013; 7(2):175-182.

Abreu C, Magro F, Vilas-Boas F, Lopes S, Macedo G, Sarmento A.

5.2 Stool isolation of *Nocardia nova* in two immunomodulated patients with inflammatory bowel diseases.

Journal of Clinical Gastroenterology 2016; 50(1):92.

Abreu C, Carvalho T, Sarmento A, Magro F.

5.3 *Nocardia* infections among immunomodulated inflammatory bowel disease patients: a review.

World Journal of Gastroenterology 2015; 21(21):6491-6498.

Abreu C, Rocha-Pereira N, Sarmento A, Magro F.

5. Other severe granulomatous infections

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SHORT REPORT

Listeria infection in patients on anti-TNF treatment: Report of two cases and review of the literature

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KEYWORDS
Listeria monocytogenes;
Anti-TNF alpha;
Inflammatory bowel disease

Abstract

Listeria monocytogenes is an aerobic gram positive intracellular bacillus, predominantly affecting pregnant women, immunocompromised patients and old individuals. Invasive listeriosis, meningitis and meningoencephalitis, bacteraemia with or without joint, eye or heart focalization are clinical manifestations of the disease. Anti-TNF-α drugs blocking the host's response against various microorganisms, particularly intracellular agents like Listeria monocytogenes, increase the risk of disease. We report two cases of *L. monocytogenes* meningitis in ulcerative colitis patients under infliximab plus steroids. One patient is HIV-1 infected. A review of reported invasive listeriosis cases under anti-TNF drugs is also showed.

1. Introduction

Over the past thirteen years the treatment of inflammatory bowel disease (IBD) has changed dramatically with the introduction of anti-tumor necrosis factor agents, namely infliximab and adalimumab. These agents are monoclonal antibodies neutralizing the biological action of tumor necrosis factor alpha (TNF-α), a critical component of the immune response.[1]

TNF-α acts as a mediator of local inflammation to contain infection and is crucial in host's response against various microorganisms, particularly intracellular agents like *Mycobacterium* spp and *Listeria monocytogenes* (*L. monocytogenes*). The risk of infection induced by anti-TNF-α agents is greater when they are used in combination with other immunosuppressive drugs.[2]

L. monocytogenes is an aerobic gram positive intracellular bacillus, predominantly affecting pregnant women, immunocompromised patients and old individuals. It is widespread in the environment, isolated from soil, vegetables, or animals and

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Table 1 Published Listeria infections in patients treated with anti-TNF drugs.

Reference/country	Year of publication	Age/ gender	Anti-TNF drug	No. of doses (therapy duration)	Indication for use	Concomitant immunosuppressive drug(s)	Type of infection	Outcome	Reintroduction of anti-TNF
Morelli J[20] **USA**	2000	67/M	Infliximab	3	CD	Prednisone (40 mg/day) Azathioprine (150/day)	Bacteraemia	Recovered	NR
Kamath BM[24] **USA**	2002	17/F	Infliximab	1	CD	Methylprednisolone (48 mg/day) 6-MP	Bacteraemia Probable meningitis	Recovered	NR
Gluck T[25] **Germany**	2002	60/F	Infliximab	6	RA	MTX CsA Prednisolone pulses	Bacteraemia Meningoencephalitis Acute cholecystitis	Death	NR
		62/F	Infliximab	2	RA	MTX CsA	Cholecystitis Brain abscess	Recovered	NR
Slifman N[8] **US**	2003	80/M	Infliximab	2	RA	Prednisone (15 mg/day)	Bacteraemia Meningitis	Death	–
		74/F	Infliximab	6	RA	Prednisone (15 mg/day) MTX (17.5 mg/week)	Meningitis	Death	–
		78/M	Infliximab	3	RA	MTX (20 mg/week IM)	Meningitis	Comatose at time of report	–
		73/F	Infliximab	2	RA	Prednisone MTX; MMF	Bacteraemia Probable meningitis	Death: acute MI, VAP followed by MOSF	–
		74/F	Infliximab	5	RA	Prednisone (3 mg/day) MTX (7.5 mg/week IM)	Bacteraemia	Recovered	–
		73/M	Infliximab	NR	NR	Prednisone MTX (50 mg/? IM)	Meningitis	NR	–
		64/F	Infliximab	1	CD	Prednisone (7.5–40 mg/day); 6-MP	Bacteraemia	Recovered	–
		39/F	Infliximab	3	CD	Prednisolone (45 mg/day) 6-MP	Bacteraemia Meningitis	Recovered with residual Paralysis of one eye	–
		20/M	Infliximab	1	CD	Methylprednisolone (100 mg/day IV for 6 weeks) Azathioprine	Meningitis	Death	–
		60/M	Infliximab	2	RA	MTX (20 mg/week IM)	Bacteraemia	Recovered	–
		NR/F	Infliximab	NR	RA	NR	Septic joint	Not recovered at time of report	Continued on infliximab
		72/M	Etanercept	NR	RA	Prednisone MTX (10 mg/?)	Bacteraemia	Death: sepsis and acute MI	–
Aparicio A[21] **Spain**	2003	57/M	Infliximab	6	PA	Prednisone (10 mg/day) MTX (15 mg/week PO)	Meningitis	Recovered	–
	2003	55/F	Infliximab	(2 years)	RA		Bacteraemia	Recovered	–

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Tweezer-Zaks[9]						Prednisone (10 mg/day)			
Israel						MTX (12.5 mg/week?)			
		48/M	Infliximab	1	CD	NR	Bacteraemia splenic abscesses	Recovered	
Joosten AA[26]	2003	41/F	Infliximab	1	CD	Prednisolone Azathioprine	Meningitis	Recovered	–
Netherlands									
Bowie V[5]	2004	73/M	Infliximab	2	RA	Prednisone 3 mg/day MTX 20 mg/week SC	Meningitis	Recovered	Infliximab discontinued
USA									
Pagliano P[22]	2004	45/?	Etanercept	(20 months)	RA	None	Meningitis	Recovered	–
Italy									
Williams G[27]	2005	37/M	Infliximab	2	CD	Prednisone (35 mg/day) Azathioprine (1.8 mg/Kg/day)	Meningitis	Recovered	Infliximab discontinued
Canada									
Cabadés F[28]	2005	71/M	Infliximab	NR	RA	Steroids MTX	Bacteraemia Encephalitis	Death	–
Spain									
De la Fuente Penco B[29]	2005	67/M	Infliximab	2	CD	Prednisone (5 mg/day) Azathioprine (100 mg/day)	Bacteraemia	Recovered	–
Spain									
Dixon W.G.[10]	2006	47/M	Infliximab	(2 months)	RA	NR	Meningitis	NR	–
UK		67/M	Etanercept	(less than 1 month)	RA	NR	Arthritis	NR	–
		60/F	Adalimumab	(14 months)	RA	NR	Arthritis	NR	–
Ramanampamonjy[30]	2006	34/M	Infliximab	2	CD	Azathioprine (2.7 mg/Kg/day)	Meningitis	Hydrocephalus, EVD, recovered	Infliximab discontinued
France									
Dederichs F[31]	2006	42/M	Infliximab	2	CD	Prednisone (20 mg/day)	Meningitis	Recovered	–
Germany									
Osuna Molina R[32]	2006	35/F	Infliximab	2	CD	NR	Meningitis	Recovered	Infliximab discontinued
Spain									
Yamamoto M[33]	2006	22/F	Infliximab	2	Still 's disease	MTX	Meningoencephalitis Brain abscess	Recovered	Infliximab discontinued
Japan									
Kesteman T[7]	2007	52/F	Infliximab	4	RA	MTX	Bacteraemia	Recovered	–
Belgium		79/M	Infliximab	(4 years)	RA	Steroids MTX	Bacteraemia Septic arthritis	Progressively recovered	–
Izbéki F[6]	2008	50/F	Infliximab	NR	CD	Methylprednisolone (32 mg/day) 6-MP (1.5 mg/Kg/day)	Meningoencephalitis	Recovered	Infliximab discontinued
Hungary									
Peña-Sagredo[34]	2008	63/F	Infliximab	24	PA	Prednisone (7.5 mg/day) MTX (10 mg/week)	Meningitis Bacteraemia	Recovered	–
Spain									
		69/F	Adalimumab	3	RA	None	Bacteraemia	Recovered	–
		63/F	Infliximab	21	RA	Prednisone (5 mg/day) MTX (12.5 mg/week)	Meningitis	Recovered	–
		56/F	Infliximab	7	RA	Prednisone (10 mg/day) MTX (10 mg/week)	Bacteraemia Peritonitis	Recovered	–
		36/F	Infliximab	21	RA	Prednisone (10 mg/day) MTX (10 mg/week)	Endophthalmitis	Not recovered	–
Ramos J[35]	2010	50/F	Infliximab	3	CD	Azathioprine 150 mg/day	Bacteraemia	Recovered	Restarted 2 months after
Spain									

(continued on next page)

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Table 1 (*continued*)

Reference/country	Year of publication	Age/ gender	Anti-TNF drug	No. of doses (therapy duration)	Indication for use	Concomitant immunosuppressive drug(s)	Type of infection	Outcome	Reintroduction of anti-TNF
									hospitalization; Follow-up of 10 months: doing well
Kelesidis T[1] USA	2010	42/F	Infliximab	5	PA	NR	Bacteraemia Endocarditis	Recovered	–
Chuang MH[36] US	2010	17/M	Infliximab	1	UC	Prednisolone (60 mg/day)? 6-MP?	Meningitis	Recovered	No

Notes: CD – Crohn's disease; UC – ulcerative colitis; RA – rheumatoid arthritis; PA – psoriatic arthritis; MTX – methotrexate; 6-MP – 6-mercaptopurine; CsA – cyclosporine A; MMF – mycophenolate mofetil; IM – intramuscular; SC – subcutaneous; M – male; F – female; NR – not reported; ? – no data; MI – myocardial infarction; VAP – ventilator-associated pneumonia; MOSF – multi-organ system failure; EVD – external ventricular derivation.

Table 2 Cerebrospinal fluid analysis and blood culture.

Cerebrospinal fluid analysis						Blood
	Cells ($\times 10^6$/L) Neutrophils %	Proteins (mg/dL)	Glucose (mg/dL)	Bacterial culture	PCR Listeria	Blood culture
Patient 1	3518/85%	323	24	*L. monocytogenes*	Positive	*L. monocytogenes*
Patient 2	2652/81%	387	50	Negative	Positive	*L. monocytogenes*

can be acquired as a food-borne infection colonizing meat, dairy products, or vegetables. Recently in the USA, an outbreak of food listeriosis, the first to be associated with cantaloupe, became one of the deadliest food-borne outbreaks in U.S. history.[3]

L. monocytogenes is controlled in infected tissues by immune defense mechanisms involving CD4 and CD8 positive T-cells and TNF-α produced by macrophages, granulocytes and dendritic cells. Immunosuppressive therapy in IBD predisposes the host to severe systemic sepsis, meningoencephalitis, cholecystitis and arthritis by this agent.[4–9]

A review of the Food and Drug Administration (FDA) Adverse Event Report System (AERS) identified 15 cases of Listeria infection in patients treated with anti-TNF therapy, mostly infliximab (14 out of 15 cases), resulting in 6 deaths.[10] A few more cases have been published to date (Table 1) but to our knowledge just one in a patient with ulcerative colitis.

We report two cases of *Listeria meningitis* in ulcerative colitis patients, one of them was HIV-1 infected, treated with infliximab and corticosteroids. A review of reported invasive listeriosis cases under anti-TNF drugs is also showed.

2. Case presentations

2.1. Patient 1

A 51-year-old woman with a 4-year history of ulcerative colitis (pancolitis) on 5-aminosalycilic acid (5-ASA) maintenance therapy was hospitalized with bloody diarrhea (>10 bowel movements per day) and fever in late February 2011.

Sigmoidoscopy on admission showed rectal and colonic mucosa with marked erythema, absent vascular pattern and deep ulcerations (Mayo endoscopic subscore 3). Abdominal X-ray excluded bowel dilation. She was started on antibiotics (ciprofloxacin and methronidazole) and IV steroids. She was negative for hepatitis B, C and HIV infection. No microbial agent was isolated (feces, blood); colon histology revealed colitis with ulcers and immunostaining was negative for cytomegalovirus (CMV). As she was unresponsive to steroid infusion and severe disease activity persisted (>10 bowel movements per day and C-reactive protein (CRP) of 55 mg/L), she received a first infliximab infusion (5 mg/kg) after latent and active tuberculosis exclusion. She had a dramatic clinical improvement, and was discharged with one bowel movement per day under steroid tapering. The second infliximab infusion was administered two weeks later. Three weeks after the second infusion, she came to the emergency department with abdominal pain, nausea and fever. She also reported perianal pain and bloody diarrhea (4 bowel movements per day).

Sigmoidoscopy showed erythema, decreased vascular pattern and mild friability (Mayo endoscopic subscore 1). The patient was admitted to the Gastroenterology Unit. One day after admission she reported mild frontotemporal headache. She was confused and signs of meningeal irritation were noted with no focal neurological findings. A laboratory workup revealed high white blood cell count (13,370$\times 10^9$/L) and CRP (215 mg/L). A head computerized tomography (CT) scan showed slight blood on the posterior horns of lateral ventricle. Lumbar puncture yielded cloudy cerebrospinal fluid (CSF) with a white blood count of 3518$\times 10^{-6}$/L, decreased glucose level and elevated total CSF protein content (Table 2). She was transferred to the Infectious Disease Department with the diagnosis of purulent meningitis under ampicillin and meropenem treatment. CSF analysis was positive for *L. monocytogenes* DNA; blood and CSF culture identified *L. monocytogenes*, and treatment was switched to ampicillin, completing 28 days of antibiotic therapy. She recovered without clinical or neurological sequelae. She is asymptomatic on 5-ASA (oral and topical) therapy. Infliximab was not reintroduced.

2.2. Patient 2

In 2008, a 69-year old Caucasian male was diagnosed with ulcerative colitis, after being admitted with a two months history of bloody diarrhea. By that time sigmoidoscopy showed continuous rectal and sigmoid mucosal edema, loss of vascular pattern, mild friability and erosions. Histology revealed architectural distortion of the colonic mucosa with polymorphic inflammatory infiltrate and multifocal activity with crypt abscess formation. After discharge he was put on maintenance therapy with 5-ASA (oral and topical). By that time total colonoscopy revealed rectal and distal sigmoid mucosa continuously involved with mild erythema and edema. Distal ileum was normal. In 2010 he had another disease flare with hospital admission and was put on IV steroids. The diagnosis of HIV-1 infection was made. CD4 cell count was 372/mmc. Rectal swabs were negative for sexually transmitted agents (*Chlamydia trachomatis* and *Neisseria gonorrhoeae*). Immunohistochemistry for CMV detection was positive in paraffin-embedded tissue sections from rectal biopsies. He was treated with IV ganciclovir 5 mg/Kg bid for 15 days and then valganciclovir 900 mg bid during another 7 days. Thiopurine therapy with azathioprine was started because of steroid dependent ulcerative colitis. He was later considered intolerant to thiopurines and on October 13, 2011 was started on anti-TNF therapy with infliximab after another severe disease flare unresponsive to steroids. Although he had a negative boosted tuberculin test, the patient was put on isoniazid therapy since October 10, 2011, because of a positive interferon-γ release assay (IGRA) test. Active tuberculosis was excluded by negative mycobacterial and image (Chest CT) exams. When infliximab was started, he had a one-year history of HIV-1 infection, under antiretroviral

therapy (ART) with emtricitabine/tenofovir/efavirenz for the last six months. HIV viral load was undetectable and CD4 cell count was 357/mmc. Second infliximab infusion (5 mg/Kg) was administered on October 27, 2011. Clinical and endoscopic remission of ulcerative colitis was achieved. On November 16, 2011 he was brought to the emergency department because he was found confused, disoriented and with signs of fecal and urinary incontinence. In the emergency room he denied abdominal pain, respiratory or genito-urinary symptoms. He was scheduled for his third infliximab infusion on November 25 and was taking 40 mg of oral prednisolone a day. He had a sub-febrile temperature and meningeal signs were excluded. High white blood cell count and CRP were revealed by a lab workup. A head CT scan showed maxillary and frontal sinusitis. He was admitted to the Gastroenterology ward and in the next morning was found agitated. Again, no meningeal signs were found. Lumbar puncture yielded cloudy CSF, with a white blood count of 2653×10^{-6}/L, decreased glucose level and increased total CSF protein content (Table 2). CSF analysis was positive for *L. monocytogenes* DNA; blood culture identified *L. monocytogenes*. He completed a twenty-one day cycle of ampicillin plus gentamicin for the first seven days and recovered without clinical neurological sequelae, being discharged on the usual antiretroviral medication and isoniazid. Four months after completing meningitis treatment, infliximab was restarted without any consequences. The patient is doing well, with sigmoidoscopy showing Mayo endoscopic subscore 0. He continues on antiretroviral therapy.

