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Corticosteroids and immunosuppressive therapy influence the result of QuantiFERON TB Gold testing in inflammatory bowel disease patients

Ulf Helwig [a,*], Michael Müller [b,1], Jürgen Hedderich [c,2], Stefan Schreiber [d,3]

[a] Medical Practice for Internal Medicine Oldenburg/University of Kiel. Neue Donnerschweer. Str. 30, 26123 Oldenburg, Germany
[b] Medical Laboratory Oldenburg, Oldenburg, Germany, Koppelstr. 7, 26135 Oldenburg, Germany
[c] Institute for Medical Statistic of the University of Kiel, Kiel, Brunswiker Str. 10, 24105 Kiel, Germany
[d] First Medical Department, University Hospital Schleswig-Holstein, Campus Kiel, Arnold-Heller-Str., 25105 Kiel, Germany

Abstract

Introduction: Latent tuberculosis infection is detected by the tuberculin skin test before treating with anti-Tumour-Necrosis factor alpha (anti TNFα) reagents. More accurate are Interferon gamma release assays (IFNγ release assays) to identify patients with latent tuberculosis. Because of a positive control in this assay, it is possible to identify those patients in which a result of tuberculosis testing is not available due to a lack of stimulation capacity of lymphocytes (indeterminate result). Patients suffering from IBD are often treated with immunosuppressive agents, which may influence the results of tuberculosis testing.
Aim: The aim is to investigate the influence of immunosuppressive agents on the outcome of IFNγ-release assay.
Methods: 50 consecutive patients were documented before introducing anti-TNF-treatment in this single centre study between April 2009 and April 2010. Data of INFγ release assay for latent tuberculosis, skin test and laboratory data and current medication were enrolled.
Results: For the period of one year data of 45 consecutive patients was available for statistical analysis. 24 patients out of 45 (corresponding to 53.3%) received at least low doses of corticoid treatment and 27 patients out of 45 (corresponding to 60.0%) received immunosuppressive agents. 13 patients out of 45 (corresponding to 28.9%) had an indeterminate result of the

QuantiFERON test. A correlation between the indeterminate result and combination therapy of corticosteroids was found. The concomitant therapy of immunosuppressive agents lead to a lower IFN release but no significance was found.

Conclusions: Steroid treatment and further combination therapy with immunosuppressive agents lead to a high risk of indeterminate QuantiFERON test.

1. Introduction

Tumour necrosis factor alpha (TNFα) is a key cytokine in the inflammatory cascade in Crohn's disease and also in ulcerative colitis. Therefore, the therapeutic blockage of TNFα is an option for both diseases.[1,2]

Additionally, TNFα is also a key cytokine in protective host defence against *Mycobacterium tuberculosis* (*M. tuberculosis*) and plays a major role in the development and maintenance of the granulome which compartmentalizes the tubercle bacilli. The therapeutic inhibition of TNFα via antibodies is associated with a 4 to 5 fold increased incidence of tuberculosis in patients latently infected with *M. tuberculosis*.[3–5] Therefore, it is recommended to exclude a latent tuberculosis infection prior to starting a TNF-alpha-inhibitor treatment. A common recommendation to exclude tuberculosis infection is to perform a chest radiography, which might exclude granulomas over the size of 1 cm, a detailed travel history and TB exposures as well as to measure the skin reaction of purified protein derivates of *M. tuberculosis* (PPD).[1,6,7]

The PPD test is a delayed-type-hypersensitivity reaction test towards a mixture of more than 200 different *M. tuberculosis* proteins. These antigens were also found in other mycobacteria and this is the reason for poor specificity in patients vaccinated with bacille Calmette-Guérin (BCG).[8] Furthermore, the reactions depend on a sufficient immune system, which is compromised in case of use of therapeutic immunosuppressive agents.[9]

