Vaccine 34 (2016) 1247–1251

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Comparison of the PRNT and an immune fluorescence assay in yellow fever vaccinees receiving immunosuppressive medication

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ARTICLE INFO

Article history:
Received 17 August 2015
Received in revised form 8 January 2016
Accepted 18 January 2016
Available online 1 February 2016

Keywords:
Yellow fever
Vaccination
Immune-compromised
Indirect Fluorescence Assay (IFA)
Plaque Reduction Neutralization Test (PRNT)

ABSTRACT

Background: The 17D-yellow fever (YF) vaccination is considered contraindicated in immune-compromised patients; however, accidental vaccination occurs. In this population, measuring the immune response is useful in clinical practice.
Methods: In this study we compare two antibody tests (the Immune Fluorescence Assay and the Plaque Reduction Neutralization Test) in a group of Dutch immune-compromised travellers with a median of 33 days (IQR [28–49]) after primary YF vaccination.
Results: We collected samples of 15 immune-compromised vaccinees vaccinated with the 17D yellow fever vaccine between 2004 and 2012. All samples measured in the plaque reduction neutralization test yielded positive results (>80% virus neutralization with a 1:10 serum dilution). Immune Fluorescence Assay sensitivity was 28% (95% CI [0.12–0.49]). No adverse events were reported.
Conclusions: All immune-compromised patients mounted an adequate response with protective levels of virus neutralizing antibodies to the 17-D YF vaccine. No adverse effects were reported. Compared to the plaque reduction neutralization test, the sensitivity of the Immune Fluorescence Assay test was low. Further research is needed to ascertain that 17D vaccination in immune-compromised patients is safe.

1. Background

1.1. Yellow fever virus and vaccine

Yellow fever (YF) is a potentially lethal viral disease caused by an RNA virus belonging to the *Flaviviridae*. In 1937, the live attenuated 17D-YF vaccine was developed. The vaccine has been proven safe and very effective: in nearly all studies, virus neutralizing antibodies develop in more than 90% of vaccinees; and antibodies appear to persist for several decades [1–3], if not lifelong [4]. Currently, international guidelines recommend vaccination against YF from nine months of age, for people traveling to or living in YF-endemic areas. After YF-vaccination, vaccinees have reported mild and transient adverse events, including fever, headache and local pain [5,6].

Serious adverse events (SAEs) rarely occur in healthy vaccinees. Around 0.3–0.4/100,000 vaccinees develop yellow fever vaccine associated viscerotropic disease (YEL-AVD), which resembles the clinical course of wild-type YF infection. An estimated 0.4–0.8/100,000 develop yellow fever vaccine-associated neurotropic disease (YEL-AND), which presents with various clinical neurological symptoms, such as Guillain Barré Syndrome and encephalitis. Anaphylactic reactions have been estimated to occur in 0.8–1.8 per 100,000 vaccinations [7–9].

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http://dx.doi.org/10.1016/j.vaccine.2016.01.037
0264-410X/

1.2. Immune-compromised individuals and the 17D-YF vaccine

In immune-compromised patients, there may be an increased risk of serious adverse events (SAEs) such as YEL-AVD and YEL-AND [10,11]. To date, the exact pathophysiological mechanism of these SAEs has not been elucidated. Possibly, they are the result of an impaired immunologic response in the host, resulting in increased viral replication. In addition to the risk of SAEs, protection against YF after vaccination may be inadequate. For example, in HIV positive patients with low CD4 counts and high viral loads, virus-neutralizing antibodies (VNAs) were less persistent and geometric mean titers (GMTs) were lower [12–14].

YF vaccination in immune-compromised patients is contraindicated in existing guidelines. Consequently, to date, no studies exist that investigate early antibody responses among patients using immunosuppressive medication. Occasionally, immune-compromised patients receive YF-vaccination accidentally, e.g. because certain immune-suppressants are not recognized as such, or because of incomplete history taking.

Two small studies have described that adverse events did not occur more frequently in this patient group compared to healthy vaccines. However, with 70 and 19 patients included, these studies were not sufficiently powered to identify rare serious adverse events. Also, it is not clear whether or not these patients were protected by neutralizing antibodies from earlier YF vaccinations [15,16].

