Safety and immunogenicity of a primary yellow fever vaccination under low-dose methotrexate therapy-a prospective multi-centre pilot study

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Original Article

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Safety and immunogenicity of a primary yellow fever vaccination under low-dose methotrexate therapy—a prospective multi-centre pilot study[1]

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Abstract

Background: More people on immunosuppression live in or wish to travel to yellow fever virus (YFV)-endemic areas. Data on the safety and immunogenicity of yellow fever vaccination (YFVV) during immunosuppression are scarce. The aim of this study was to compare the safety and immunogenicity of a primary YFVV between travellers on methotrexate and controls.

Methods: We conducted a prospective multi-centre controlled observational study from 2015 to 2017 in six Swiss travel clinics. 15 adults (nine with rheumatic diseases, five with dermatologic conditions and one with a gastroenterological disease) on low-dose methotrexate (≤20 mg/week) requiring a primary YFVV and 15 age and sex-matched controls received a YFVV. Solicited/unsolicited adverse reactions were recorded, YFV-RNA was measured in serum samples on Days 3, 7, 10, 14, 28 and neutralizing antibodies on Days 0, 7, 10, 14, 28.
Results: Patients´ and controls' median ages were 53 and 52 years; 9 patients and 10 controls were female. 43% of patients and 33% of controls showed local side effects ($P = 0.71$); 86% of patients and 66% of controls reported systemic reactions ($P = 0.39$). YFV-RNA was detected in patients and controls on Day 3–10 post-vaccination and was never of clinical significance. Slightly more patients developed YFV-RNAaemia (Day 3: $n = 5$ vs $n = 2$, Day 7: $n = 9$ vs $n = 7$, Day 10: $n = 3$ vs $n = 2$, all $P > 0.39$). No serious reactions occurred. On Day 10, a minority of vaccinees was seroprotected (patients: $n = 2$, controls: $n = 6$). On Day 28, all vaccinees were seroprotected.
Conclusions: First-time YFVV was safe and immunogenic in travellers on low-dose methotrexate. Larger studies are needed to confirm these promising results.

Key words: immunosuppression, yellow fever vaccine, travel

Introduction

The number of individuals using immunosuppressive agents is increasing. The administration of live vaccines, such as the yellow fever vaccine (YFVV), to immunosuppressed patients bears the risk of uncontrolled replication of the attenuated viral vaccine strain with an acute clinical infection.[1] Therefore, most international guidelines state that YFVV is contraindicated in patients on immunosuppression.[2–4] However, yellow fever is a serious disease. While the majority of infections have an asymptomatic course, more than 50% of those developing serious symptoms die.[5,6] In the light of recent yellow fever virus (YFV) outbreaks, this is alarming as many immunosuppressed people are left unprotected.

Furthermore, the evolution of treatment strategies has often tremendously improved patients' quality of life and a growing number of persons on immunosuppressive medications living in non-endemic areas engage on international trips, including destinations endemic for YFV. For the consulted specialist the concept of 'first, do not harm' is the leading principle and risks and benefits have to be carefully weighed against each other on an individual basis.[7]

Also in individuals without immunosuppression, the risks and benefits of YFVV have to be balanced as rarely serious adverse events, such as YFVV-associated visceral disease (YEL-AVD) can occur.[8–10]

According to the Swiss vaccination recommendations and a limited number of other recently published guidelines, YFVV may be administered to patients on low-dose methotrexate (MTX) therapy (≤20 mg/week) after a careful risk–benefit assessment.[11–13] The Swiss recommendation has been based on common practice in several Swiss travel centres, but not on solid data. Data on YFVV in immunosuppressed individuals are extremely scarce and mostly based on inadvertent vaccination, retrospective studies and booster vaccinations.[14] As explored in a systematic review by Croce et al. no serious adverse event occurred when a booster dose of YFVV was given during methotrexate therapy. During a YFV vaccination campaign in Peru a more than 20 times higher risk of YEL-AVD was found to be associated with one particular vaccine lot compared to other vaccine lots. Among 42 742 vaccinees who received a vaccine from this specific lot, five developed a YEL-AVD. Amongst these, was a 49-year-old female with a history of rheumatoid arthritis and systemic lupus erythematodes who started treatment with MTX and dexamethasone 4 days after vaccination (dosage unknown). The patient died from YEL-AVD. This was the only serious adverse event the authors identified for YFVV in patients on MTX.

In this pilot study, we compared safety (adverse events, RNAaemia) and immunogenicity (antibody production) after YFVV among travellers on low-dose MTX therapy and controls. To our knowledge, this is the first prospective controlled study on primary YFVV in an immunosuppressed cohort.