An alternative diagnostic approach is to test the interferon gamma release as a specific reaction of T-cells on stimulation with *M. tuberculosis*.[10] At the moment, there are two assays available to test ex vivo the IFNγ release on peripheral mononuclear cells (ELISpot) or in whole blood stimulation (QuantiFERON).[11] The purpose of these testing systems is a higher specificity because of absent or false positive reaction against other mycobacteria and BCG vaccination. Furthermore, it has a higher sensitivity and the possibility to detect an indeterminate result via a positive reaction control.[11,12]

The therapeutic strategy in Crohn's disease and ulcerative colitis is the use of corticosteroids in the acute phase of inflammation and immunosuppressive agents like azathioprine and 6-mercaptopurine or methotrexate for maintenance of the remission phase.[1] In case of treatment failure or fulminant inflammation, the therapeutic options are anti-TNFα antibodies (Infliximab, Adalimumab or Certolizumab).[1,6,7,13] Hence, patients are often under immunosuppressive therapy before starting an anti-TNFα-therapy.

The immunosuppressive therapy may influence also the test of latent tuberculosis.

In the performance of the tuberculin skin test a positive control is not implicated, whereas a false indeterminate result cannot be detected.

Recently, several studies have documented the benefit of IFNγ release assay in a large number of patients in comparison to the tuberculin skin test.[12,14–18] In these cohorts combination of immunosuppressive therapy and corticosteroid treatment is rare and indeterminate results are not often. The usage of immunosuppressive agents and corticosteroids prior to anti-TNFα-therapy is a standard procedure in Germany.

Therefore, we investigated a consecutive prospective study in patients suffering from Crohn's disease and ulcerative colitis with indication of anti-TNFα-use. We assessed the rate of indeterminate results of QuantiFERON test and its association with corticosteroid and immunosuppressive treatment.

2. Patients and methods

50 consecutive patients with indication for an anti-TNF-therapy were documented in this single centre study. 45 patients fulfilled all documentation criteria. Other patients had an IFNγ-releasing test performed in another laboratory without available data on mitogen and antigen stimulation. Consequently, we excluded these patients from all statistical analysis.

2.1. Ethics statement

This study was approved by the ethical committee of Lower Saxony, Hanover, Germany and was performed in concordance to Helsinki declaration.

2.2. QuantiFERON test

Laboratory parameters including liver enzymes, CRP, white blood cell count, haemoglobin, thrombocytes etc. were performed prior to the start of anti-TNF-treatment. During this blood collection, venous blood was obtained for the QuantiFERON assay. The QuantiFERON assay (Cellestis, Victoria, Australia) was performed according to the manufacturer's instruction.

In short: one ml of whole venous blood was added to each of the three assay kit-tubes (nil, mitogen, antigen). The tubes were transferred via courier to the performing laboratory for assay. After incubation for 24 h at 37 °C, samples were centrifuged and supernatant was taken to perform the IFNγ-ELISA.

Indeterminate results were defined as increase less than 0.5 IU/ml after mitogen stimulation. A positive test was defined as increase of IFNγ to over 0.35 IU/ml after tuberculosis antigen stimulation.

2.3. Statistic analysis

The primary point was to determine the influence of indeterminate results of the QuantiFERON test by immunosuppressive agents. Results from 45 patients were analysed using SPSS Software (version 18.0). Based on Kolmogorov–Smirmov tests normal distribution could not be assumed. Therefore we used median and quartile values (box-whisker plots) for data description. Differences between some subpopulations were tested with the Mann–Whitney U-Test with a significance level set at 5%. The probability for a valuable test was predicted by a logistic regression model. For the logistic regression we included corticoid therapy immunosuppression, blood cell count of erythrocytes, leucocytes, thrombocytes, C-reactive protein, iron serum level, sex and the diagnosis of Crohn's disease or ulcerative colitis.

The dose depending curve (Fig. 1) is based on the results of the logistic regression where corticosteroids and immunosuppression are the main factors.

