**Randomized clinical trial to assess the protective efficacy of a *Plasmodium vivax* CS synthetic vaccine**

**Supplemental Material**

**Appendix 2**

**Paraclinical Safety Tests**

Safety paraclinical tests were taken at the time of recruitment (selection), at the first immunization (month 0), after the first immunization (month 1), before the second (month 2), after the second immunization (month 3), before the third (month 6) and after the third (pre-challenge).

Paraclinical safety alterations during immunizations totaled 191 and consisted of: Anemia 24% (46), hematuria 15% (29), hyperglycemia 12% (23), proteinuria 9% (17), prolonged partial thromboplastin time 8 % (16), eosinophilia 7% (14), elevation of glutamic pyruvic transaminase 7.3% (14), prolongation of thrombin times 6% (11), elevation of indirect bilirubin without alteration of the AST/ALT pattern 2% (4), glutamic oxaloacetic transaminase elevation 1% (2), Leukocytopenia 3% (6), Leukocytosis 2% (4), Neutropenia 2% (4).

|  |
| --- |
| **Indirect bilirubin up to 0.83mg / dL** |
|  | **CS1018** | **CS1025** | **CS1030** | **CS1535** |
| Selection  | 0.08 | 0.13 | 0.28 | 0.28 |
| Lab Control Month 0 | 0.34 | 0.10 | 0.88 | 0.04 |
| Lab Control Month 1 | 0.02 | 0.06 | 0.2 | 0.22 |
| Lab Control Month 2 | 0.1 | 0.86 | 0.12 | 0.13 |
| Lab Control Month 3 | 0.16 | 0.05 | 0.58 | 0.1 |
| Lab Control Month 6 | 0.9 | 0.10 | 0.11 | 0.88 |
| Lab Control Pre- challenge  | 0.11 | 0.08 | 0.02 | 0.01 |

**Table 4.** Indirect bilirubin in the paraclinical follow-up of immunized volunteers who presented an adverse event (AE)

Renal function:

There was no alteration in renal function: creatinine and urea nitrogen values. All values were within the Protocol's standard ranges.

Glycemia:

There was a glycemia elevation in 17 volunteers in the selection paraclinical and on the first day of immunization. However, despite the request to present fasting for volunteers, some paraclinical were not taken on an empty stomach. Volunteers took their glycemia levels when they had time disposition. To determine glycemia, it was pertinent to measure fasting glucose levels or with a glucose tolerance curve.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Selection** | **Grade** | **Lab Control Month 0** | **Grade** |
| **CS1001** | 121 | 1 |  |  |
| **CS1003** | 104 |  | 126 | 2 |
| **CS1015** | 123 | 1 | 99 |  |
| **CS1018** | 113 | 1 | 110 | 1 |
| **CS1036** | 110 | 1 |  |  |
| **CS1037** | 110 | 1 |  |  |
| **CS1506** | 111 | 1 | 117 | 1 |
| **CS1535** | 87 |  | 156 | 2 |
| **CS1537** | 77 |  | 124 | 1 |
| **CS1538** | 94 |  | 152 | 2 |
| **CS1547** | 95 |  | 119 | 1 |
| **CS1549** | 97 |  | 130 | 2 |
| **CS1553** | 87 |  | 112 | 1 |
| **CS1554** | 90 |  | 121 | 1 |
| **CS1565** | 111 | 1 | 119 | 1 |
| **CS1570** | 101 |  | 125 | 1 |
| **CS1572** | 92 |  | 113 | 1 |
| **CS1575** | 97 |  | 112 | 1 |
| **CS1581** | 110 | 1 | 110 | 1 |

**Table 5.** Glycemia in the paraclinical follow-up of immunized volunteers who presented an AE.

Transaminases:

Grade 1 transaminase elevation (ALT and AST) (elevation 1.1-2.5 ULN) occurred in 7 volunteers (CS1575, CS1025, CS1030, CS1031, CS1538, CS1570, CS1581).

Elevated Glutamic Pyruvic Transaminase:

Grade 1 elevation (elevation 1.1-2.5 ULN) occurred in 7 volunteers. Bearing in mind that the reference values ​​vary according to whether you are male or female. In men's case, the reference value is Up to 40 U / L; therefore, a Grade 1 AE corresponds to an elevation greater than 44 U / L in the case of men (CS 1575). In the case of women, the reference value is up to 32 U / L. Therefore, AE Grade 1 corresponds to values ​​higher than 35 (CS1025, CS1031, CS1538, CS1570, CS1581). Altered values ​​at the time of selection are not related to immunizations due to temporality; subsequent elevations may or may not be related.

It should be noted that the volunteer CS1565 and CS1538 presented elevations in glutamic pyruvic transaminase from the beginning of the study before the administration of the immunization. Both belong to the experimental group. Volunteers CS1025, CS1030, CS1031, CS1575, CS1581 were part of the experimental group. Volunteer CS1570 belonged to the control group.

|  |
| --- |
| **Glutamic Pyruvic Transaminase U / L** |
| Moment | **CS1025** | **CS1031** | **CS1538** | **CS1565** | **CS1570** | **CS1575** | **CS1581** | **Grado** |
| Selection | 31 | 31 | 39 | 39 | 26 | 29 | 28 | 1 |
| Lab Control Month 0 | 33 | 24 | 50 | 25 | 13 | 40 | 28 | 1 |
| Lab Control Month 1 | 54 | 22 | 61 | 22 | 20 | 41 | 23 | 1 |
| Lab Control Month 2 | 22 | 48 | 53 | 19 | 15 | 41 | 36 | 1 |
| Lab Control Month 3 | 25 | 31 | 59 | 29 | 12 | 27 | 27 | 1 |
| Lab Control Month 6 | 38 | 52 | 19 | 23 | 28 | 80 | 19 | 1 |
| Lab Control pre-challenge | 26 | 14 | 72 | 31 | 53 | 37 | 15 | 1 |

**Table 6.** Glutamic Pyruvic transaminase in the paraclinical follow-up of immunized volunteers who presented an AE.

Elevation of glutamic oxaloacetic transaminase:

There was no evidence of elevated SGOT in any male volunteers from the moment of recruitment until the safety paraclinical after the third immunization.