Methods

Design

This was a prospective multi-centre controlled observational study. Adults (≥18 years) seeking travel advice in six Swiss travel centres (Aarau, Basel, Bern, Geneva, Lausanne, Zurich) and requiring a primary YFVV were included. The decision on whether YFVV was administered was based on the general indication for YFVV: either trip to a yellow-fever endemic country and/or YFVV mandatory for border crossing. Inclusion criteria: adults currently on low-dose MTX (≤20 mg/week) therapy and age (same 10-year age-span) and sex-matched controls. Controls were looked for in parallel to patients' enrolment. Exclusion criteria: contraindication for YFVV, pregnancy, current treatment with other immunosuppressive agent; alemtuzumab or rituximab in the last year, tumour necrosis factor (TNF)-blocking therapy in past 3 months, any immunocompromising condition in controls, other immunocompromising condition than the condition for which low-dose MTX was prescribed in patients, age < 18 years, known previous YFVV. All vaccinees received a subcutaneous injection of the attenuated 17D YFV vaccine (STAMARIL®, Sanofi, France). All participants received the YFVV and the travel advice for free. In addition, they received 30 Swiss francs for each clinical visit to cover for their travel and time expenditures.

Table 1. Baseline characteristics in MTX patients and control group

MTX group ($n = 15$)	Control group ($n = 15$)	*P* value[a]	
Age in years, median (IQR)	53 (31–55)	52 (32–59)	0.53
Female, *n* (%)	9 (60)	10 (67)	0.71
Caucasian, *n* (%)	14 (93)	14 (93)	0.99
BMI, median (IQR)	24.2 (22.5–26.7)	23.7 (21.5–26.8)	0.88
Current smoker, *n* (%)	3 (21)	6 (40)	0.43
Other chronic conditions[b], *n* (%)	4 (27)	6 (40)	0.70
Additional immunosuppressant[c], *n*	1	0	
Concomitant vaccination, median (range)	1 (0–2)	1 (0–3)	0.49

BMI: body mass index, IQR: interquartile range and MTX: methotrexate.
[a]Mann–Whitney *U* test, Chi-squared test or Fisher exact test, as applicable.
[b]Other chronic conditions include: $n = 1$ diabetes mellitus type 1, $n = 3$ pulmonary diseases, $n = 4$ cardiovascular diseases, $n = 1$ neurological disease and $n = 5$ other diseases.
[c]One individual on 15 mg MTX/week concomitantly received prednisolone 2.5 mg orally/day.

Ethical approval was obtained from the respective ethics committees; each patient provided written informed consent. ClinicalTrials.gov Identifier: NCT02383680.

Safety assessment

Solicited and unsolicited reactions were documented during clinical visits on Days 0, 3, 7, 10, 14 and 28 and in diary cards. Details on assessment of adverse events are given in Supplementary 1. YFV-RNAaemia was measured in serum samples obtained on Days 3, 7, 10, 14 and 28 using the RealStar® Yellow Fever Virus RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany). The analytical sensitivity is 0.69 copies/µl (95% confidence interval (CI): 0.41–1.56 copies/µl) and the cut-off for quantification was 10 copies/µl.

Immunogenicity assessments

Neutralizing antibodies were measured with the plaque neutralization assay in serum samples collected on Days 0, 7, 10, 14 and 28 in the Leiden University Medical Center Laboratory, the Netherlands. Details are given in Supplementary 2.

Sample size

This project was planned as a pilot study; thus, no sample size calculation was performed. Based on the numbers of patients included in the study by E.F. Wheelock and W. A. Sibley, who measured YFV viraemia and antibody development after YFVV in 15 healthy young adults, we decided to include 15 patients on low-dose MTX and 15 controls.[15]

Statistical analysis

Cases and controls were compared with a Fisher exact test/Chi-squared test and a Mann–Whitney *U* test, as appropriate. A mixed linear model was used to adjust for potential confounders. Analyses were carried out using Stata/IC 13.1 (Stata Corp, Texas, USA).

Results

Thirty-two participants were enrolled between 2015 and 2017 (16 with MTX, 16 controls). Two participants were excluded due to a prior YFVV based on neutralizing antibodies on Day 0, one in each group. One MTX patient lost his diary card; therefore, no information on local and systemic reactions was available. Baseline and medical characteristics are presented in Table 1. Twelve MTX patients received the YFVV because they were travelling to a YFV-endemic area, and three as it was required for border-crossing.

Out of the 15 MTX patients, nine patients had a rheumatic condition (four rheumatoid arthritis, three psoriatic arthritis, one juvenile arthritis, one sarcoidosis), five had a dermatologic condition (three psoriasis, one neurodermatitis, one atopic dermatitis) and one patient had ulcerative colitis. Median MTX dosage was 12.5 mg (IQR 10–15, range 7.5–20). Patients received MTX for a median duration of 3.0 years (IQR 1.5–7.1, range 34 days-16 years). MTX was not interrupted before or during YFVV administration.