3. Results

45 patients fulfilled all criteria to enter the statistical analysis. 19 male and 26 female were included; 34 patients suffered from Crohn's disease and 11 patients from ulcerative colitis. 27 patients out of 45 (corresponding to 60.0%) received immunosuppressive therapy. 24 patients out of 45 (corresponding to 53.3%) received at least a low doses of corticosteroid treatments and 21 patients out of

Table 1 Characteristics of patients.

Characteristics of patients		45
	Crohn's disease	34
	Ulcerative colitis	11
Sex		
	Male	19
	Female	26
QuantiFERON results		
	Positive for tuberculosis	1
	Negative for tuberculosis	31
	Indeterminate result	13
Corticosteroid treatment		
	Yes	24
	No	21
	15 mg or more/day	18
Immunosuppressive therapy		
	Yes	27
	No	18
Combined immunosuppressive and corticosteroid therapy		15

45 (corresponding to 47%) patients were free of corticosteroid treatment. Further patient data is presented in Table 1.

One patient out of 45 (corresponding to 2.2%) was tested positively, 31 patients out of 45 (corresponding to 68%) were tested negatively and 13 patients out of 45 representing 28.9% showed an indeterminate result of the QuantiFERON test. The logistic regression model has shown corticoid treatment as being the main independent risk factor to achieving indeterminate results (p = 0.002).

To describe the difference between the patient group receiving corticosteroid, corticosteroids and immunosuppression in combination or no concomitant medication we used the Mann–Whitney-U-test. The indeterminate results were associated with corticosteroid treatment and stronger association was found in patients with combined immunosuppressive therapy and corticosteroid treatment. These findings depend upon the dose. A daily dose of 15 mg or more showed a high risk for indeterminate results.

We analysed the stimulation capacity in dependence of immunosuppressive therapy.

Patients with immunosuppressive therapy and corticoid treatment over 15 mg/day had a significant lower stimulation capacity for IFNγ-release after mitogen stimulation than patients under immunosuppressive therapy and less than 15 mg corticoids per day (Fig. 2). Also, without immunosuppressive therapy, the IFNγ-release was reduced under corticoid treatment.
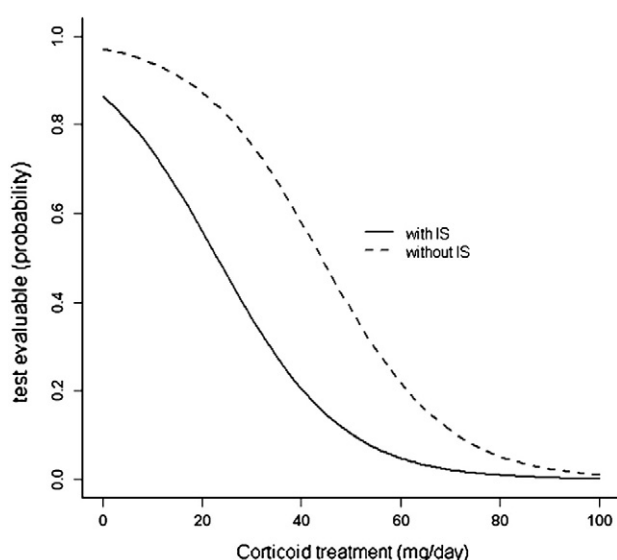

Figure 1 Dose depending curve of risk for indeterminate results of the IFNγ-release test. Data bases on the logistic regression model. Odds ratio is 5.3 (confidence interval is 0.68–42.50).

