Long-term immunogenicity after yellow fever vaccination in immunosuppressed and healthy individuals

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ABSTRACT

Background: The live-attenuated yellow fever vaccine (YFV) is generally contraindicated in immunosuppressed patients. Our aim was to investigate if immunosuppressive therapy impairs the long-term protection against yellow fever virus in patients who had received YFV prior to the start of their immunosuppressive therapy.

Methods: Our study examined 35 healthy individuals and 40 immunosuppressed patients with autoimmune diseases or organ transplants. All individuals had received YFV prior to the onset of their immunosuppression. We analysed the long-term influence of the immunosuppressive therapy on the YFV protective immunity by measuring neutralising antibodies (NA) with the Plaque Reduction Neutralisation Test (PRNT). We assessed risk factors for a negative PRNT result (titre below 1: 10) and their influence on the magnitude of the NA.

Results: A median time interval of 21.1 years (interquartile range 14.4–31.3 years) after the YFV in all patients, a total of 35 immunosuppressed patients (88%) were seropositive (PRNT $\geq$ 1:10) compared to 31 patients (89%) in the control group. The geometric mean titres of NA did not differ between the groups. The duration of an underlying rheumatic disease was the only risk factor found for a lower magnitude of NA. An insufficient level of NA was found in nine subjects (12%) who had received a single dose of YFV (in one subject, the number of YFV doses was unknown).

Conclusion: The use of an immunosuppressive drug started after the administration of the YFV did not affect long-term persistence of NA. A second dose of YFV may be necessary to secure long-term immunity.

1. Introduction

The 17D yellow fever vaccination (YFV) shows a short-term seroconversion rate exceeding 95% in immunocompetent individuals and provides highly effective and durable immunity against yellow fever (YF) [1,2]. Since no curative treatment is available, vaccination is strongly recommended for those living in or

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travelling to areas in which YF is endemic. As it is the case with other live-attenuated vaccines the YFV is generally contraindicated in immunosuppressed patients because there may be an increased risk of uncontrolled viral replication with subsequent serious adverse events [3]. The number of immunosuppressed travellers wishing to visit YF-endemic regions is increasing. For travel medicine consultants, the situation is challenging when facing immunosuppressed travellers because neither do they want to interfere with the travel plans, nor do they want to endanger the immunosuppressed traveller by administering YFV and risking serious side effects [4]. In those with a previous YFV the amount of neutralising antibodies (NA) in the sera can be measured to determine whether or not they still have protective titres [2]. A study has shown that protective antibody levels may persist up to 30–35 years in healthy individuals [5]. But in immunosuppressed individuals, antibody levels may be less persistent [6–8].

Studies covering the efficacy and safety of YFV in immunosuppressed patients have mainly focussed on the immunological response of patients receiving the vaccine while under immunosuppressive therapy [9].

However, in patients who become immunosuppressed after YFV administration, little is known about how and to what extent immunosuppressive drugs influence the long-term preservation of the protection acquired from the vaccination. This seems particularly important in light of the ongoing controversy on whether protective immunity is due to long-lived plasma cells or is antigen-driven [10,11]. Despite some controversy, the favoured concept of long-lived plasma cells has been used as framework for the underlying theory [12–14].

NA are recognised as the key mediators of immunity to cytopathic viral infections, preventing the replication and spread of the virus and also acting as a surrogate for protective immunity against the YF virus [2]. Nevertheless, it has been shown that the immune response to the YFV includes a variety of components of innate and adaptive immunity [15]. In case of exposure to the wild-type virus, or revaccination after a previous YFV, these NA are reinforced in the immune response by specific B and T memory cells [2,16,17].

The maintenance of a persistent level of secreted antibodies is dependent on the constant antibody secretion by long-lived plasma cells [18]. The underlying mechanism of the preservation of those long-lived plasma cells has been intensely debated in recent years. At present, the most widely accepted theory argues that plasma cells have the capacity to live indefinitely in so-called "survival niches", mostly located in the bone marrow [12–14]. Once the plasma cell precursors, known as plasmablasts, have reached the end-stage of differentiation, their maintenance remains independent of persistent antigen, T cells or memory B cells [19–21].

As survival niches are limited in number, there is steady competition between newly generated plasmablasts and resident plasma cells [22]. The excessive number of plasmablasts producing autoantibodies generated in patients with active autoimmune disease can lead to the expulsion of vaccine-induced plasma cells and a reduction of Immunoglobulin G (IgG) titres in the serum [19,23].