3. Discussion

We report two cases of *L. meningitis* in ulcerative colitis patients older than 50 years under infliximab plus steroids; one of them was HIV infected. Predisposing factors for Listeria infection are older age (>75 years), pregnancy, diabetes mellitus, immunosuppression, liver failure, HIV infection, chronic alcohol abuse, and splenectomy. These conditions may increase the incidence of listeriosis to as high as 210 cases per 100,000 (as compared with 0.7–3 per 100,000 cases in healthy individuals), and mortality to thirty percent.[11] As shown in animal studies of experimental Listeria infection, those treated with anti-TNF agents displayed a premature death with large numbers of bacterial copies per cell before and during Listeria infection.[12]

The causal association between anti-TNF-α agents and listeriosis was not showed in the placebo-controlled clinical trials. However, in October 2001, the FDA issued a new warning about the risk of tuberculosis, invasive fungal infections and other opportunistic infections, such as listeriosis and pneumocystosis in the labeling of infliximab.[13] Nevertheless, most patients are immunocompromised due to their chronic inflammatory illness and concurrent immunosuppressive therapy. This aspect makes it difficult to assess the weight of each risk factor. One of our patients was HIV infected, a known predisposing condition for Listeria infection. This is, to our knowledge, the first case of Listeria infection in an HIV-positive patient under anti-TNF-α therapy. Meanwhile, he had, by the time of Listeria infection, a CD4 cell count of 350/mmc, so it is hard to say if HIV was responsible for the condition. Although the experience in treating HIV infected patients with anti-TNF is limited, probably these drugs may be given with benefits, without affecting the CD4 cell level or viral load.[14]

TNF-alpha antagonists have been used successfully in HIV positive patients with rheumatologic conditions.[15] There are two cases reporting concomitant HIV and Crohn's disease successfully treated with infliximab, with and without antiretroviral therapy.[16,17] Long-term effects of TNF-alpha antagonists in HIV positive patients need further study. Opportunistic infections in IBD patients were associated with corticosteroids (OR 3.4), azathioprine (OR 3.1), or infliximab (OR 4.4) with the highest incidence within three months of starting this drug.[18] Considering the study of Fidder et al., the only independent risk factor for infection in patients treated with infliximab was concomitant treatment with steroids (OR 2.69; 95CI 1.18–6.12, p=0.018).[19] The relative risk of infection is greater in IBD patients older than 50 years old (OR 3.0; 95% CI 1.2–7.2).[18]

The first *L. monocytogenes* infection complicating infliximab treatment in IBD was reported in 2000 in a Crohn's disease patient.[20] In 2006, Dixon et al. reported in a rheumatoid arthritis cohort, three cases of listeriosis in 7664 patients treated with anti-TNF-α agents.[10] This displays an incidence rate of approximately three cases per 10,000 patient-years, which is much higher than the rate of listeriosis infection in the general population. Infliximab-linked Listeria infections have also been reported in patients with psoriatic arthritis and rarely in ulcerative colitis and juvenile rheumatoid arthritis.[21]

In immunocompromised patients it is not clear whether Listeria infections originate from contaminated ingested food or from chronic fecal carriage.[1] In our patients a possible dietary source for the Listeria was not identified. Cases of listeriosis related to anti-TNF-α therapy have been showed. Meningitis and meningoencephalitis, bacteraemia with or without joint, eye or heart focalization were reported.[8,22,23] To our knowledge a total of forty-three cases of invasive Listeria infection associated with anti-TNF-α were reported (Table 1). Thirty eight were related to infliximab, three to etanercept and two to adalimumab. Twenty-three patients had rheumatoid arthritis, fourteen Crohn's disease, three psoriatic arthritis, one ulcerative colitis and a last one Still's disease. From the thirty-seven patients, all but two (one on etanercept and another on adalimumab) were taking at least one concomitant immunosuppressive agent. The infection developed soon after therapy with three or less infusions in sixty three percent of patients. The mean age of this group was fifty-six years, and if we exclude the two cases involving teenagers, the mean age rises to fifty-eight years. Our two patients are older than fifty years. Listeria infection was neurological in fifty eight percent of patients with twenty cases of meningitis and two brain abscesses. Bacteraemia without known focalization was described in eleven patients and bacteraemia with focalization in nine, namely joint (four patients), gallbladder, heart, eye, spleen, and peritoneum, in one patient each. Fifteen died as direct consequence of listeriosis; six of those patients correspond to the older reports. Since 2005 no deaths have been reported.

Physicians should be aware of the possibility of Listeria infection in anti-TNF-α treated individuals. Early recognition of listeriosis as a potential complication of treatment with these agents may help decrease its high morbidity and mortality.

During infection the anti-TNF-α agent was discontinued in all but one patient. The decision to reintroduce anti-TNF-α agents after infection should left up to the clinician and specialist in Infectious diseases and always discussed with the patient.[2]

Conflict of interest

We declare that we have no conflicts of interest.

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5.2 Stool Isolation of *Nocardia nova* in two immunomodulated patients with inflammatory bowel diseases.

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Stool Isolation of *Nocardia nova* in Two Immunomodulated Patients With Inflammatory Bowel Diseases

To the Editor:

We report 2 middle-aged female patients with inflammatory bowel disease under immunomodulation therapy (azathioprine and pulses of steroids) from whom *Nocardia nova*, a pathogenic specie belonging to *Nocardia asteroides*, was repeatedly isolated from the stool. To our knowledge this is the first report of *N. nova* being isolated from stools of patients with inflammatory bowel disease under immunomodulation, without evidence of extraintestinal disease. The *Nocardia* was isolated in Lowenstein medium culture in 4 stool samples over 3 months (months 0 to 2) in one patient and in 3 samples in the other patient, who was then treated with cotrimoxazole, resulting in negative culture, respectively, for 17 and 10 months of follow-up; thus, it is hard to assume a laboratorial contamination. *Mycobacterium tuberculosis* infection was excluded in both patients. As there was absence of extraintestinal disease we considered colonization resulting from inhalation of spores or swallowed sputum.[1] The natural history and the risk of clinical disease and dissemination of the bacteria under immunosuppression is not reported in the literature. In 1 patient, because colonic biopsy histologic examination revealed epithelioid granuloma, although negative for the presence of *Nocardia* DNA (and for *M. tuberculosis*), we chose to treat for 2 months with cotrimoxazole and then repeated treatment for the first 5 months when anti-TNF therapy was introduced, as anti-TNF has been shown to increase the incidence of nocardiosis.[2] The identification of *Nocardia* strains at the species level is difficult,[3] but we should highlight that this procedure seems to be essential as pathogenic and antibiotic sensibility may be different according to the species isolated.[4,5] We continue to follow-up both patients closely, and appreciate comments regarding treatment options and follow-up.

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The authors declare that they have nothing to disclose.

Obscure Gastrointestinal Bleeding in the Setting of Portal Hypertension

To the Editor:

Gastrointestinal (GI) bleeding is a common cause for hospital admission, but rare etiologies can present a challenge when using conventional diagnostic techniques, especially in a patient with a complex surgical history. A pancreaticoduodenectomy (PD), or Whipple procedure, involves extensive manipulation of the pancreas, duodenum, and their accompanying vasculature. The portal vein is frequently manipulated during the surgery, and such damage can lead to long-term and possibly insidious prehepatic portal hypertension, which can result in diffuse bleeding throughout the GI tract.[1] Diagnostic testing may be inconclusive in cases in which bleeding does not localize to a single source.

A 67-year-old woman with a history of PD for a benign pancreatic cystic mucinous ductal lesion 2 years ago presented with a recurrent episode of bloody stools. She had a positive tagged red blood cell scan identifying a small bowel source from the transferring hospital. Previously, she presented with melena a year after surgery that required blood transfusion. She underwent multiple transfusions in the subsequent year, with her hemoglobin ranging from 5.5 to 10.2 g/dL. Evaluation was extensive and included 2 colonoscopies, 3 upper esophagogastroduodenoscopies, video capsule endoscopy, CT angiogram, small bowel enteroscopy, and antegrade single-balloon endoscopy. These studies occasionally demonstrated active bleeding, but a clear source was not identified.

On this admission, she presented with a multiple-day history of maroon-colored stools. Her exam was unremarkable except for melenic stool on rectal exam and orthostatic hypotension. Initial hemoglobin was 6.9 g/dL (after multiple transfusions at the transferring hospital). After being transfused packed red blood cells and on stabilization in the ICU, she underwent diagnostic testing. A nuclear medicine tagged RBC scan showed intermittent intraluminal GI bleeding in the small bowel. CT angiogram revealed no evidence of bleeding or of vascular abnormality; colonoscopy was grossly normal, and esophagogastroduodenoscopy was significant for a small angiectasia or arteriovenous malformation that was coagulated with an argon beam. Single-balloon endoscopies revealed the same arteriovenous malformation but it was believed noncontributory.

She continued to have grossly bloody bowel movements and required

The authors declare that they have nothing to disclose.

5. Other severe granulomatous infections

5.3 Nocardia infections among immunomodulated inflammatory bowel disease patients: a review.

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REVIEW

Nocardia infections among immunomodulated inflammatory bowel disease patients: A review

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Abstract

Human nocardiosis, caused by *Nocardia* spp., an ubiquitous soil-borne bacteria, is a rare granulomatous disease close related to immune dysfunctions. Clinically can occur as an acute life-threatening disease, with lung, brain and skin being commonly affected. The infection was classically diagnosed in HIV infected persons, organ transplanted recipients and long term corticosteroid treated patients. Currently the widespread use of immunomodulators and immunossupressors in the treatment of inflammatory diseases changed this scenario. Our purpose is to review all published cases of nocardiosis in immunomodulated patients due to inflammatory diseases and describe clinical and laboratory findings. We reviewed the literature concerning human cases of nocardiosis published between 1980 and 2014 in peer reviewed journals. Eleven cases of nocardiosis associated with anti-tumor necrosis factor (TNF) prescription (9 related with infliximab and 2 with adalimumab) were identified; 7 patients had inflammatory bowel disease (IBD), 4 had rheumatological conditions; nocardia infection presented as cutaneous involvement in 3 patients, lung disease in 4 patients, hepatic in one and disseminated disease in 3 patients. From the 10 cases described in IBD patients 7 were associated with anti-TNF and 3 with steroids and azathioprine. In conclusion, nocardiosis requires high levels of clinical suspicion and experience of laboratory staff, in order to establish a timely diagnosis and by doing so avoid worst outcomes. Treatment for long periods tailored by the susceptibility of the isolated species whenever possible is essential. The safety of restarting immunomodulators or anti-TNF after the disease or the value of prophylaxis with cotrimoxazole is still debated.

Key words: Nocardiosis; Immunomodulation; *Nocardia* spp.; Inflammatory diseases

Core tip: Opportunistic infections in immunomodulated patients with inflammatory diseases has gained renewed interest because of the new biological therapies. Concerning inflammatory bowel disease, in particular anti-tumor necrosis factor drugs, turned granulomatous infection diseases a real risk. The awareness and knowledge about nocardiosis, a rare but severe granulomatous infection, is probably lacking for the majority of doctors treating these patients. Our aim is to increase the awareness about the infection and review the published cases in this particular group of patients. We would like that our reads increase knowledge about clinical manifestations and up-to-date treatment, be aware of the risk of the disease and when to suspect nocardiosis.

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INTRODUCTION

Human nocardiosis is generally recognized as an opportunistic disease close related to immune dysfunctions, however any host may be affected. The infection can range from a sub-clinical infection to acute life-threatening disease[1].

Classically the infection was more common in patients living with human immunodeficiency virus (HIV) infection, organ transplant recipients and those on long-term corticosteroid therapy[2]. Concurrent use of immunosuppressants, preexisting pulmonary diseases and diabetes mellitus are also associated with increased risk of nocardiosis[3].

The incidence of *Nocardia* infection is low, nevertheless early diagnosis and treatment in immunosuppressed patients is essential, due to its high morbidity and mortality[4]. Nocardia infection causes granulomatous diseases and differential diagnosis should be made with more frequent granulomatous diseases, like tuberculosis[5] and Crohn's disease. After the introduction of anti-tumor necrosis factor drugs (TNF-α) an increase in the incidence of granulomatous infections, including nocardiosis[5] was noticed.

Our purpose is to focus on the descriptions of nocardiosis in immunomodulated patients due to inflammatory diseases and to review published cases in this setting.

RESEARCH

We searched PubMed, B-On, OVID databases for articles till November 2014, using these key words alone or in combination: "*Nocardia* spp.", "nocardiosis", "immunosuppressed patients", "nocardia diagnosis", "nocardia treatment", "nocardia sensibility", "inflammatory bowel disease", "Crohn Disease", "ulcerative colitis", "anti-TNF therapy". We selected review articles of nocardiosis and 14 articles of case reports all in English language except one case report, all together 50 articles.

NOCARDIA SPP: THE BACTERIA AND PATHOGENIC MECHANISMS

Nocardia species are ubiquitous soil-borne aerobic microorganisms which belong to a large group of bacteria, aerobic actinomycetes, with more than 80 different species of *Nocardia* identified, of which at least 33 species are pathogenic[6]. The majority of *Nocardia* infections are caused by inhalation, but some may be acquired by percutaneous inoculation after direct contact with soil. *Nocardia* species can spread hematogenously from lung parenchyma, particularly within the upper lobes, or from cutaneous infection sites to the brain, kidneys, joints, bones, soft tissues and eyes causing disseminated nocardiosis[7]. Bacteria dissemination has been related to immunocompromising conditions as cell-mediated response and macrophages function[2]. Therefore, patients under corticosteroids, in which macrophage and T-cell function are decreased, and patients treated with infliximab, an inducer of apoptosis of macrophages and T cells, are at risk of developping nocardiosis[8]. The need for continuous immunosuppressive therapy, disseminated disease and central nervous system involvement[9] are factors associated with poor prognosis. In a review of 10 cases of nocardiosis occurring in rheumatic patients 6 out of 10 had disseminated disease when their pulmonary lesion was diagnosed[10].

CLINICAL ASPECTS

Nocardiosis may have several clinical presentations[7]. (1) pulmonary Nocardiosis: in more than two-thirds of cases the lungs are the primary site of nocardial infection; the onset of the disease may be subacute or chronic and it is not distinguished by any specific signs or symptoms. Fever, weight loss, anorexia, dyspnea, cough, and haemoptysis[2] may be present. Radiographic findings of lung involvement may include single or multiple nodules, lung masses (with or without cavitation), reticulonodular infiltrates, interstitial infiltrates, lobar consolidation[7] (Figure 1). Brain imaging should be performed in all patients with pulmonary nocardiosis as cerebral dissemination is frequent and the bacteria seems to have a special tropism for neural tissue[9]; (2) cerebral Nocardiosis: CNS is involved in approximately 20 percent of

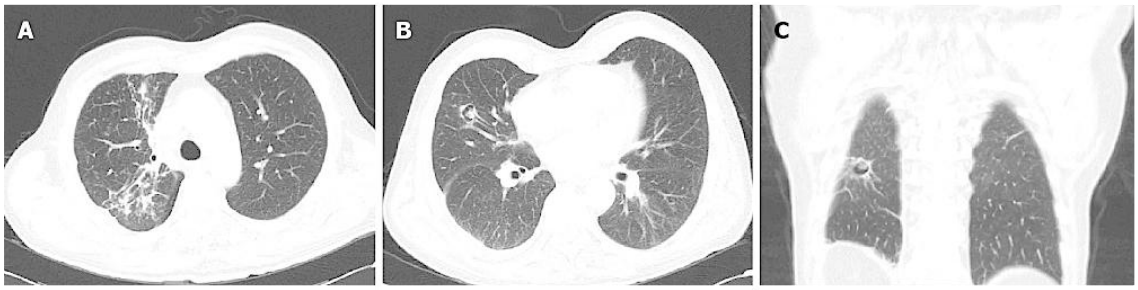

Figure 1 Torax computed tomography scan 73-year-old man with disseminated nocardiosis (cutaneous, pulmonary and cerebral involvements). A: Consolidations in upper right pulmonary lobe; B: Cavitation with heterogenous filling in right upper pulmonary lobe; C: Coronal image showing a cavitation in right lower pulmonary lobe.

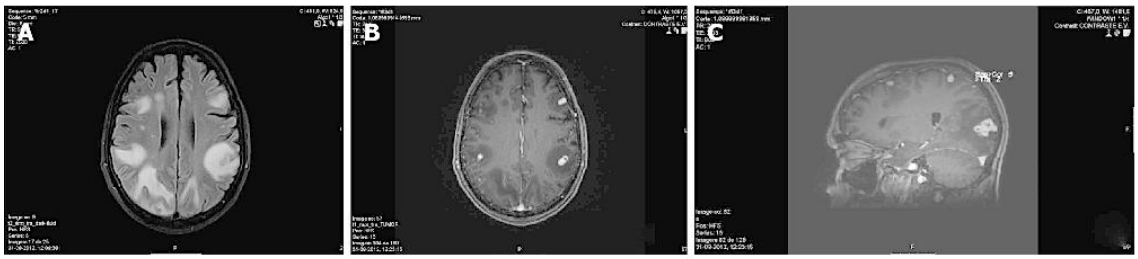

Figure 2 Cerebral magnetic resonance of 73-year-old man with disseminated nocardiosis (cutaneous, pulmonary and cerebral involvements). A: Axial T2 FLAIR (Fluid attenuated inversion recovery). Multiple focal lesions in bilateral fronto-parietal subcortical white matter, with proeminent edema and mass effect resulting in sulcal effacement; B: Post-gadolinium T1 3D-MPRAGE, axial reformat. Ring enhancing lesions were depicted in post-contrast images; C: Post gadolinium T1 3D-MPRAGE, sagittal reformat. Ring enhancing lesions were depicted in post-contrast images, forming clusters in right occipital lobe.

nocardiosis and in 44 percent of disseminated cases[9]. Most commonly it results from dissemination of infection from a pulmonary or cutaneous site. Cerebral lesions are parenchymal abscess that can occur in any region of the brain[9] (Figure 2). Signs and symptoms of nocardial brain abscess are diverse and nonspecific: fever, headache, meningismus, seizures, and/or focal neurologic deficits. Nocardial meningitis is rare and can occur with or without an associated brain abscess[11]. The clinical presentation is a subacute or chronic meningitis and the cerebrospinal fluid is similar to other bacterial meningitis[11]; and (3) skin and cutaneous Nocardiosis: cutaneous disease most commonly results from direct inoculation of organisms into the skin after trauma in immunocompromised individuals. Primary infections usually present as superficial painless cellulitis or abscess with localized lymphadenopathy, and progress slowly[7].

Disseminated nocardiosis is defined as two or more noncontiguous sites of involvement that may or may not include a pulmonary focus. There are no pathognomonic signs or symptoms of nocardiosis. The infection should be suspected in any patient who has brain, soft tissue, or cutaneous lesions, and a concurrent or recent pulmonary lesion. Pulmonary nocardiosis may mimic an exacerbation of an underlying lung disease, like chronic obstructive pulmonary disease[12] and pulmonary sarcoidosis[13]. Nocardiosis may be misdiagnosed as tuberculosis (since upper lobe involvement is common and *Nocardia* spp. are weakly acid fast), invasive fungal disease and malignancy[2].