Concomitant immunosuppression
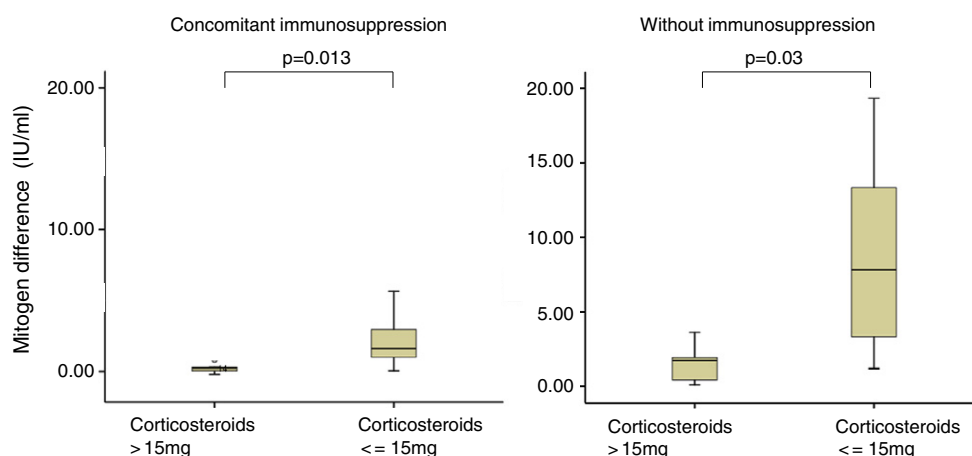
Without immunosuppression

Figure 2 Stimulation capacity of whole blood stimulation. Results are shown as difference between basal IFNγ release and after stimulation with mitogen. Data are differentiated for immunosuppressive agents and co-medication of more than 15 mg or less than 15 mg corticosteroids (prednisolone equivalent) per day. Data are shown as median and interquartile ranges.

4. Discussion

The presented study shows the effect of anti-inflammatory agents on the outcome of testing the latent tuberculosis infection. Corticosteroid treatment increases the risk for indeterminate results. Moreover, the combination of immunosuppressive medication with corticosteroids further increases the risk for an indeterminate result of the QuantiFERON test. Until now it is unclear, if this is due to the general immunologic situation of patients suffering from Crohn's disease or ulcerative colitis or not. In the past the suspicion of modified immunologic reactions against tuberculosis agents in inflammatory bowel disease was discussed (Fig. 3).[19–21]

The therapeutic strategy against Crohn's disease and ulcerative colitis is the use of corticosteroids in the acute phase of inflammation and immunosuppressive agents like azathioprine and 6-mercaptopurine or methotrexate for maintenance of remission phase. In patients with fistula disease and severe inflammation or failure to corticoid and or immunosuppressive therapy the treatment with anti-TNF-antibodies like Infliximab, Adalimuab or Certolizumab will be induced.

This therapeutic strategy reflects the high rate of patients with immunomodulatory treatment in the presented cohort (63%).

In the presented patient group, 28.9% had an indeterminate result of the IFNγ-release assay. However, in the presented patient population there was only one patient tested positively for latent tuberculosis infection. We cannot comment on the risk for undiagnosed latent tuberculosis reaction because of the low number of patients with latent tuberculosis.

We performed risk factors for the development of an indeterminate result and found that the corticosteroid therapy is the main risk factor for false negative results. The risk increases with higher doses of corticosteroids and is supported by immunosuppressive agents. In the combination therapy with immunosuppressive agent corticoid, doses of 15 mg/day or more often lead to indeterminate results. This is related to the reduced stimulation capacity of lymphocytes when patients receive corticoid treatment and is
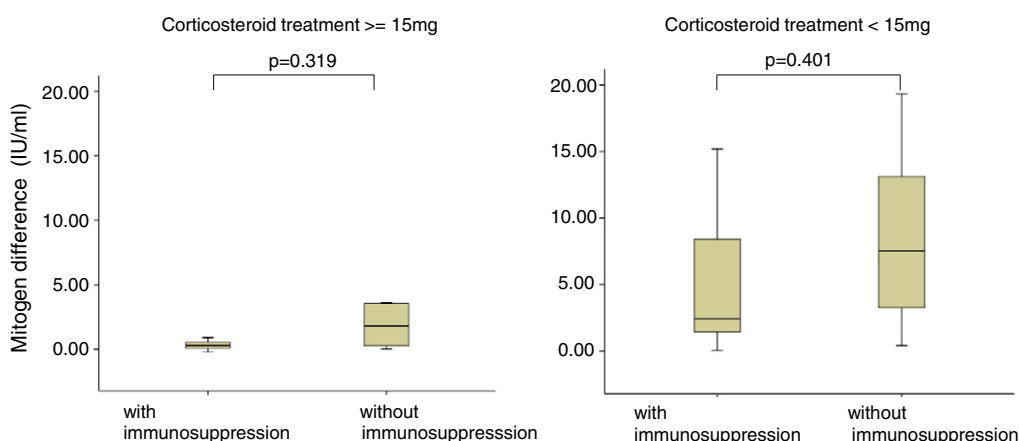
Corticosteroid treatment >= 15mg