Once plasmablasts have become long-lived plasma cells and settled in their survival niches, they constitute a difficult target for medication. Since it has been shown that plasma cells do not undergo DNA synthesis, it is not surprising that neither cyclophosphamide nor mycophenolate are able to lower their numbers [24–26]. In the bone marrow of rats, methotrexate was able to reduce the levels of CXCL-12 protein, which is known as an integral component of the survival niche, but, as Hoyer et al. have pointed out, CXLC-12 is not essential for the survival of plasma cells [27,28]. Other anti-inflammatory drugs such as dexamethasone also appear to have no effect on antibody production [22]. The lack of CD20 on the surface of plasma cells additionally means that rituximab has no target and thus no effect on long-lived plasma cells [19]. Most studies investigating the effects of immunosuppression on long-lived plasma cells, however, have employed mouse models; in humans, only short time periods (up to 21 days) were investigated [24–27].

Summing up, based on the concept that long-lived plasma cells are independent of B- or T-cells, immunosuppressive drugs do not seem to have an impact on the production of NA. On the other hand, if the alternative concept applies, and repeated re-exposure to antigen in immune-complexes is needed, the immunological memory may be influenced by long-term immunosuppression.

We conducted this study in order to assess the long-term preservation of humoral protection against the YF virus in patients who are currently under immunosuppressive therapy and who had been vaccinated prior to the onset of their immunosuppression. In the hypothesis of absence of a booster, we focused on NA as a surrogate for long-lived plasma [2].

2. Methods

2.1. Study population and design

The data in this study has been collected as part of a larger study from the same research group, referred to below as the "tetanus study", which was conducted as a multicentre prospective cohort study in six rheumatology and two travel clinics in Switzerland. (For further details, see reference [29]). Patient recruitment took place at the following Swiss rheumatology clinics: Cantonal Hospital of Aarau, University Hospital of Basel, University Hospital of Bern, Geneva University Hospitals, Cantonal Hospital of St. Gallen, University Hospital of Zurich. Controls and patients were recruited in two travel clinics: Travel Clinic at the Epidemiology, Biostatistics and Prevention Institute, University of Zurich, Travel Clinic of the Swiss Tropical and Public Health Institute in Basel.

Study subjects under immunosuppressive therapy and the control group for the cross-sectional study were taken from the already existing pool and complemented with patients from the Travel Clinic in Zurich (Fig. 1). Immuosuppression was defined according to Table 1 in Eperon et al. [30]. In total, blood samples from 75 subjects were analysed in the study: 60 blood samples were obtained from the larger tetanus study conducted between 2014 and 2016, and 15 blood samples were gathered between 2013 and 2017 in the Zurich Travel clinic. The vaccination status of all participants was checked and documented. The criterion for inclusion in the study was a record of a previous YFV. In both groups, the date of YFV was determined from vaccination cards or personal memories (n = 23 were reported from personal memories; n = 13 in the group of patients with immunosuppressive therapy). Patients were considered immunosuppressed if they had started an immunosuppressive therapy between the YFV and the blood draw. Start date of immunosuppression was based on patientś memories.

2.2. Ethical approval

The ethics committees of Aarau, Bern, Geneva, Nordwestschweiz, St. Gallen and Zurich approved the study. (Reference numbers Aarau EK: 2013/062, Bern: 182/13, CCER 2016-00218, EKNZ 257/13, EKSG 13/138, KEK-ZH 2013-0188). All participants signed an informed consent form prior to enrolling in the study.

2.3. Laboratory analysis

Serological responses to the YFV can be measured through neutralisation of the virus by antibodies with the Plaque Reduction

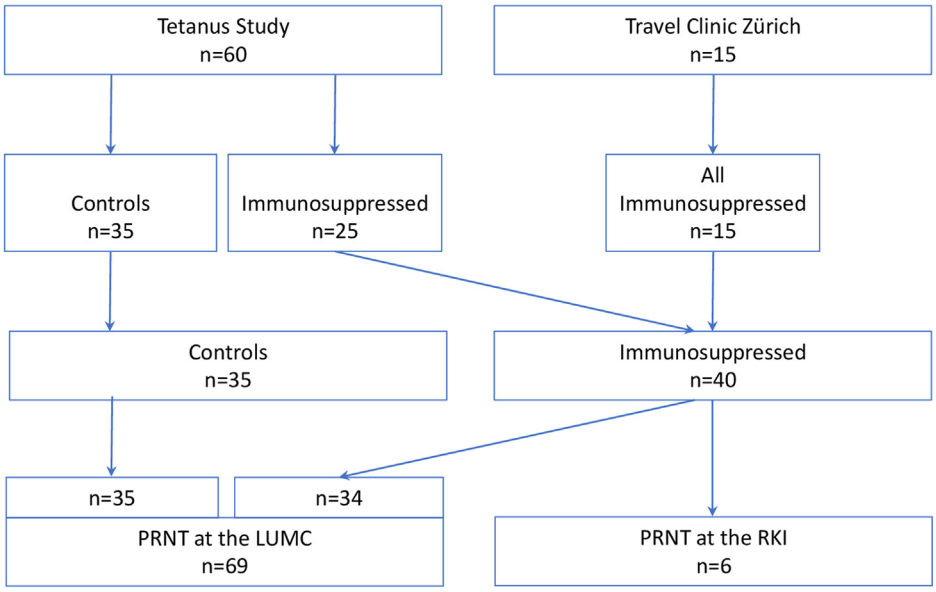

Fig. 1. Flowchart of recruitment and analysis Abbreviations: LUMC = Leiden University Medical Center; n = number of participants investigated; PRNT = plaque reduction neutralisation test; RKI = Robert Koch Institute.