NOCARDIA LABORATORIAL DIAGNOSIS

Nocardia spp. appear as delicate, filamentous, branching gram-positive rods in clinical specimens[6]. The bacteria, like *Mycobacterium*, *Corynebacterium*, *Rhodococcus*, *Gordona* and *Tsukamurella* , members of the Nocardiform actinomycetes subgroup, are all variably acid-fast on appropriate staining[7]. However acid-fast staining property of *Nocardia* is often lost in older cultures[1]. Growing of *Nocardia* species in culture is slow and incubation should be carried out for at least two weeks, and ideally cultures should be maintained for 4-6 wk before they are red as negative. Therefore, when *Nocardia* infection is suspected, the laboratory should be notified for specific culture media and staining procedures. For instance some sputum decontamination solutions are toxic to *Nocardia* spp., particularly sodium hydroxide, N-acetylcysteine and benzalkonium chloride[2]. Curiously *Nocardia* spp. only rarely can be recovered in blood cultures despite frequent hematogenous dissemination[14]. Most cases of bacteremia are associated with central venous catheters or other endovascular devices[14]. Nocardia species identification is essential as not all species are pathogenic and different *Nocardia* species and strains often have markedly different

Table 1 Usual susceptibility patterns of common *Nocardia* species in human diseases

Nocardia species	Susceptibility	Resistance	Ref.
N. asteroids sensu stricto	TMP-SMX, TGC, Amikacin Imipenem (64%-98%)	TGC	Lerner *et al*[2] Sorrel *et al*[43]
N. farcinica	Amikacin TMP-SMX, Minocycline	Tobramycin TMP-SMX (80%), TGC	Wallace *et al*[21] Lerner *et al*[2] Uhde *et al*[44]
N. nova	TMP-SMX, TGC, Imipenem, Amikacin Clarithromycin (96%)	TMP-SMX and TGC (53%)	Uhde *et al*[44] Wallace *et al*[21] Larruskain *et al*[46]
N. brasiliensis	TMP-SMX, Amikacin TGC (88%-100%), Imipenem (20%-30%)	Ceftriaxone (81%)	Uhde *et al*[44] Sorrel *et al*[43]
N. transvalensis	TMP-SMX (88%), Imipenem (90%), TGC (50%)	Amikacin	Sorrel *et al*[43] McNeil *et al*[47]
N. otitidiscaviarum	Amikacin, Minocycline	TMP-SMX	Lerner *et al*[2]

TMP-SMX: Trimethropim-sulfamethoxazol; TGC: Third generation cephalosporins.

susceptibility patterns[15]. Identification of the species is difficult when using routine phenotypic tests but identification based on conventional phenotypic and enzymatic tests enables for the rapid identification of the most common[16]. Alternatively, polymerase chain reaction (PCR) for identification of *Nocardia* spp. permits faster results than the conventional methods[17]. Susceptibility patterns of Nocardia varies among different species; the most common patterns of sensibility and resistance are detailed on Table 1. Results of laboratorial antimicrobial susceptibility testing of *Nocardia* should be interpreted with caution because few studies correlated *in-vitro* data with clinical outcome. Nevertheless, it should be pointed that susceptibility of all *Nocardia* spp. to trimethoprim plus sulphamethoxazole, amikacin and linezolid has been confirmed by several studies[6,15,18-20], whereas susceptibilities to beta-lactams, other aminoglycosides, ciprofloxacin and clarithromycin varied markedly[15]. From 93 *Nocardia* isolates in clinical specimens, belonging to 15 strains of *Nocardia* spp., activity of beta-lactams was variable, with 89% of isolates being susceptible to imipenem, 84% to amoxicillin + clavulanate, 55% to ceftriaxone, 50% to amoxicillin and 9% to piperacillin + tazobactam[15]. High-level of resistance to beta-lactams, including ceftriaxone and imipenem, was found in reference strains of *N. brasiliensis, N. otitidiscaviarum* and *N. niigatensis*[15]. Also *N. farcinica* characteristically demonstrates resistance to third-generation cephalosporins and is often resistant to imipenem[15,21,22]. Antimicrobial susceptibility testing is recommended as a guide to therapy for severe disease, refractory cases and for patients who are intolerant to treatment with trimetroprim-sulphamethoxazole[15]. Susceptibility testing is particularly important in patients infected with *Nocardia* species that have high frequencies of antimicrobial resistance, such as *N. farcinica*. Drugs to be tested by microdilution are: amikacin, amoxicillin-clavulanate, ceftriaxone, ciprofloxacin, clarithromycin, imipenem, linezolid, minocycline (which predicts doxycycline susceptibility), sulfamethoxazole or trimethoprim-sulfamethoxazole, and tobramycin[23].

THERAPY

Trimethoprim-sulphamethoxazole (TMP-SMX) is the first line option and can therefore be used as an initial empirical treatment in patients with extensive disease, including brain abscess. It has been reported that this drug has excellent penetration into most tissue compartments, including the central nervous system, and high serum concentrations even after oral administration (recommended oral dose: 2.5-5 mg/kg of the trimethoprim component orally twice daily). Concerning endovenous TMP-SMX the doses should be similar to the treatment of pneumocystosis (15 mg/kg per day of the trimethoprim component in two to four divided doses). There have been few reports of patients failing to respond to TMP-SMX[24]. Although, in patients with life-threatening disease and in those failing treatment, sulfonamide levels should be monitored. A sulfonamide level measured two hours after a dose should have a serum concentration between 100 and 150 mcg/mL[2]. If the patient is allergic to sulfonamides, desensitization should be performed. For patients infected with sulfonamide-resistant *Nocardia* spp. or those who are allergic to sulfonamides, imipenem (500 mg IV every six hours) plus amikacin (7.5 mg/kg iv every 12 h) is an option.

Cutaneous infections

Oral TMP-SMX or amoxicillin-clavulanic acid are the drugs of choice for 1 to 3 mo in the case of mild cutaneous disease. Immunocompromised patients must be treated for a minimum of six months to one year.

Severe nocardia infections

Severe nocardiosis refers to some cases of pulmonary disease, all cases of disseminated disease or central nervous system disease, and infections involving more than one site in immunocompromised patients. In severely ill patients combined therapy with endovenous

Table 2 Clinical forms of Nocardiosis related to anti-TNF therapy in inflammatory bowel disease, rheumatic and psoriatic patients

Clinical form	Anti-TNF					
	IFX or ADA duration of therapy	Age	Associated treatment	Nocardia isolation	Outcome	Ref.
Cutaneous						
IBD-P	IFX -3 infusions	45	No	*Nocardia* spp.	Favourable	Singh *et al*[30]
	IFX- 1,5 yr	61	No	*Nocardia* spp.	Favourable	Ali *et al*[31]
R-P	IFX -3 yr	70	Metothrexate + steroids	*N. otitidiscaviarum*	Favourable	Fabre *et al*[32]
Pulmonary						
IBD-P	IFX - 8 mo -6 infusions	77	Steroids	*N. asteroids*	Favourable	Stratakos *et al*[33]
	IFX - 3 infusions	53	Azathioprine + steroids	*N.cyriacigeorgica*	Favourable	Parra *et al*[34]
	IFX - 6 mo	81	6-mercapto-purine	*Nocardia spp*	Favourable	Saleemuddin *et al*[35]
R-P	ADA - 4 mo	63	Steroids (DPOC)	*N. asteroids*	Favourable	Doraiswamy *et al*[48]
Disseminated						
R-P	ADA[1]- 4 mo	63	Metotrexate	*N. farcinica*	Favourable	Wendling *et al*[38]
P-P	IFX[2]- 2 mo	66	Alefacet 6 mo before	*N. farcinica*	death	Al-Tawfiq *et al*[37]
IBD-P	IFX - 5 infusions	73	Prednisolone methrotexate	*N. asteroids*	Favourable with sequelae	Sidney *et al*[49]
Hepatic						
IBD-P	IFX ≤ 1 mo	23	Steroids	*N. farcinica*	Favourable	Nakahara *et al*[36]

[1]Previously was treated with 3 mo of etanercept; [2]Diabetic patient. IBD-P: IBD-patients; R-P: Rheumatologic patients; P-P: Psoriatic patients.

drugs with activity against Nocardia, like amikacin, imipenem, meropenem, ceftriaxone or cefotaxime is advisable[7]. Initially treatment with two intravenous drugs is recommended by some authors[2,6]. For more severe infection, even three intravenous drugs should be used[6]. When the severe infection does not involve CNS initial treatment may consist of TMP-SMX (15 mg/kg iv of the trimethropim per day in two to four divided doses) plus amikacin (7.5 mg/kg iv every 12 h). An alternative would be imipenem (500 mg IV every 6 h) plus amikacin[2]. When there is CNS disease TMP-SMX plus imipenem is an option. Amikacin may be associated in case of multiorgan involvement.

SURGERY

In several settings surgical intervention may be needed: (1) cerebral or some large soft tissue abscesses that do not respond to antibiotic therapy[25]; (2) empyemas and mediastinal infection with fluids; and (3) pulmonary nocardiosis complicated by pericarditis, which is almost always fatal if pericardial drainage is not performed[26].

TREATMENT DURATION

The optimal duration of antimicrobial treatment for severe disease has not been clearly settled. Drugs should be switched to oral medication 3 to 6 wk after initial endovenous therapy and maintained for at least 6 to 12 mo in the case of cerebral or extensive disease. Immunocompromised patients may require longer courses of initial IV therapy (3 to 6 mo, depending on the extent of the disease, and clinical response) and the addition of amikacin, or a carbapenem or ceftriaxone may be advisable. All immunocompromised patients (except those with isolated cutaneous infection) and all patients with CNS involvement should be treated for at least one year[2].

RISK OF NOCARDIA RELAPSES

Because of the relapsing nature of *Nocardia* infection, long duration antimicrobial treatment is recommend. The need for continuation therapy in those who need re-introduction of immunosupressors is not well settled. Some authors recommend prolonged oral maintenance therapy to prevent relapse of nocardiosis in patients who continue to be immunosuppressed as a result of their disease or treatment[27]. TMP-SMX is the drug of choice but protection is not complete and it is not known the most appropriate regimen[27]. Thus, in patients whose immunosuppression cannot be reversed, a maintenance regimen of TMP-SMX one single strength tablet daily if the *Nocardia* isolate is susceptible to TMP-SMX is the choice[28]. Alternative maintenance regimens have not been systematically evaluated, although doxycycline 100 mg daily is a possible alternative.

NOCARDIOSIS IN PATIENTS UNDER ANTI-TNF-α

Nocardiosis is an infrequent complication in patients with chronic inflammatory diseases under anti- TNF-α agents. A total of eight cases of nocardiosis were identified among approximately 300000 patients treated with anti-TNF agents with a rate of 3.55 and 0.88 per 100000 treated patients with infliximab or etanercept, respectively[5,29]. To our knowledge 10 cases were published (Table 2). Following infliximab therapy three cutaneous cases of nocardiosis were published: two in IBD patients[30,31]. and the other in a rheumatic patient[32]. Three pulmonary nocardiosis cases[33-35] and one hepatic nocardiosis[36] have been reported in IBD patients under infliximab therapy. Disseminated nocardiosis was described following

Table 3 Clinical cases of nocardia disease in inflammatory bowel disease patients: Literature review

Ref.	IBD	Age (yr)	Sex	Medication	*N. species*	Clinical form	Treatment (duration)	Evolution
Vohra *et al*[40]	CD	16	F	6-Mercaptopurine 6 wk steroids	*N. asteroids*	Brain abcess; calf abscess	TMP-SMX + ceftriaxon: not established	Favourable
Stack *et al*[41]	UC	68	M	Cyclosporine, steroids	*N. asteroids*	Pulmonary (abcess)	Amikacin + cefotaxime-3 wk followed by cefuroxime 3 mo	Favourable
Singh *et al*[30]	CD	45	M	Infliximab 6 wk	*N.* spp. (*polymerase chain reaction*)	Cutaneous	TMP-SMX, 3 yr	Favourable
Stratakos *et al*[33]	CD (DM)	77	F	Infliximab 8 mo, steroids	*N asteroids* (+*Pneumocystis jiroveci*)	Pulmonary	TMP-SMX, 6 mo	Favourable
Parra *et al*[34]	CD	53	F	Infliximab, azathioprine, steroids	*N. cyriacigeorgica*	Pulmonary	TMP-SMX + amikacin + imipnem - 6 wk followed by TMP-SMX, 7.5 mo	Favourable
Arora *et al*[42]	UC	61	F	Azathioprine, steroids	*N. nova*	Cutaneous, abscess: brain lung, renal, pancreatic	TMP-SMX, 1 yr	Favourable, remission 2 yr after treatment
Nakahara *et al*[36]	CD	23	M	Infliximab, < 3 wk, steroids	*N. farcinia*	Liver nocardiosis	TMP-SMX for? not known	Favourable
Ali *et al*[31]	CD	61	M	Infliximab > 1 yr	*N.* spp.	Cutaneous	TMP-SMX for 6 mo	Favourable, restarted anti-TNF after therapy
Saleemuddin *et al*[35]	CD	81	M	Infliximab (3 mo) 6-mercapto-purine	*Nocardia* spp.	Pulmonary	TMP-SMX for? not known	Favourable; 5 mo after restarted anti-TNF under TMP-SMX; ok 1 yr after diagnosis
Sidney *et al*[49]	CD	73	F	Infliximab (5 infusions) Prednisolone methotrexate	*Nocardia asteroids*	Disseminated: Pulmonary cerebral	TMP-SMX for? not known	Favourable with sequelae

TMP-SMX: Trimethoprim- sulfamethoxazole; DM: Diabetes mellitus; CD: Crohn's disease; UC: Ulcerative colitis.

infliximab treatment in one case of psoriasis[37] and in another of rheumatoid arthritis[38]. One patient with disseminated nocardiosis did not survived: he was a man, 66-year-old, on infliximab due to psoriasis[37]. Outside immunologic diseases it was reported a disseminated nocardiosis in a patient with alcoholic hepatitis treated with etanercept[39].

NOCARDIOSIS IN PATIENTS WITH IBD

In patients with inflammatory bowel diseases to the best of our knowledge nine cases had been reported. Three cases were reported in patients under immunomodulation (steroids in two associated with 6-mercaptopurine/azathioprine and in one with cyclosporine[40-42]) and two of them were disseminated. Six other cases were associated with anti-TNF: two cutaneous forms were under anti-TNF alone[30,31] and four with anti-TNF combined with immunomodulators (steroids, thiopurines), with pulmonary disease in three cases and hepatic disease in one[33-36] (Table 3).

Seven out of nine patients had a diagnosis of Crohn colitis with median age of 49 (61 if not considering the teenager) years-old and 5 were male. All but two patients, with cutaneous forms, were under two or more immunomodulatory drugs. Six patients were under steroids and six under anti-TNF. Pulmonary nocardiosis was the most common clinical form of Nocardiosis, described in 44% of patients. *N asteroids* was isolated in 3 patients, *N. farcinica*, *N. cyriacigeorgica* and *N. nova* in one case each. Two patients restarted anti-TNF therapy: one under TMP-SMX, 5 mo after starting Nocardia therapy and the other after 6 mo of therapy with TMP-SMX.

CONCLUSION

Nocardiosis is an uncommon disease caused by a Gram positive bacteria with acid-fast staining proprieties and diagnosis requires high levels of suspicion and experience of laboratory staff. The clinical impact of the disease is partly unknown, suggesting an underestimating of the real role of nocardiosis in human diseases. Persons under immunomodulation or immunossupressive therapy require, as those with HIV, organ transplant re-

cipients, patients with pulmonary diseases and diabetes mellitus, special attention concerning the risks of nocardiosis. Remarkably double or triple immunosuppression seems to represent a higher risk for the disease. When concerning patients treated with anti TNF alone, just two cases of cutaneous forms were described. The most common clinical presentations are pulmonary and cutaneous, but the bacteria has the ability to disseminate and affect any organ, in particular the central nervous system. Laboratorial diagnosis is based on the identification of the bacteria (that growths slowly) on biological products, rarely on blood, and in alternative by PCR. The optimal duration of antimicrobial treatment for severe disease is not established but a prolonged course (one year) is advisable, because of the relapsing nature. There are several unanswered questions in nocardiosis infection as the safety of restarting immunomodulators or anti-TNF or the value of prophylaxis with TMP-SMX claiming urgent attention from physicians and investigators devoted to infection and immunological diseases.

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5. Other severe granulomatous infections

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6. Severe intracellular DNA Herpes virus infections

6. Severe intracellular DNA Herpes virus infections

The third aim of this thesis concerned severe Herpes virus infections among patients treated with immunomodulators, biologics or steroids.

First we describe the case of a young lady treated with azathioprine and steroids for an ulcerative colitis who had a disseminated cutaneous herpes simplex virus infection resulting from a reactivation of HSV type1. She was successfully treated with intravenous acyclovir. In the literature, we found five cases of serious herpes simplex infection in IBD patients, all but one treated with steroids alone or in association with azathioprine; two of them died.

Concerning varicella zoster virus (VZV), we describe a varicella pneumonia in a young male treated with azathioprine and infliximab for Crohn's Disease. Because a severe adult respiratory distress syndrome developed, he needed inotropic support and mechanical ventilation for 11 days. He recovered and infliximab was re-started two months later. In the literature twenty cases of primary varicella infection have been reported in IBD patients and five of them died.

Two studies were conducted:

6.1 Disseminated cutaneous herpes simplex infection in a patient with Crohn's disease under azathioprine and steroids: first case report and literature review.

Journal of Crohn's & colitis 2014; 8(4):326-330.

Santos-Antunes J*, Abreu C*, Magro F, Coelho R, Vilas-Boas F, Andrade P, Lopes S, Macedo G.

*the first and second authors contributed equally in the design, conception, analysis and paper writing

6.2 Varicella complicated by severe pneumonia and shock in an immunosuppressed Crohn's disease patient under azathioprine and anti-tumour necrosis factor alpha.

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Abreu C, Santos L, Magro F.

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SHORT REPORT

Disseminated cutaneous herpes simplex infection in a patient with Crohn's disease under azathioprine and steroids: First case report and literature review

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Abstract

Immunosuppressive treatments used in the management of Inflammatory Bowel Disease, namely steroids, thiopurines and anti-TNF drugs, raise the risk of acquiring opportunistic infections. However, most of these infections are mild and self-limited, not requiring specific therapy or suspension of the immunosuppressors. We report a case of disseminated cutaneous herpes simplex infection in a patient with Crohn's disease under steroids and azathioprine.

1. Introduction

The treatment of Inflammatory Bowel Disease (IBD) is associated with an increasing use of immunomodulators, being steroids, thiopurines and anti-TNFα drugs commonly prescribed. Despite a better control of the disease with these drugs, there is a higher risk of infection and, concerning viral agents, benign infections may become severe or disseminated. We report the case of a young female with double immunosuppression (corticosteroids and

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azathioprine) due to Crohn's disease, who developed a disseminated Herpes Simplex Virus (HSV) infection, resulting from a reactivation of latent HSV. A review of the literature regarding HSV in patients with IBD is included.

2. Case description

A 21 years old female patient was admitted with abdominal pain and diarrhoea, with 8 bowel movements per day, without rectal bleeding or fever. Blood tests were remarkable for microcytic anaemia (haemoglobin of 10.9 g/dL) and increased inflammatory markers, namely C-Reactive Protein of 87 mg/L and 13.000 × 10^9 leukocytes per litre. Colonoscopy showed aphthous ulcers in the terminal ileum and in some areas of the transverse colon, with histology showing active chronic colitis. Entero-CT scan showed inflammatory changes in the last ileal loop with an extension of 3 cm. With these elements, a diagnosis of Crohn's disease was made, and the patient started immunosuppression with intravenous steroids and azathioprine. She was discharged asymptomatic one week later under azathioprine (AZA) 50 mg/d and prednisolone 40 mg/d.

