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An extra priming dose of hepatitis A vaccine to adult patients with rheumatoid arthritis and drug induced immunosuppression – A prospective, open-label, multi-center study

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ABSTRACT

Background: Previous studies have indicated that a pre-travel single dose of hepatitis A vaccine is not sufficient as protection against hepatitis A in immunocompromised travelers. We evaluated if an extra dose of hepatitis A vaccine given shortly prior to traveling ensures seroconversion.

Method: Patients with rheumatoid arthritis (n = 69, median age = 55 years) treated with Tumor Necrosis Factor inhibitor(TNFi) and/or Methotrexate (MTX) were immunized with two doses of hepatitis A vaccine, either as double dose or four weeks apart, followed by a booster dose at six months. Furthermore, 48 healthy individuals, median age = 60 years were immunized with two doses, six months apart. Anti-hepatitis A antibodies were measured at 0, 1, 2, 6, 7 and 12 months.

Results: Two months after the initial vaccination, 84% of the RA patients had protective antibodies, compared to 85% of the healthy individuals. There was no significant difference between the two vaccine schedules. At twelve months, 99% of RA patients and 100% of healthy individuals had seroprotective antibodies.

Conclusion: An extra priming dos of hepatitis A vaccine prior to traveling offered an acceptable protection in individuals treated with TNFi and/or MTX. This constitutes an attractive pre-travel solution to this vulnerable group of patients.

1. Introduction

Rheumatic arthritis (RA) is a chronic inflammatory disease with severe impairment on physical activity and social life. The new biological drugs that have been introduced over the last decades have reduced symptoms of the disease and inhibited the progressive structural damage to the joints, resulting in improved physical activity and quality of life [1]. The biological drugs are effective by themselves but have been shown to have an enhanced effect when combined with other disease modifying anti-rheumatic drugs, particularly methotrexate (MTX) [1,2]. Before the introduction of biological drugs, patients suffering from RA were unlikely to undertake remote traveling due to the disability caused by the disease. With improved quality of life, they are more prone to travel to all parts of the world including areas with moderate or high risks of hepatitis A. A recent study by Schwartz et al. indicated that immunocompromised travelers visit the same risk areas during approximately the same length of time as non-immunocompromised travelers and are also represented among travelers visiting friends and relatives (VFR) [3]. Although hepatitis A vaccination is recommended to travelers going to high risk areas, less than fifty percent of frequent travelers reported previous hepatitis A vaccination in a recent study conducted in the five most populated countries in

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Europe and approximately half of the participants were not aware of the correct number of injections to complete their vaccination, indicating an increased susceptibility to infection [4]. The estimated incidence of a symptomatic hepatitis A infection in unprotected Scandinavian travelers varies between 2 and 40/100 000 person months depending on the destination. The highest risk is seen among VFR's [5,6]. Although there is no evidence for a worse outcome of a hepatitis A infection in immunocompromised individuals, there are reports on sustained viral shedding in individuals on immunosuppressive drugs, with the subsequent risk of prolonged outbreaks [7,8]. Furthermore, the risk of hospitalization and fatality is enhanced following a hepatitis A infection with increasing age [9–12]. Since a significant number of RA patients on immunosuppressive drugs are middle aged or older it is of vital importance to offer them proper protection against hepatitis A when traveling to endemic areas.

Hepatitis A vaccines have been proven to be effective to prevent disease in healthy adults even when given close to exposure [13–16]. In immunocompromised adults on the other hand, studies following the first dose have overall shown reduced seroconversion rates of 10–62% and low antibody concentrations [17–21]. After the booster dose at 6 months, acceptable seroconversion rates of 82–95% has been reported for groups of patients with various forms of immunosuppression [17,20–22], although in liver and kidney transplant recipients it is often considerably lower [18,19,23]. To the best of our knowledge only our previous study has reported on the effect of hepatitis A vaccination for a homogenous group of patients, i.e. RA patients on biological drugs or Methotrexate (MTX) alone or in combination. That study clearly illustrated a prolonged time to achieved seroprotection (≥20 mIU/mL) in all groups, regardless of treatment, from 10% to 33% one and six months after the first vaccine dose. With a lower definition of immunity (≥10 mIU/mL) seroconversion increased from 29% to 45% between one and six months after the initial vaccination, though patients on Tumor Necrosis Factor inhibitors (TNFi) only, seroconverted earlier (seroprotective antibody levels in 73% at one month and 60% at six months). Furthermore, it was shown that RA patients on immunosuppressive drugs had the ability to generate a booster response, since they demonstrated an increased seroconversion rate of 83% and higher geometric mean concentrations (GMC) of anti-HAV antibody following a second dose after 6 months. This indicated that an early extra hepatitis A vaccine dose might induce a more rapid seroconversion [21]. A three dose hepatitis A vaccination regime has been tried in three previous studies on HIV positive patients and showed significant higher GMCs after the full vaccination compared to two doses, but the early response was not assessed [24–26].