Table 1
Overview of the demographic and clinical characteristics.

	All (n = 75)	Immunosuppressed (n = 40)	Non-immunosuppressed (n = 35)	P-value°
Age at vaccination (years), median (IQR)	30.5 (25.1–44.4)	36.1 (25.5–48.2)	28.7 (24.7–38.2)	0.14
Age at blood draw (years), median (IQR)	61.4 (46.4–69.2)	60.9 (48.7–69.6)	62.1 (38.7–68.8)	0.27
Female n(%)	46 (61.3)	24 (60.0)	22 (62.9)	0.99
BMI, median (IQR)	24.9 (22.3–26.9)	25.2 (23.8–27.4)	24.4 (21.0–26.2)	0.06
Nationality Swiss, n(%)	49 (78.7)	32 (80.0)	27 (77.1)	0.99
Country of birth Switzerland, n(%)	38 (50.7)	16 (40.0)	22 (62.9)	0.99
Non-rheumatic chronic disease, n(%)	31 (42.9)	22 (57.9)*	9 (25.7)**	0.005
Number of YFV doses, n(%)				0.80
1	57 (80.3)	30 (80.0)	27 (81.8)	
2	10 (14.1)	5 (13.2)	5 (15.2)	
3	4 (5.6)	3 (7.9)	1 (3.0)	
Mean	1.3	1.3	1.2	0.79
Time since last YFV (years), median (IQR)	21.1 (14.4–31.3)	20.6 (14.4–31.5)	23.4 (14.4–31.1)	0.82
Duration of immunosuppressive drug intake (years), median (IQR)		4.6 (0.9–8.4)		

Abbreviations: n = number of patients investigated, YFV = yellow fever vaccine, IQR = interquartile range.
°Mann-Whitney *U* test or Fisher exact test.
* Non-rheumatic chronic diseases include: cancer n = 3 (all considered in remission at time point of blood draw: breast cancer, Hodgkin lymphoma, spinocellular cancer), cardiovascular n = 9 (coronary heart disease, hypercholesterolemia, hyperlipidemia, hypertension, mild mitral valve insufficiency, peripheral artery occlusive disease), kidney disease n = 5 (chronic renal failure, renal insufficiency, renal problems due to lupus, thin basement membrane disorder), lung disease n = 2 (asthma, pulmonary insufficiency).
** Non-rheumatic chronic diseases include: cancer n = 1 (all considered in remission at time point of blood draw: prostate cancer), cardiovascular n = 4 (history of heart attack, hypercholesterolemia, hypertension), diabetes n = 1, liver disease n = 1 (unknown liver disease), lung disease n = 1 (chronic bronchitis).

Neutralisation Test (PRNT), which is the standard technique for assessing the humoral response to the YFV [2]. For this study, the PRNT were performed at two different laboratories, the Robert Koch Institute Berlin (RKI) and the Leiden University Medical Center (LUMC).

At the RKI, the neutralisation titre (NT) represents the highest serum dilution able to induce a 90% reduction (PRNT90) of plaque counts in cells infected by the 17D YF strain [31]. In the LUMC laboratory, protection was defined as the occurrence of at least an 80% (PRNT80) reduction of plaque count in a 1:10 serum dilution according to the design of the PRNT, as previously described by De Madrid and Porterfield and modified for the LUMC PRNT test setup [32–34]. In both laboratories, a titre of 1:10 was the accepted threshold for protection given that a NT of ≥1:10 is generally believed to be a serological surrogate of protection against wild-type YF virus [35]. The LUMC additionally reported endpoint titres in IU/ml using the 1st International Reference Preparation of Anti-Yellow Fever Serum (National Institute for Biological Standards and Control, UK); the method for the calculation is described by Cohen et al. [34,36].

Since the RKI used a higher plaque-reduction cut-off (PRNT90), it was decided that all patients with a PRNT ≥1:10 can be included in the group with seropositive results in the PRNT measured by the LUMC for the following statistical analysis.