After 3 weeks on treatment she was readmitted with diarrhoea (4 bowel movements per day) and fever (max = 38.5 °C). Fever was interpreted in the context of a flare, and she was again treated with intravenous steroids. At day 3 of admission, her clinical status deteriorated and higher fever (max = 40 °C) was elicited with poor response to antipyretics. C-Reactive Protein ascended to 200 mg/L. Antibiotic therapy was begun empirically, with ceftriaxone and metronidazole. An abdomino-pelvic CT scan excluded the presence of intra-abdominal abscess. Hemocultures and urocultures were negative. IgG antibodies against Herpes Simplex Virus 1 (HSV1) and Cytomegalovirus (CMV) were both positive, with negativity for IgM; IgG and IgM antibodies for Epstein-Barr Virus (EBV) and HSV2 were negative.

On the 3rd day, physical examination was remarkable for papular lesions in the extensor face of both legs. During the following days of hospitalization, they spread and evolved, presenting at the 5th day papular, vesicular and pustular lesions, with different stages of evolution, in the lower and upper limbs (Figs. 1 and 2), trunk, back (Fig. 3), face and scalp, suggesting chickenpox. A swab from the vesicular cutaneous lesions looking for HSV and varicella-zoster virus (VZV) DNA was done at that time. Meanwhile, intravenous acyclovir was prescribed as the patient persisted with high-grade fever being double immunosuppressed (which was stopped at this moment) and under antibiotics. Serology showed again IgG positivity for both VZV and HSV1, consistent with the previous infection with these two viruses. The swab became positive for HSV1-DNA and negative for VZV-DNA. A diagnosis of disseminated cutaneous herpes virus infection in the context of double immunosuppression (corticosteroids and azathioprine) was established. There were no mucosal lesions in the lips, mouth or genitals.

The patient became afebrile after the second day of acyclovir and she was treated for 14 days: the first seven days intravenously, followed by oral therapy.

Fig. 2 Vesiculo-papular rash in the arms.

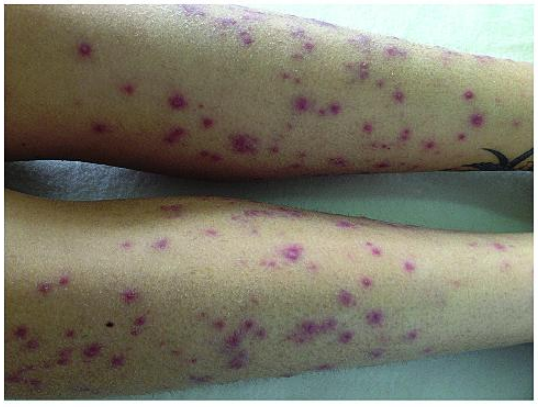

Fig. 1 Vesiculo-papular rash in the legs.

Fig. 3 Close-up image of the back of the patient.

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3. Discussion

We present an atypical case of disseminated cutaneous herpes infection in a patient with Crohn's disease under low-dose of azathioprine and steroids, requiring the suspension of immunosuppressants and the prescription of antivirals against HSV-1. This virus is transmitted by close contact, and primary infections are usually acquired during childhood and adolescence, whereas infection with HSV-2 is responsible for most genital herpes, being mostly sexually transmitted. After the first exposure, HSV become latent in neural cells, and nearly 90% of the adult population has serological markers of previous contact. While these viruses are normally localized in the skin or mucosa of the lips and genitals, with immunosuppressive therapy or in critical ill patients[1] other organs may be affected, being oesophagus the most common gastrointestinal location.[2]

HSV does not seem to be responsible for the aetiology or flares of CD. An experimental study that searched for a vast group of pathogens in colonic mucosa by PCR-based methodologies did not found this virus in any patient with this disease.[3] On the other hand, in UC, super-infections with HSV, with a putative role in the origin of a flare (or, at least, favouring a poorer outcome), were reported.[4]

Patients with IBD may have a higher prevalence of opportunistic or severe infections, due to the immunosuppressive therapy that is increasingly applied. This risk is higher with cumulative therapies,[5] malnutrition, surgery and, probably, by an immunological derangement inherent to the disease.[6,7] In a recent Japanese prospective study, 9.1% of 570 patients with IBD developed opportunistic infections, particularly on those older than 50 years-old and taking azathioprine[6]; HSV and VZV were the main agents observed but, and in contrast to what we showed, all HSV infections were limited to one location (mainly face and genitals).

It is important to highlight that HSV-related disease, even in immunocompromised patients, is normally subclinical or very mild, not warranting either discontinuation of therapy or systemic antivirals. The vast majority of cases of clinical herpes infection in patients with IBD are very limited and benign,[5,6,8–13] as can be analysed in Table 1. Nevertheless, serious events as herpes hepatitis and encephalitis in patients with IBD are also reported, almost all in patients under steroids, with a high rate of mortality[14–18] — Table 2.

Concerning the drugs that our patient was exposed to, azathioprine was found to raise the risk of benign herpes flares independently of steroids or infliximab, in a prospective study in patients with IBD,[8] but it is important to stress that the median time between the initiation of azathioprine and such flares was 76 months. In contrast, in our patient the clinical onset was severe and azathioprine was prescribed only for 3 weeks (and in a suboptimal dose, about 1 mg/kg of body weight per day), which is not enough to even reach its maximum immunosuppressant activity and therefore it may have little contribution for the development of this infection. The reason why azathioprine may contribute to HSV reactivation is not clear, but this drug promotes an immunological derangement in Natural killer cells and CD4 and CD8 lymphocytes, which is known to be crucial in preventing viral infections. Regarding steroids, the literature about its use in IBD and viral infections is very scarce,[19] and the true risk of these agents is very difficult to assess, since it is almost always associated with other maintenance immunomodulators.

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Table 1 Published works that included mild herpes simplex infections in IBD patients.

Reference	Type of publication	Cases (n)	IBD	Drugs at the time of HSV disease (n)	HSV type	Primary vs reactivation	Type of infection	Age/ Gender	Outcome
Toruner M[5]	Observational	18	CD/ UC	Thiopurines (5), steroids (2), none (?), others?	NR	NR	Oesophagus, extremities, face	NR	NR
Naganuma M[6]	Prospective	29	CD/ UC	Azathioprine (14); more than one immunosupressor (18)	NR	NR	Face (26), genitals (2), extremities (1)	NR	NR
Seksik P[8]	Prospective	NR	CD/ UC	NR	NR	NR	Genital and skin	NR	NR
Baumgart DC[12]	Prospective	1	NR	Adalimumab, others?	NR	NR	Limited cutaneous	NR	NR
Lopez-Negre JL[13]	Case report	1	CD	Infliximab	NR	NR	Peri-anal vesicles	31/F	NR
Schreiber S[16]	RCT	3	CD	Certolizumab (2), placebo + other? (1)	NR	NR	Limited, location not specified	NR	NR
Sandborn WJ[17]	RCT	5	CD	Certolizumab (4), placebo plus other? (1)	NR	NR	Limited, location not specified	NR	NR
Sciaudone G[18]	Case report	1	UC	Infliximab	HSV-1	NR	Erythema multiforme plus vesicles in the lips	19/F	Good

IBD — Inflammatory Bowel Disease.
UC — ulcerative colitis.
CD — Crohn's disease.
NR — not reported.

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Table 2 Published cases of serious herpes simplex infections in IBD patients.

Reference	Type of publication	IBD	Drugs at the time of HSV disease	HSV type	Primary vs reactivation	Type of infection	Age/ gender	Outcome
Shlien RD[14]	Case report	UC	Prednisolone 40 mg/d (2 weeks)	HSV-1	Primary	Hepatitis	16/F	Dead
Seksik P[15]	Case report	UC	Prednisone 40 mg/d (8 days)	HSV-1	Primary	Hepatitis	37/M	Dead
Alimohamadi SM[16]	Case report	UC	Prednisolone 10 mg/d, AZA 75 mg/d	NR	Unknown	Encephalitis	22/F	Good
Robineau O[17]	Case report	CD	AZA	HSV-1	NR	Meningoencephalitis	28/F	Good
Francois-Dufresne A[18]	Case report	CD	Prednisone 50 mgnupdb, AZA 150 mg/d	HSV-1	Possible reactivation	Pneumonia	44/M	Sequela

IBD — Inflammatory Bowel Disease.
UC — ulcerative colitis.
CD — Crohn's disease.
AZA — azathioprine.
NR — not reported.

One class of immunomodulators that is being increasingly used in patients with IBD is anti-TNF agents. A recent meta-analysis studied the risk of opportunistic viral infections in patients with Crohn's disease taking biological therapies.[20] The authors only found two Randomized Controlled Trials (RCTs), both in patients with Crohn's disease taking Certolizumab, that describe HSV infections under biologics,[9,10] reporting a total of 6 patients with mild HSV disease. Overall, this meta-analysis found no significant difference in the frequency of HSV infection in patients taking biological therapies (RR = 1.67, CI = 0.46–6.12).

Besides the several cases of benign presentation similar to those found in the general population, in the literature, we can find a case report of a more exuberant dermatological manifestation caused by this virus, namely a HSV-related *erythema multiforme* in a patient with ulcerative colitis taking infliximab[11] — Table 1. However, disseminated skin involvement with multiple papules and pustules as our case, particularly in a patient with Crohn's disease, was not previously reported. Furthermore, it must be highlighted that our patient was young and her presentation was due to a reactivation of latent HSV, since she had history of labial herpes and positive IgG for HSV1. Additionally, she was only on AZA for 3 weeks (combined with steroids) and had a very atypical presentation: besides the disseminated infection with widespread lesions all over the body surface, she had no oral or genital involvement and the lesions were completely painless.

Our patient was treated with acyclovir, a nucleoside analogue that inhibits HSV replication by acting in the viral polymerase after uptake, that is the preferred drug for the treatment of these conditions. In an immunosuppressed patient, intravenous administration for 2 weeks is advisable.

Herein, it is showed a unique and atypical case of HSV reactivation as disseminated cutaneous herpes in a patient with Crohn's disease under steroids, that was also taking azathioprine for 3 weeks. It is crucial to have in mind the raised risk of severe viral infections, even if the mechanism is not yet understood, and to have a high level of suspicion to detect atypical presentations, since an early diagnosis is warranted for the success of the treatment.

Conflict of interest

The author declares that there is no conflict of interest.

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6.2 Varicella complicated by severe pneumonia and shock in an immunosuppressed Crohn's disease patient under azathioprine and anti-tumour necrosis factor alpha.

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Letter to the Editor

Letter to the Editor

Varicella Complicated by Severe Pneumonia and Shock in an Immunosuppressed Crohn's Disease Patient Under Azathioprine and Anti-Tumour Necrosis Factor Alpha

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We report a life-threatening varicella pneumonia in a young man treated with azathioprine and infliximab for Crohn's disease. On timeline he reported a 3-day history of fever, headache, and a progressive, exuberant, cutaneous vesicular rash. He had had contact with a child with varicella, was a smoker, and had no past history of varicella. Anti- varicella Ig G was negative. Work-up was relevant for hypoxaemia [pO_2 = 66 mmHg] while breathing room air, thrombocytopenia [64 000/µl], elevated lactate dehydrogenase [716 UI/l], and C-reactive protein of 44.5 mg/l. Chest radiographs showed diffuse bilateral alveolar infiltrates. A severe adult respiratory distress syndrome developed and he required inotropic support and mechanical invasive ventilation for 11 days. After intravenous acyclovir and aggressive supportive management, the patient was discharged [Day 17] without remarkable sequelae. Infliximab was re-started 2 months after varicella and he is doing well 4 months thereafter.

Varicella tends to be severe in adults, and those on corticosteroids and/or on combination of immunosuppressors seem to be at higher risk.[1] Clinical manifestations in the immunosuppressed host can include ongoing development of lesions over weeks, haemorrhagic skin lesions, pneumonia, hepatitis/acute hepatic failure, neurological disease, or widespread disease with disseminated intravascular coagulation.

Twenty cases of primary varicella infection have been reported in inflammatory bowel disease [IBD] patients[1] and, among the five who died, three had pulmonary involvement. Smoking and male sex[2] are additional risk factors for the development of varicella zoster virus [VZV] pneumonia. Several cases of pulmonary varicella in inflammatory bowel disease [IBD] patients [Table 1] have been reported, and we would like to stress that vaccination must be done in those negative for VZV infection. The attenuated vaccine [Varivax@] is given in two doses, 4 weeks apart, at least 3–4 weeks before beginning immunosuppressors [including prednisolone unless not higher than 2 mg/kg for > 14 days] or more than 3 months after discontinuing these drugs[3] [1 month if isolated steroid therapy].

Our patient was not vaccinated because of ongoing azathioprine therapy. In such cases, specific immunoglobulin for varicella [VZIG] should be administered as soon as possible until 10 days after exposure [125 IU/10 kg intramuscularly to a maximum of 625 IU [5 vials]].[4] If VZIG is not available, intravenous immunoglobulin [IVIG] may be used, as it has been shown to have high levels of varicella-specific IgG. Prophylaxis with valacyclovir [1 g orally three times daily] is advised by some experts for 3–22 days, if VZIG is not available.[5] If varicella shows up, prompt admission for intravenous acyclovir is mandatory. Most situations of primary VZV infection favour the withdrawal of immunosuppression, as we did. In conclusion, this case highlights the risk of severe VZV primary infection in a doubly immunosuppressed patient and the need for early recognition and aggressive management.

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Conflict of Interest

The authors have no conflict of interest to declare.

Author Contributions

CA and FM contributed equally to the concept and design of the study, did the draft of the article, and revised it critically for important intellectual content; CA did the acquisition and interpretation of data. LS also revised the article critically for important intellectual content.

Table 1. Isolated case reports of varicella with pulmonary involvement in IBD patients.

Author, year	Age/sex	IBD	Therapy	Presentation	Pulmonary Xray/CT scan	Therapy	Evolution	Outcome
Deutsh, 1995	15/M	CD	6-MP [6 weeks after stopping prednisolone]	3 day of symptoms, rhinorrhoea, dry cough, wheezing, cutaneous rash, progressive respiratory distress	Diffuse interstitial infiltrates	Acyclovir intravenous	Intensive care, mechanical ventilation on D3	Died D27 of admission. Autopsy: ARDS and DIC
Vergara, 2001	27/M	CD	Azathioprine	Fever 40°C, cutaneous disseminated rash, hypoxaemia, renal and hepatic insufficiency	Diffuse interstitial infiltrate	Acyclovir intravenous	Intensive care admission, multi-organ dysfunction	Died in a few hours
Bernal, 2003	40/F	CD	Azathioprine	Fever 38°C, rash	Diffuse interstitial infiltrate	Acyclovir intravenous, 14 days	Favourable	Normal Xray at week 6, resumed azathioprine
Lemyze, 2003	18/F	CD	Azathioprine [for 9 months]	3 day of symptoms, thoraco-abdominal pain, fever [40°C], cutaneous disseminated rash, dyspnoea, no cough	Diffuse interstitial infiltrate. CT scan: micronodular opacities	Acyclovir intravenous, 10 days	Favourable	Recovered
Leung, 2004	26/M	CD	6-MP + infliximab [9 days after first dose], prednisolone	Fever 38.4°C, chills, nausea, vomiting, abdominal pain, rash	Unknown	Acyclovir intravenous on D2	Respiratory failure on the subsequent days; intubation, mechanical ventilation; hepatic, cardiac, renal failure; DIC	Died D4
Tougeron, 2006	33/M	CD	Azathioprine, infliximab, prednisolone 10 mg/day	2 day of symptoms, thoraco-abdominal pain, fever [38.2°C], cutaneous disseminated rash, hepatitis	Interstitial infiltrate on medium lobe, then diffuse	Acyclovir intravenous, 10 days	Hepatitis, severe cytolysis; dyspnoea and hypoxaemia D2 after admission; favourable after that	Recovered
Springfeld, 2009	25/M	CD	Azathioprine	3 days of symptoms, rash on the back, trunk, and arms, abdominal pain, hepatitis	Unknown	D1: prednisolone 75 mg/day, D2: imipnem, D3:acyclovir intravenous, D5: added foscarnet	Fulminant hepatitis, disseminated rash; dyspnoea; intensive care admission; D3 mechanically ventilated; D5 shock, severe ARDS	Died D6 [multi-organ failure: liver, lung, renal]
Monaghan, 2010	65/M	CD	Azathioprine [1 week of treatment], prednisolone 35 mg/day	Fever, headache, shortness of breath, nausea, vomiting, abdominal discomfort, hypotension, tachypnoea, vesiculopustular rash on forehead and trunk	Unknown	Antibiotics, antifungals, acyclovir, activated protein C, steroids	Hepatic failure and shock; intensive care admission; D3 mechanical ventilation	Recovered
Wuber, 2010	?	?	Infliximab	Pneumonia				Died

IBD, inflammatory bowel disease; CT, computed tomography; M, male; F, female; CD, Crohn's disease; 6-MP, 6-mercaptopurine; ARDS, adult respiratory distress syndrome; DIC, disseminated intravascular coagulation; D, day.

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6. Infectious prevention

7. Infectious prevention

The fourth aim of this thesis was to develop a practical approach to screening, monitoring and prescription of immunizations and chemoprophylaxis on patients about to start biologics, based on the risks identified on goal 1, 2 and 3. The purpose is to reduce the infectious risk and raise the safety of these therapies.

Two studies were conducted:

7.1 Immunisations in Crohn's disease: who? why? what? when?

Best Practice & Research of Clinical Gastroenterology 2014; 28(3):485-496.

Magro F, Abreu C.

7.2 Screening, prophylaxis and counselling before biological therapies: a practical approach

Digestive and Liver Diseases*, 2017 – in press accepted manuscript*

Abreu C, Sarmento A, Magro, F.