Volunteer CS1538, who belonged to the experimental group, presented Grade 1 elevation of the SGOT in control paraclinical after the first and third immunization.

|  |
| --- |
|  **Glutamic Oxaloacetic Transaminase (SGOT) Woman Reference value: 8-39 U/L** |
|  | **CS1538** | **Grade** |
| Selection | 34 | 1 |
| Lab Control Month 0 | 40 | 1 |
| Lab Control Month 1 | 51 | 1 |
| Lab Control Month 2 | 40 | 1 |
| Lab Control Month 3 | 43 | 1 |
| Lab Control Month 6 | 23 | 1 |
| Labo Control pre-challenge | 46 | 1 |

**Table 7.** Glutamic Oxalacetic Transaminase in the paraclinical follow-up of immunized volunteers who presented an AE.

Clotting times:

Prothrombin time: (standard value 12-15 sec)

There was a slight prolongation considering a Grade I adverse event in 9 volunteers, of which eight corresponded to the experimental group (CS1006, CS1015, CS1028, CS1031, CS1036, CS1506, CS1565, CS1575) and 1 to the control group (CS1037).

However, two volunteers who belong to the experimental group and one who belongs to the control group presented prolonged selection paraclinical.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  **PT VN** **12-15 sec** | **CS1006** | **CS1015** | **CS1028** | **CS1031** | **CS1036** | **CS1037** | **CS1506** | **CS1565** | **CS1575** |
| Selection | 8.3 | 9.5 | 13.7 | 8.4 | 13.6 | 14.2 | 9.7 | 8.3 | 9.6 |
| Lab Control Month 0 | 8.2 | 9.3 | 9.1 | 8.4 | 8.2 | 8.70 | 9.5 | 8.6 | 8.3 |
| Lab Control Month 1 | 9.8 | 9.2 | 8.7 | 10.2 | 8.5 | 10.3 | 8.2 | 9.2 | 9.3 |
| Lab Control Month 2 | 8.6 | 10.8 | 10.4 | 9.8 | 10.5 | 8.3 | 11.2 | 9.4 | 10.0 |
| Lab Control Month 3 | 11.0 | 9.8 | 9.9 | 11.1 | 9.8 | 9.5 | 9.5 | 11.0 | 11.0 |
| Lab Control Month 6 | 8.7 | 9.9 | 9.2 | 8.5 | 8.5 | 9.8 | 9.0 | 11.1 | 8.9 |
| Lab Control pre-challenge | 9.8 | 9.0 | 9.3 | 8.2 | 9.8 | 9.6 | 11.8 | 10.2 | 7.5 |
| **Grade** | 1 | 1 | 4 | 1 | 4 | 4 | 1 | 1 | 1 |

**Table 8.** Prothrombin time in the paraclinical follow-up of immunized volunteers who presented an AE.

Partial Thromboplastin Time (PTT):

(normal value 25-35 sec)

There was an alteration in the partial thromboplastin time values, following the Protocol's adverse event values in 14 volunteers.

10 volunteers (CS1023, CS1028, CS1031, CS1038, CS1506, CS1511, CS1537, CS1547, CS1553, CS1565) belonged to the experimental group 4 volunteers (CS1037, CS1549, CS1554, CS1574).

However, 4 volunteers of the 14 who presented prolongation of the TTP showed this alteration in the selection paraclinical. Of these three volunteers correspond to the experimental group and one to the control group.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **PTT VN 25-35 sec** | **CS1023** | **CS1028** | **CS1031** | **CS1037** | **CS1038** | **CS1506** | **CS1511** | **CS1537** | **CS1547** | **CS1549** | **CS1553** | **CS1565** | **CS1574** |
| **Selection** | 31.1 | 36.5 | 31.8 | 36.7 | 22.3 | 24.8 | 22.9 | 30.2 | 26.8 | 28.3 | 25.9 | 28.4 | 25.3 |
| **Lab Control Month 0** | 24.8 | 25.3 | 28.6 | 22.70 | 22.80 | 28.8 | 22.3 | 28.2 | 27.4 | 24.8 | 23.0 | 23.7 | 25.8 |
| **Lab Control Month 1** | 26.9 | 23.3 | 28.6 | 26.7 | 26.1 | 24.3 | 26.7 | 23.6 | 23.6 | 22.2 | 24.2 | 27.1 | 28.1 |
| **Lab Control Month 2** | 25.3 | 29.9 | 26.2 | 26.6 | 27.1 | 27.2 | 26.5 | 25.3 | 27.7 | 28.1 | 22.0 | 26.1 | 18.2 |
| **Lab Control Month 3** | 23.1 | 25.4 | 27.1 | 26.2 | 27.1 | 25.1 | 26.1 | 26.1 | 26.1 | 22.1 | 23.2 | 24.5 | 22.8 |
| **Lab Control Month 6** | 25.3 | 27.9 | 30.1 | 25.5 | 32.0 | 31.0 | 27.9 | 26.5 | 30.7 | 35.7 | 28.1 | 25.1 | 27.7 |
| **Lab Control pre-challenge** | 22.8 | 24.6 | 22.3 | 24.9 | 28.5 | 31.9 | 31.8 | 34.5 | 37.7 | 39.3 | 34.0 | 32.9 | 32.9 |
| **Grade** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **Grade** |  |  |  |  |  | 1 |  |  | 2 | 2 |  |  |  |

**Table 9.** Partial thromboplastin time in the paraclinical follow-up of immunized volunteers who presented an AE.

Hemogram:

Anemia:

The Protocol's reference values to categorize anemia are Hb less than or equal to 12g / dL in women and less than or equal to 13.5g / dL in men.