2.4. Statistical analysis

Descriptive statistics were used to summarise the demographic and clinical characteristics of the study subjects. Comparisons of the study subjects with seropositive or seronegative results in the PRNT were carried out using the Fisher's exact test for categorical variables, or the Mann-Whitney-*U* test for continuous variables. As risk factors for an outcome in the PRNT of <1:10, the following candidate characteristics were considered:

sex, age at blood draw, age at vaccination, length of time between vaccination and serology, number of previous YFV doses, rheumatic disease, type of rheumatic disease, duration of rheumatic disease, intake of immunosuppressive drugs and duration of drug intake. Furthermore, we analysed if those risk factors showed a correlation with the magnitude of the NA, expressed as the log10 of the reciprocal of the neutralisation titre value (referred to below as "antibody titre") for which we only used the results from the LUMC because the PRNT titres from the RKI were not directly comparable. We excluded subjects without an exact titre in the PRNT from this part of the analysis (n = 5; four with a result over the upper limit and one below the lower limit of the PRNT). To investigate the influence of the risk factors on the magnitude of the antibody titre, we used either the Mann-Whitney *U* test for categorical risk variables or the Spearman rank test for continuous risk variables. Correlations between the magnitude of the antibody titre and the risk factors were calculated with Spearman's Rank Correlation coefficient (Spearman's rho).

P values of <0.05 were defined as statistically significant. Statistical analysis was carried out using Stata (Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

Table 2
Demographic and medical details of patients under immunosuppressive therapy.

ID	Sex	Age at blood take	Age at vaccination	Underlying disease	Drugs	Years of Drug intake	Number of YFV	Years since last YFV	PRNT titre	PRNT IU/ml	Lab	PRNT Interpretation
71	F	46	36	Crohn's disease	Azathioprine (na)	3.6	1	10.5*	32	8.3	LUMC	Positive
66	F	66	23	Kidney transpl.	Cyclosporine, Myco.	na	1	42.7	32	5.4	LUMC	Positive
73	F	65	46	Kidney transpl.	Myco., Tacrolimus	4.6	3	18.4	32	4.2	LUMC	Positive
77	F	69	49	Kidney transpl.	Myco., Rapamune	20	1	20.1*	26	na	RKI	Positive
63	M	58	48	Liver transpl.	Myco., Tacrolimus	na	3	10.6	32	9.3	LUMC	Positive
70	M	64	43	MS	Interferon, Predni. (na)	7.6	3	20.6*	40	na	RKI	Positive
76	F	39	19	MS	Dimethylfumarat	1.6	1	19.9	75	na	RKI	Positive
31	F	63	23	pPsA	Apremilast, RTX	2.2	na	39.9*	0.0	0.0	LUMC	Negative
24	M	40	24	pPsA	Chlo., MTX (15 mg), Myco., Predni. (5 mg)	4.9	na	15.9*	17.5	2.3	LUMC	Positive
32	M	50	23	pPsA	MTX (20 mg), Predni.(1.25 mg)	0.3	1	26.9*	67.7	8.0	LUMC	Positive
69	M	71	51	pPsA	MTX (10 mg)	na	2	20.4*	21	4.5	LUMC	Positive
20	M	60	27	pPsA	IFX	5.5	1	33.5	70.9	9.4	LUMC	Positive
74	F	56	44	pPsA	Secukinumab	0.7	1	11.4*	28	3.7	LUMC	Positive
21	F	69	29	RA	Abatacept, Predni. (2.5 mg)	6.8	1	40.0	27.1	3.6	LUMC	Positive
35	F	71	52	RA	Adalimumab	11.4	1	18.9	11.2	1.3	LUMC	Positive
39	F	68	54	RA	Chlo., Sulfasalazine	13.2	2	14.4	35.6	4.8	LUMC	Positive
61	F	75	50	RA	Chlo., Predni.(5 mg), Tocilizumab	9.2	2	25.0	14.5	1.8	LUMC	Positive
02	F	69	46	RA	MTX (10 mg), Predni. (2.5 mg)	0.7	1	22.7	106.3	12.9	LUMC	Positive
03	M	42	25	RA	MTX (15 mg)	0.1	1	16.8	1.5	0.2	LUMC	Negative
26	F	75	66	RA	MTX (10 mg), Predni.(5 mg), Tocilizumab	10.0	1	8.6	29.2	3.9	LUMC	Positive
36	M	72	38	RA	MTX (15 mg)	0.6	1	34.8	45.8	5.4	LUMC	Positive
56	F	56	43	RA	MTX (10 mg)	12.7	1	12.7*	29.6	3.6	LUMC	Positive
68	M	61	28	RA	MTX (15 mg)	9.5	2	33.1*	32	na	RKI	Positive
33	M	81	57	RA	Leflunomide, MTX (na)	0.3	1	23.6	23.2	2.7	LUMC	Positive
64	F	42	27	RA	Golimumab, MTX (na)	na	1	14.6*	9	1.8	LUMC	Negative
27	M	66	40	RA	Golimumab, Predni. (na), Tocilizumab	5.1	1	26.2	32.8	4.4	LUMC	Positive
67	F	67	21	RA	Golimumab, Predni. (2 mg)	1.2	1	46.2*	25	na	RKI	Positive
65	F	56	31	SLE	Lef., Predni. (2 mg)	na	1	24.4*	8	2.3	LUMC	Negative
30	F	50	43	SpA	Adalimumab, Predni. (25 mg)	0.1	1	7.4	905.7	107.2	LUMC	Positive
34	M	65	27	SpA	Adalimumab	0.2	1	37.9	37.5	4.4	LUMC	Positive
05	M	46	30	SpA	Etanercept	10.4	1	15.6	9.2	1.1	LUMC	Negative
29	F	55	48	SpA	Golimumab	2.7	1	7.3	524.9	62.2	LUMC	Positive
60	F	51	na	SpA	Golimumab, Lef., Predni. (5 mg), RTX	2.4	1	na	18.2	2.2	LUMC	Positive
23	F	32	9	SpA	IFX	5.6	1	22.5	46.9	6.2	LUMC	Positive
25	F	46	25	SpA	IFX	4.6	1	21.5	38.1	5.1	LUMC	Positive
28	M	54	23	SpA	IFX	0.2	1	31.5	41.6	4.9	LUMC	Positive
72	F	25	16	Ulcerative colitis	Azathioprine (100 mg), IFX	1.6	1	9.4	98	na	RKI	Positive
75	M	44	36	Ulcerative colitis	Azathioprine (50 mg)	na	2	7.8	18	5.4	LUMC	Positive
19	F	69	34	Vasculitis	IFX, Predni. (0.3 mg)	11.8	1	35.2	22.7	3.0	LUMC	Positive
22	M	76	49	Vasculitis	MTX (na), Predni. (na), RTX	6.3	1	26.6	32.8	4.4	LUMC	Positive