7. Infectious prevention

7.1 Immunisations in Crohn's disease: who? why? what? when?

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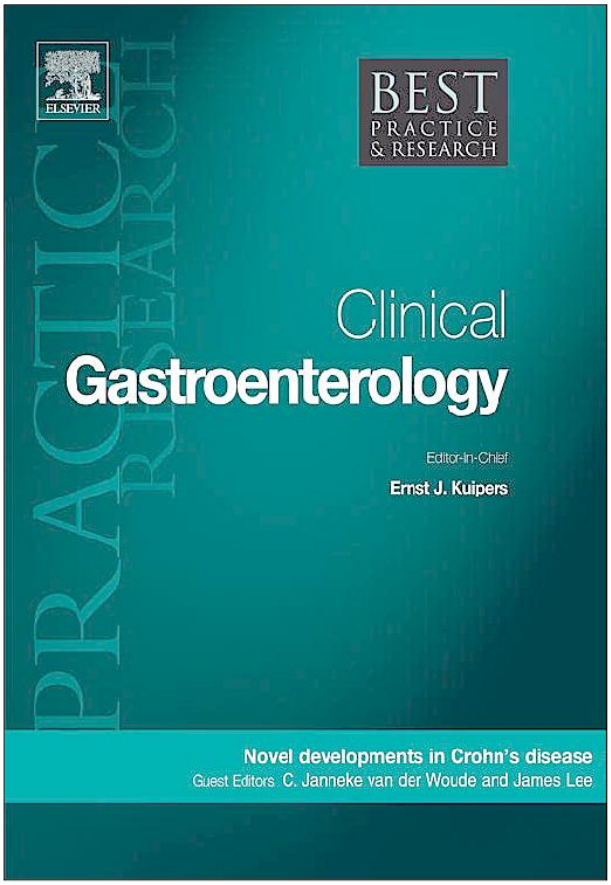

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Immunisations in Crohn's disease: Who? Why? What? When?

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Crohn disease
Inflammatory bowel disease
Immunosuppression
Immunomodulator medication

A B S T R A C T

Immunosuppression induced by drugs increase the risk of infections in Crohn's disease (CD) patients. The vaccination rate in CD patients is usually low due to inaccurate information concerning the safety and efficacy of vaccines. Vaccines and immunoglobulins, are artificial ways of protection from common infectious diseases and they have had a major effect on mortality. Herein we detail the need of protection induced by vaccines of measles, varicella, Zoster, papillomavirus, shingles, pneumococcal invasive disease, influenza, hepatitis A and B in CD at diagnosis and during the course of the disease even during immunosuppression periods but with different singularities. Vaccination in CD travellers and the matters related to immunization of household healthy members of immunosuppressed patients are also discussed.

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7. Infectious prevention

Introduction

Immunisations, vaccines and immunoglobulins, are artificial ways of protection from common infectious diseases. With the exception of safe water no other modalities have had such a major effect on mortality reduction and population growth than vaccination [1]. Immunosuppression by drugs [2] and CD by itself, increases the risk of infections [3]. CD patient is considered immunosuppressed when is taken one or more of these: glucocorticoids, more than 20 mg/day of prednisolone or equivalent, calcineurin inhibitors (specific T-cell inhibitors) like cyclosporine, tacrolimus, antiproliferative drugs (cytotoxic drugs) as thiopurines, cyclophosphamide, methotrexate, chlorambucil, mycophenolate mofetil (MMF) and anti TNF alfa [4–6].

Vaccination rates are frequently low in inflammatory bowel diseases (IBD) patients [7]. Nineteen percent of this study population had been vaccinated against influenza, 3% against pneumococcal pneumonia, 22% against hepatitis B and 5% against varicella. Of those who had travelled, 9% had been vaccinated for hepatitis A and 1% for yellow fever. This under vaccination may occur because clinicians have insufficient or inaccurate information concerning the safety and efficacy, of such vaccines [8], unawareness of the infection risk and concerns about the hypothetical exacerbation of disease activity after vaccination [5]. Herein, we will be focused on practical aspects of immunization of adults, older patients, children and infants born from mother's immunomodulated. Vaccines from attenuated *versus* inactivated agents will be discussed concerning indications and contraindications, table-time of administration, and efficacy. For susceptible patients protection from common agents like measles, varicella and shingles, pneumococcal invasive disease, hepatitis A and B will be detailed. Vaccination in CD travellers and the issues related to immunization of household healthy members of immunosuppressed patients will be discussed (Table 1).

Who and why?

Immunization schedules for adults should be considered for each CD patient, according to his age and individual risk. At CD diagnosis a complete review of previous immunization is mandatory and all patients with incomplete series should commence catch-up vaccination [4]. Soon or later CD patients may go through immunomodulation [9] and ideally vaccination should precede immunomodulators for better efficacy. A compromised immune system may be unable to mount a sufficient response to immunization, and the protection afforded by the vaccine may be lessened [10]. Nevertheless, when this didn't happen immunization should be done even though immunosuppression has been done in the hope that some protection will be reached. The goal is to decrease morbidity and mortality from vaccine preventable diseases and there is now an amount of evidence that vaccines are not related with CD flares [5].

What?

Adult CD patients should be protected for the common agents like measles, varicella, influenza (annual vaccine), tetanus, diphtheria, polio, hepatitis A and B and also pneumococcus. Pneumococcus vaccine for adults (see below) follows different schedules for IBD patients when compared with the healthy adults. Furthermore, IBD vaccinations have some peculiarities, they need sometimes an extra dose (for instance for hepatitis B) or different formulations like inactivated vaccines instead of attenuated in polio and typhoid vaccines in those under immunomodulation. As shingles seems more common in IBD patients under anti TNF drugs or thiopurines [11] and may be more severe, zoster vaccination for those with 60 or more years old becomes more relevant.

When?

Whenever possible immunization should be done before immunomodulation: at least 2 weeks before for inactivated vaccines and at least 4 weeks for attenuated vaccines. If not possible, vaccination should be done in the less immunosuppressed periods and whenever possible vaccine protection

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Table 1
Vaccines in adult persons with Crohn's disease.[e]

Vaccine	At CD diagnosis	Under immunosuppression[a]	Frequent vaccination schedule
Common vaccines			
Hepatitis A	☑	☑	2 doses: 0–6 to 12 mo.
Hepatitis B	☑	☑	3 doses: 0–1–6 mo. or 0–7–21 days and one year (Same schedules if hepatitis A+ B vaccine)
Td, Tdap	☑	☑	One dose every 10 years; one of this vaccine should be Tdap
Humanpapillomavirus	☑(11–26 years)	☑(11–26 years)	3 doses: 0–1 – 6 mo
Influenza-inactivated	☑	☑	One dose every year
Influenza-live attenuated	☑	×	–
Measles, mumps, and rubella–live	☑	×	2 doses: 0–4 weeks
Pneumococcal conjugate (PCV13)	☑	☑	One dose: ideally before PPSV23; if after PPSV23: ≥1 year
Pneumococcalpolysaccharide (PPSV23)	☑	☑	One dose repeated each 5 years until 65 of age; separated ≥8 weeks if after PCV13
Polio-inactivated (Salk)	☑	☑	3 doses: 0–1 and 12 mo or separated by ≥4weeks
Varicella–live	☑	×[c]	2 doses: 0–4 weeks
Zoster–live	☑(50–59 years)[d] ☑(≥60)	×	One dose
Travel related vaccines			
Cholera, oral (inactivated)	☑	☑	2 doses if >6 years: 0–1 week Repeat each 2 years if needed
Hepatitis A	☑	☑	Same as above
Japonese encephalitis (inactivated)	☑	☑	2 doses: 0–4 weeks; if booster needed: one dose >1 year after the first
Meningococcal, conjugate (A,C,Y, W135) (MCV4)	☑(2–55 years)[b]	☑(2–55 years)[b]	One dose; repeated after 5 years if protection still needed
Polio, inactivated (Salk)	☑	☑	One dose (if > 10 years after a complete schedule vaccination); same as above if not vaccinated before
Tick borne encephalitis	☑	☑	3 doses (0, 1–3 mo, 6–15 mo)
Typhoid, inactivated	☑	☑	One dose; each 2,5–3 years
Yellow fever	×	×	One dose; each 10 years if needed

☑Administer if patient is not current with recommendations for adult immunocompetent persons in same risk and age categories; × – contraindicated.

[a] If not vaccinated previously.

[b] More than 55 years: meningococcal polysaccharide vaccine (A,C, Y, W135), (MPV4).

[c] Varicella vaccine is admitted for ACIP if low level immunosuppression (treatment with prednisone <2 mg/kg with a maximum of ≤20 mg/day; methotrexate ≤0.4 mg/kg/week; azathioprine ≤3 mg/kg/day; or 6-mercaptopurine ≤1.5 mg/kg/day).

[d] Recommended by IDSA guidelines; contraindicated by ACIP recommendations.

[e] Adapted from the protocol immunomodulation and risk of Infection Consult (IRIC), Centro Hospitalar S. João-Porto, Portugal.

(quantification of antibodies) should be done. This is particularly true in old age groups because vaccine efficacy decline with age [12,13].

Vaccinations of adult patients with CD

For those who had been vaccinated against measles, mumps, rubella, polio, hepatitis B, tetanus toxoid and reduced diphtheria toxoid (Td), booster of tetanus and diphtheria toxoids should be done every ten years. For American Committee of Immunisations Practice (ACIP) one of the tetanus boosters should include pertussis (Tdap), independent of the age group or medical condition [14,15].

7. Infectious prevention

Inactivated vaccines

Combined Tetanus toxoid and reduced Dipheteria toxoid (Td) and combined Tetanus toxoid, reduced Dipheteria toxoid and acellular Pertussis (Tdap)

Recommendations

Td vaccine is considered safe for immunosupressed patients and boosters are recommended each 10 years by CDC and WHO regardless of immunosuppression. For adults current guidelines from ACIP recommend a booster vaccination with Tdap after the age of 18 years [15].

Response

Response to tetanus immunization in IBD patients is not clear, since there are studies [16] that have shown that there is a normal response to the tetanus vaccine, while others [17] suggesting an impaired anti-tetanus response. A few evaluated booster response rates and geometric mean titre (GMT) of antibodies following administration of tetanus boosters in IBD patients. One of them included 59 IBD patients categorized by their level of immunosuppression (ie, no therapy, immunomodulator monotherapy, biologic monotherapy, or combined immunomodulator and biologic therapy). Serum antibody levels and GMTs were measured at baseline and near 4 weeks after vaccination. All IBD patients who were not on immunosuppression achieved protective tetanus titres as well as 78% of those on combined therapy ($P = .01$) [18]. To best of our knowledge no cases of tetanus vaccine failure in IBD patients were, so far, published on the literature. The safety and immunogenicity of the pertussis booster in patients with IBD was studied by Dezfoli et al. [18] in 25 patients with IBD being serum antibody titres against pertussis measured at baseline and 4 weeks after vaccination. Response rates between patients without medication and those on anti TNF monotherapy showed no differences but in those on immunomodulator monotherapy the response to one of the pertussis antigen was lower, and post-vaccination protective titres of pertussis filamentous Haemagglutinin (FHA) were lowest on combination therapy. So, CD patients should receive a Tdap vaccine ideally before initiation of immunomodulators [18].

Hepatitis B vaccine

Recommendations

All IBD patients should be screened for hepatitis B at diagnosis [19]. HBs Ag-positive patients would receive anti-viral prophylaxis before starting immunosuppressants and those HBs Ag negative and not vaccinated should be vaccinated against HBV at diagnosis or, if it was not possible at the diagnosis, in remission periods of CD [20]. Primary adult vaccination consists of three or more intramuscular doses administered intramuscularly (delthoid muscle) at 0, 1, and 6 months; alternative vaccination schedules (0, 1, and 4 months or 0, 2, and 4 months) elicit dose-specific and final rates of seroprotection similar to those obtained with a classical schedule [21]. Recently Gisbert et al. showed that a double dose administration (0, 1 and 2 months) was associated with a higher response rate in IBD patients [19]. The combined hepatitis A and B vaccine (Twinrix®) may be used for vaccination of persons aged with more than 16 years in the same schedule. A four-dose schedule, approved by FDA, is administered at 0, 7, and 21–30 days, followed by a dose at 12 months (MMWR, 2007). Nothdurft et al. compared Twinrix® administered at 0, 7, and 21 days with hepatitis A at day 0 plus hepatitis B vaccine administered at 0, 7, and 21 days. Both groups received a booster dose at 12 months. The seroprotection rate against hepatitis A and hepatitis B were comparable [22]. Nevertheless, some authors point that seroprotection is higher when the combined vaccine is administered [23].

Response

The response rate of HBV vaccination is influenced by medication and disease activity [20] and by smoking, obesity, genetics [24–26], age and gender [27,28]. By age of 60 years protective levels of antibody developed in only 75% of vaccinated [27] but for those with less than 40 years old a protective antibody response is achieved in more than 90% after the third dose [29]. A tendency towards a lower rate of response to vaccine is usually found in the immunosuppressed patients [19]. The response may

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be lower in IBD patients, especially on those receiving anti-TNF therapy [19] and in active IBD patients compared to patients in remission (41% versus 63%) [20]. Although in one study in IBD patients under 6-mercaptopurine the response of HBV vaccination was similar compared to controls [16]. Adequate serologic response to immunization is usually considered when, one to two months after vaccination, antibodies to HBs antigen are higher than 10 UI/L (WHO, ACIP/CDC) and should be evaluated in all IBD patients. For those immunosuppressed some authors consider an effective immune response if antibodies to HBs antigen are more than 100UI/L [19,20]. Patients with an inadequate response may receive extra doses of vaccine but studies are lacking to determine if booster immunizations are needed [30]. Although 25%–50% of the patients that did not respond to a primary three-dose vaccine they responded to an additional dose, and 44%–100% respond to a second three-dose course. Three additional doses of hepatitis B vaccine in the case of inadequate immune response to the three dose vaccine [31] was also recommended. Others suggested revaccination with a 3 dose regimen using a double dose [32].

Hepatitis A vaccine

Recommendations

Probably more than 30% of the adult population in developed countries didn't had contact with hepatitis A virus (HAV) [33]. This infection has easy transmission by food and water and the disease may be much more severe in adults than in children, so vaccine protection for those who are seronegative for HAV seems advisable. Some groups like men that have sex with men, intravenous drug users, travellers to developing regions, and persons with chronic hepatitis B or C virus have a higher risk of the disease [33]. Vaccine is inactivated and safe, administered intramuscularly into the deltoid muscle, two doses, separated by 6–12 months [21]. The combined hepatitis A and hepatitis B vaccine may be done, as already said.

Response

Seroconvertion is elicited in virtually everyone after the second doses and protective levels of anti-HAV could be present for more than 25 years in adults and for more than 14–20 years in children [34]. In a prospective controlled study in 66 paediatric IBD patients, of whom 49 were taking thiopurines and/or steroids, the rate of seroconversion was similarly high after the second dose (>97%) [35].

The absolute lower limit of anti-HAV required to prevent HAV infection has not been define [30]. In vitro studies using cell-culture-derived virus indicated that low levels of antibody (e.g., <20 mIU/mL) can be neutralizing [36]. Some concerns about the efficacy of the vaccine in hepatic transplant patients and patients with chronic liver disease [37,38] still exist. It is not yet established if it is necessary to quantify the antibodies levels of hepatitis A vaccine for those immunosuppressed, since the protect value is not clearly defined and the sensibility of the tests are variable [30].

Influenza vaccines

Recommendations

Influenza infection may result in serious illness in immunocompromised individuals, and may be followed by medical complications. ACIP recommends annual influenza vaccination for all immunosuppressed patients, as well as for everybody with 6 or more months of age [39]. Inactive influenza vaccine (two influenza A and one influenza B strains) revealed to be a safe and effective in both children and adults with chronic diseases [40,41]. More recently inactivated influenza vaccines with 4 antigens (two influenza A and 2 influenza B strains) are available in some countries and may become more common in the near future. Protection against the type B flu strain may be especially important for children since influenza B causes a substantial number of illnesses, hospitalizations and deaths in the paediatric setting. At the moment no preferential recommendation was made for any type of inactivated vaccine or brand of licensed influenza vaccine over another by ACIP [39]. Inactivated influenza vaccine, high-dose, [60 μg of each vaccine antigen (180 μg total)] has no special recommendation by ACIP for those aged 65 years or more or immunosuppressed patients. Live attenuated nasal vaccine, quadrivalent or trivalent, are not recommended for CD immunomodulated treated patients or those older than 49 years [39].

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Persons who live with or care for patients leaving with immunomodulated CD should receive inactivated flu vaccine [39].

Response

Most studies about the efficacy of the influenza vaccine in IBD patients have been carried out in children. Mamula et al. [42] compared response to influenza vaccine in children with IBD with controls and found less effective responses to one-third of antigens, and patients on combination treatment, had an impaired response to two-thirds of antigens. Lu et al. [43] found similar response rates to influenza vaccinations in IBD children regardless of their immunosuppressive therapy and similar conclusion was found in a recent study in IBD adults patients on thiopurines treatment that were able to mount an effective immune response to the vaccine [16].

Pneumococcal vaccines

Recommendations

Pneumococcal infection is responsible for more deaths than all other vaccine-preventable bacterial disease. Chronic immunosuppressive therapy and chronic illness are well-known risk factors for pneumococcal infection and therefore these patients should be vaccinated. Before October 2012 the vaccine indicated for immunosuppressed patients was the 23-valent polysaccharide (PPSV23). A one-time revaccination dose of PPSV23 is recommended 5 years after the first dose for immunocompromised persons. All adults are eligible for a dose of PPSV23 at age 65 years, regardless of previous PPSV23 vaccination; however, a minimum interval of 5 years between PPSV23 doses should be maintained [44]. Current indication by ACIP for immunosuppressed patients naïve to pneumococcal vaccine is to vaccinate with the 13-valent conjugate vaccine, a much more immunogenic vaccine but with only 13 serotypes, followed at least 8 weeks after by the 23-valent polysaccharide vaccine (PPSV23). Given the high burden of invasive pneumococcal disease caused by serotypes in PPSV23, but not in PCV13, broader protection might be provided through use of both pneumococcal vaccines. This latter vaccine should be repeated 5 years later if the first dose is administered before 65 years old, due to antibody decline. For those who previously have received $\geq$1 doses of PPSV23 should receive a PCV13 dose $\geq$1 year after the last PPSV23 dose. For those who require additional doses of PPSV23, the first such dose should be given no sooner than 8 weeks after PCV13 and at least 5 years after the most recent dose of PPSV23.

Response

Several studies point that patients on combined immunomodulator and biologic therapy had a significantly lower response rate compared to non-immunosuppressed patients to PPSV23 vaccine [41,45,46]. Antibody responses to the pneumococcal vaccine in 96 patients with IBD showed a decreased response in patients receiving a biologic agent alone or in combination with thiopurines compared to patients receiving only 5-aminosalicylic acid [47]. In a prospective cohort study, Dotan et al. evaluated the effects of thiopurines on immune response in 28 IBD patients vaccinated with PPSV23 [16]. In this study thiopurines, at doses commonly used in IBD patients, did not diminish immune response to the pneumococcal vaccine.