Thirteen volunteers presented a slightly low hemoglobin value ​​ranging from 11.2 - 11.8 g / dL in 10 women and three men. Among 9 volunteers (CS1005, CS1012, CS1013, CS1028, CS1511, CS1537, CS1549, CS1574, CS1581), 10 presented Grade 1 classification and only 2 volunteers were found in Grade 2. Among the men, only one volunteer presented Grade I anemia (CS1572).

|  |
| --- |
| **Anemia** |
|  | **CS1005** | **CS1006** | **CS1012** | **CS1013** | **CS1028** | **CS1511** | **CS1537** | **CS1547** | **CS1549** | **CS1572** | **CS1575** | **CS1574** | **CS1581** |
| Selection | 12.5 | 12.50 | 12.00 | 10.80 | 12.30 | 11.50 | 12.70 | 14.70 | 12.30 | 12.90 | 13.70 | 12.80 | 12.10 |
| Lab Control Month 0 | 12.70 | 12.60 | 11.20 | 11.80 |  | 11.80 | 12.20 | 14.50 | 12.10 | 13.20 | 14.00 | 12.20 | 12.10 |
| Lab Control Month 1 | 13.20 | 12.40 | 11.70 | 11.60 | 12.40 | 11.40 | 11.60 | 13.50 | 11.30 | 12.80 | 14.10 | 12.20 | 10.40 |
| Lab Control Month 2 | 12.80 | 12.50 | 11.10 | 11.50 | 11.60 | 11.20 | 12.10 | 14.20 | 10.60 | 13.30 | 13.70 | 12.00 | 11.20 |
| Lab Control Month 3 | 11.30 | 12.00 | 11.20 | 12.60 | 13.00 | 11.10 | 12.30 | 14.90 | 11.50 | 12.10 | 16.10 | 12.20 | 11.30 |
|
| Lab Control Month 6 | 12.50 | 12.40 | 11.50 | 11.90 | 11.60 | 11.60 | 12.00 | 14.20 | 10.70 | 12.80 | 13.30 | 11.90 | 11.50 |
| Lab Control pre-challenge | 13.00 | 13.20 | 11.80 | 13.10 | 12.60 | 11.30 | 11.90 | 14.50 | 12.10 | 13.10 | 14.00 | 12.00 | 12.30 |
| **Grade** | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 |

**Table 10.** Hemoglobin in the paraclinical follow-up of immunized volunteers who presented an AE

Thrombocytopenia:

Thrombocytopenia was not evidenced in the volunteers during the immunizations, considering the reference values ​​of the Protocol.

Leukocytopenia:

He had Grade 1 leukopenia (3500-2500 cells / mm3), 3 volunteers (CS1006, CS1013 and CS1025). Volunteer CS1006 and CS1013 belonged to the experimental group.

|  |
| --- |
| **Leukocytopenia (Reference value: <3500)** |
|   | **CS1006** | **CS1013** | **CS1025** |  |
| Selection | 7.40 | 4.30 | 5.10 | **Grade** |
| Lab Control Month 0 | 3.40 | 7.10 | 4.80 | 1 |
| Lab Control Month 1 | 3.60 | 3.30 | 5.30 | 1 |
| Lab Control Month 2 | 3.20 | 6.40 | 6.20 | 1 |
| Lab Control Month 3 | 4.00 | 4.40 | 3.50 | 1 |
| Lab Control Month 6 | 3.10 | 10.60 | 7.00 | 1 |
| Lab Control pre-challenge | 2.80 | 4.30 | 5.30 | 1 |

**Table 11.** Leukopenia in the paraclinical follow-up of immunized volunteers who presented an AE

Leukocytosis:

Three volunteers presented Grade I leukocytosis at four times, following the reference values ​​of the Protocol. Volunteers CS1015, CS1535 and CS1537 belonged to the experimental group.

|  |
| --- |
| **Leukocytosis (Reference values >10.800)** |
|  | **CS1015** | **CS1535** | **CS1537** | **Grade** |
| Selection | 5.5 | 12.10 | 9.4 | 1 |
| Lab Control Month 0 | 7.20 | 11.40 | 11.80 | 1 |
| Lab Control Month 1 | 10.90 | 8.90 | 6.70 | 1 |
| Lab Control Month 2 | 5.60 | 8.00 | 5.80 |  |
| Lab Control Month 3 | 4.60 | 8.60 | 6.50 |  |
| Lab Control Month 6 | 7.00 | 10.30 | 8.20 |  |
| Lab Control pre-challenge | 4.70 | 7.90 | 8.70 |  |

**Table 12**. Leukocytosis in the paraclinical follow-up of immunized volunteers who presented an AE

Lymphopenia:

Lymphocyte levels below the ranges determined by the Protocol that characterize lymphopenia were not evidenced in volunteers during immunizations.