Underlying disease: MS = multiple sclerosis, pPsA = peripheral psoriatic arthritis, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SpA = spondyloarthritis (ankylosing spondylitis or non-radiographic axial spondyloarthritis), transpl. = transplantation, Vasculitis = ANCA-associated vasculitis.
Drugs and doses: Azathioprine (dose in mg per day P.O.), Chlo. = chloroquine, IFX = infliximab, Lef. = leflunomide, MTX = methotrexate (dose in mg per week s.c.), Myco. = mycophenolate, Predni. = prednisone (dose in mg per day P.O.), RTX = rituximab.
Other abbreviations: ID = identification number, LUMC = Leiden University Medical Center, n = number of subjects, na = data not available, PRNT = plaque reduction neutralisation titre.
* = date of YFV was reported orally.

3. Results

3.1. Study population

40 subjects were included in the group of patients under immunosuppressive therapy (60.0% female, median age 60.9 years (IQR 48.7–69.6 years) and 35 in the control group (62.9% female, median age 62.1 years (IQR 38.7–68.8 years). An overview of the demographic and clinical characteristics of the study subjects can be found in Table 1. All study subjects had received at least one injection of the 17D YFV; 10 subjects had received two YFV doses, and four subjects had received three doses. In four participants it was not clear how many doses they had received, and in three participants no date of the YFV could be determined. The median length of time since the last YFV was 20.6 years (IQR 14.4–31.5 years) in the patient group and a median of 23.4 years (IQR 14.4–31.1 years) in the control group.

Table 2 shows the demographic and medical details of patients under immunosuppressive therapy. Of the 40 subjects included in the group of patients under immunosuppressive therapy, 36 had an autoimmune disease, including rheumatic diseases (rheumatoid arthritis, axial spondyloarthritis (ankylosing spondylitis, non-radiographic axial spondyloarthritis), psoriatic arthritis, vasculitis (ANCA-associated vasculitis)), inflammatory bowel diseases (Crohn's disease or ulcerative colitis) or multiple sclerosis. Four patients took immunosuppressive therapy due to organ transplantation (kidney or liver). 92.5% (n = 37) of patients were still under immunosuppressive therapy at the time point of the blood draw and one started with low-dose prednisone (5 mg/day) before the YFV. The median duration of immunosuppressive medication intake between the last YFV and blood draw was 4.6 years (IQR 0.9–8.4 years).

55.0% of patients had received at least two immunosuppressive drugs following YFV. The most commonly used agents were corticosteroids (19.7%), followed by methotrexate (16.9%), infliximab (8.5%), golimumab (7.0%), mycophenolate mofetil (7.0%), others: abatacept, adalimumab, apremilast, azathioprine, cyclosporine, dimethyl fumarate, etanercept, leflunomide, sirolimus, rituximab, secukinumab, tacrolimus, tocilizumab (each less than 5%).

Despite hydroxychloroquine and sulfasalazine being considered as immunomodulatory and not immunosuppressive, we included the patient with the ID number 39 due to the underlying rheumatic disease. Also, the patient with the ID number 26 was not removed from analysis even if he was taking prednisone at the time of the vaccination. Since it was a low-dose therapy, we felt that it would not alter the outcome. Nevertheless, when excluding these subjects from analysis the results did not change.