With the current recommendations of two doses of vaccine given 6 months apart, immunocompromised patients cannot rely on full protection against hepatitis A if undertaking a journey within a few months from initiation of vaccination. Instead, it has been suggested to use either intramuscular immunoglobulin (Ig) as prophylaxis or an extra priming dose of hepatitis A vaccine prior to traveling [21,27], although there has been no evidence of the latter alternative being effective until now.

1.1. Aim of the study

The aim of this study was to evaluate if an extra priming dose of hepatitis A vaccine given prior to traveling would ensure seroprotection in immunocompromised patients, at least comparable to that of intramuscular Ig in healthy historical individuals.

2. Materials and methods

2.1. Study population and design

This prospective open-label study was conducted in 4 different medical centres in Sweden. Adult patients (≥18 years) with RA who received regular treatment with MTX and/or immunomodulating biological drugs and who had an intention to travel abroad were enrolled. One patient on immunomodulating biological drugs was treated with an Interleukin 6 inhibitor (tocilzumab) and all others with TNFi (adalimumab, etanercept, infliximab, certolizumabpegol and golimumab), henceforth all abridged as TNFi. Patients were excluded from participating if treated with Rituximab within 9 months prior to enrolment or if they previously had had an infection with hepatitis A or had been vaccinated with a hepatitis A vaccine. Baseline (BL) data were collected from prior blood tests, including IgG levels and C-reactive protein (CRP), via a self-evaluation questionnaire concerning daily function (Health Assessment Questionnaire; HAQ, range 0–3) and from the patients' medical files. If conceivable, disease activity was described using the 28-joint Disease Activity Score (DAS, range 0–9) and CRP levels.

The patients of each of the three anti-RA treatment modalities (TNFi, TNFi + MTX and MTX) were all stratified into receiving intramuscular hepatitis A vaccine according to two different vaccine schedules. One group was given an initial simultaneous double dose, one injection in each arm, followed by a booster dose after 6 months (2 + 1 schedule). The other group was given two doses with one month apart followed by a booster dose at months 6 (1 + 1 + 1 schedule).

Additionally, two groups of healthy adults, divided by age (less than 40 years old or ≥ 50 years), were enrolled as reference. They were vaccinated with hepatitis A vaccine according to the standard 2 dose vaccination regime at 0 and 6months (1 + 1 schedule) (Fig. 1).

From all participants a signed written informed consent was obtained. The study was approved by the regional ethic committee of Stockholm, Sweden (2007/1126-31/3), and conducted according to the International Conference on Harmonization- Good Clinical Practice (ICH-GCP). The study was registered in the Clinical trials register (Clin.gov.trails NCT01446978).

2.2. Vaccines

Two different brands of inactivated hepatitis A vaccines were used, Havrix® (GlaxoSmithKline, Rixensart, Belgium) and Epaxal® (Crucell Switzerland AG, Bern, Switzerland). Havrix, 1 ml, contains 1440 enzyme-linked immunosorbent assay units inactivated hepatitis A virus and Epaxal®, 0.5 ml, contains at least 24 IE inactivated hepatitis A virus absorbed to a virosome. The two different vaccines are considered equally immunogenic and thus they were used according to the normal medical routine at each medical center [28].

2.3. Sample analysis

Serum samples were collected at the first day of vaccination and thereafter at months 1, 2, 6, 7 and 12. The serum samples at month 1 and 6 were drawn before additional vaccine doses were given. Total IgG and IgM antibodies against hepatitis A (anti-HAV) were determined with an enzyme-linked immunosorbent assay (ELISA) E10 of Mediagnost (Reutlingen, Germany) in all samples from our study subjects and all healthy individuals ≥ 50 years. Due to technical issues in the younger healthy subjects, the ELISA was only applied to samples from months 0, 1 and 2. The combined results of IgG and IgM were expected to be more sensitive and to give an earlier indication of vaccine response. The seroprotective cut-off for this analysis was per protocol anti-HAV ≥31 mIU/mL.

Additional analysis of all blood samples was done with the chemiluminescent immuno assay (CMIA) Architect system HAVAb-IgG from Abbott (Wiesbaden, Germany) at the Karolinska University Laboratory, Stockholm, Sweden, measuring only anti-HAV IgG. Anti-HAV titers were quantified against a standard curve based on simultaneously analysis of a set of standards (AxSym HAVAB 2.0 Quantative Standard Calibrators, Abbott) and titers ≥20 mIU/mL were considered seroprotective.