Papillomavirus vaccine

Recommendations

In immunosuppressed patients like HIV infected and transplanted patients, the infection with human papillomavirus (HPV) and the development of cervical dysplasia and cancer is higher than in healthy controls [48,49]. High-risk types of HPV (eg, types 16 and 18) are associated with 70% of all cervical and anogenital cancers [50]. In women with IBD several studies have shown that their risk for cervical pap smear abnormalities and HPV infection is three to five times higher than well match controls [51–53]. Nevertheless other studies have not found an association between IBD and cervical dysplasia unless patients are under immunosuppressive therapy or they smoke [54,55]. The first vaccine for cervical cancer was introduced in 2006 and is a quadrivalent human papillomavirus vaccine

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(HPV4) given in a three dose series over a 6-month period. It is indicated for the prevention of disease caused by HPV types 16, 18, 6 and 11. It is recommended for girls 11–12 years, with a 'catch up' for unvaccinated women aged 13–26 years. After this first vaccine a bivalent human papillomavirus (types 16 and 18 of HPV – Cervarix®) vaccine was licensure, only for women, with the same schedule of administration and contraindications [56].

Recently, the ACIP expanded its guidelines and vaccine (HPV4) recommendations to include males aged 9–26 years, if they have other high risk factors like HIV infection, homosexual activity or immunosuppression [57]. The vaccine should be administered ideally prior to initiation of sexual activity [58].

Response

A study concerning the immune response of HPV vaccine in patients (9–26 years-old) with IBD under immunosuppressive agents compared with normal control subjects is ongoing (ClinicalTrials.gov identifier: NCT00727636). A small study of 30 girls and women with IBD who were on immunomodulator or biologic therapy received the vaccine and the immunologic response to the HPV4 vaccine was assessed. More than 90% of the patients became seropositive for each of the 4 serotypes, and post-vaccination geometric median titres (GMT) of antibodies were comparable to those of historical controls [59]. Moreover, HPV4 vaccine was safe and immunogenic in most women with IBD who were on immunomodulated therapy.

Attenuated vaccines

Measles, mumps and rubella vaccine (MMR)

Recommendations

Measles remains a common disease in many parts of the world, including restricted areas in Europe, Asia, the Pacific, and Africa. In Europe, most of the measles cases result from international travel. People who get infected in other countries bring the disease that can be spread quickly. As the vaccine is available in combination with mumps and rubella antigens we will refer always measles vaccine as the MMR vaccine. Students attending colleges or other post-high school educational institutions, healthcare personnel, and international travellers are high risk groups and the administration of 2 doses of MMR vaccine separated by 4 weeks [60] is recommended. Special attention should be paid for travellers to countries were measles is endemic. Protection results from vaccination or from the disease itself.

At CD diagnosis if there is no known protection to measles, serology should be done and vaccination might be considered at least one month before beginning immunomodulation, although there are no studies investigating its use in non immunomodulated patients with IBD [61]. For those under immunosuppressed therapy vaccination should only be done at least 3 months after stopping immunomodulation. If a CD patient not protected to measles comes into contact to a patient with measles, immunoglobulin for protection should be done, no more than 5 days after the contact [60]. Furthermore, susceptible close contacts of these patients should be vaccinated [18].

Precautions for MMR vaccine include recent ($\leq$11 months) receipt of an antibody-containing blood product, moderate or severe illness with or without fever, history of thrombocytopaenia or thrombocytopenic purpura, and tuberculin skin testing. If a tuberculin test need to be performed, it should be administered before, simultaneously with, or at least 4–6 weeks after administration of MMR vaccine because MMR vaccine is a cause of false negatives in tuberculin test. Also, in persons with a personal or family history of seizures of any aetiology, additional precaution for MMR plus varicella vaccine (MMRV) should be taken [60].

Varicella vaccine

Recommendations

Varicella zoster virus (VZV) causes chickenpox, a common disease in children, and may cause a more severe disease in adults. Reactivation can occur after a period of latency, resulting in herpes

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zoster, commonly referred to as shingles. Disseminated disease and severe complications and death may occur. In two cohorts of IBD patients under immunomodulation VZV were the most common viral infections [62,63]. Immunosuppression increases the risk of VZV infection and herpes zoster in patients with CD [11]. TNF-α inhibits replication of VZV and VZV antigen expression and it has been shown that blocking TNF-α by monoclonal antibodies completely inhibits this antiviral activity [64].

Primary prevention with the varicella vaccine for immunocompetent adults without evidence of varicella immunity is done with two doses of attenuated vaccine separated by four to eight weeks. Vaccine is derived from the Oka strain of live, attenuated VZV [65]. Serologic testing is recommended to all patients with CD without a clear history of chickenpox or vaccination before initiating immunosuppression. For those naive for VZV, vaccination should be consider at least one month before the beginning of immunosuppression.

Being an attenuated vaccine, patients under immunomodulated therapy should not be vaccinated. VZV vaccine appears to be safe to administer for those patients on corticosteroids after a one-month hiatus. For others immunomodulators a 3-month period is needed before administrating of live vaccines [5,66]. Patients with CD treated with immunomodulators and recent varicella exposure should get protection with passive antibody, that is, varicella zoster immunoglobulin (VZIG) given within 10 days of exposure [67]. A carefully observation should be maintained over the next 4 weeks for evidence of the disease, and antiviral therapy should be started immediately in case of varicella [66].

Zoster vaccine

Recommendations

For healthy persons the incidence and severity of zoster increase with age, in association with the decreased in cellular immunity. Age is the most important risk factor for zoster [65]. Patients with IBD, especially those on immunomodulated therapy, are at higher risk for herpes zoster compared with the general population [11]. The zoster vaccine is similar to the varicella vaccine but at least 14 times more potent then varicella vaccine and is administered in one dose [65]. The vaccine is recommended for individuals older than 60 years not vaccinated against varicella, to prevent herpes zoster and to reduce the severity of the complications of herpes zoster infection namely post herpetic neuralgia (PHN) [65]. For patients with CD older than 60 years, the vaccine should be prescribed at least 2 weeks (and for others authors 4 weeks) before starting immunomodulation therapy. According to ACIP recommendations, patients under therapy that induce a low level of immunosuppression [short-term corticosteroid therapy (<14 days), low doses of methotrexate (<0.4 mg/kg/week), azathioprine (<3.0 mg/kg/day), or 6-MP (<1.5 mg/kg/day)] may not be considered sufficiently immunosuppressed, and administration of the zoster vaccine may not be contraindicated [65].

For those who are aged 50–59 years and varicella positive prior to initiation of immunomodulation or being treated with low-dose immunomodulation Zoster vaccination is recommended by Infectious Diseases Society of America (IDSA) guidelines published in 2013 but not by ACIP [65]. In addition, by IDSA recommendations, zoster vaccination could be considered prior to initiation of immunosuppression for patients aged 13–49 years with a chronic immune-mediated or inflammatory disorder who have a history of varicella or who are seropositive despite no previous varicella vaccination; however, safety and effectiveness data are lacking (Rubin, IDSA guidelines 2013). As antiviral for herpes virus may interfere with the replication of the VZV-based zoster vaccine, persons under chronic acyclovir, famciclovir or valacyclovir should discontinue these medications at least 24 hours before zoster vaccine [65] and for the next 14 days following vaccination [65].

Response

Vaccine efficacy was accessed in a phase 3 vaccine trial (Shingles Prevention Study), a double-blind randomized placebo-controlled trial involving 38,546 healthy adults older than 60 years old. The vaccine reduced the risk for developing zoster by 51% and prevented PHN in 67% persons. In general, with increasing age at vaccination declines the vaccine efficacy, but the vaccine retained efficacy against severity of zoster better than against zoster itself. For persons older than 80 years the efficacy against zoster was 18%, but the efficacy against PHN was 39% [68]. Zoster vaccine is not contraindicated

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for household contacts of immunosuppressed patients, however if a localized rash develop, the immunosuppressed contacts should avoid rash exposure and the vaccinated person should keep the rash covered [65].

Special conditions

Vaccines for children with CD and infants born from mothers treated with biologics during pregnancy

As anti-TNF-α agents are frequently prescribed during pregnancy and since both infliximab and adalimumab have been shown to cross the placenta and are detectable in cord blood, precautions should be taken concerning vaccination of these infants. In fact, measurable levels of infliximab have been detected in the blood of infants up to 6 months after birth [69,70] and the same was found for adalimumab but not for certolizumab pegol [71]. So, BCG and rotavirus vaccine, both attenuated vaccines, should not be given during the first 6 months of these infants or should not be administered until the biological agent is no longer measurable in the circulation of the offspring [72–74]. BCG may be administered after 6 months and MMR and varicella vaccines, in the usual schedule, at 12 and 12–15 months respectively. Rotavirus vaccine should not be prescribed, since according to ACIP recommendations and due to the risk of intussusception, the first dose of vaccine should be done at maximum age of 14 weeks and 6 days (Centers for Disease Control and Prevention [75]. In children with IBD treatment at diagnosis with exclusive enteral nutrition offers an opportunity to update and optimize the patients' vaccination status. Special care should be taken for not prescribe immunomodulators agents before a month after the administration of attenuated vaccines.

Vaccines for travellers

Recommendations

Inactivated vaccines (parenteral typhoid, hepatitis A and B vaccines, japonese encephalitis, tick borne encephalitis, polio (SalK), oral cholera, meningococcal) should follow the usual recommendations for the healthy travellers, according to travel medicine experts. Studies are lacking concerning efficacy of these vaccines in CD patients with the exception of hepatitis.

Attenuated vaccines (yellow fever, oral typhoid, measles, oral polio) should follow the usual contraindications for the immunomodulated traveller. Being yellow fever vaccination mandatory for the entrance in some sub-saharian Africa countries and recommended for other African and south America countries if the traveller is under immunomodulators a vaccination waiver should be given by a vaccination Centre, certified by WHO. The patient should be adverted about the risk of yellow fever disease in the absence of the vaccine and how to protect from insects bites or even advise not to travel.

Measles is still endemic in some countries and immunization is more relevant for the traveller to areas in developing. Two doses of vaccine, separated by 4 weeks, are advisable for adult travellers who are not natural immunised nor vaccinated [60]. Oral typhoid and oral polio vaccines should be replaced by the inactivated vaccines in the immunomodulated CD travellers.

Vaccination of household contacts of the immunosuppressed CD patient

Vaccination of household contacts of CD immunosuppressed patients rises some concerns. Rotovirus vaccination is not contraindicated among immunosuppressed household contacts, but meticulous hand washing should be practiced after changing the diaper of a recently vaccinated infant [18]. For household members vaccinated against varicella or zoster contact precautions should be observed in the case of a rash develops following vaccine administration; these precautions should be continued until resolution of the rash [18]. The oral polio vaccine should not be given to household contacts of immunosuppressed patients as the virus is shed in the faeces and may inadvertently vaccinate the person under immunomodulators (herd immunity).

Conflict of interest

The authors declare no conflict of interest.

Research agenda

- Large prospective randomized studies have to be conducted to show the effect of imunomodulators and anti TNF in the vaccine response rate.
- Prospective randomized studies in IBD patients with Zoster vaccine are necessary .
- Research should focus on the amount of immunosuppression that

should be achieved for contraindicate live vaccines

Practice points

- Patients with CD are at increased risk for a variety of vaccine-preventable diseases.
- Inactive vaccines are considered safe but may be less effective in patients under imunomodulators
- Live vaccines are contraindicated in CD patients under imunomodulators therapy.
- Vaccines should be thought and prescribed ideally before immunosuppression to maximize efficacy and avoid contraindications.
- In IBD immunomodulated patients an attenuated vaccine should only be done 3 months after stopping immunomodulation and these drugs should be resumed only after 4 weeks after vaccination.
- Vaccine indications and contraindications in CD treated patients may change with time and knowledge should be up-to-date

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7.2 Screening, prophylaxis and counselling before biological therapies: a practical approach

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Review Article

Screening, prophylaxis and counselling before the start of biological therapies: A practical approach focused on IBD patients

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ABSTRACT

The standard of care in the management of immune-mediated inflammatory conditions relies on immunomodulators, glucocorticoids, and biologicals (including anti-tumour necrosis factor –α and other monoclonal antibodies). These agents have an overall favourable benefit/risk ratio; however, they modulate the immune response as part of their mechanisms of action, and therefore they may increase the risk of developing infections, particularly in older patients or in patients with concomitant corticosteroids. Some of these infections may be preventable by immunization, chemoprophylaxis or counselling. AIM: screening for and monitoring infections throughout these therapies is so mandatory to ensure patients' safety. Still, standardized guidelines focused on these procedures have yet to be established. This review aims to fill such a gap. The authors searched for articles published in English from 2009 until 2017 using PUBMED, with the terms "immunomodulators", "biological drugs", "anti-TNF α", "inflammatory bowel diseases", "immunomediated inflammatory diseases", "risk of infection", "infection prevention", "screening", "immunization", "tuberculosis", "latent tuberculosis", "listeriosis", "endemic mycosis", "*Pneumocystis jiroveci* pneumonia", "granulomatous infection", "varicella", "herpes virus", "hepatitis B", "hepatitis A", "hepatitis C" and identified the journal articles. Based on the literature and in their own experience the authors established recommendations and a practical guide for infections' screening, monitoring and prevention before and during immunomodulatory and biological therapies.

1. Introduction

The current therapeutic strategies employed in the management of immune-mediated inflammatory conditions include medication with immunomodulators, glucocorticoids and, more recently, biologicals (monoclonal antibodies, namely anti-tumour necrosis factor α [anti-TNFα]). These agents have an overall favourable benefit/risk ratio; however, they do impose a high risk of infections development [1–4]. In fact, and according to current guidelines [5], patients on glucocorticoids (prednisolone 20 mg/day or equivalent for two weeks or more), immunomodulatory drugs and biological agents should be considered immunocompromised.

Due to the infectious risk, screening for infection and monitoring, as well as prevention based on immunizations practices and counselling, are important issues related to the treatment of inflammatory immunomediated patients.

Despite the importance of a rigorous baseline assessment and routine-based monitoring, standardized guidelines focused on these procedures have yet to be established.

This review gathers together screening, prophylactic and monitoring procedures that can be used as a practical guide by those who treat immune-mediated inflammatory diseases' patients in order to reduce the infection risk before and during immunomodulatory and biological therapies.

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Table 1
Checklist for the medical interview.

Medical history	Check
Tuberculosis or tuberculosis exposition	
Respiratory disorders	
Hepatic disorders	
Cardiovascular disorders	
Joint symptoms	
Neurologic diseases and symptoms	
Hematologic disorders	
Diabetes mellitus	
HIV infection	
Other sexual transmitted diseases	
Immunizations	**Check**
BCG (data)	
Hepatitis A	
Hepatitis B (data) anti HBs quantified >10 UI/mL	
Measles/MMR	
Pertussis Booster of Tdap[a]	
Pneumococcal:conjugate –Polissacharidea – (data)	
Influenza (annual)	
Papilomavirus	
Varicella	
Others	**Check**
Malignancies (type, data, treatment)	
Cancer screenings (according to age and sex)	
Pregnancy issues	

[a] Booster of *Tetanus diphteria* and acellular *Pertussis*.

1.1. Search strategy and selection criteria

We searched for articles published in English from 2009 until 2017 using PUBMED. A search with the terms "immunomodulators", "biological drugs", "anti-TNF α", "inflammatory bowel diseases", "immunomediated inflammatory diseases" and "infection", "risk of infection", "infection prevention", "screening", "immunization", "tuberculosis", "latent tuberculosis", "listeriosis", "endemic mycosis", "*Pneumocystis jiroveci* pneumonia", "granulomatous infection", "varicella", "herpes virus", "hepatitis B", "hepatitis A", "hepatitis C" and identified the journal articles. We also read some other papers cited in these articles.

2. The clinical interview

A detailed clinical interview focused on the patient's medical history should be carried out before the prescription of immunomodulatory drugs and/or biologicals—a checklist for such an interview is depicted on Table 1. One should keep in mind that the utilization of immunosuppressive drugs in the past may carry and additional risk of infection [6,7]. Moreover, patients afflicted with diabetes or chronic pulmonary obstructive syndrome are particularly prone to suffer infections [8], as are patients with renal insufficiency. Tuberculosis (TB) is a key issue in the field: patients should disclose whether they were previously diagnosed with latent or active TB, and should depict any known contact with TB patients. Moreover, patients should also disclose whether and when they had TB screening tests in the past – either tuberculin skin tests, interferon gamma release assays (IGRAs) or pulmonary X-rays – as well as the result(s) obtained. Previous surgeries should also be disclosed, as well any places were the patient has lived in or travelled to where there is an intermediate or high risk for TB, or where endemic mycosis (histoplasmosis, coccidoidomicosis) or *Strongyloides stercoralis* infections are reported. Previous allergic reactions should also be inquired during this interview, as well as the gynaecologic and obstetric history of female patients. Sexual risk behaviours and sexual-transmitted diseases (STDs) must also be disclosed. Regarding patients' personal habits, smoking and alcohol intake should be taken into consideration. Finally, patients' immunization history should be carefully checked.

3. Patients' counselling

3.1. General counselling

All risks and alarm signs should be carefully explained to the patient during his/her first appointment. Patients should be instructed to seek medical care should any of the following symptoms occur: weight loss, excessive sweating, asthenia (in the absence of a known cause), fever, respiratory signs, and neurologic, cutaneous or articular signals developed during the treatment. Contact with TB-diagnosed patients during their contagious phase and activities such as revolving the ground in mycosis-endemic areas should be avoided [9]. Moreover, and to prevent the occurrence of *Listeria* sp. and *Salmonella* sp. infections, patients should not consume raw eggs, unpasteurized milk products, hot dogs or deli meats (unless reheated at high temperatures), and uncooked meat/fish [9]. *Clostridium difficile* associated colitis is a risk for immunosuppressed patients and the IBD patient; a minority of cases are acquired from other patients, so enteric precautions should apply in the case of colonized or ill patients with the bacteria, especially for inpatients [10]. Unpasteurized milk products should also be avoided in areas where there it the risk of brucellosis. Persons whom are not immune to varicella (*i.e.*, have not been vaccinated and never had the disease) should avoid contact with varicella patients. The establishment of a close and confidence-based relationship between the patient and the medical team is a fundamental step at this point.

3.2. Travel-related counselling

Whenever a patient who is about to start or already on biological therapy has plans to travel to tropical areas (where yellow fever, malaria and other infectious risks are a concern), he/she should have an appointment at a travel medicine clinic, ideally with an IBD-dedicated infeciologist, at least four weeks before travelling. With the exception of the attenuated vaccines, all the other prophylactic measures usually applied to non-immunocompromised patients should be applied to patients on immunosuppressive drugs. Special care should be taken to check for drug-to-drug interactions between prophylactic measures and immunosuppressive medication. High risk destinations and adverse live conditions that, according to the IBD-dedicated gastroenterologist, may worse the disease, should best be avoided.