Neutropenia:

Three volunteers from the experimental group had mild neutropenia (CS1013, CS1006, CS1025)

|  |  |  |  |
| --- | --- | --- | --- |
| **Time** | **CS1006** | **CS1013** | **CS1025** |
|  Selection  | 6.00 | 2.40 | 2.50 |
| Lab Control Month 0 | 1.80 | 4.50 | 2.00 |
| Lab Control Month 1 | 1.90 | 1.20 |  |
| Lab Control Month 2 | 1.10 | 3.90 |  |
| Lab Control Month 3 | 1.90 | 2.60 | 1.30 |
| Lab Control Month 6 | 1.60 | 7.50 |  |
| Lab Control pre-challenge | 1.10 | 2.70 | 3.60 |
| **Grade** | 1 | 1 | 1 |

**Table 13.** Neutropenia in the paraclinical follow-up of immunized volunteers who presented an AE

Eosinophilia:

Within the reported values, five volunteers had Grade I eosinophilia, and one volunteer had Grade II eosinophilia at four times. Four volunteers belonged to the control group (CS1018, CS1554, CS1572, CS1574) and 5 to the experimental group (CS1023, CS1511, CS1535, CS1538, CS1569).

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **CS1018** | **CS1023** | **CS1511** | **CS1535** | **CS1538** | **CS1554** | **CS1569** | **CS1572** | **CS1574** |
| Selection | **0** | 728,0 | **0** | 1089,0 | 888,0 | 344,0 | 288,0 | 882,0 | 768,0 |
| Lab Control Month 0 | 1332,0 | 0 | 84,0 | 228,0 | 288,0 | 87,00 | 110,0 | 448,0 | 2765,0 |
| Lab Control Month 1 | 602,0 | 0 | 0,0 | 1157,0 | 0 | 0 | 116,0 | 171,0 | 2160,0 |
| Lab Control Month 2 | 0 | 0 | 249,0 | 560,0 | 0 | 552,0 | 0 | 58,0 | 2263,0 |
| Lab Control Month 3 | 62,0 | 210,0 | 0,0 | 258,0 | 0 | 345,0 | 348,0 | 0 | 1932,0 |
| Lab Control Month 6 | 0 | 0 | 675,0 | 0 | 0 | 711,0 | 1050,0 | 462,0 | 0 |
| Lab Control pre-challenge | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Grade** | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |  |

**Table 14.** Eosinophilia in the paraclinical follow-up of immunized volunteers who presented an AE.

Urinalysis:

Proteinuria:

Proteinuria was found in 13 volunteers. Of these, 3 had proteinuria in paraclinical on the day of immunization.

Proteinuria was classified as Grade 1 (trace) in 4 volunteers (CS1006, CS1036, CS1037, CS1047); Grade 2 (+) in 5 volunteers (CS1001, CS1005, CS1012, CS1030, CS1038) and Grade 1 and 2 in 4 volunteers (CS1003, CS1015, CS1018, CS1031).

Of these, 8 volunteers belonged to the experimental group (CS1001, CS1006, CS1015, CS1030, CS1031, CS1036, CS1038, CS1547) and 5 to the control group (CS1003, CS1005, CS1012, CS1018, CS1037)

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|   | **CS1001** | **CS1003** | **CS1005** | **CS1006** | **CS1012** | **CS1015** | **CS1018** | **CS1030** | **CS1031** | **CS1036** | **CS1037** | **CS1038** | **CS1547** |
| Selection | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| Lab Control Month 0 | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Pos |  | Neg | Neg | Neg | Neg |
| Lab Control Month 1 | Pos | Neg | Pos | Pos | Pos | Pos | Neg | Neg | Pos | Neg | Neg | Neg | Neg |
| Lab Control Month 2 | Neg | Pos | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Pos | Pos | Neg | Pos |
| Lab Control Month 3 | Neg | Neg |  | Neg | Neg | Neg | Pos | Neg | Neg | Neg | Neg | Neg | Neg |
| Lab Control Month 6 | Neg | Pos |  | Neg | Neg | Neg | Neg | Neg | Pos | Neg | Neg | Pos | Neg |

**Table 15.** Proteinuria in the paraclinical follow-up of immunized volunteers who presented an AE

Glycosuria:

He presented severe glycosuria (500mg / dL) in volunteer CS1584, subsequently diagnosed with diabetes mellitus (HbA1c: 8.1%).

Hematuria:

Hematuria was mild in 14 volunteers at 26 moments classified as Grade I. In two moments, it was classified as Grade II (Lab control month 0 in CS1569 and Lab control pre-challenge in CS1038).

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Selection** | **Lab Control Month 0** | **Lab Control Month 1** | **Lab Control Month 2** | **Lab Control Month 3** | **Lab Control Month 6** | **Lab Control pre-challenge** |  |
|  **Code**  | **Erythrocytes** | **Erythrocytes** | **Erythrocytes** | **Erythrocytes** | **Erythrocytes** | **Erythrocytes** | **Erythrocytes** | **Classification EA** |
| CS1005 | 0 | - | - | 5 - 8 xc | NA | NA | - | Grade I |
| CS1012 | 0-2xc | 3-5xc | - | - | - | - | - | Grade I |
| CS1013 | 4-6xc | NA | 5 xc | 5 xc | 1 xc | 1 xc | eumorphs | Grade I |
| CS1023 | \*- | 0-1xc | - | - | - | - | - | Grade I |
| CS1031 | - |  | 0-2 xc | - | - | - | - | Grade I |
| CS1037 | 0-2xc | 0 | 3 | 5-8 xc | - | - | - | Grade I |
| CS1038 | - | - | - | - | - | - | 8-12xc | Grade I |
| CS1506 | 0-1xc | - | - | - | - | - | 0 eumorphs | Grade I |
| CS1535 | 0-2xc | - | - | - | - | 2 xc | 2 xc | Grade I |
| CS1537 | - | 0-2 xc | 5 xc | - | - | - | - | Grade I |
| CS1549 | 0 | 0 | 0 | 0 | - | 0 | 2 xc | Grade I |
| CS1565 | 0-2 xc | - | - | - | - | - | 2xc | Grade I |
| CS1569 | 1-3xc | >25 xc eu, 1-3xc dis | - | - | - | 5xc | 0 | Grade I-II |
| CS1575 | 2-4 xc | 2 xc | 5 xc | - | - | - | - | Grade I |

**Table 16**. Hematuria in the paraclinical follow-up of immunized volunteers who presented an AE

**Clinical manifestations after the Infectious Challenge (CHMI)**

As expected, there were symptoms and signs of malaria infection.