A high proportion of subjects had anti-HAV levels above 31 mIU/mL

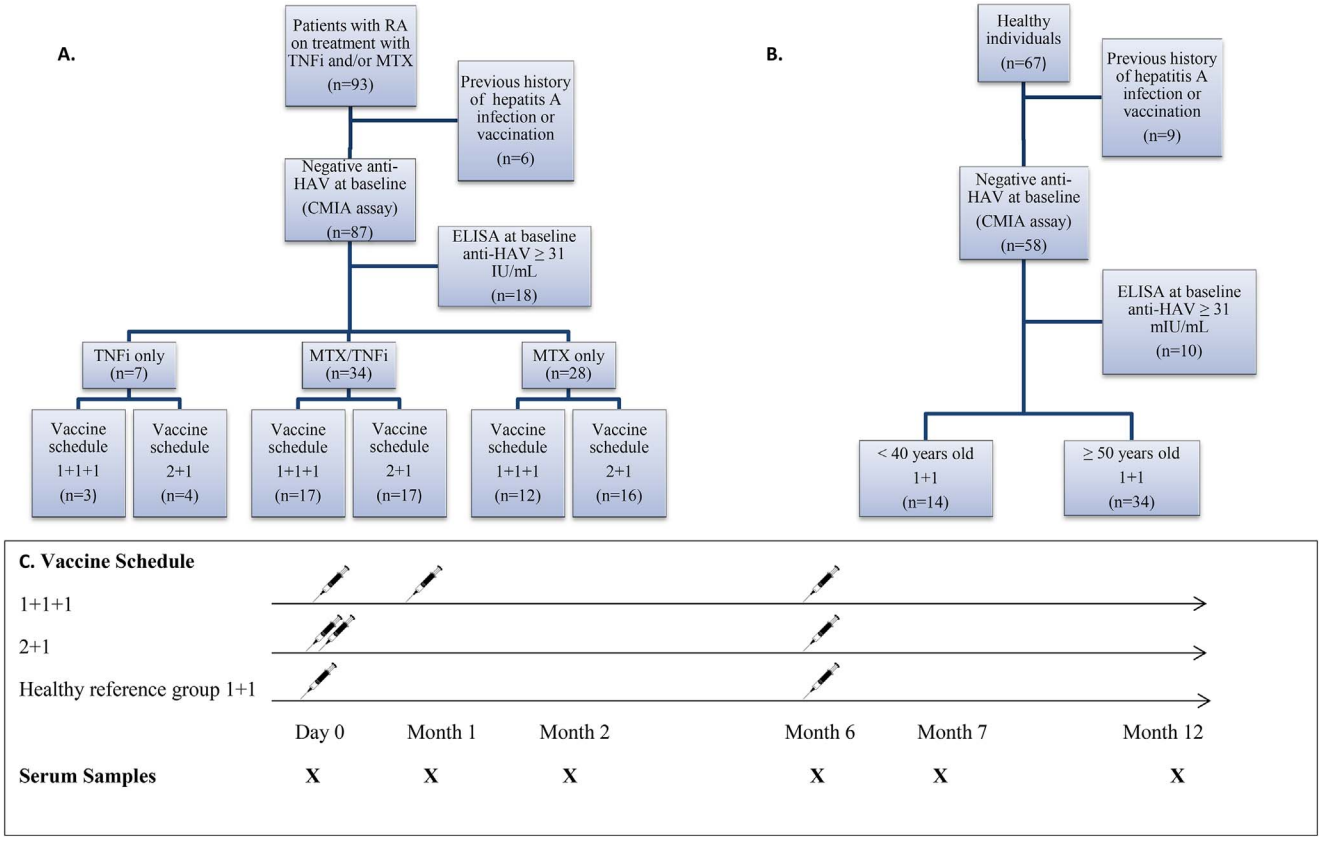

Fig. 1. A and B: Study schedule for patients with rheumatic disease and for a reference group of healthy individuals; C: Vaccination and serum sampling schedule.

at BL in the ELISA although they were negative in the CMIA and to our best knowledge had no previous immunity to hepatitis A. They were regarded as subjects with false positive BL results. Thus, we performed an additional qualitative analysis of the antibody response pattern to assess the time until seroconversion. This analysis was done on samples from all RA patients and was based on the combined results from the Abbott CMIA and the Mediagnost ELISA, using three different definitions for seroconversion. Samples from cases negative at BL in the CMIA assay were classified as seroconverted if the following anti-HAV antibody changes were found in the ELISA: (1) doubling of BL value, i.e. ≥ 100% rise of anti-HAV concentration from BL, and achievement of at least 31 mIU/mL, (2) achievement of 31 mIU/mL and an anti-HAV rise from BL of at least 30% (= rise greater than ELISA intratest variation) or (3) achievement of 20 mIU/mL and an anti-HAV rise from BL of at least 30%. For all cases an anti-HAV titer ≥20 mIU/mL in the CMIA assay was considered seroprotective.

2.4. Safety

At every visit, all subjects were asked by the research nurse to report any kind of adverse effect (AE) and any unscheduled contact with health care providers in between visits for vaccination or serum sampling. The reported AEs were noted in the case report form and any severe AEs were to be immediately reported to the Swedish Medical Product Agency and to the pharmaceutical companies.