No significant differences were found between study groups with regard to sex, BMI, age at blood draw, age at vaccination, number of previous YFV doses and time elapsed since the last YFV (Table 1).

3.2. Immunogenicity

The vast majority (88%, n = 66, 95%CI = 80–96%) in both groups showed a PRNT of ≥1:10. In the immunosuppressed group, 88% of individuals (n = 35, 95%CI = 77–98%) had a PRNT of ≥1:10, and in the control group 89% (n = 31, 95%CI = 77–100%) (Fig. 2). No differences between patients and the control group were identified in geometric mean titres (GMT) (GMT 33.3 (95%CI = 22.7–48.9) versus GMT 31.3 (95%CI = 18.8–52.3)).

Antibody titres had a median of 4.4 IU/ml (IQR 2.3–5.4 IU/ml) in the immunosuppressed group and a median of 5.6 IU/ml (IQR 2.0–10.4 IU/ml) in the control group. Across both groups there was no correlation between antibody titres and the time since vaccination.

Table 3 shows the characteristics of patients and healthy controls with a seronegative PRNT. No evidence was found that the selected risk factors – sex, age at blood draw, age at vaccination, length of time between vaccination and serology, number of previous YFV doses, rheumatic disease, type of rheumatic disease, duration of rheumatic disease, intake of immunosuppressive drugs and duration of drug intake – influenced the seropositivity (Table 4). Even if, as a subgroup, the intake of a cytotoxic immunosuppressive drug was analysed separately as a risk factor, no difference was found. However, all participants (whether immunosuppressed or not) who were not seropositive had only received a single YFV dose in the past (Table 4). All those who had received 2 doses or more were seropositive.

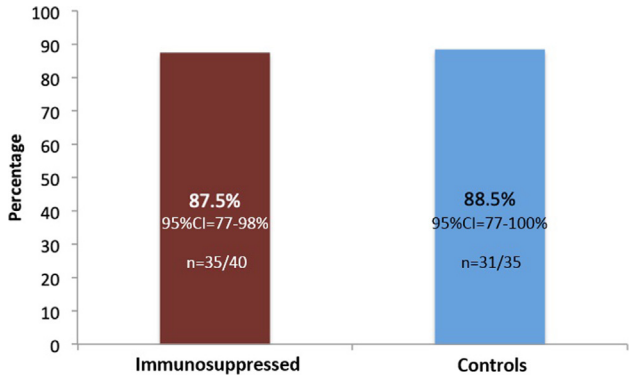

Fig. 2. Percentage of seropositivity in immunosuppressed patients and controls after a median of 21 years after the last YFV.

Table 3
Demographic and medical details of patients and healthy controls with a seronegative PRNT.

ID	Sex	Age at blood draw	Age at vaccination	Underlying disease	Drugs	Years of drug intake	Number of YFV	Years since last YFV	PRNT titre	PRNT IU/ml	Lab	PRNT
31	F	63	23	pPsA	Apremilast, RTX	2.2	na	39.9*	<0.01	<0.01	LUMC	Negative
03	M	42	25	RA	MTX (15 mg)	0.1	1	16.8	1.5	0.2	LUMC	Negative
64	F	42	27	RA	Golimumab, MTX (na)	na	1	14.6*	9	1.8	LUMC	Negative
65	F	56	31	SLE	Leflunomid, Predni. (2 mg)	na	1	24.4*	8	2.3	LUMC	Negative
05	M	46	30	SpA	Etanercept	10.4	1	15.6	9.2	1.1	LUMC	Negative
10	F	47	28				1	19.4*	6.8	1.0	LUMC	Negative
18	F	77	66				1	11.4	0.07	0.01	LUMC	Negative
44	F	26	8				1	17.8	3.7	0.5	LUMC	Negative
47	M	77	25				1	44.5	4.7	0.54	LUMC	Negative

Underlying disease: pPsA = peripheral psoriatic arthritis, RA = rheumatoid arthritis, SLE = systemic lupus erythematosus, SpA = spondyloarthritis (ankylosing spondylitis).
Drugs and doses: MTX = methotrexate (dose in mg per week s.c.), Predni. = prednisone (dose in mg per day P.O.), RTX = rituximab.
Other abbreviations: ID = identification number, LUMC = Leiden University Medical Center, na = data not available, PRNT = plaque reduction neutralisation titre.
* = date of YFV was reported orally.

Table 4
Risk factors for PRNT < 1/10.