4. Tests to be performed before therapy and their rationale

Before starting biological drugs, patients should go through a battery of screening and diagnosis tests, which are described below and listed in Table 2. A blood analysis – including a complete blood count, liver transaminases, serum creatinine levels and serological tests to some infectious agents – is required at this point. STDs, such as syphilis, HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), *herpes simplex virus* type 2 (HSV2), should also be screened for. Moreover, and given the increasing number of adults who are not immune to the hepatitis A virus (HAV), the presence of anti-HAV IgG should also be evaluated. The presence of antibodies to the common herpesvirus, such as *Epstein–Barr virus* (EBV), *cytomegalovirus* (CMV), *herpes simplex virus* type 1 (HSV1) and *varicella-zoster virus* (VZV), should also be assessed. In the absence of measles vaccination or previous infection, anti-measles IgG antibodies should also be assayed.

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Table 2
Laboratorial work-up before immunosuppressive prescription.

Analytical data	Observations	Check
Hepatitis B virus - Anti-HBs quantified* - HBs antigen, anti-HBs and - Anti-HBc**	*For those vaccinated **For all the others (see text)	
Hepatitis C virus (anti-Hepatitis C)		
Human immunodeficiency virus (anti-HIV)		
Hepatitis A virus (anti-HAV IgG)		
Epstein–Barr virus (anti-EBV)	anti VCA (IgG, IgM), EBNA, EA-D	
Citomegalovirus (anti-CMV IgG and IgM)		
Herpes virus (anti-HSV 1 and 2: IgG and IgM)		
Varicella-zoster virus (anti VVZ IgG)		
Syphilis (VDRL or TPPA)		
In particular circumstances		
Anti-measles Ig G	If not vaccinated and without past measles disease	
Anti-JC virus	Before natalizumab prescription	

VCA—viral capsid antigen; EBNA—Epstein Barr nuclear antigen; EA-D—early antigen D; VDRL—venereal disease research laboratory; TPPA—*Treponema pallidum* antigen; JC—John Cunningham virus.

5. Dealing with test' results

5.1. Lymphopenia and neutropenia

Neutropenia or neutrophils' functional abnormalities – which are related to innate immunity – are known to increase the patients' risk of suffering a number of infections, namely from enteric Gram-negative bacteria, *Staphylococcus* sp. and fungi (*Candida* spp., *Aspergillus* spp., *Mucor* spp.) [11]. On the other hand, lymphopenia – especially if prolonged in time – and abnormal T cells may increase the patients' risk of suffering infections from herpes virus, progressive multifocal leukoencephalopathy associated to John Cunningham (JC) virus, and infections from *Mycobacterium* spp, *Nocardia* spp., *Listeria* sp., *Cryptococcus* spp., *Histoplasma capsulatum*, *Toxoplasma gondii* and *Strongyloides stercoralis* [11]. Moreover, an absolute lymphocyte count below 500 lymphocytes/μL is a risk factor for severe infections [12] and a contra-indication for tofacitinib (used in the treatment of rheumatoid arthritis). Patients' haematological profile should be routinely monitored (the suggestion is each two weeks if lymphocytes are close to 500/μL, and at least each two weeks for the first four weeks of a drug prescription that may frequently cause leukopenia) as its variations may contribute to increase the risk of developing infections among those on immunosuppressive drugs.

5.2. Syphilis

If a patient has a positive VDRL (venereal disease research laboratory) test but no symptoms, he/she should be diagnosed with latent syphilis. The appropriate treatment is a single intramuscular penicillin injection if the patient has at least one negative VDRL result in the previous 12 months; otherwise, latent syphilis should be treated with three weekly injections of penicillin [13]. All sexual partners should be screened for syphilis and treated accordingly.

5.3. HIV

An HIV test should always be requested when in the presence of a sexual transmitted disease (STD), and treatment must be started upon a positive result. It should be stressed that HIV infection is not a contraindication for biological therapy – namely with anti-TNFα agents – when the patient is being treated and his/her clinical situation is stable [14].

5.4. HBV and HCV

Patients who have not been vaccinated against HBV should be screened using hepatitis B surface antigen (HBsAg) antibody (anti HBs quantified) and anti-hepatitis B core (anti-HBc) IgG before being placed on immunosuppressive drugs. Following a positive HBV serological test, a sensitive HBV DNA assay should be performed. HBsAg-positive patients with HBV-DNA above 2000 UI/mL should be treated according to the current guidelines (which can be found at the European Association for the Study of the Liver [EASL] [15], Asian–Pacific Association for the Study of the Liver [APASL] [16] and American Association for the Study of Liver Diseases [AASLD]) [17]. HBsAg-positive patients with HBV-DNA below 2000 UI/mL, or HBsAg-negative patients with a positive result for anti-HBc IgG, are also at risk of HBV reactivation during immunosuppressive therapies [18]. This risk depends on the serological profile of the patient, his/her medical condition and which immunosuppressive drugs are to be used. The risk is higher for patients on anti-CD20 therapies, like rituximab and ofalumumab, mostly used for the treatment of haematological and rheumatic diseases, but is still considerable for patients on anti-TNFα, other biological drugs, antracyclines and glucocorticoids. Therefore, routine monitoring and application of prophylactic measures is mandatory for HBs antigen-positive and/or anti-HBc-positive patients who will be placed on immunosuppressive drugs or chemotherapy [19]. Table 3 depicts the procedures advocated by EASL, ECCO (European Crohn and Colitis Organization), APASL and AASLD in these situations. Accordingly, the American Gastroenterology Association (AGA) 2015 recommendations state that anti-HBc IgG positive patients, irrespective of their HBs Ag status, should receive prophylactic treatment before being placed on any biological therapy and anthracyclines derivatives (*e.g.*, doxorubicin, epirubicin). Moreover, those that are to be placed on systemic glucocorticoids for a period longer than four weeks should be treated if they are HBs Ag positive or if they are supposed to receive a dosage higher than 10 mg per day of prednisolone or equivalent [18].

The antiviral drug used in the prophylaxis should have a high genetic barrier, should be started before the immunosuppressive drugs' therapy and should be maintained for a minimum period of six months after the immunosuppressive drugs' discontinuation, or for a minimum period of 12 months if B cell-depleting agents were used. AGA do not recommend the use of this routine-based antiviral prophylaxis in patients whose risk for HBV reactivation is low (*i.e.*, less than 1%): patients on traditional immunosuppressive agents (*e.g.*, azathioprine, 6-mercaptopurine, methotrexate) on intra-articular steroids, on oral glucocorticoids for a short period (less than one week), or on less than 10 mg prednisolone or equivalent if the patient is HBsAg negative and anti-HBc positive. Moreover, and against former recommendations, AGA does not recommend HBV DNA monitoring for the decision of preemptive therapy as an alternative to antiviral prophylaxis [18].

Concerning hepatitis C, HCV-RNA should be assayed whenever HCV antibodies are detected. Should the patient have a positive result occurs in the former, he/she should be treated for HCV. As far as the current knowledge goes, there is no known risk of HCV reactivation with immunosuppressive drugs, and therefore a positive result in HCV testing is not a contra-indication for immunosuppres-

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Table 3
Screening, monitoring and prophylactic antiviral therapy concerning HBV status according to different guidelines.

	AASLD, 2009	ECCO, 2013	APASL, 2015	EASL, 2017
Screening a) before IST	HBs Ag and anti-Hbc for high risk of HBV infection	Hbs Ag, anti-HBc, anti-HBs for all; HBV-DNA if HBsAg positive	HBs Ag and anti-HBc for all	HBs Ag, HBs atc and anti-Hbc for all; HBV-DNA if isolated anti-HBc positive
b) during IST (each 1–6 month)		HBV serology and HBV DNA every 1–3 months	Monitoring ALT and HBV-DNA	Anti Hbc positive: monitoring HBV-DNA/ALT 1–3 months
Prophylactic-anti viral therapy	HBs Ag positive	HBs Ag positive; anti Hbc positive and HBV-DNA positive:	HBs Ag positive; anti Hbc positive and HBV-DNA positive:	HBs Ag positive; anti Hbc positive and HBV-DNA positive; anti-HBc positive: if high risk of HBV reactivation (>10%) consider also if: long duration of IST, limited compliance to monitoring or unknown risk of viral reactivation for new biologicals
Timing	From onset to 6 months after stopping IST	Best start 2 weeks before IST to at least 12 months after stopping IST	From onset to 12 months after stop IST	From onset to 12 to 18 months after stop IST
Nuc(s)	Lamivudine; preferred ETV/TDF particularly for those treated >12 months	Nucleoside/nucleotide analogues with high barrier to resistance (ETV/TDF)	lamivudine; ETV/TDF/TAF	ETV/TDF/TAF Lamivudine may be used although few cases of HBV exacerbation due to LAM resistance have been reported.

AASLD—American Association for the study of Liver Diseases; ECCO—European Crohn and Colitis Organization; EASL—European Association for the Study of Liver. APASL—Asian Pacific Association for the study of liver. IST—immunosuppressive therapy. Nuc(s)—nucleoside(s). ETV—entecavir; TDF—tenofovir; TAF—tenofovir alafenamide.

sive therapy [20]. Still, and as HCV is a treatable and curable disease, its screening should definitively be done.

Finally, biological agents should not be prescribed to patients who have liver dysfunction classified as Child–Pugh class B and higher [21].

5.5. Herpesvirus (EBV, CMV, HSV, VVZ)

The prescription of thiopurines has been associated to fatal early post-mononucleosis lymphoproliferative diseases in young men (less than 35 years-old) seronegative for EBV [22,23]. In fact, a Crohn's disease patient on azathioprine was reported to have an EBV primoinfection which developed into infectious mononucleosis, haemophagocytic lymphohistiocytosis (HLH) and a B-cell lymphoproliferative disorder [3]. Accordingly, HLH has been associated to EBV in medically-immunosuppressed patients [24]. Therefore, thiopurines' alternatives should be considered for EBV-negative patients [20].

CMV primoinfection or reactivation can occur in patients on immunosuppressive drugs, and may cause retinitis, pneumonia, encephalitis, and other invasive infections [25]. Accordingly, a prospective case-control report has described an association between severe steroid-refractory inflammatory bowel disease and CMV infection [25,26]. The knowledge of the CMV sero-status before the treatment of the immune-mediated inflammatory disease is thus a useful tool in the differential diagnosis.

VZV has been associated to significant morbidity and mortality in immunocompromised patients. After an acute infection (varicella), VZV persists in a latent state in autonomic ganglia, dorsal nerve roots and cranial nerves [27], and might later reactivate as zoster. Severe forms of VZV infection with retinian necrosis have been described in patients treated with fingolimod [28] and natalizumab [29]. Moreover, severe forms of varicella have been associated to anti-TNFα therapy [30–32]. Therefore, all patients who have not been vaccinated and who do not have a previous definitive diagnosis of chickenpox should be assayed for the presence of VZV antibodies before initiating immunosuppressive drugs; in the presence of a negative test, vaccination should be offered and completed at least four weeks before initiating immunosuppressive drugs [33]. For persons 60 years old or older without a history of chickenpox there is no need to check VZV antibodies and shingles vaccine should be offered in the absence of a medical contra-indication [34].

5.6. Measles

Patients who test negative for measles' antibodies should be vaccinated before initiating immunomodulation. If the vaccine is somehow contraindicated, and if the patient has contact with a person with measles, immunoglobulin should be administered within six days of exposure, as it may provide some protection or modify the clinical course of the disease [35].

5.7. TB

5.7.1. Screening

Patients should be screened for latent tuberculosis (LTB) before starting immunosuppression. TB reactivation is a risk not only for patients on anti-TNFα, but also for patients on glucocorticoids, leflunomide, teriflunomide, mitoxantrone and alemtuzumab (anti-CD52). Before vedolizumab and ustekinumab Food and Drug Administration (FDA) suggests TB screening, until the risk is clearly settled. Currently, there are no gold standard tests for TB screening. In this context, several guidelines (ECCO [5], National Institute for Health and Care Excellence [NICE] [36], World Health Organization [WHO] [37], and also national recommendations concerning LTB diagnosis) advocate the use of a tuberculin skin test (TST) and/or an IGRA test (Quantiferon or TB-spot.TB), and a pulmonary X-ray. The performance of TST and an IGRA test raises the diagnosis sensitivity and has been the authors' choice [38]. For immunocompromised patients, a single positive result in any of these tests supports an LTB diagnosis, as in the context of immunosuppression missing an LTB diagnosis poses a greater risk than the potential hepatotoxicity resulting from the prophylactic TB therapy. Concerning results of IGRA tests that remain indeterminate, should probably be better to proceed as they were positive.

Screening for LTB during biological therapy is controversial. Still, and having into account the serious risk posed by an active TB in immunocompromised individuals, some authors advocate a routine-based TB surveillance during and after anti-TNFαtherapy [39]. Conversely, other authors believe that TB screening should tailored-based and adjusted to the risk of developing TB [40]. What-

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ever the option is, TB screening should undoubtedly take place whenever a contact with a TB patient occurs.

5.7.2. LTB treatment

The WHO guidelines suggest different alternatives – considered to be equivalent – for LTB treatment: six-months isoniazid, nine-months isoniazid, or three-months of weekly rifapentine plus isoniazid. Other options include three to four months' isoniazid plus rifampicin, or three to four months' rifampicin alone [41]. NICE recommendations, on the other hand, recommends as treatment three-months isoniazid (with pyridoxine) and rifampicin, or six months isoniazid (with pyridoxine) [36]. Patients should be closely monitored throughout the treatment as there is the risk of hepatotoxicity. After one month of LTB therapy is generally considered safe to start biological therapy [5,42].

6. Immunizations

Upon the diagnosis of an immune-mediated inflammatory condition, the patients' previous immunizations should the carefully reviewed, and any missing vaccine should be promptly updated [43]. Adult patients should be protected against common agents, which are briefly mentioned in Table 4. Current evidence suggests the absence of a relationship between vaccines and immunomediated disease flares [5].

Vaccination should ideally precede the onset of therapy with immunosuppressive drugs to ensure efficacy: inactivated vaccines should be administered at least two weeks before immunomodulatory therapies, and attenuated vaccines at least four weeks before. Still, and should that fails to occur, immunization using inactivated vaccines can be carried out while the patient is on immunosuppressive drugs, as some protection is expected to be achieved, and, whenever possible, the quantification of antibodies should be performed after the vaccination. Attenuated live vaccines, on the other hand, should not be administered during immunosuppression or biological therapies given the risk of reactivation of the vaccines' attenuated virus. Physicians should keep in mind that immunizations' recommendations may change over time, and should ensure they are updated with the latest indications.

Overall, vaccination should be applied outside periods of exacerbation of the immune-mediated disease (four to six weeks after a relapse in the case of multiple sclerosis), more than three months after immunoglobulin usage, and ideally more than six months after anti-CD20 agents like rituximab [44].

6.1. Inactivated vaccines

6.1.1. Pneumococcal

Streptococcus pneumoniae is the most common bacterial cause of pneumoniae and may also be responsible for invasive diseases such as bacteraemia and meningitis. Vaccination against this agent is recommended for children, persons who are 65 years or older, and patients on immunosuppressive agents, among others. Concerning the latter (immunocompromised patients over 19 years of age), the Advisory Committee on Immunization Practices (ACIP) guidelines recommends the following: patients who are naïve to pneumococcal vaccine should receive the conjugated vaccine (PCV13) and, at least eight weeks later, the polysaccharide vaccine (PPSV23) [45]; those who had been previously vaccinated with PPSV23 should receive, one dosage of PCV13, at least one year after PPSV23 [46]. The PPSV23 should be re-inoculated once within five years in patients who have been vaccinated for the first time before 65, and in patients turning 65 and for whom more than 5 years had elapsed since the last dose [45].

6.1.2. Influenza

Influenza infection might be followed by serious complications, especially in immunocompromised individuals. As so, the ACIP recommends that these individuals should be vaccinated against influenza once a year [47]. The inactive influenza vaccine (two influenza A and one influenza B strains) is known to be safe and effective both in children and adults with chronic diseases [48]. An inactivated influenza vaccine with four antigens (two influenza A and two influenza B strains) may become available in the near future. Rituximab-treated patients are known to have a weak response (in terms of antibodies production) to the influenza vaccine; still, these patients should nevertheless be vaccinated [49].

6.1.3. HBV and HAV

All patients who have a seronegative result concerning HBV (*i.e.*, a negative or low-titer HBsAb result) should be vaccinated against this agent. The HBV vaccine is safe, but its response may be significantly reduced in those on immunosuppressive drugs, and therefore an intensified vaccination protocol may be required. Protection is considered to be achieved if a concentration of anti-HBs higher than 10 mUI/mL is detected one to two months after vaccination. Patients who have not quantified the anti-HBs after vaccine administration, should do it; vaccine should be repeated if the anti-HBs concentration falls below 10 mUI/mL. According to several opinions expressed in the current literature, the re-inoculation can be done in one, two or three doses (20 μg) [50,51]. Alternative vaccination schedules (at 0, 1, and 4 months or 0, 2, and 4 months) have been approved and are known to elicit dose-specific and final rates of seroprotection similar to those obtained with a classical schedule [52].

The vaccine against HAV should be applied in two doses, separated by six to 18 months. Alternatively, individuals over 16 years may be inoculated with a combined HAV/HBV vaccine (Twinrix®) using one of the following schedules: 0, 1 and 6 months, or 0, 7, 21–30 days and 12 months (approved by FDA) [53]. Seroconversion is elicited in virtually everyone after the second dose, and antibodies to HAV can remain for more than 25 years in adults [54]. The absolute lower limit of anti-HAV required to prevent HAV infection has not been defined and, accordingly, neither has the vaccine's protective value. Antibodies quantification is not recommended as the sensitivity of the current tests is known to be variable.

6.1.4. Papillomavirus

The human papillomavirus (HPV) has been associated with cervical, vulvar and vaginal cancer in females, penile cancer in males, and anal cancer and oropharyngeal cancer in both genders [55]. Moreover, HPV is known to be related with cervical pre-cancers, including cervical intraepithelial neoplasia grade 2 or 3 and adenocarcinoma *in situ* (≥CIN2). Immunosuppressive drugs have been associated to an increased incidence of HPV-associated warts or condylomata [56]. Importantly, HPV infection can be prevented with a highly efficacious conjugated vaccine, although its application does not exclude the need of regular screening for cervical cancer in women.