2.5. Statistics

Confidence intervals of a proportion was calculated with VassarStat [29]. All other basic descriptive and analytic statistics, as well as GMCs with 95% CI, were calculated in SPSS, version 22. GMC calculations were done from the log10 transformed antibody values. In those samples with negative anti-HAV antibody results half of the lower limit of detection was used, but if value below the lower limit of detection was reported the actual value was used. Graphs were constructed in Excel. Differences in seroconversion rates were analyzed with a two-sided Pearson Chi square test or Fischer's Exact test. The level of statistical significance was set at $p < 0.05$.

3. Results

3.1. Demographics

We enrolled 93 patients with RA and 67 healthy individuals. Among those, 6 patients and 8 healthy individuals had anti-HAV ≥ 20mIU/ml at baseline in the CMIA assay. One healthy individual had an unreasonable rise in antibody titers one month after the first vaccine dose. All these 15 subjects were considered to have had a previous history of hepatitis A infection or previous vaccination and were thus excluded from further analysis (Fig. 1a and b). The final study population consisted of 87 patients with RA (74% women) with a median age of 50 (range 40–70) years and 58 healthy individuals (55% women) with a median age of 60 (range 19–73) years. Twenty of the healthy individuals were less than 40 years old. The majority of the RA patients were treated with a combination of TNFi and MTX (47%), while 43% were on MTX alone and 10% were treated with TNFi only. The DAS-28 score in combination with CRP indicated that all patients had a low disease activity at BL. The self-estimated HAQ score suggested that the study subjects consisted of a quite physically active cohort (Table 1). In 18 of the RA patients and 10 of the healthy individuals the BL blood samples were positive (32–50 mIU/mL) in the ELISA or missing. These individuals had to be excluded from the originally intended analysis of the ELISA results and could only be used in the qualitative analysis. Thus, the final results are based on 69 RA patients, 86% women with a median age of 55 (40–70) years, and 48 healthy individuals (56% women) with a median age of 52 (19–73) years. Their BL data did not

Table 1
Baseline characteristics of 87 hepatitis A naïve patients with rheumatic arthritis (RA) and 58 healthy individuals. Characteristics according to vaccine schedule and immunosuppressive treatment at baseline is presented in addition. TNFi = Tumor Necrosis Factor inhibitor. MTX = Methotrexate. All variables are in medians unless stated otherwise.

	RA patients	Immunosuppressive treatment			Vaccine schedule		Healthy individuals	Healthy individuals < 40 years	Healthy individuals ≥50 years
		TNFi	TNFi + MTX	MTX	1 + 1 + 1	2 + 1			
Number	87	9	41	37	42	45	58	20	38
Age (yrs)	55 (40–70)	53 (48–68)	55 (40–70)	55 (40–66)	56.5 (40–70)	53 (40–68)	60 (19–73)	21.5 (19–38)	63.5 (51–73)
Female (%)	74 (85%)	8 (89%)	33 (81%)	33 (89%)	36 (86%)	38 (84%)	32 (55%)	12 (60%)	20 (51%)
Duration of the disease (yrs)	12(1–43)	13.5 (6–30)	12 (1–43)	10 (2–42)	10 (1–43)	12 (2–42)			
Time span since last TNFi-infusion (days)	7 (0–46)	6 (0–19)	7(0–46)		7 (0–36)	5.5 (0–46)			
MTX weekly dose (mg)		15 (3.75–25)		15(3.75–25)	18.75 (7.5–25)	17.5 (3.75–25)	15(7.5–25)		
DAS-28 [a]	2.66 (0.77–5.4)	3.79 (2.47–5.4)	2.55 (0.77–4.81)	2.67 (1.08–5.04)	3.77 (1.08–5.4)	2.53 (0.77–4.82)			
HAQ [b]	0.5 (0–2.88)	0.38 (0.13–1.75)	0.5 (0–2.25)	0.38 (0–2.88)	0.5 (0–2.88)	0.5 (0–2.25)			
C-reactive protein (mg/l)	2.3 (1–42)	2 (2–4.7)	2 (1–42)	3.1 (1–17)	2.3 (1–27)	2.45 (1–42)			
Immunoglobulin G (g/l)	11.4 (7–18.5)	11.6 (9.3–17.9)	11.8 (7–18.5)	10.7 (8.1–14.7)	11 (7–18.5)	11.9 (8.1–17.9)			
Prednisone (no of patients) [c]	28	3	15	10	16	12			
NSAID (no of patients) [d]	8	1	5	2	4	4			
Salazopyrin (no of patients)	7	1		6	2	5			
Anti-malarial drugs (no of patients)	1			1		1			
Other immunomodulation/-suppressive drugs (no of patients)	2 [e]	1		1		2			

[a] 28-joints Disease Activity Score (DAS-28, 0–9).
[b] Health Assessment Questionnaire (HAQ, 0–3).
[c] Prednisone daily dose (median 5, range 1.88–7.5 mg).
[d] NSAID = Non Steroidal Anti Inflammatory Drugs.
[e] Leflunomid and civlosporin.

differ significantly from the original BL data in Table 1 (data not shown).