Risk factors	All (n = 75)	PRNT < 1/10 (n = 9)	PRNT ≥ 1/10 (n = 66)	P-value*
Female, n(%)	46 (61.3)	6 (66.6)	40 (60.6)	0.99
Age at vaccination (years), median (IQR)	30.5 (25.1–44.4)	27.8 (25.7–30.8)	32.1 (24.7–45.7)	0.24
Age at blood draw (years), median (IQR)	61.4 (46.4–69.2)	47.6 (42.5–63.6)	62.1 (46.6–69.2)	0.39
Length of time between vaccination and serology (years), median (IQR)	21.1 (14.4–31.3)	17.8 (15.6–24.4)	22.5 (12.7–31.5)	0.82
Number of YFV doses, n(%)				0.75
1	57 (80.3)	8 (100.0)	49 (77.8)	
2	10 (14.1)	0	10 (15.9)	
3	4 (5.6)	0	4 (6.3)	
Rheumatic disease	31 (41.3)	5 (55.6)	24 (36.4)	0.41
Type of rheumatic disease, n(%)				0.27
RA	14 (18.7)	2 (22.2)	12 (18.2)	
SpA	8 (10.7)	1 (11.1)	7 (10.6)	
pPsA	6 (8.0)	1 (11.1)	5 (7.6)	
Vasculitis	2 (2.7)	0	2 (3.0)	
SLE	1 (1.3)	1 (11.1)	0	
Duration of rheumatic disease (years), median (IQR)	7.2 (0.9–13.7)	8.5 (0.4–15.6)	7.0 (1.1–13.4)	0.83
Intake of immunosuppressive drug, n(%)	40 (53.3)	5 (55.6)	35 (53.0)	0.59
Duration of immunosuppressive drug intake (years), median (IQR)	4.6 (0.9–9.3)	2.2 (1.1–6.3)	4.7 (1.1–9.2)	0.56

Type of rheumatic disease: RA = rheumatoid arthritis, SpA = spondyloarthritis (ankylosing spondylitis or non-radiographic axial spondyloarthritis), pPsA = peripheral psoriatic arthritis, Vasculitis = ANCA-associated vasculitis, SLE = systemic lupus erythematosus.
Other abbreviations: IQR = interquartile range, n = number of subjects, PRNT = plaque reduction neutralisation titre, YFV = yellow fever vaccine.
* Mann-Whitney *U* test or Fisher exact test.

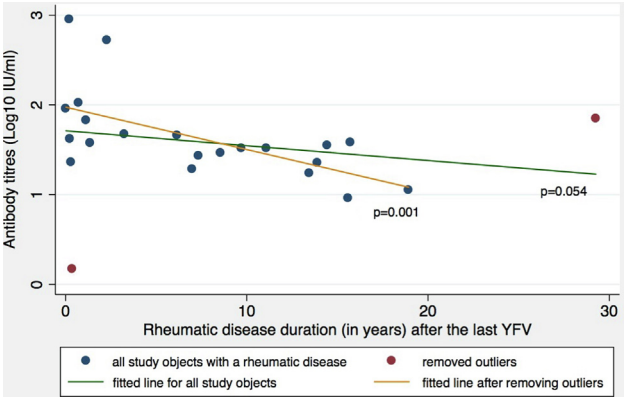

Fig. 3. Antibody titres (log10 IU/ml) against the rheumatic disease duration (years) Abbreviations: YFV = yellow fever vaccination. The correlation between serum titre and time since vaccination was calculated with the Spearman's Rank Correlation coefficient. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

When we examined the influence of the risk factors on the magnitude of NA we found a small negative association for rheumatic disease duration since receipt of YFV on the level of NA (Spearman's rho: −0.41; p-value of 0.054). After removing two outliers from the group – one with a very low PRNT level and one with a very long-lasting rheumatic disease – the correlation was significant (Spearman's rho: −0.68; p = 0.001) (Fig. 3).

Since we found no association between the above-mentioned risk factors and seropositivity and only an association for duration of rheumatic disease on the magnitude of NA in univariate analysis, we refrained from building a multivariate model.

4. Discussion

After comparable time periods since YFV, we found no significant difference in seropositivity in patients under immunosuppressive therapy (88% seropositivity) and healthy controls (89% seropositivity). The same result was found in a paper by Ferreira et al., which investigated the long-term effect of disease-modifying anti-rheumatic drugs on the duration of protective immunity induced by the YFV in patients with rheumatoid arthritis. The authors found that a therapy with conventional synthetic immunosuppressive drugs did not show a significant difference in the time-dependent decline of NA compared to the control group; the combination with biological immunosuppressive drugs showed a significantly lower seropositivity rate in the PRNT between 5 and 9 years after vaccination [37]. Due to the low number of patients in our study with that combinational therapy, this finding could not be reproduced.

The seropositivity rates in our study are similar to those found in other papers, for example, a study which found a seropositivity of 87% in elderly subjects, 60 years old or older, with a median time of 3.3 years since the last vaccination; and a Brazilian study in which a seropositivity of 85% was found in subjects aged 18 to 83 years who were vaccinated more than 12 years earlier [1,38]. The seroconversion rate after the yellow fever vaccination is stated to be around 95% in most studies [1,39]. Consequently, a proportion of our negative results in seropositivity may be due to a primary failure to seroconvert after the YFV.