Solicited local and systemic reactions were comparable between patients and controls. 6/14 MTX patients and 5/15 controls reported a solicited local side effect ($P = 0.71$). 12/14 MTX patients and 10/15 controls reported a solicited systemic reaction ($P = 0.39$). The most severe solicited reactions were a 30 mm in diameter local erythema in an MTX patient and a 20 mm erythema in a control. One MTX patient developed a self-limiting fever on Day 6 (max. 38.7°C).

We observed 11 unsolicited adverse events (four in MTX patients, seven in controls). All were of mild or moderate nature. One unsolicited mild adverse event (headache) in a control subject was possibly associated with the YFVV. No serious adverse events occurred. No reactivation of an underlying condition was observed.

Slightly more subjects on MTX developed RNAaemia after YFVV without statistical significance between the two groups (Figure 1). RNAaemia was present in MTX patients and controls between Day 3 and Day 10 post-vaccination. RNAaemia was <10 copies/µl in all participants at all time points with three exceptions: three MTX patients had an RNAaemia of 10, 10.4 and 15.3 copies/µl on Days 7, 3 and 7.

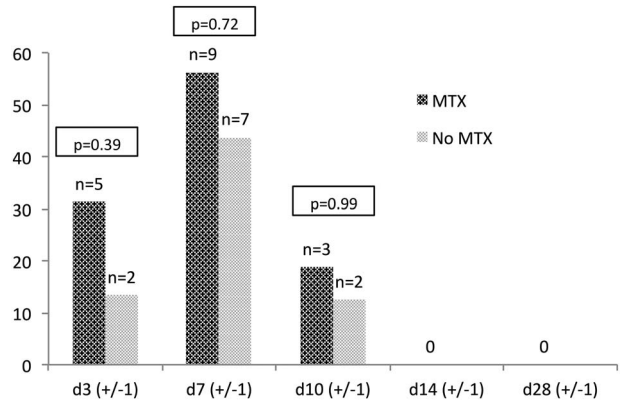

Figure 1. Percentages of participants with yellow fever RNAaemia over time *P*-values were calculated with the Fisher's exact test. Day 3: YFV-RNAaemia information of one control missing, Day 14: YFV-RNAaemia information of one MTX patient missing. Percentages show the percentage of MTX patients and controls with YFV-RNAaemia at each time point. YFV-RNAaemia was defined as ≥10 copies per microliter.

On Day 10 in both groups, the majority of vaccinees was not seroprotected (13 (87%) MTX patients and 9 (60%) controls, $P = 0.22$, Figure 2A and B). Out of four participants not protected on Day 14, two were on MTX and two were without MTX. On Day 28, all participants had protective antibody titres. The median titre was higher in controls across all time points (Figure 2C and D). Neither smoking, BMI, comorbidities, nor ethnicity confounded the result.

Discussion

First-time YFVV was safe and immunogenic in all 15 patients with autoimmune conditions on low-dose MTX therapy. YFV-RNAaemia was low in all participants at all time points. It may, however, take longer until MTX patients develop a protective immune response after YFVV. Roukens et al. observed delayed antibody responses after YFVV in older travellers.[16,17] In the elderly, in contrary to our results, higher RNAaemia levels were detected.

Although MTX may accumulate in cells and cause apoptosis in vitro, immunological studies suggest that low-dose MTX (≤25 mg/week) may have a less immunosuppressive but a more pure anti-inflammatory effect, e.g. numbers of circulating B cells, CD4 cells, CD8 cells and the CD4/CD8 ratio have been shown to be similar in rheumatoid arthritis patients with and without MTX.[18–20] Additionally, the *in vitro* immunoglobulin synthesis was not impaired.[21] These immunological findings (demonstrating no strong immunosuppressive effect of low-dose MTX) and the results from our study, point towards the safety of YFVV in patients on low-dose MTX. However, due to our small sample size we cannot exclude to have missed serious adverse events that could have occurred in a larger population. According to the World Health Organization (WHO), healthy adults are protected by a single YFVV for life and they do not need YFV booster vaccinations.[22] The WHO recommendation has been controversially discussed as especially individuals living in non-endemic settings do not receive natural boosters and may not be seroprotected for long time periods. Kareko et al.[23] recently