3.2. Vaccine

Forty two of the RA patients were vaccinated with the 1 + 1+1 schedule and 45 with the 2 + 1 schedule. All healthy individuals were vaccinated according to the regular 1 + 1 schedule (Fig. 1). Two of the centers used Havrix and vaccinated 28 patients. Due to local changes of routine one center first vaccinated 59 patients and 16 healthy individuals with Epaxal and the following 20 healthy individuals were vaccinated with Havrix. The final 22 healthy subjects were enrolled at the fourth center and vaccinated with Epaxal.

3.3. Immunogenicity

3.3.1. ELISA-assay

Based on the ELISA's pre-defined antibody level (≥31mIU/mL) seroprotection was achieved in 64% (44/69) of the RA patients at one month and in 84% (61/69) after two months (Fig. 2).

In the 2 + 1 group, who had already received two doses at their first visit, 68% (25/37) had seroconverted at month 1 compared to 60% (19/32) in the 1 + 1+1 group, who had only received one dose at that time. The corresponding results at one month for the healthy subjects, who had received one priming dose, were 94% (44/47) ($p < 0.005$ in relation to the 2 + 1 group and $p < 0.001$ in relation to the 1 + 1+1 group). On the other hand, at two months, when all RA patients had received two priming doses, seroconversion rate in the 1 + 1+1 group was 90.6% (29/32) and 86.5% (32/37) in the 2 + 1 group. When dividing the healthy controls into younger (< 40 years) and older (≥50 years) subjects, seroconversion rate was 93% (13/14) and 91% (31/34) at month 1 and 100% (14/14) and 79% (26/33) at month 2, respectively.

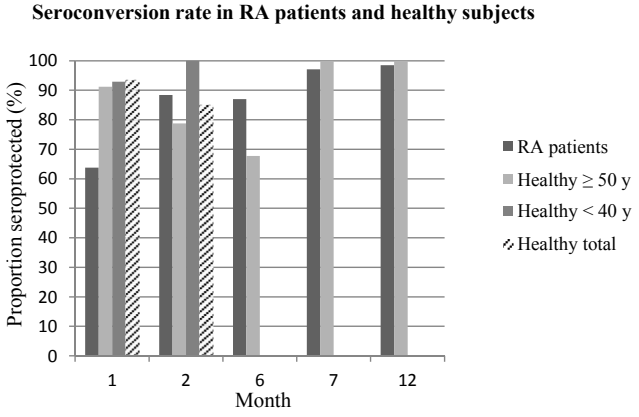

Fig. 2. Proportion of patients with rheumatic arthritis (RA) and healthy individuals with anti-HAV ≥ 31 mIU/ml (analysed with the E10 ELISA (Mediagnost, Reutlingen, Germany) at 1, 2, 6, 7 and 12 months after vaccination with hepatitis A vaccine. The healthy individuals were vaccinated with two doses (1 × 2 schedule) at 0 and 6 months and the RA patients were given one additional dose at time 0 (2 + 1schedule) or at month 1 (1 + 1 + 1 schedule). Results based on the originally intended analysis population (i.e. without false positive base line cases).

One month after the booster dose 99% (67/68) of the RA patients and 100% (33/33) of the controls ≥ 50 years had seroconverted, and at the 12 month follow up 99% of the RA patients and 100% of the older healthy controls still had protective antibody concentrations (Fig. 2).

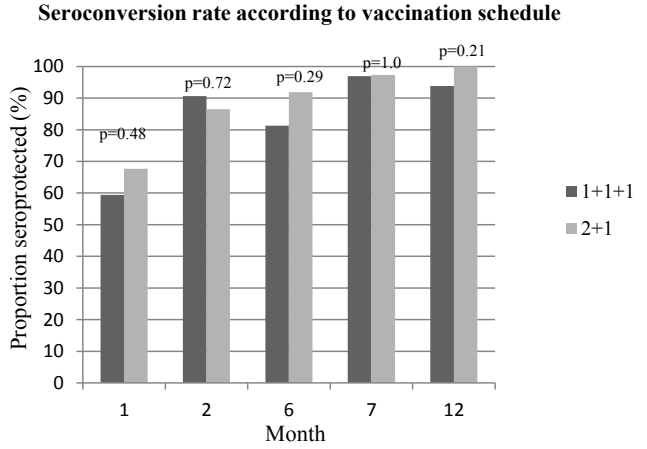

Fig. 3. Proportion of patients with rheumatic arthritis (RA) with anti-HAV ≥ 31 mIU/ml (analysed with the E10 ELISA (Mediagnost, Reutlingen, Germany) after vaccination with three doses of hepatitis A vaccine either with two primary doses followed by a booster dose at 6 months (2 + 1 schedule) or three separate doses given at time 0,1 month and 6 months (1 + 1 + 1 schedule). Results based on the originally intended analysis population (i.e. without false positive base line cases).

Overall, there was no significant difference in seroconversion rates in relation to the vaccine regime in the RA-group (Fig. 3) or in relation to the immunosupressive treatment at baseline.