Arthralgia: Of the 32 volunteers exposed to the infectious challenge, on eight occasions, they reported arthralgia in the face-to-face and telephone medical follow-ups after the infectious challenge. 75% of the time was related to the infectious challenge, and 12.5% ​​of the time possibly and probably related.

Deterioration: On 16 occasions, the volunteers exposed to the infectious challenge reported deterioration, which was related in 43% (7/16) of the cases, possibly in 13%, and probably in 43% (7/16) infectious challenge.

Diaphoresis: On one occasion, a volunteer presented diaphoresis with hypotension (TA: 80/50), which responded to intravenous fluids.

Diarrhea: On five occasions, some volunteers had diarrhea which was found to be possibly related in 40% (2/5), in 40% (1/5) probably related, and 20% (1/5) probably unrelated to the infectious challenge.

Abdominal pain: On seven occasions, they had abdominal pain. Of these 28% (2/7) presented in the epigastrium and 72% (5/7) it was not specified, also in 28% (2/7) of the cases were considered probably related, in 14% (1/7) possibly related, in 28% (2/7), probably not related, in 14% (1/7) not related to the infectious challenge; in one case it was not specified.

Headache: Headache was reported on 48 occasions after the infectious challenge, of these: in 16% (8/48), it was considered related, in 29% (14/48), it was considered probably related, in the 20% (10/48) was considered possibly related, in 18% (9/48) it was considered probably unrelated, in 8% (4/48) it was deemed to be unrelated, there is no data for 6% (3 / 48).

Arm pain: In 85% (6/7), it was considered not related to the infectious challenge, and in 15% (1/7), it was considered related to the infectious challenge.

Chills: On 21 occasions, the volunteers had chills. In 76% (16/21), it was considered related, in 4% (1/21) probably related, in 4% (1/21) possibly related, and in the 14% (3/21) probably unrelated.

Fever: On 20 occasions, the volunteers presented fever after the infectious challenge. It was considered in 85% (17/20) related and in 15% probably unrelated (3/20).

Insomnia: On one occasion, insomnia occurred, which was considered possibly related to the infectious challenge.

General discomfort: On 41 occasions, the volunteers presented general discomfort. It was considered: in 39% (16/41) related, in 7% (3/41) probably related, in 7% (3/41) possibly related, in 20% (8/41) probably not related, in 2% (1/41) not related to the infectious challenge. However, in ten cases (24%), the general malaise was not related to the infectious challenge.

Myalgias: On 23 occasions, the volunteers presented myalgias. It was considered: in 61% (14/23) related, in 9% (2/23) probably related, in 17% (4/23) possibly related, probably 13% (3/23) unrelated to the CHMI.

Nausea: On 20 occasions the volunteers had nausea, it was considered: in 5% (1/20) related, in 50% (10/20) probably related, in 20% (4/20) possibly related, in 15% (3/20) probably unrelated, in 10% (2/20) unrelated.

Itching: On two occasions, itching was reported, which was not related to the infectious challenge or treatment.

Urticaria: On two occasions, the volunteers presented urticaria (20 minutes), which was not related to the CHMI but rather to the xenodiagnosis. Urticaria lasting 4 min, unrelated to the CHMI, was also reported on one occasion.

Blurred vision: Blurred vision was reported on six occasions. It was considered: in 83% (5/6) possibly related, and in 17% (1/6) probably not related to the CHMI.

Others: 29 findings were reported as others which are recognized as alterations in the area of ​​exposure to xenodiagnosis, the clinical picture of dyspnea and cough in treatment with salbutamol, viral vision, emesis, dizziness, myalgia of the lower limbs, dyspnea predominantly nocturnal, possible origin psychosomatic, cyst in the left ovary, rhinorrhea and earache, mild dizziness, dry cough, and vomiting.

