Duration of immunity in volunteers eight years after a yellow fever vaccine dose-response study

Reinaldo de Menezes Martins, Maria de Lourdes S. Maia, Sheila Maria Barbosa de Lima, Tatiana Guimarães de Noronha, Janaina Reis Xavier, Luiz Antonio Bastos Camacho, Elizabeth Maciel de Albuquerque, Roberto Henrique Guedes Farias, Thalita da Matta de Castro, Akira Homma and Collaborative Group for Studies on Duration of Immunity of Yellow Fever Vaccine
Introduction
Global shortage of YF vaccine

- Unpredictable outbreaks increase suddenly the demand for YF vaccine, and production targeting routine vaccination may not meet the needs for mass campaign vaccination. Currently, there are only four WHO prequalified YF vaccine producers, of which only two are big producers. In addition, YF vaccine is produced by laborious traditional methods, is very cheap, so it is not attractive to new producers. Alternative vaccines, employing more modern technologies, have not yet been developed, although actively searched
Remembering a pioneer study, 1940-41

- Minimum immunizing dose (500 mid)
- Immunogenicity of different substrains and choose of seed-lot (17D – NY104)
- Dose and immune response (dose-response)
- Routes of delivery and immune response (IM, SC, ID)
- Age and immune response
First dose-response study with 17DD vaccine (Lopes et al, 1988)

• In response to the need to increase production by diluting the vaccine, in 1988 Bio-Manguinhos did a YF 17DD vaccine dose-response study in adults, with very high seroconversion rates with doses from 2000 PFU (plaque forming units) to 200 PFU, and lower seroconversions below this dose. However, the small number of participants on the study arms precluded the adoption of smaller doses of yellow fever vaccine
Second dose-response study (Martins et al, 2013, N = 900, and 749 were AP)

• Following the YF vaccine shortage in the 2008 epidemic in Brazil, Bio-Manguinhos in 2009 did a randomized dose-response study with the YF 17DD vaccine administered in the usual mean dose of 27,476 IU (full dose, reference), and in decreasing doses: 10,447 IU, 3013 IU, 587 IU, 158 IU and 31 IU, by the usual subcutaneous route and volume (0.5mL). The decreasing doses were obtained by dilution in the laboratory of the producer and the lots in test had industrial quality
Doses down to 587 IU had similar immunogenicity to the full dose, whereas the lowest doses - 158 IU and 31 IU - were inferior. Moreover, seropositivity of volunteers who had seroconverted and had not been revaccinated was maintained for at least 10 months, except on 31 IU dose group.

Thirty days after vaccination, for groups ≥ 587 IU/dose, 4.2% of the initial cohort of groups ≥587 UI/dose were revaccinated for being seronegative at 30 days or 10 months. For the 158 IU/dose and 31 IU/dose group, 13.5% and 43.9%, respectively, were revaccinated for this reason.
Complementary study Campi-Azevedo et al, 2014

• A complementary study on a subset of participants of the previous study, evaluated the cellular immune response to the YF vaccine and concluded that doses ≥3,013 IU had immunological responses equivalent to the standard mean dose of 27,476 IU
Current study
Objective

• To evaluate the duration of immunity 8 years after administration of reduced doses of the YF 17DD vaccine in the dose-response study of 2009, by measuring the level of neutralizing antibodies, and so to give support to the use of fractionate doses
Methods (1)

• This is a cohort study, in young healthy male adults, military recruits, who received the YF 17DD vaccine during the dose-response study in 2009. The target group comprised participants who were seronegative before vaccination on the dose-response study in 2009 and who were not revaccinated. Participants seronegative to YF at 30 days and at 10 months after vaccination were revaccinated with the standard dose and were not included in the current study. Those who went in military missions or travelled or lived in endemic areas were analyzed separately.
Methods (2)

• Participants were contacted by phone calls or in home visits, and blood was collected at Fiocruz, or if necessary at home or in a safe place, after applying the Consent Form.

• Questioning about revaccination was done at least two times: by the initial phone call and personal interview before blood collection. Participants were also asked about confirmation of participation on the dose-response study in 2009, about travels in military missions, or travel to endemic areas.