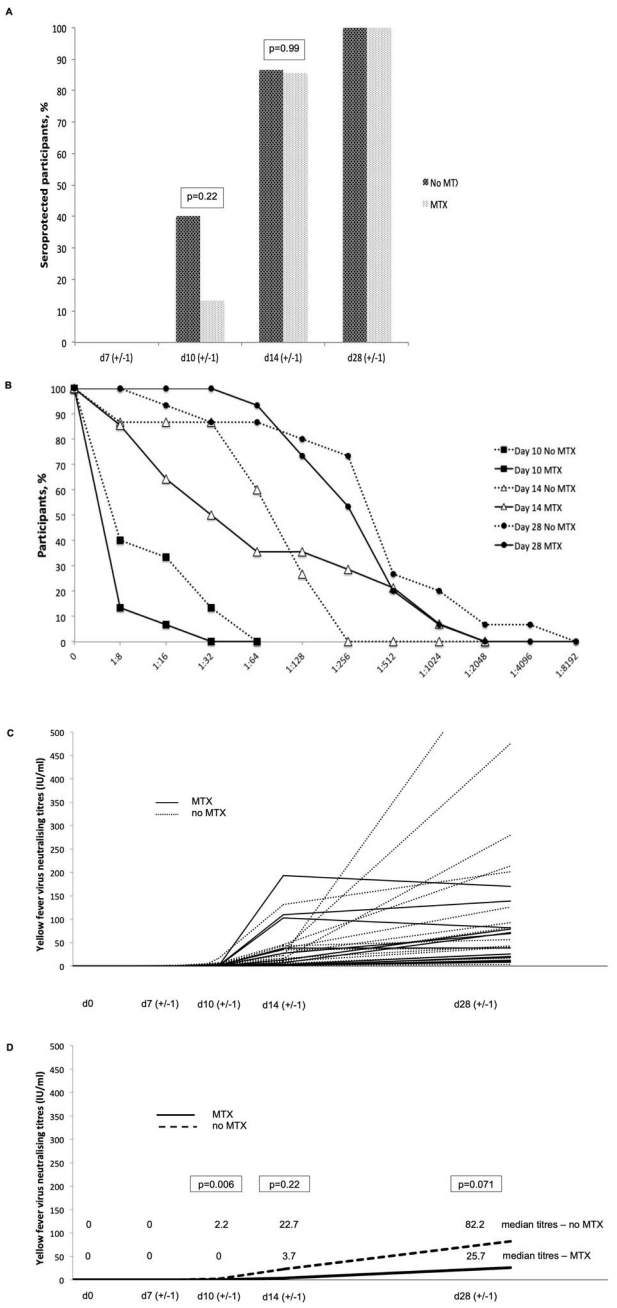

Figure 2. Immunogenicity development in MTX patients and controls over time. (A) The graph shows the percentages of seroprotected MTX patients and controls at time points 7, 10, 14 and 28 days. (B) Reverse cumulative distribution curves of yellow fever neutralizing antibody titres (serum dilution at which 80% virus was neutralized in plaque reduction neutralization test) for Day 10, Day 14 and Day 28. (C) The graph depicts titres in IU/ml of each participant. (D) The graph depicts the median titre in MTX patients and the median titre in controls. *P*-values were calculated with the Mann–Whitney *U* test and Fisher's exact test.

reported that vaccinees most likely become seronegative between 3 and 12 years after YFVV. In line with these findings, Lindsey et al. found that post-YFVV antibody titres showed a time-dependent decrease; 94% of vaccinees were seroprotected if they had received a single YFVV less than 10 years before antibody

measurement, and 82% if they had been vaccinated more than 10 years prior to serum collection.[24]

In our study, 1 month after vaccination all patients with and without MTX were seroprotected. However, we found significantly lower neutralizing titres in participants on MTX on Day 28 (82.2 vs 25.7 IU/ml, $P = 0.071$). As neutralizing antibodies wane over time, vaccinees on MTX, starting off from lower levels, may lose seroprotection more rapidly. The antibody development over time will have to be studied in patients who received a primary YFVV during MTX therapy in the future. As a practical recommendation, we do recommend to measure neutralizing antibodies in individuals who received their primary YFVV during MTX treatment before the next planned trip to an YFV-endemic area. They may require a booster YFVV.

Larger studies are needed to confirm these promising results, which will allow patients in endemic areas as well as travellers on low-dose MTX to such areas to be safely protected from yellow fever. Considering the increasing risk of YFV infections after the recent outbreaks, hopefully many additional international recommendations can be adapted in the near future allowing physicians to vaccinate patients on low-dose MTX against YFV.[25]

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Supplementary Data

Supplementary data are available at *JTM* online.

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Conflict of Interest Statement

None of the authors has any conflict of interest, commercial or otherwise, to declare.

Authors´ Contributions

All authors contributed to either study design, participant recruitment, statistical analysis or data interpretation, literature research and either drafting or revising the manuscript for important intellectual content. All authors approved the final version of the manuscript and they agree to be accountable for all aspects of the work.

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