Two patients with RA were lost to follow up at month 2 and 12 respectively. Additionally 2 blood samples from RA patients and 5 from the healthy individuals failed to be collected or were lost.

GMC's based on the ELISA analysis, with corresponding confidence intervals (CI), are displayed in Table 2.

3.3.2. CMIA-assay

The CMIA assay (with a pre-defined immunity level ≥20 mIU/mL) confirmed that all patients and healthy individuals enrolled in the analysis were hepatitis A naïve.

3.3.3. Qualitative analysis

The cumulative seroconversion rates in our study population based on our qualitative analyses are presented in Table 3. The overall agreement between the three different definitions varies between 86 and 99%.

3.4. Safety

The vaccination was well tolerated. Overall, 23% of the participants reported any side effect. Local pain or hematoma was reported by 6% and 17% reported general symptoms like muscle pain, sensation of fever, tiredness or headache. The reported side effects lasted for a maximum of a few days and were all well-known side effects of the vaccines used. No long lasting and no severe or serious adverse events were reported.

Table 3
Qualitative analysis. Cumulative seroconversion after hepatitis A vaccination in patients with RA based on three alternative interpretations of the hepatitis A antibodies measured by Mediagnost E10 ELISA. Seroconversion is considered if by definition (1) a doubling of BL value, i.e. ≥ 100% rise of anti-HAV concentration from BL and achievement of at least 31 mIU/mL, (2) an achievement of 31 mIU/mL and an anti-HAV rise from BL of at least 30% or (3) achievement of 20 mIU/mL and an anti-HAV rise from BL of at least 30%. For all cases an anti-HAV titer ≥20 mIU/ml in CMIA assay was considered seroprotective. Agreement is calculated based on the comparison of definition 1 and 3.

	Definition 1 (%)	Definition 2 (%)	Definition 3 (%)	Agreement (%)
Month 1	56.3 (45.9–66.3)	60.9 (50.4–70.5)	70.1 (59.8–78.7)	86.2 (77.4–91.9)
Month 2	80.5 (70.9–87.3)	90.8 (82.9–95.3)	96.6 (90.3–98.8)	83.9 (74.8–90.2)
Month 6	90.8 (82.9–95.3)	95.4 (88.8–98.2)	97.7 (92.0–99.4)	93.1 (85.8–96.8)
Month 7	96.4 (90.3–98.8)	97.7 (92.0–99.4)	98.8 (93.8–99.8)	97.7 (92.0–99.4)
Month 12	97.5 (92.0–99.4)	97.7 (92.0–99.4)	98.8 (93.8–99.8)	98.9 (93.8–99.8)

4. Discussion

In this study an extra priming dose of hepatitis A vaccine resulted in 88% seroprotection after two months in RA patients with drug-induced immunosuppression (TNFi and/or MTX). At month 12, half a year after the final booster dose, 99% seroprotection was seen. The results support the strategy to give pre-travel vaccinations, rather than immunoglobulin, to this group of patients.

4.1. Comparison with previous studies

Previous studies have shown that in patients with drug induced immunosuppression last minute vaccination against hepatitis A shortly prior to traveling is not reliable since a considerable number (36–92%) are unprotected one month after the initiation of vaccination [18–21]. However, after a booster dose seroconversion rates have improved in the majority of studies in immunocompromised patients [18–21]. The present study, with an extra primary vaccine dose given to this vulnerable group, shows a fairly good response rate already two months after the primary vaccination (88%).

When designing the study we expected a 1 + 1+1 schedule to generate higher GMC's due to a likely booster effect of a second primary dose on an already primed immune system. Such an early booster effect has previously been demonstrated in healthy individuals with for example combined hepatitis A and B vaccine [30] as well as in organ transplants with influenza vaccine [31,32] and hepatitis A vaccine in HIV positives [25]. The 2 + 1 schedule was added since many travelers present with short notice for pre-travel consultation and might not have time for two separate doses of vaccine. An increased antigen dose has proven to give higher GMC's for other vaccines, such as influenza vaccine [32–34]. Thus, we expected a double initial dose to give an increased early outcome while the 1 + 1+1 schedule was expected to be superior over time. However, no significant differences in

Table 2
Geometric Mean Concentrations (GMC) of anti-HAV. The results of the Mediagnost Anti-HAV E10 ELISA. The protective level of anti-HAV was defined as ≥ 31 mIU/mL. The lower limit of detection (LLD) was 5 mIU/mL. The results presented are based on patients with a negative result at baseline with the Architect system (CMIA assay). The results are given as GMC's of anti-HAV antibodies (mIU/ml) with 95% confidence intervals (CI) at month 0, 1, 2, 6, 7 and 12 based on Log10. GMC's was calculated using half of the LLD for negative sera, but if reported value below LLD, the actual value was used. NA = not available. Lost samples are excluded from the analysis.