• The study was held from March 2017, to September 2017, about 8 years after the dose-response study.
Laboratory methods

• Were the same used on the primary study: neutralization by the same Laboratory (Latev) and same cut-off (>2.7 mUI/mL)
Ethics approval and GCP

• The study protocol and final report was approved by the Research Ethics Committee of the National Institute of Infectology Evandro Chagas, and by an Independent Data Safety Monitoring Committee. It followed all requirements of the Helsinki Declaration, the Brazilian Research Ethics Codes and of Good Clinical Practices from the America`s Document and of the International Conference on Harmonization. Identification and promotion of vaccination of seronegatives to YF 8 years after vaccination was a clear benefit for participants.
Results
Study inclusion steps

Dose-response study in 2009
N = 900

Possible participants
N = 786

Included but not eligible: N = 4
Not eligible: N = 99
Refusal: N = 82
No longer lives in Rio de Janeiro: N = 35
Died: N = 8
Impossible to localize: N = 122
Areas of urban conflict: N = 66

Included: blood sample collected
N = 370

Susceptible to YF before YFV
N = 319

Susceptible result, by laboratory recommendation: N = 1

27,476 IU Available: N = 68
10,447 IU Available: N = 51
3013 IU Available: N = 67
587 IU Available: N = 59
158 IU Available: N = 50
31 IU Available: N = 23

Revaccinated: N = 89
Unprotected at 10 months: N = 18
Unprotected at 30 days and no serology at 10 months: N = 7
### GMTs at sequential times

<table>
<thead>
<tr>
<th>GROUP</th>
<th>N (T = 318)</th>
<th>Before vaccination</th>
<th>At 30 days</th>
<th>At 10 months</th>
<th>At 8 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>27,476 IU</td>
<td>68</td>
<td>154.4</td>
<td>16,472.3</td>
<td>4,627.6</td>
<td>1,682.0</td>
</tr>
<tr>
<td>10,447 IU</td>
<td>51</td>
<td>150.0</td>
<td>13,346.1</td>
<td>4,388.9</td>
<td>2,166.7</td>
</tr>
<tr>
<td>3013 IU</td>
<td>67</td>
<td>132.3</td>
<td>13,609.4</td>
<td>4,670.8</td>
<td>1,602.7</td>
</tr>
<tr>
<td>587 IU</td>
<td>59</td>
<td>146.7</td>
<td>13,209.2</td>
<td>5,052.9</td>
<td>2,394.4</td>
</tr>
<tr>
<td>158 IU</td>
<td>50</td>
<td>139.7</td>
<td>13,171.0</td>
<td>4,955.8</td>
<td>1,651.2</td>
</tr>
<tr>
<td>31 IU</td>
<td>23</td>
<td>119.3</td>
<td>15,897.6</td>
<td>4,872.7</td>
<td>4,086.6</td>
</tr>
<tr>
<td>p (for differences among groups)</td>
<td>0.639</td>
<td>0.570</td>
<td>0.940</td>
<td><strong>0.025</strong></td>
<td></td>
</tr>
</tbody>
</table>
Proportion of seropositivity of participants on the dose-response study 8 years after vaccination, by vaccine group

<table>
<thead>
<tr>
<th>Group</th>
<th>Seropositive participants (neutralizing antibodies &gt;2.7 log10 mUI/mL)</th>
<th>Total tested</th>
<th>p-value (comparisons to the reference vaccine)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td>27,476 IU</td>
<td>56</td>
<td>82.4</td>
<td>71.2; 90.5</td>
</tr>
<tr>
<td>10,447 IU</td>
<td>44</td>
<td>86.3</td>
<td>73.7; 94.3</td>
</tr>
<tr>
<td>3013 IU</td>
<td>54</td>
<td>80.6</td>
<td>69.1; 89.2</td>
</tr>
<tr>
<td>587 IU</td>
<td>55</td>
<td>93.2</td>
<td>83.5; 98.1</td>
</tr>
<tr>
<td>158 IU</td>
<td>40</td>
<td>80.0</td>
<td>66.3; 90.0</td>
</tr>
<tr>
<td>31 IU</td>
<td>22</td>
<td>95.7</td>
<td>78.1; 99.9</td>
</tr>
<tr>
<td>Total</td>
<td>271</td>
<td>85.2</td>
<td>80.8; 88.9</td>
</tr>
</tbody>
</table>