Corticosteroid treatment < 15mg

Figure 3 Stimulation capacity of whole blood stimulation. Results are shown as difference between basal IFNγ release and after stimulation with mitogen. Data are differentiated for >=15 mg corticosteroids (left side) and less than 15 mg corticosteroids (right side) (prednisolone equivalent per day). Differences are shown between co-medication with immunosuppressive agents. Data are shown as median and interquartile range.

further reduced in case of concomitant therapy with immunosuppressive agents.

The stimulation capacity is significantly higher in patients under immunosuppressive therapy when the daily corticoid dose is less than 15 mg prednisolone equivalent.

However, the presented study population might show a low rate of latent tuberculosis infections compared to other studies,[14,15,22] but, nonetheless, our data represents the low incidence of tuberculosis infection in the northwest of Germany.[23] Hence, this data is comparable to i.e. the North American population where 1.5% positive QuantiFERON tests were found.[24]

In the presented study a high rate of an indeterminate results occurred compared to the studies of Schoepfer at al. and Qumseya et al. with a maximum of 10% of indeterminate results.[18,24] One explanation for this difference is the higher rate of treatment with immunosuppressive agents in combination with a high dose of corticosteroids prior to starting anti-TNF-therapy in the presented population. This might be due to the German recommendation of use of anti-TNF-therapy in patients with refractory disease to immunosuppressive agents or corticosteroids.[6,7]

A couple of months ago Papay et al. published predictors of indeterminate results of IFNγ release assay whereas in one cohort the indeterminate results were 13.7% and in an IBD-cohort only 7.7%.[17,25] In the IBD-cohort a total of 71.6% were under immunosuppressive therapy; 47.1% under thiopurines and MTX, 33.7% under steroids and 8.7% under TNF-α inhibitors. However, in the IBD-cohort no data on the combined therapy with immunosuppressive agents and corticosteroids was available whereas in the second cohort data on corticosteroids is available and leads to similar results as the main risk factor for indeterminate results of the IFN-gamma release assay. Moreover we could show in the presented study an influence depending on the dose of corticosteroids on the results of QuantiFERON-test.

In conclusion, we can confirm the data of Papay et al. that corticosteroid treatment is the main risk factor for an indeterminate result of IFN-γ release assay. Moreover, we found a high correlation between the combination of immunosuppressive agents and corticosteroids in the presented study. Furthermore, we could show an influence depending on the dose of corticosteroids on the indeterminate results of IFNγ release assay. Therefore, we recommend reducing the corticosteroid treatment to less than 15 mg prednisolone per day if corticosteroid treatment is combined with immunosuppressive agents before starting the QuantiFERON-test. If the clinical situation of the patient requires a high dose of corticosteroid treatment and does not allow a dose reduction of corticosteroids, we recommend excluding latent tuberculosis infection by chest-x-ray and detailed medical history.

The study-setup and the low patient number do not allow a comment on the risk for false negative results and no comparison to the tuberculin skin test.

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