Study group	Month 0	Month 1	Month 2	Month 6	Month 7	Month 12
RA patients	NA	48 (39–59)	84 (69–102)	94 (75–118)	432 (348–535)	217 (177–265)
Healthy individuals		106 (83–136)	80 (64–102)	72 (50–106)	920 (680–1245)	551 (386–787)
< 40 years		155 (118–204)	129 (98–171)	150 (91–246)	NA	NA
≥ 50 years		80 (62–103)	56 (45–71)	48 (30–75)	930 (859–1268)	447 (302–662)

seroconversion rate or GMC levels were observed between the two different vaccine schedules.

The high overall seroconversion rate of 99% following the completed vaccination in our RA patients is consistent or even higher than reported in previous studies in immunosuppressed individuals [18–21,24,25]. Although the RA patients in our study develop lower GMC than the healthy individuals, seroprotection seems to persist for at least six months following a full vaccination schedule. Few other studies have in fact demonstrated persistent antibody levels in immunocompromised individuals. One study in organ transplant recipients reported 59% and 26% seroprotection after two years in liver and kidney transplants, respectively [23] and in the only previous study on strictly RA patients, 86% of those who initially seroconverted still had anti-HAV ≥20 mIU/mL at 24 months [21]. In a third study by Cheng et al. HIV positive men were vaccinated with two or three doses of Hepatitis A vaccine. Five year follow up data showed persistent seroprotection in 88% and 94% respectively, and GMCs were higher in the group given three doses of vaccine [26]. Thus, for a last minute vaccination either a 2 + 1 or a 1 + 1 + 1 schedule seems feasible in this group of patients.

To the best of our knowledge no other study has assessed the effect of a three dose vaccine regime against hepatitis A in patients with drug induced immunosuppression. Though, three previous studies have evaluated the effect of an extra priming Hepatitis A vaccine dose given one month after the initial dose in HIV positive individuals [24–26]. In the study by Launay et al., the three dose vaccine schedule produced higher GMC's than the two dose regime at week 24 and had a higher seroprotection rate at week 8 compared to week 4, indicating booster ability with the extra vaccine dose [25]. In the study by Tseng et al., a three dose regime was compared with the standard two dose regime in HIV –positive and HIV-negative individuals. Although the response rate was improved by the three dose regime in HIV-positives, the GMC's in HIV-positive individuals were significantly lower than in the HIV-negative subjects for both dose regimes [24]. This is consistent with the results from our study after a full vaccine regime. Though, at two months, when all RA patients had received their extra priming vaccine dose, no significant difference was seen in GMC's between the RA patients and the healthy individuals (Table 2), supporting the fact that a three dose vaccine regime to patients with drug induced immunosuppression offers an early and acceptable protection against hepatitis A.

In the study by Askling et al. only 10% of RA patients on MTX and/or TNFi had seroconverted (cut of ≥20 mIU/ml) one month after a single dose of hepatitis A vaccine [21], compared to 59% in the 1 + 1 + 1 group in our study. One possible explanation for this might be that different methods were used to analyze IgG and IgM anti-HAV in the two studies, Microparticle EIA and ELISA respectively. Additionally, in our study we had a higher proportion of women and the median age was slightly lower. Since female gender and low age is considered to have a positive influence on the immune response following vaccination [35,36], this might partly explain our results.

Due to the high median age in the RA patients and the older healthy controls in our study, the impact of age needs to be further considered. In a study by D'Acremont et al., 100% of participants less than 30 years of age had seroconverted one month after a single dose of hepatitis A vaccine compared to 70% of subjects between 50 and 60 years and 60% in those older than 60 years of age [35]. Although our older healthy controls exhibit a higher seroconversion rate (91%) after the first vaccine dose than reported by D'Acremont, there was an initial trend of lower antibody concentrations than in the younger healthy individuals. One can assume that the lower GMC's in the RA patients is partly an age-related effect, but it cannot be the only explanation since there is a clear difference between RA patients and older healthy individuals from month 7 and onwards (Table 2).

It has previously been recommended to use immunoglobulin (Ig) as prophylaxis for certain immunosuppressed patients, when the effect of the vaccine is thought to be insufficient [20,21]. [37] The protective efficacy of Ig is estimated to be 80–90% initially and declining over time [37–40]. Depending on the preparation and the given dose, protection can be expected to last four to six months [37]. When comparing a single dose of Ig 0,06 ml/kg with hepatitis A vaccine (approximately half the amount of antigen as standard today) in healthy adults, Shouval et al. showed 100% seroprotection one week after the Ig administration, 92% at four weeks, but none of the Ig recipients displayed seroprotective antibodies after week 20. The vaccine group on the other hand had a 100% seroconversion rate the earliest at week 5, but after 6 months all still had seroprotective antibodies and after receiving a booster dose the GMC peaked [41]. Thus, in healthy individuals, after approximately one month, vaccine and Ig offers similar protection. In reality Ig is not always available due to production difficulties, and in some countries it has been abandoned as recommended pre-exposure prophylaxis due to the risk of transmission of infectious diseases [37]. Thus, vaccine is the only alternative in many situations and for longer or repeated journeys vaccine would in any case be the preferable choice. Although we have not compared the vaccine effect directly with Ig, the high seroprotection rate in our study indicate that a three dose vaccine regime will offer at least an equal protection as Ig against hepatitis A after two months and after the third dose, i.e. the booster dose, an even higher protection that will last longer.