P = 0.159 (All groups)
Geometric mean antibody titers (GMT in mIU per mL), ratios between GMT from lower doses and the full dose, and corresponding 95% C.I., by vaccine group (cut-off 501 mUI/mL)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>GMT</th>
<th>95% CI</th>
<th>Ratio to reference vaccine</th>
<th>95% CI of ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>27,476 IU</td>
<td>68</td>
<td>1682.0</td>
<td>1235.6; 2289.7</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>10,447 IU</td>
<td>51</td>
<td>2166.7</td>
<td>1457.4; 3221.1</td>
<td>1.29</td>
<td>0.79; 2.10</td>
</tr>
<tr>
<td>3013 IU</td>
<td>67</td>
<td>1602.7</td>
<td>1173.2; 2189.4</td>
<td>0.95</td>
<td>0.62; 1.47</td>
</tr>
<tr>
<td>587 IU</td>
<td>59</td>
<td>2394.4</td>
<td>1758.2; 3260.8</td>
<td>1.42</td>
<td>0.92; 2.20</td>
</tr>
<tr>
<td>158 IU</td>
<td>50</td>
<td>1651.2</td>
<td>1144.6; 2382.1</td>
<td>0.98</td>
<td>0.61; 1.57</td>
</tr>
<tr>
<td>31 IU</td>
<td>23</td>
<td>4086.6</td>
<td>2605.2; 6410.5</td>
<td>2.43</td>
<td>1.35; 4.36</td>
</tr>
<tr>
<td>Total</td>
<td>318</td>
<td>1949.8</td>
<td>1698.2; 2290.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P = 0.025
Scatter plot of neutralizing antibody titers by group, 8 years after vaccination
Further analyses
Missions, travels

- At 8 years after vaccination, the seropositivity rate of those who joined military mission and those who did not were similar ($p = 0.648$) and the difference of geometric mean titers of neutralizing antibodies was not significant ($p = 0.466$). The same was true for those who had travelled or lived in endemic areas: for rates of seropositivity ($p=0.098$), and for geometric mean titers ($p = 0.631$)
• A group of 70 volunteers (22%) had titers of neutralizing antibodies higher in 2017 than in 2009. The variability within PRNT tests is well known, and recent validation of the test at our viral technology laboratory demonstrated that variations until three-fold could be expected in intra- and inter-assays. Therefore, increases above this range may mean exposure to the virus or revaccination. For this reason, the laboratory recommended to exclude one result, reducing the total from 319 to 318.
• Assuming the group with antibody titers higher in 2017 than in 2009 included individuals revaccinated or exposed to natural infection, data analysis was conducted disregarding their results. The total seropositivity of those who remained was 81% (200/247) and differences among groups were of magnitude similar to the whole cohort, and not statistically significant ($p = 0.235$). On the same line, the analysis of variance of antibody neutralizing titers among groups had no statistical significance ($p = 0.171$), although the 31 IU group had still the highest level of antibodies.
• There was no statistically significant association between participants who went into missions and those with higher level of antibodies in 2017 than in 2009 (p=0.484). The same was true for those who went on travels or lived in endemic areas (p=0.118)
Inclusion of these groups is justified

• Disregarding from analysis all these groups (travel in military mission after the study of 2009, municipality to which the participant traveled or lived had recommendation for yellow fever vaccination, and GMT higher in 2017 than in 2009), there remained 156 participants. Results of analysis of seropositivity and GMTs comparing groups are similar and coherent with what has already been presented. So, the inclusion of all these groups on according to protocol analysis is justified
Discussion
Comparison of studies on duration of immunity

- A major difficulty for evaluation of duration of immunity is the diversity of methodologies used for evaluation of seroprotection or seropositivity: intracerebral inoculation in weaned mice, neutralization test in suckling mice, protection test in mice, LNI - Log neutralization index - and plaque reduction neutralization test - PRNT. PRNT may have different endpoints (% reduction of plaques), 90, 80, 75 or 50. The cut-offs for seropositivity in general are 1/10, but 1/20, 1/50 or 2.9 mIU/mL were also used [Gotuzzo et al, 2013]. Only 1 study investigated the contribution of cellular immune responses to duration of immunity [Campi-Azevedo et al, 2016]
Seropositivity and level of neutralizing antibodies according to time after yellow fever vaccination in 2 studies employing identical laboratorial methods