1.3. Available tests and cross reactivity

Various serologic tests are available to measure the presence and amount of VNAs in vaccinees. Currently, the Plaque Reduction Neutralization Test (PRNT) is considered as gold standard [17,18]. More commonly, however, the Indirect Immune-fluorescence Assay (IFA, EuroImmun (Lübeck, Germany)) is used, because of lower costs and processing time and because the PRNT was not routinely available for clinical use.

The IFA test for IgG antibodies has been found to be highly sensitive, but not specific compared to the PRNT in an early study by Monath et al. [18]. An explanation for lower specificity is that cross-reactions between various *flaviviridae* can occur with IFA, resulting in more false positive results [19,20]. According to a more recent study that comprised 150 serum samples, the IgG IFA using EuroImmun Biochip technology had a high sensitivity and specificity (both 95%) compared to the PRNT as gold standard [21].

1.4. Study objective

In the past years, we occasionally encountered immune-compromised travellers from various hospitals across the country (including our own) who had been accidentally vaccinated against YF despite having an absolute contra-indication. In addition, several patients received YF-vaccinations in our clinic not by accident, but because they had planned to travel to highly endemic areas, despite medical advice not to do so. In these situations, an individual decision whether or not to vaccinate was made, weighing the risk of SAEs (depending on the time interval, dose and type of medication used) against that of acquiring yellow fever (based on traveler vaccination guidelines and previous risk estimates [22,23]). We therefore had the unique opportunity to study the immune response in this group of patients. To gain insight into the immunologic response in this population, we tested the hypothesis that patients using immunosuppressive medication would have a sub-optimal immunologic response to the 17D-YF vaccine.

2. Methods

2.1. Ethics

The protocol and consent forms for this study were approved by the Medical Ethics Committee of the Academic Medical Center (MEC AMC).

2.2. Recruitment of samples

We included all available post vaccination samples from travellers using immunosuppressive medication who had been vaccinated for the first time with a 17-D-204 YF vaccine (Stamaril or Arilvax) between 2004 and 2012. We collected demographic data (age, sex) and clinical data (medical history, time interval, type and dose of medication, previous vaccinations, adverse events, days between last vaccination dose and sampling). Patients who had stopped the immunosuppressive medication ≥three months prior to vaccination were excluded. Additionally, we analyzed stored sera of healthy vaccinees who had been vaccinated subcutaneously between 2005 and 2007 with a comparable time interval between vaccination and blood sampling. These sera were stored at −20 °C from sampling until determination of the NAb titer.

2.3. Adverse events

Adverse events were self-reported. A physician was available 24/7 in case of adverse events following vaccination.

3. Serology

3.1. Immune Fluorescence Assay (IFA)

Serum samples taken approximately one month or longer after YF 17-D vaccination were sent to the Department of Virology at the Erasmus Medical Center Rotterdam, the Netherlands, to measure yellow fever IgG responses by IFA using the EuroImmun assay. Sera were diluted in 1:10, 1:32, 1:100, 1:320, and 1:1000 and incubated on a biochip with YFV infected cells. Biochips are glass slides with YFV-infected cells cut into millimeter-sized fragments, after fixation and gamma-irradiation [21]. Incubation was performed using the Titerplane technique, allowing all Biochips to come into contact with the reagents simultaneously [21]. Sera which reacted in the YFV IgG IFA with a titer of 1:00 were also analyzed for other flavivirus-specific antibodies as controls (tick-borne encephalitis virus (TBEV) strain K32, West Nile virus (WNV) strain NY, and a Japanese encephalitis virus (JEV) strain). In the second reaction step, after 30 min of incubation at 20 °C, the slides were washed with washing buffer before the incubation with the fluorescein isothiocyanate-conjugated anti-human IgG binds to the human antibody. Finally, after 30 min, the slides were washed again and covered by a cover slide before being analyzed using a fluorescence microscope at a wavelength of 488 nm. Titers of >1:100 for IgG were considered positive.

3.2. Plaque Reduction Neutralization Test (PRNT)

PRNTs were performed by the Department of Infectious Diseases at the Leiden University Medical Center (LUMC), where the test is routinely performed. The technique described by De Madrid and Porterfield (1969) was used, modified for the LUMC PRNT test setup [24]. Vero cells were seeded in six-well plates (Corning Inc., USA) and cultured to obtain a monolayer. Heat-inactivated post vaccination sera were tested in two-fold dilutions up to 1:8192, all assayed in duplicate. One hundred Plaque Forming Units (PFUs) of 17D-YF were added to each serum dilution. After one-hour incubation on