Routine HPV vaccination (quadrivalent [4vHPV], bivalent [2vHPV] and the nonovalent [9vHPV]), is usually administered to 11- to 12-years-old infants in two doses at least six months apart. When HPV vaccination is started later (between the ages of 15 and 26), three doses are needed to achieve seroprotection [57]. The 9vHPV, 4vHPV or 2vHPV vaccines can be administered up to the age of 26 years; ACIP recommends the administration of three doses of either 9vHPV or 4vHPV vaccine in those who have not been vaccinated previously and are immunocompromised (including those with HIV infection) [58].

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Table 4
Vaccination in adults suffering from immuno-mediated inflammatory diseases.[d]

Vaccine	At diagnosis	Under immunosuppression (IS)	Routine vaccination schedule
Common vaccines			
Hepatitis A	√	√	2 doses: 0–6 to 12 month
Hepatitis B	√	√	3 doses: 0–1 and 6 month or 0–7 and 21 days and one year (same schedule applies to HAV/HBV vaccine)
Td, Tdap	√	√	1 dose every 10 years[#] one of this vaccine should be Tdap
Human papillomavirus	√(11–26 years)	√(11–26 years)	at diagnosis if 9–14 years: 2 doses at 0–6 month Under IS: 3 doses at 0, 1 and 6 month
Influenza-inactivated	√	√	1 dose every year
Influenza-live attenuated	√	**X**	–
Measles, mumps, rubella (MMR)—live*	√	**X**	2 doses: 0–4 weeks
Pneumococcal conjugate (PCV13)	√	√	1 dose: ideally before PPSV23; if after PPSV23: ≥1 year
Pneumococcal polysaccharide (PPSV23)	√	√	Before 65 years: 1 dose repeated 5 years after and at 65 of age, if an interval of at least 5 years from the last dose; after 65 years: 1 dose Timeline after PCV13 shot: ≥8 weeks after if under IS >6–12 months if without IS
Polio-inactivated (salk)	√	√	3 doses: 0–1 and 12 month or separated by ≥ 4weeks
Varicella—live	√	**X**	2 doses: 0–4 weeks
Zoster—live	√(50–59 years)[b] √(≥60)	**X**[a]	1 dose
Travel related vaccines			
Cholera, oral (inactivated)	√	√	>6 years: 2 doses 0–1 week Repeat each 2 years if needed
Japonese encephalitis (inactivated)	√	√	2 doses: 0–4 weeks; if booster needed: one dose >1 year after the initial one
Meningococcal, conjugate (A,C,Y,W135) (MCV4)	√(2–55 years)[c]	√(2–55 years)[c]	1 dose; repeated after 5 years if protection is still needed
Polio, inactivated (salk)	√	√	1 dose (if >10 years after a complete schedule vaccination); same as above if not vaccinated before
Tick borne encephalitis, inactivated (FSME-IMMUN®)	√	√	3 doses (0, 1–3 month, 6–15 month) first booster if needed at 3 years
Typhoid, inactivated	√	√	1 dose; each 2 to 3 year if needed
Yellow fever	√	**X**	1 dose is enough for life-long protection (WHO,2016)

√—administer if patient is not current with recommendations for adult immunocompetent persons in same risk and age categories.
[#]Portuguese recommendations state Td booster at 10, 25, 45 and 65 and then each 10 years.
X—contra-indicated.
*If not vaccinated previously.
[a]Zoster vaccine is admitted for ACIP if low level immunosuppression (treatment with prednisone <2 mg/kg with a maximum of ≤20 mg/day; methotrexate ≤0.4 mg/kg/week; azathioprine ≤3 mg/kg/day; or 6-mercaptopurine ≤1.5 mg/kg/day).
[b]Recommended by IDSA guidelines; contraindicated by ACIP recommendations.
[c]More than 55 years: meningococcal polysaccharide vaccine (A,C, Y, W135) (MPV4).
[d] Adapted from the protocol of Immunomodulation and risk of Infection Consultation (IRIC), Centro Hospitalar S João-Porto, Portugal.

6.2. Live vaccines

6.2.1. Varicella

Varicella vaccination consists of two doses of an attenuated vaccine administered four to eight weeks apart. For patients with immune-mediated inflammatory diseases, vaccination should be considered at least one month before being placed on immunosuppressive drugs, one month after discontinuing steroids, or three months after discontinuing other immunosuppressive drugs (including anti-TNFα therapy) [5]. Some authors [59] have reported a good response (in terms of tolerance) to the VZV vaccine in children with IBD on 6-mercaptopurine or infliximab. Still, a throughout knowledge of the risks and benefits of the varicella vaccine in patients on immunosuppressive drugs awaits larger prospective studies; until then, patients should be to tested for VZV as early as possible after diagnosis, and those previously unexposed to VZV should be vaccinated according to the timeline depicted above. Patients exposed to varicella who do not have anti-VZV antibodies and for whom the vaccine is contra-indicated should be treated with varicella zoster immunoglobulin (VZIG) within 10 days of the exposure [60]. These patients should also be carefully observed during the following four weeks, and antiviral therapy should be started immediately should varicella develops [5].

6.2.2. Zoster

The administration of a zoster vaccine has been approved for patients who are VZV-positive and at risk of developing herpes zoster (*i.e.*, the elderly, 60 years of age or older or, according to Infectious Diseases Society of America [IDSA], 50 years or older). This vaccine consists on a single dose; patients should be inoculated at least four weeks before being placed on immunosuppressive drugs [34]. Moreover, this vaccine should not be administered within one

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month after discontinuing immunosuppressive drugs [34]. However, and according to ACIP, it can be administered to patients on low doses of immunosuppressive drugs, such as azatioprine, metothrexate and steroids (Table 4).

Zoster vaccine seroprotection is fallible for those older than 70 years; still, it remains effective against this disease-associated pain [61]. Recently, a new vaccine against zoster – a subunit-inactivated vaccine – has been developed. This vaccine is now on its phase three trial, which is being conducted in adults aged 70 years or older, and its administration is made in two doses two months apart. This vaccine's efficacy against herpes zoster has been settled at 91.3%, whereas efficacy against post-herpetic neuralgia has been settled at 88% [62].

6.2.3. *Measles, mumps and rubella (MMR)*

Patients with immune-mediated inflammatory diseases who are not naturally immunized nor have been vaccinated while children against measles, mumps and rubella, should be inoculated twice with four weeks apart. This inoculation should not be made while the patient is on immunosuppressive drugs. If an unprotected patient has contact with a measles-infected person, he/she should be placed on immunoglobin within six days of the contact [35]. Moreover, susceptible close contacts should be vaccinated ("cocooning" strategy) [63]. The MMR vaccine should be postponed if the person has been recent (*i.e.*, in the previous 11 months) receipt of an antibody-containing blood product, has moderate or severe illness with or without fever, has history of thrombocytopenia or thrombocytopenic purpura, and performed a tuberculin skin testing. Concerning the latter, a tuberculin test should be done before, simultaneously with, or at least four to six weeks after MMR vaccination, as this vaccine can cause false negatives in the test [35].

7. Other prophylactic treatments: *Pneumocystis jiroveci pneumonia* (*Pjp*)

Pjp is a severe disease and its mortality is greater among HIV-uninfected individuals. Risk factors for Pjp development are known to include glucocorticoid therapy, lymphopenia (total lymphocyte count, <600 cells/mm^3),age greater than 55 years [64], and defects in cell-mediated immunity [65]. ECCO recommends prophylactic treatment in patients on three immunomodulators, or two when one is a calcineurin-inhibitor; the first-line agent for Pjp prophylaxis is Cotrimoxazole (960 mg administered three times a week) [66]. All the other prophylactic treatments are second-line alternatives, and include Atovaquone (1500 mg once a day, should be taken with fatty food) or Dapsone (100 mg a day). One should keep in mind that cotrimoxazole intolerance often predicts dapsone intolerance [65]. The optimal moment to discontinue prophylaxis is also yet to be established: whereas some authors advocate discontinuation one month after stopping immunosuppressive drugs [67], others prefer to prolong prophylaxis for up to three months, as there is a remaining risk for Pjp during immune reconstitution phase.

8. Conclusions

The new and more aggressive therapeutic strategies employed in immune-mediated inflammatory diseases – which include an earlier and more prolonged use of biological drugs – are a challenge for clinicians. Patients should be carefully examined before being placed on immunosuppressive drugs: risk factors for developing infectious diseases, demographic and epidemiological variables, past medical history and comorbidities should be enquired and considered in detail. Moreover, a thought-out screening and a routinely-based surveillance can actually reduce the risk of reactivation of some infectious diseases (such as TB, herpetic infections and endemic mycosis), whereas vaccination and chemoprophylaxis can protect from others. It is important to stress that a detailed evaluation before immunomodulation may prevent later therapeutic interruptions. The treatment of latent tuberculosis and the prevention of hepatitis B virus reactivation, should be done before biologics whenever they apply. Other prophylaxis (such as herpes virus reactivation, meningococcal infection prevention) should be done according to the biologic prescribed (for instance alemtuzumab demands herpes virus prevention [68], eculizumab demands meningococcal immunization [69]). Considering *Pneumocystis jiroveci* infection prevention, it should be done according to the association of therapies prescribed (immunomodulators or immunomodulators and biologicals) that are considered a risk for the disease.

We hereby propose an evidence-based practical guide for clinicians that aims to standardize patients' monitoring before and during biological therapies. A multidisciplinary approach, involving MDs specialized in infectious diseases and pneumology, pharmacists, microbiologists, general practitioners and nurses, is a key asset. Patients should be clearly informed, committed to the treatment and should have easy access to the medical team. Patients' surveillance is crucial, as some drug-related risks become known long after the approval and prescription of a specific drug. An up-to-dated knowledge is of an utmost importance, as pharmacovigilance-implemented modifications to therapies and new guidelines for immunization are continuously being published. An attentive medical team working closely with informed and committed patients is thus very important for the success of these treatments.

Conflict of interest

FM served as speaker and received honoraria from Merck Sharp & Dohme, Abbvie, Vifor, Falk, Laboratorios Vitoria, Ferring, Hospira and Biogen.

CA served as speaker and received honoraria from Pfizer, Janssen and Biogen.

AS has no conflict of interest to declare.

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8. Discussion

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The focus of this Thesis was infection and risk of infection in Portuguese patients treated with anti-TNFα, alone or in association with immunomodulators and/or glucosteroids. Granulomatous and intracellular infections were the main concern.

In the cohort studied, the incidence of tuberculosis per patients/year during therapy with infliximab, adalimumab or etanercept was very high, compared with other series. The severity of most of the cases, 60% of which being extra pulmonary, the interruption of anti-TNFα therapy and cases of immune reconstitution inflammatory syndrome (IRIS) associated with the withdrawal of anti-TNF´s were a big concern. As active TB in the field of immunosuppression results generally from the reactivation of latent forms, the diagnosis and treatment of latent TB is crucial. Although, despite a more accurate diagnosis of latent TB on the last years, active TB is still diagnosed (data not showed).

It is estimated that TB screening before anti-TNFα therapies reduces the risk of active TB in about 80-90% of the cases[26,55]. Two situations concur to this: patients may be new infected with TB or the diagnosis of latent TB may be a "false" negative result. The first part is hard to control and depends on the incidence of TB in the general population and the exposure to TB patients. The second one is the result of not having gold standard tests for the diagnosis of latent TB neither tests with high predictive value for progression to active TB. In our study, TST was the test with higher sensitivity (compared with IGRA's test) irrespective of concurrent or previous immunomodulator or biological therapy or before these treatments. Thus, despite the specificities of the cohort studied (BCG vaccinated at birth, living in an intermediate incidence area of TB and not including people younger than 18 years old), the results obtained are interesting especially considering the current opinion that IGRA's tests should replace the oldest TST. Although both TST and IGRA's represent indirect markers of *M. tuberculosis* exposure and indicate a cellular immune response to this pathogen, having both low predictive value for progression to active TB [56]Nowadays, a shortage of TST exists due to the increasing trend of its replacement by IGRA's tests that are much more expensive and thus more appealing to the pharmacology industry. Of course TST has limitations: may have a positive result due to repeated BCG vaccination after the first year of life, has inter-reader variability and, like in IGRA's, conversions and reversions are observed [57].

Another interesting finding of our study was that IGRA's in patients already immunosuppressed despite having a higher specificity had also a lower sensitivity that can result in the sub-diagnosis of latent TB. Thus, in the absence of a test that can predict the risk of reactivating a latent TB, probably should be better to favour tests with higher sensitivity. The recommendations of the National Institute for Health and Care Excellence (NICE), concerning immunosuppressed patients, is that the optimal strategy for latent TB diagnosis is to offer an IGRA as a first-line test and then use TST (≥ 5 mm) as a second-line test in IGRA negative cases[58].

Concerning the screening for TB during treatment with anti-TNFα, this is even more difficult issue to solve and a lot of recommendations have been proposed[59-64]. In our cohort, despite the ongoing therapeutic immunosuppression TST became positive in 12 out of 46 patients across 26 months. Curiously, all but one TST conversion occurred on the first three screening tests, turning the boosting effect rather improbable. In the same population, T-SPOT.TB turned positive in 7 patients. The concordance between TST and IGRA positive results was fable. On the follow-up, IGRA tests reverted frequently, and QFT-GIT results, when positive, were close to the cut-off values. In fact, IGRA's are highly dynamic in nature, with high rates of conversions and reversions when repeated tests are performed[56]. Concerning for instance serial testing of health care workers, TST seems preferable to the IGRAs due to high rates of conversions and reversions of IGRA's which are harder to interpret[65].

The question, yet unsolved, is how to screen for TB during anti-TNFα therapy: in a regular schedule or according to epidemiology? performing a TST or and an IGRA test? Use the usual or other cut-off values? It is generally accepted that TST is considered positive at an induration of five or more millimetres for immunosuppressed patients and at 10 or more millimetres for persons not immunosuppressed. Similarly, should we work on establishing a different cut-off value for IGRA tests for persons under immunomodulators or biological drugs? Moreover, in populations that have been BCG vaccinated at birth lower rates of IGRA positivity compared with non-vaccinated population have been elicited[66]. This situation may introduce an extra difficulty for IGRA tests results for those that were BCG vaccinated. Thus, it seems that several answers to the question of rescreening for TB will be expectable, according to the specificities of the studied population. According to our results and the actual knowledge, we would suggest screening for TB during anti-TNF therapies (and other drugs with risk of TB reactivation) for those with a negative TB screening at baseline. The suggestion is to do this first screening after six to nine months of therapy, due to the higher risk of infection in the first months of ant-TNFα therapy, and then annually. A TST would probably be the best strategy. When TST is not available, an IGRA test should be used. After some negative screenings, the

decision to maintain TB screening yearly would be tailor made, according to demographic and clinical issues. Male gender and long duration of the immunomediated disease should favour the screening. A TST conversion or a positive test result in the absence of active disease will be an indication for latent TB treatment; at this point ongoing TB surveillance will be based on clinical and epidemiological factors.

Considering other granulomatous infections, a real risk was found in our cohort. There is no simple way to reduce the risk, as several and different factors may be in its origin, such as: food (considering *Salmonella, Listeria, Brucella*), air-borne (*Legionella, Coxiella*), environment (*Coxiella*, endemic mycosis, *Nocardia*). Thus, accuracy for an early diagnosis is essential.

Persistent intestinal colonization with *Nocardia nova* was a concern in our cohort as therapeutic immunosuppression may give rise to dissemination and disease. Although, the need of screening for these agents is not stated.

Intracellular infections caused by herpes DNA virus may reactivate due to several reasons, including immunomodulators (thiopurines analogs and glucosteroids, for instance) and biological therapies[67,68]. Some of these infections may be severe, as were the case of disseminated herpes simplex virus infection and varicella pneumonia described in our cohort. Besides varicella-zoster and zoster vaccines and the prophylactic prescription of acyclovir under certain drugs (like tofacitinib and alemtuzumab) no other prevention is available. The risk may be higher under particular situations that may occur together, such as immunosuppressive drugs in association, advanced age, rheumatic diseases. Define a score for risk concerning herpes virus reactivation would be useful to stratify risk and proceed according to it.

Considering the global risk of infection, outside TB screening and latent TB treatment, counselling concerning food, environment and travel is useful. Also, screening for infections (such as hepatitis A, B and C, HIV, syphilis, DNA-virus), immunizations (against *Pneumococcus*, influenza, hepatitis A and B, *Bordetella pertussis* among others) and sometimes prophylaxis (for *Pneumocystis jirovecii* disease and herpes infection) are important issues to reduce this risk. A practical protocol to guide this workup is included in this work.

9. Final remarks

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In the real world of a Portuguese cohort of adult patients treated with immunmodulators and biological drugs, mainly anti -TNF α, TB was the core of opportunistic and severe diseases. The majority of these TB cases likely resulted from reactivation of latent infection. Therefore, screening and treatment of latent TB is essential to mitigate the risk. The suboptimal performance of the tests used for TB diagnosis (TST and IGRA's), including uncertainties concerning the interpretation of test results in patients already immunosuppressed, the complexity of these patients considering the disease to be treated, comorbidities, age and epidemiology, among other variables, increase the difficulties of latent TB diagnosis.

Of note, the sensitivity of TST was higher than the sensitivity of IGRA's (and that from T-SPOT.TB higher than that from QFT-GIT) in all the situations: before anti-TNF α or in the switch of anti -TNF α and even during anti-TNF α prescription. Against the actual mainstream, probably would be advisable to wait for better tests for latent TB diagnosis before replace the old TST.

Concerning latent TB field, we need a high predictive test able to identify those at highest risk of progressing to active TB and for whom therapy for latent TB would be beneficial. Such a test may probably rely on the identification of biomarkers or biosignatures, especially gene expression signatures that are still far from the real word[69].

For now, on the setting of TB screening during anti-TNF α prescription, based in our results we suggest to screen on the first years of treatment all TST and IGRA's negative patients and then do the screening in a tailor based way, according to perceived TB risk. Considering that TST and IGRA's are not optimal tests, both should be considered. Although, due to shortage of TST one will probably rely on IGRA tests; T-SPOT.TB, due to higher sensitivity in our sample than Quantiferon, should probably be preferred.

There is also risk of other severe granulomatous and intracellular infections, as we have diagnosed in our cohort. Thus, precautions concerning food and environment risks are important, as well as an active surveillance of signals and symptoms, to treat infections as early as possible.

We believe that screening, prevention of infection (including immunizations) and counselling in patients about to star biologics or immunomodulators due to an auto

inflammatory disease is a useful task on infectious risk mitigation, and we herein propose a practical protocol.

Finally, the risk of infection may be even partially unknown for the most recent therapies, so is crucial to maintain an attentive surveillance.

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