<table>
<thead>
<tr>
<th>Interval since vaccination, Collaborative study</th>
<th>N</th>
<th>SP %</th>
<th>95% CI</th>
<th>Mean Log10 mUI/mL</th>
<th>95% CI Log10</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-45 days</td>
<td>165</td>
<td>95.2</td>
<td>90.7; 97.9</td>
<td>3.94</td>
<td>3.85; 4.04</td>
</tr>
<tr>
<td>1-4 years</td>
<td>114</td>
<td>99.1</td>
<td>95.2; 100.0</td>
<td>3.48</td>
<td>3.40; 3.56</td>
</tr>
<tr>
<td>5-9 years</td>
<td>83</td>
<td>88.0</td>
<td>79.0; 94.1</td>
<td>3.34</td>
<td>3.23; 3.45</td>
</tr>
<tr>
<td>10-11 years</td>
<td>138</td>
<td>86.2</td>
<td>79.3; 91.5</td>
<td>3.23</td>
<td>3.15; 3.30</td>
</tr>
<tr>
<td>≥12 years</td>
<td>190</td>
<td>90.0</td>
<td>84.8; 93.9</td>
<td>3.32</td>
<td>3.23; 3.40</td>
</tr>
<tr>
<td>(B) 8 years after vaccination, current study</td>
<td>318</td>
<td>85.2</td>
<td>80.8; 88.9</td>
<td>3.29</td>
<td>3.23; 3.36</td>
</tr>
</tbody>
</table>
Attention!

- It should be clear that, although all groups had similar seropositivity 8 years after vaccination, the 158 IU/dose and the 31 IU/dose groups are still inferior, because they had more primary vaccination failures, especially the lowest dose group. But, for those who seroconverted, and remained seropositive 10 months later, whichever the dose, about 85% of them remained seropositive, and ranging from 80% to 95% across groups of vaccine dose.
Selection bias?

• For groups with doses ≥587 IU, only 4.4% of the participants on the 2009 study were revaccinated due to seronegativity at 30 days or 10 months after vaccination, so the remaining participants included on the current study are not strongly biased towards higher seroconverters. Importantly, sustained seropositivity of reduced doses was similar to the reference vaccine.

• However, for the 31 IU group the included participants could have been selected for high responders, introducing bias. The 158 IU group was closer to the groups with higher doses.
Conclusions (1)

• At least 80% of the subjects who had seroconverted after yellow fever vaccination with doses from 27,476 IU down to 31 IU showed seropositivity comparable to that of the full dose after 8 years. Consistently, the levels of antibody titers in the reduced-dose groups were also comparable to those of the full-dose group. However, the lowest doses (158 IU and 31 IU) had more primary seroconversion failures, so they should continue to be considered inferior to the full dose vaccine.
Conclusions (2)

• Groups of decreasing doses from the dose-response study of 2009 have rates of seropositivity to yellow fever ranging from 80.0% to 95.7%, 8 years later. All are acceptable and comparable to other studies of duration of immunity in adults with the full dose.
Conclusions (3)

- Geometric mean titers of neutralizing antibodies to yellow fever are similar across groups, except for the 31 IU group, in which it is higher. All groups had the lower 95% confidence intervals above the cut-off for seropositivity
Conclusions (4)

• As we did not explore subgroups who could respond less well to the YF vaccine, the current study supports the use of yellow fever vaccine in fractionated doses and the minimum dose recommended by WHO, 1000 IU.
Funding

• This research was funded by an award from Wellcome Trust and did not receive any specific grant from other funding agencies in the public, commercial, or not-for-profit sectors
Collaborative Group for Studies on Duration of Immunity of Yellow Fever Vaccine

- Suelen Manhães Pessanha, Maria Letícia Borges dos Santos, Robson Leite de Souza Cruz, Dayana Cristina Vieira de Souza, Ricardo Cristiano Brum, Clara Lucy de Vasconcelos Ferroco, Deborah Araújo da Conceição, Leonardo Secundino, Olindo Assis Martins Filho, Ana Carolina Campi Azevedo
Thank you for your attention!