4.2. Methodological considerations

In this study we have measured anti-HAV with two different assays, the CMIA Architect system from Abbott and the ELISA E10 of Mediagnost. The Architect system only measures IgG and is therefore not appropriate to measure an early antibody response post vaccination [21]. Thus, we analyzed our sera mainly with the Mediagnost ELISA that measures both IgG and the early IgM response and, therefore, is more sensitive to determine the early immunological response. Although the standard anti-HAV ELISA assays measuring total anti-HAV (IgG + IgM) have in healthy individuals generally shown high precision and specificity (C Herzog, personal communication), we unfortunately had an increased background activity in our results with the ELISA E10, causing a fifth of BL test results to be above the seroprotective level of 31 mIU/ml. Similarly, high background activity has been reported in autoimmune disease patients with other laboratory tests. We do, however, strongly believe these individuals to be truly HAV naïve based on the results of the CMIA assay, on the careful pre inclusion interviews and on the antibody reaction after vaccination. Nevertheless, we decided to exclude the subjects with false positive BL values from the primary analysis, even though we considered the ELISA to have reliably measured the ensuing vaccine-induced immune response. In the intention to include all RA patients in the analysis we moreover looked at the data from a more qualitative perspective and defined three alternative seroconversion algorithms based on the course of the anti-HAV response (Table 3). When considering the most conservative of these definitions (definition 1) the result support the encouraging outcome from the primary analysis, i.e. that at least 80% of RA patients with drug-induce immunosuppression have developed seroprotective anti-HAV levels after two months, and with the less strict definition 3 more than 96% seroconverted early.

4.3. Limitations

One limitation of this study was the lack of a blinded control group of RA patients on immunosuppressive drugs given the standard two dose vaccine regime. However, we believed that this would have been unethical since a prior study had clearly shown that a single vaccine dose gave a very low primary response rate and, in addition, several of the participants had the intention of traveling within the timespan of the study. Instead, we included a healthy control group receiving the standard two dose schedule, representing for comparison the expected

response rate in a healthy population of similar age. We chose to divide the healthy controls into two age groups, with the older group corresponding age-wise to our RA patients. The younger controls on the other hand were mainly to document the immunogenicity of the hepatitis A vaccines in young adults, thus verifying our methods.

Another limitation of the study was the fact that we had to use two different methods to determine anti-HAV levels. The more sensitive assay, the ELISA, had some undefined background activity at BL that forced us to exclude 18 RA patients and 10 healthy individuals in the final analysis. The CMIA was not sensitive enough with even a large proportion of healthy young adults being still seronegative one month after vaccination which is not consistent with the general experience in previous studies [27,28,35]. The background activity in the ELISA was seen in samples from both RA patients and healthy individuals, so it could not solely be a result of the rheumatic disease, as discussed above. In the end, the study lost power and it was not possible to determine whether an initial double dose or two separate doses was the superior regimen. Neither was the number of participants enough to conclude if any BL immunosuppressive treatment modality was more likely to result in a lower seroconversion rate than the others.

4.4. Strengths

This study is the first study of a three dose hepatitis A vaccine regime in patients with drug-induced immunosuppression, and is a first important indicator of how to deal with this growing and vulnerable group of patients, especially when they present for consultation at short notice prior to a journey. Furthermore, the diverse results of the two different anti-HAV assays used in this study, point to the importance of measuring the early antibody response, IgM, as well as IgG, when determining the early immunological response of a vaccine.

5. Conclusions

A vaccine regimen against hepatitis A with two primary doses provides seroprotection equally to intramuscular immunoglobulin's in patients with drug-induced immunosuppression after approximately two months. If traveling is conducted within less pre-travel time it is reasonable to recommend intramuscular immunoglobulin's to ensure protection. After the third dose at six months a prolonged protection is to be expected, though this must be confirmed with follow up data. Furthermore, a larger study determining whether a 2 + 1 or a 1 + 1 + 1 schedule is superior would be desirable as well as additional studies on the three dose vaccine schedule in other groups of immunocompromised patients.

Authors' contribution

HHA and LR conceived and designed the study. AR, HHA and LR where involved in data acquisition. AR drafted the article. CH, AR and HHA was responsible for analysis and interpretation of data. GF has advised on methodical issues and interpretation. HHA, TN, CH and LR revised the article critically. All authors approved the version to be submitted.

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Conflicts of interest

AR, LR, GF, TN and HHA declare no conflicts of interest.

CH: worked until 2011 as clinical research scientist for Crucell Switzerland AG (formerly Berna-Biotech), he declares no conflicts of interest.

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