**Table 17. Clinical manifestations after the infectious challenge**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Categorized Adverse Event** | **Description** | **Relationship to the Challenge** | **Relationship to Treatment** | **Total** |
| **Arthralgia** | **NA** | Definitely related | Not related | 5 |
|  |  |  | Probably unrelated | 1 |
|  |  | Possibly related | Not related | 1 |
|  |  | Probably related | Not related | 1 |
|  | **Total NA** |  |  | **8** |
| **Total Arthralgia** |  |  |  | **8** |
| **Decay** | **NA** | Definitely related | Not related | 7 |
|  |  | Possibly related | Not related | 2 |
|  |  | Probably related | Not related | 7 |
|  | **Total NA** |  |  | **16** |
| **Total Decay** |  |  |  | **16** |
| **Diaphoresis** | **Hypotension 80/50 responded to treatment with intravenous fluids** | NA | NA | 1 |
|  | **Total, hypotension 80/50 responded to treatment with intravenous fluids** |  | **1** |
|  | **NA** | Definitely related | Probably unrelated | 1 |
|  | **Total NA** |  |  | **1** |
| **Total Diaphoresis** |  |  |  | **2** |
| **Diarrhea** | **NA** | Possibly related | Not related | 1 |
|  |  |  | Possibly related | 1 |
|  |  | Probably unrelated | Not related | 1 |
|  |  | Probably related | Not related | 1 |
|  |  |  | Possibly related | 1 |
|  | **Total NA** |  |  | **5** |
| **Total Diarrhea** |  |  |  | **5** |
| **Abdominal****pain** | **Abdominal pain in the epigastrium** | Probably unrelated | Not related | 1 |
|  | **Total, Abdominal pain in the epigastrium** |  |  | **1** |
|  | **epigastric pain** | Probably unrelated | Probably related | 1 |
|  | **Total, epigastric pain** |  |  | **1** |
|  | **NA** | NA | NA | 1 |
|  |   | Not related | Possibly related | 1 |
|  |  | Possibly related | Not related | 1 |
|  |  | Probably related | Possibly related | 1 |
|  |  |  | Probably related | 1 |
|  | **Total NA** |  |  | **5** |
| **Total Abdominal pain** |  |  |  | **7** |
| **Headache** | **Global mild headache** | Probably unrelated | Probably related | 1 |
|  | **Total Global mild headache** |  |  | **1** |
|  | **Migraine-like headache** | Probably unrelated | Not related | 1 |
|  | **Total Migraine-like headache** |  |  | **1** |
|  | **NA** | Definitely related | Not related | 6 |
|  |  |  | Possibly related | 1 |
|  |  |  | Probably unrelated | 1 |
|  |  | NA | NA | 3 |
|  |  | Not related | Not related | 2 |
|  |  |  | Possibly related | 1 |
|  |  |  | Probably related | 1 |
|  |  | Possibly related | Not related | 9 |
|  |  | Probably unrelated | Not related | 6 |
|  |  |  | Probably unrelated | 1 |
|  |  | Probably related | Not related | 14 |
|  | **Total NA** |  |  | **45** |
|  | **Refers mild headache** | Possibly related | Not related | 1 |
|  | **Total Refers mild headache** |  |  | **1** |
| **Total Headache** |  |  |  | **48** |
| **Arm pain** | **Pain in mosquito exposure area** | Not related | Not related | 1 |
|  | **Total pain in mosquito exposure area** |  | **1** |
|  | **Pain in the area of exposure to xenodiagnosis** | Not related | Not related | 1 |
|  | **Total pain in the area of exposure to xenodiagnosis** |  | **1** |
|  | **Pain in the area of exposure to xenodiagnosis** | Not related | Not related | 1 |
|  | **Total pain in the area of exposure to xenodiagnosis** |  | **1** |
|  | **Pain in the area of exposure to xenodiagnosis** | Not related | Not related | 1 |
|  | **Total pain in the area of exposure to xenodiagnosis** |  | **1** |
|  | **Pain in the area of exposure to xenodiagnosis** | Not related | Not related | 1 |
|  | **Total pain in the area of exposure to xenodiagnosis** |  | **1** |
|  | **NA** | Definitely related | Not related | 1 |
|  | **Total NA** |  |  | **1** |
|  | **Patient who had an accident with a polisher with lesion in the left hand thenar region** | Not related | Not related | 1 |
|  | **Total Patient who had an accident with a polisher with lesion in the left hand thenar region** | **1** |
| **Total arm pain** |  |  |  | **7** |
| **Chills** | **NA** | Definitely related | Definitely related | 1 |
|  |  |  | Not related | 14 |
|  |  |  | Probably unrelated | 1 |
|  |  | Possibly related | Not related | 1 |
|  |  | Probably unrelated | Not related | 3 |
|  |  | Probably related | Not related | 1 |
|  | **Total NA** |  |  | **21** |
| **Total Chills** |  |  |  | **21** |
| **Fever** | **NA** | Definitely related | Not related | 16 |
|  |  |  | Probably unrelated | 1 |
|  |  | Probably unrelated | Not related | 2 |
|  | **Total NA** |  |  | **19** |
|  | **Unquantified fever** | Probably unrelated | Probably unrelated | 1 |
|  | **Total Unquantified fever** |  | **1** |
| **Total Fever** |  |  |  | **20** |
| **Insomnia** | **NA** | Possibly related | Not related | 1 |
|  | **Total NA** |  |  | **1** |
| **Total Insomnia** |  |  |  | **1** |
| **Malaise** | **Flu-like symptoms** | Not related | Not related | 1 |
|  | **Total flu-like symptoms** |  |  | **1** |
|  | **Viral infection** | Probably unrelated | Not related | 1 |
|  | **Total viral infection** |  |  | **1** |
|  | **Malaise, flu-like symptoms** | Possibly related | Not related | 1 |
|  | **Total malaise, flu-like symptoms** |  | **1** |
|  | **NA** | Definitely related | Not related | 14 |
|  |  |  | Probably unrelated | 2 |
|  |  | NA | NA | 10 |
|  |  | Possibly related | Not related | 1 |
|  |  | Probably unrelated | Not related | 6 |
|  |  | Probably related | Not related | 3 |
|  | **Total NA** |  |  | **36** |
|  | **Malaise** | Probably unrelated | Probably unrelated | 1 |
|  | **Total again after three days general malaise begins** |  | **1** |