Interestingly, all patients under immunosuppressive therapy with seronegative results in the PRNT had an underlying rheumatic disease. One the one hand, this was highly likely since 77.5% of the group of patients had a rheumatic disease. But it also has been shown that plasmablasts producing autoantibodies in patients with active autoimmune disease take up some amount of the survival niches in the bone marrow and possibly displace some of the vaccine-induced plasma cells, which could have played a part in the lowered magnitude in NA and the consequent seronegativity [19,23].

By measuring the immune response with the PRNT, the focus is only on one part of the immune response. But, aside from the persistent number of NA produced by long-lived plasma cells, YFV induces the production of a large amount of memory B and T cells, which are able to respond rapidly to a subsequent exposure to the wild-type virus [2]. Therefore, although the immunosuppressive therapy seems to have no effect on the production of NA, the question remains as to whether they are capable of providing full protection in case of exposure to the wild-type YF virus. In one study, hamsters were passively immunised with sera derived from hamsters inoculated with an inactivated or live-attenuated YF virus. The study demonstrated that NA alone were capable of giving full protection against the challenge from the YF virus [40]. But it was also suggested that CD8 + T cells may be important in comple-

menting NA in viral clearance after an intracerebral challenge in murine models [41,42].

However, the findings on (life-) long-persistent NA after YFV cannot be extrapolated to all vaccines as most vaccines need booster doses to replenish the (antigen driven) plasma cell pool via B and T memory cell co-stimulation.

Summing up, even though NA seem to be the primary mechanism of protection, the question remains as to whether they provide full protection from exposure to wild-type YF virus in humans. The roles of other components of the immune system are not yet completely clarified, which is especially important for patients whose immune systems have been altered by immunosuppressive therapy.

Overall, the high rate of seropositivity in both groups, even after a median length of time of 21.1 years since the last YFV, is consistent with other findings, which have shown that NA may persist 30–35 years [5].

No significant decline in the magnitude of NA over time was found, which contrasts with some studies but which can probably be explained by the high median age of our sample group (61.4 years), since two other studies examining NA in elderly people also detected no significant decline in this age group [1,43,44].

However, in nine out of 75 participants we received a negative result for seropositivity in the PRNT. In those individuals, the time elapsed since the last vaccination was more than ten years (median: 17.8 years) and two were over 60 years old at the time of blood draw (median 47.6 years) (Table 4). All of the study subjects with a PRNT <1:10 had received only one YF vaccination (one patient did not know the number of YFV doses received). In contrast, all of our study's subjects who had received two or more vaccinations had a PRNT $\geq$1:10 (Table 4). The median time span since the last vaccination in the subjects with a seropositive PRNT was even longer (median 22.5 years) than in those with a seronegative PRNT (median 17.8 years) (Table 4). A similar result was also seen in a study from Lindsey et al., in which all subjects with two or more YFV doses had a positive PRNT regardless of the time elapsed since the last vaccination [7].

The limitations of our study include its small sample size and also that the results of the PRNT came from different labs. NA are currently seen as the best surrogate for assessing the protection against the YFV, and measuring NA with the PRNT is considered the standard technique [2]. But even though a NT of $\geq$ 1:10 is generally regarded as protective, a clear cut-off correlating with protection to the YFV is lacking [2,33,35]. Additionally, due to safety reasons, both laboratories (RKI and LUMC) use the 17D vaccine strain, not a wild-type strain in the PRNT to measure the neutralisation. Moreover, we cannot exclude that the proportion with a primary vaccine failure differed in the two groups; this may have affected our results. Furthermore, we cannot rule out that some of the study subjects had received a natural booster through exposure to wild-type YF virus while travelling to an YF-endemic country. Since some of the information regarding the YFV has been reported orally it also cannot be excluded that there are some faults related to dates of the YFV or the number of YFV doses.

5. Conclusion

We found no evidence that the use of an immunosuppressive medication started after YFV has an influence on the long-term preservation of YF immunity. But in nine subjects (12%), an insufficient level of NA was found, and all nine had only received one dose of YFV (with one subject having an unknown number of YFV doses). Combining our results and those from other authors [1,7,45], the findings suggest that even if the proportion of primary vaccine non-responders is low, a second dose of YFV may still be beneficial to secure long-term immunity.

We advise checking NA in patients with rheumatic diseases, even in vaccinated subjects who are not currently on an immunosuppressive therapy. In further investigations, it would be worthwhile to see whether this correlation is confirmed in a study with a larger sample size.

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Author contributions

All authors contributed to either study design, participant recruitment, statistical analysis or data interpretation, 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.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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