|  | **General malaise spontaneous resolution** | Possibly related | Not related | 1 |
|  | **Total refers to general discomfort yesterday, spontaneously resolved** |  | **1** |
| **Total Malaise** |  |  |  | **41** |
| **Myalgia** | **NA** | Definitely related | Not related | 13 |
|  |  |  | Probably unrelated | 1 |
|  |  | Possibly related | Not related | 4 |
|  |  | Probably unrelated | Not related | 3 |
|  |  | Probably related | Not related | 2 |
|  | **Total NA** |  |  | **23** |
| **Total Myalgia** |  |  |  | **23** |
| **Other** | **Alterations in the xenodiagnosis exposure area** | Not related | NA | 1 |
|  | **Total Alterations in the xenodiagnosis exposure area** |  | **1** |
|  | **A clinical case of more than one month of evolution consisting of dyspnea associated with dry cough, apparently without triggers. Treated with salbutamol inhaler.** | Probably unrelated | NA | 1 |
|  | **Total, A clinical case of more than one month of evolution consisting of dyspnea associated with dry cough, apparently without triggers. Treated with salbutamol inhaler.** | **1** |
|  | **Flu-like symptoms** | Not related | NA | 1 |
|  | **Total, Flu-like symptoms** |  |  | **1** |
|  | **Pain at mosquito exposure site** | Not related | NA | 1 |
|  | **Total, pain at mosquito exposure site** |  | **1** |
|  | **Vomiting** | Definitely related | NA | 1 |
|  | **Total, vomiting** |  |  | **1** |
|  | **With fever** | Probably unrelated | NA | 1 |
|  | **Total, with fever** |  |  | **1** |
|  | **Dizziness** | Probably unrelated | NA | 1 |
|  | **Total dizziness** |  |  | **1** |
|  | **Myalgia, only in lower limbs** | Probably unrelated | NA | 1 |
|  | **Total, myalgia, only in lower limbs** |  |  | **1** |
|  | **NA** | Not related | NA | 7 |
|  |  | Probably unrelated | NA | 2 |
|  | **Total NA** |  |  | **9** |
|  | **Dyspnea, predominantly nocturnal in the sitting and ulnar position, is not exacerbated by exercise nor associated with respiratory symptoms. Possible psychosomatic origin is questioned, and it is decided to continue follow-up.** | Not related | NA | 1 |
|  | **Dyspnea, predominantly nocturnal in the sitting and ulnar position, is not exacerbated by exercise nor associated with respiratory symptoms. Possible psychosomatic origin is questioned, and it is decided to continue follow-up.** | **1** |
|  | **Flu-like symptoms** | Probably unrelated | NA | 1 |
|  | **Total Flu-like symptoms** |  |  | **1** |
|  | **Flu-like symptoms** | Probably unrelated | NA | 1 |
|  | **Total Flu-like symptoms** |  |  | **1** |
|  | **Probably of gynecological origin** | Not related | NA | 1 |
|  | **Total, Probably of gynecological origin** |  | **1** |
|  | **Cyst on the left side under follow-up by gynecology** | Not related | NA | 1 |
|  | **Cyst on the left side under follow-up by gynecology** |  | **1** |
|  | **Reports that dizziness is associated with the intake of primaquine.** | Probably unrelated | NA | 1 |
|  | **Total, Reports that dizziness is associated with the intake of primaquine.** |  | **1** |
|  | **Hyaline rhinorrhea / otalgia** | Not related | NA | 1 |
|  | **Total, Hyaline rhinorrhea /otalgia** |  |  | **1** |
|  | **Dizziness (feeling)** | Possibly related | NA | 1 |
|  |  | Probably unrelated | NA | 1 |
|  | **Total, dizziness (feeling)** |  |  | **2** |
|  | **Mild dizziness (feeling)** | Probably unrelated | NA | 1 |
|  | **Total Mild dizziness (feeling)** |  |  | **1** |
|  | **Dry cough** | Probably unrelated | NA | 1 |
|  | **Total, dry cough** |  |  | **1** |
|  | **Vomiting** | Probably related | NA | 1 |
|  | **Total, vomiting** |  |  | **1** |
| **Total NA** |  |  |  | **29** |
| **Nausea** | **NA** | Definitely related | Possibly related | 1 |
|  |  | Not related | Not related | 1 |
|  |  |  | Possibly related | 1 |
|  |  | Possibly related | Not related | 1 |
|  |  |  | Possibly related | 1 |
|  |  |  | Probably unrelated | 1 |
|  |  |  | Probably related | 1 |
|  |  | Probably unrelated | Possibly related | 1 |
|  |  |  | Probably related | 1 |
|  |  | Probably related | Not related | 7 |
|  |  |  | Probably related | 3 |
|  | **Total NA** |  |  | **19** |
|  | **Nausea accompanying epigastric pain** | Probably unrelated | Probably related | 1 |
|  | **Total, Nausea accompanying epigastric pain** |  |  | **1** |
| **Total Nausea** |  |  |  | **20** |
| **Pruritus** | **1-week clinical picture of generalized itching, predominantly in the back.** | Not related | Not related | 1 |
|  | **Total, 1-week clinical picture of generalized itching, predominantly in the back.** | **1** |
|  | **Pruritus in the xenodiagnosis area** | Not related | Not related | 1 |
|  | **Total, Pruritus in the xenodiagnosis area** |  | **1** |
| **Total Pruritus** |  |  |  | **2** |
| **Urticaria (20 minutes)** | **Pruritus in the xenodiagnosis area** | Not related | Not related | 1 |
|  | **Total, Pruritus in the xenodiagnosis area** |  | **1** |
|  | **Maculopapular rash at the mosquito exposure site** | NA | NA | 1 |
|  | **Total, Maculopapular rash at mosquito exposure site** |  | **1** |
| **Total Urticaria (20 minutes)** |  |  | **2** |
| **Urticaria (4 minutes)** | **Pruritus in the exposure area** | NA | NA | 1 |
|  | **Total, Pruritus in the exposure area** |  |  | **1** |
| **Total Urticaria (4 minutes)** |  |  |  | **1** |
| **Blurred vision** | **after taking antimalarials** | Probably unrelated | Possibly related | 1 |
|  | **Total, after taking antimalarials** |  | **1** |
|  | **NA** | Possibly related | Not related | 3 |
|  |  |  | Possibly related | 2 |
|  | **Total NA** |  |  | **5** |
| **Total blurred vision**  |  |  |  | **6** |
| **Total, general** |  |  |  | **259** |

**Results of paraclinical in Infectious Challenge**

The alterations in safety paraclinical during the CHMI were 27. They consisted of hyperglycemia 4% (1), the elevation of glutamic-pyruvic transaminase 19% (5), the elevation of indirect bilirubin without alteration of the AST / ALT pattern 4% (1), anemia 41 (11), leukopenia 11% (3), leukocytosis 11% (3), lymphopenia 4% (1), hematuria 7% (2). According to the FDA classification of AE, 85% of paraclinical AE during the infectious challenge correspond to Grade 1 and the remaining 15% to Grade 2. Safety paraclinical after the CHMI were taken on day 33 of the challenge.

Paraclinical results were registered in the RedCap database; the reference values ​​were obtained from the Protocol. All recorded values ​​were analyzed, and the paraclinical test alterations were classified according to the Protocol.

Glycemia: A fasting sample was taken. Therefore, the values ​​were found in normal ranges. Volunteer CS1575 had blood glucose levels of 140 mg/dl, which would lead to a Grade I adverse event.

Creatinine and BUN: There was no elevation evidence to ranges considered an AE according to the protocol classification.

Transaminases: They presented Grade 1 elevation of Pyruvic Glutamic Transaminase, five volunteers of which 3 were women, and 2 were men. No elevation of Oxaloacetic Transaminases was evidenced in any of the volunteers after the challenge.

|  |  |  |  |
| --- | --- | --- | --- |
| **Volunteer** | **Value ALT (U/L)** | **Sex** | **Grade** |
| CS1025 | 40 | Woman | 1 |
| CS 1538 | 58 | Woman | 1 |
| CS 1565 | 43 | Woman | 1 |
| CS1015 | 54 | Man | 1 |
| CS1575 | 74 | Man | 1 |

**Table 18**. Alteration in Glutamic Pyruvic and Oxaloacetic Transaminases

**Direct bilirubin:** No elevation was evidenced in post-challenge paraclinical.

Indirect bilirubin without alteration of the AST / ALT pattern: Only volunteer CS1506 presented Grade 1 elevation.

|  |  |
| --- | --- |
| **Paraclinical** | **Value** |
| Bilirubin indirect | 0.87 |
| ALT | 27 |
| AST | 29 |

**Table 19.** Indirect bilirubin alteration without compromise of the AST / ALT pattern

Prothrombin Time- Partial Thromboplastin Time: There were no alterations in clotting times

**Blood count**

On day 33, post-CHMI paraclinical tests indicated:

Anemia: 11 volunteers had mild anemia. According to the reference values ​​of the protocol for the classification of the AE, seven volunteers (CS1005, CS1006, CS1554, CS1565, CS1570, CS1574, CS1581) had Grade 1 anemia (Hb 11-12 g / dL) and four volunteers (CS1511, CS1537, CS1549, CS1569) Grade 2 (Hb 9.5-10.9 g / dL):

|  |  |  |
| --- | --- | --- |
| **Volunteer** | **Hemoglobin** | **Grade** |
| CS1005 | 11.8 | 1 |
| CS1006 | 11.7 | 1 |
| CS1511 | 10.6 | 2 |
| CS1537 | 10.3 | 2 |
| CS1549 | 10.8 | 2 |
| CS1554 | 11.4 | 1 |
| CS1565 | 11.2 | 1 |
| CS1569 | 10.4 | 2 |
| CS1570 | 11.6 | 1 |
| CS1574 | 11.8 | 1 |
| CS1581 | 11.0 | 1 |

**Table 20**. Alteration in Hb values

Leukopenia: Grade 1 leukopenia was evidenced in three volunteers (CS1006, CS1028, CS1037).

|  |  |  |
| --- | --- | --- |
| **Volunteer** | **Leucocytes /ul** | **Grade** |
| CS1006 | 2800 | 1 |
| CS1028 | 3000 | 1 |
| CS1037 | 3000 | 1 |

**Table 21**. Leukopenia post-CHMI paraclinical follow-up.

Leukocytosis: Grade 1 leukocytosis was evidenced in three volunteers (CS1012, CS1547, CS1570).

|  |  |  |
| --- | --- | --- |
| **Volunteer** | **Leucocytes /ul** | **Grade** |
| CS1012 | 11.5 | 1 |
| CS1570 | 11.2 | 1 |
| CS1547 | 11.2 | 1 |

**Table 22**. Leukocytosis in post-CHMI paraclinical follow-up

Lymphopenia: In post-challenge paraclinical, only volunteer CS1006 had Grade 1 lymphopenia (896).

Thrombocytopenia: Thrombocytopenia was not evidenced.

**Urine test**

Glycosuria: in the RedCap report, there is no evidence of altered glucose values ​​in urine.

Proteinuria: no proteinuria was evidenced in any of the volunteers post-CHMI.

Hematuria: Volunteer CS1013 and volunteer CS1553 presented Grade 1 hematuria.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  **Selection**  | **Lab Control Month 0** | **Lab Control Month 1** | **Lab Control Month 2** | **Lab Control Month 3** | **Lab Control Month 6** | **Lab Control pre-challenge** | **Challenge** |
|  **Code**  | **Erythrocytes:** | **Erythrocytes:** | **Erythrocytes:** | **Erythrocytes:** | **Erythrocytes:** | **Erythrocytes:** | **Erythrocytes:** | **Erythrocytes:** |
| CS1013 | 4-6xc |  - | 5.00 | 5 | 1 | 1 | eumorphs | 5 eumorphs |
| CS1553 | - | - | - | - | - | - | - | 1 eumorphs |

**Table 23**. Hematuria in post-challenge paraclinical follow-up

However, volunteer CS1013 from the selection paraclinical presented Grade I hematuria. There was likely no relationship between the hematuria post-CHMI and the investigation (immunizations and CHMI). Volunteer CS1553 presented Grade I hematuria on the lower levels.