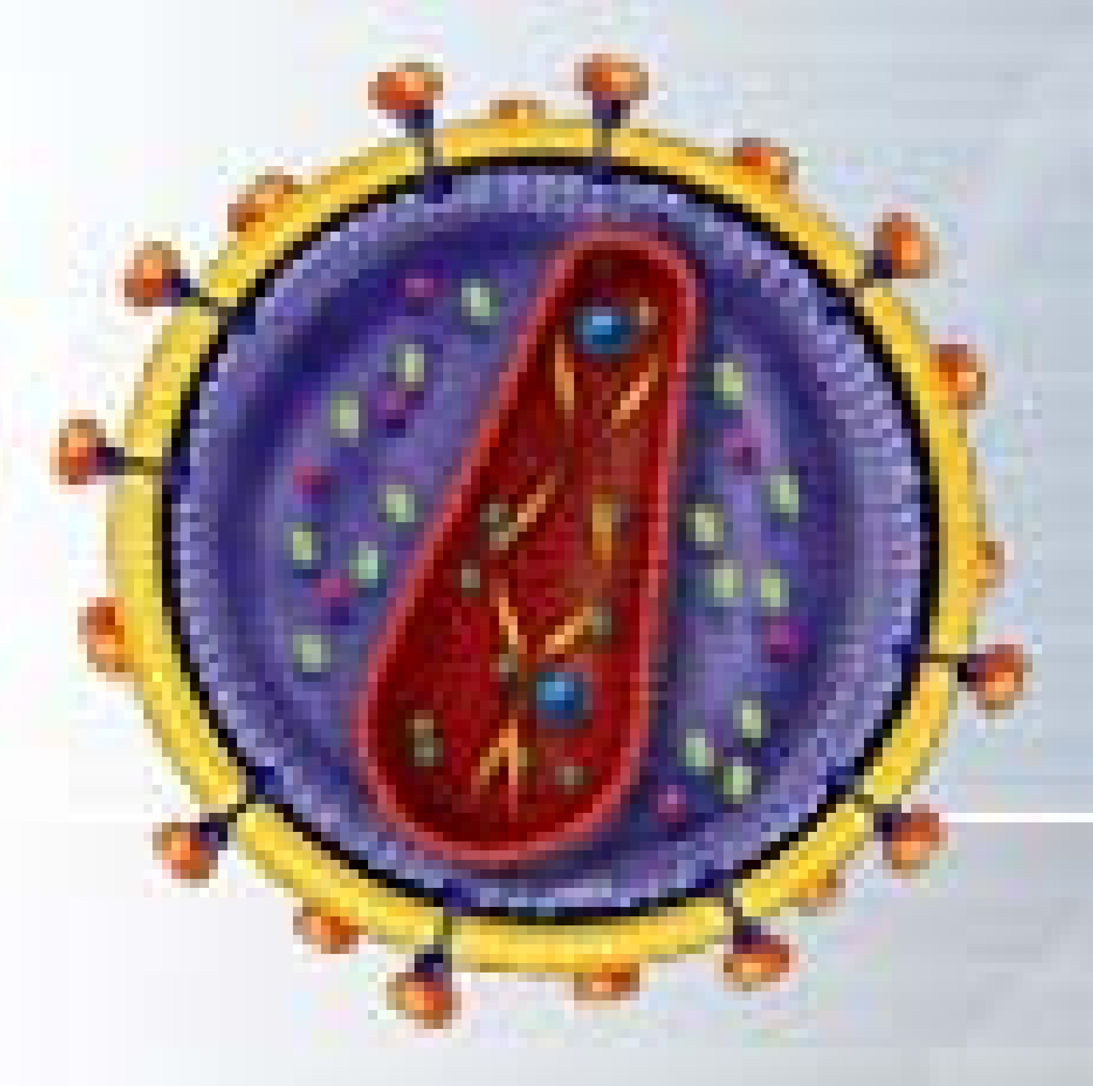

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Effects of HAART Interruption in Acquired Humoral and Cellular Responses to Recommended Vaccines

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ABSTRACT (I)

Background: HIV infection causes dysfunction in humoral and cellular responses to recommended vaccines. These responses worsen with immunosuppresion and improve with HAART. The effects of treatment interruption on these responses are unknown.

Methods: We performed a single-centre prospective, randomized, double blind, placebo-controlled clinical trial to evaluate the effect of a vaccination program in successfully treated HIV-infected adults on HAART and after its interruption. We included 26 HIV-positive adults with CD4 $\geq$ 500/mm^3 and plasma viral load < 200 copies/mL who were randomized to receive during 12 months either 10 commercial vaccines or placebo. Vaccination program included: hepatitis B (months 0, 1, 2, and 6), influenza (month 1), pneumococcal (month 2), hepatitis A (months 4 and 10), chickenpox (months 4 and 6), measles-mumps-rubella (month 8), and diphteria-tetanus (monht 10). On month 12 HAART was interrupted during 6 months, and evolution of immune responses to vaccinations agents were analysed on the whole cohort and compared between groups. Humoral responses were evaluated with ELISA commercial assays (with quantitative and qualitative results) on months 12 and 18. Cellular responses were analysed with ELISPOT and AMPLISPOT assays on months 12, 15 and 18.

ABSTRACT (II)

Results: Effect of HAART interruption was evaluated in 25 patients (12 on placebo group and 13 on vaccination group). Although vaccinated group presented higher levels of antibodies against the different agents on month 12, evolution of immune responses after HAART interruption was similar in both groups. Antibodies against rubella, *S. pneumoniae* and tetanus presented a significant decrease ($p \leq 0.001$), meanwhile titers against diphteria decreased not significantly. In the qualitative analysis some patients presented a negativization of their serology: 1/24 for varicella, 3/24 for mumps, 2/7 for *S. Pneumoniae*, 2/23 for tetanus and 4/13 for diphteria. Humoral responses against the other agents evaluated remained stable during the interruption period. By contrast, cellular responses presented a general trend (not significant) to increase after HAART interruption, except for Hepatitis B.

Conclusions: Interrupting HAART may cause dysfunction in acquired humoral responses to vaccines, even decreasing antibody titers to "unprotective" levels in some patients. The potential risk of suffering these infections should be evaluated in large randomized studies.

BACKGROUND (I)

- Guidelines recommend that HIV-infected adults be vaccinated against several agents because they can present more severe diseases when infected and share risk factors for other preventable infections[1].

- Recommended vaccines in HIV-infected adults include those against Tetanus, Influenza, Pneumococcal, Hepatitis A (HAV) and Hepatitis B (HBV). Others like measles, mumps and rubella vaccines are recommended depending on the epidemiological context. Nowadays vaccination against varicella (VVZ) is not recommended in adults, but it will probably change in the future[2].

BACKGROUND (II)

- However, HIV infected patients present impaired responses to vaccines, both humoral and cellular, due to dysfunction in T and B lymphocytes[3-6].

- Responses to vaccination worsen as immunosuppression progresses and can improve with HAART[7-11].

- When HAART is interrupted, HIV reappears and CD4-T cells count falls again. The effect of HAART interruption over previously acquired responses is unknown.

SUBJECTS AND METHODS (I)

STUDY DESIGN AND SUBJECTS:

• VAC-01 is a single-centre prospective, randomized, double blind, placebo-controlled clinical trial to evaluate the effect of a vaccination program in successfully treated HIV-infected adults on HAART.

• Twenty-six HIV positive adults were randomized to receive during 12 months either a vaccination program (VG) (**Figure 1**) or placebo (PG). Inclusion and exclusion criteria are collected in **Tables 1** and **2**.

FIGURE 1: Vaccination program

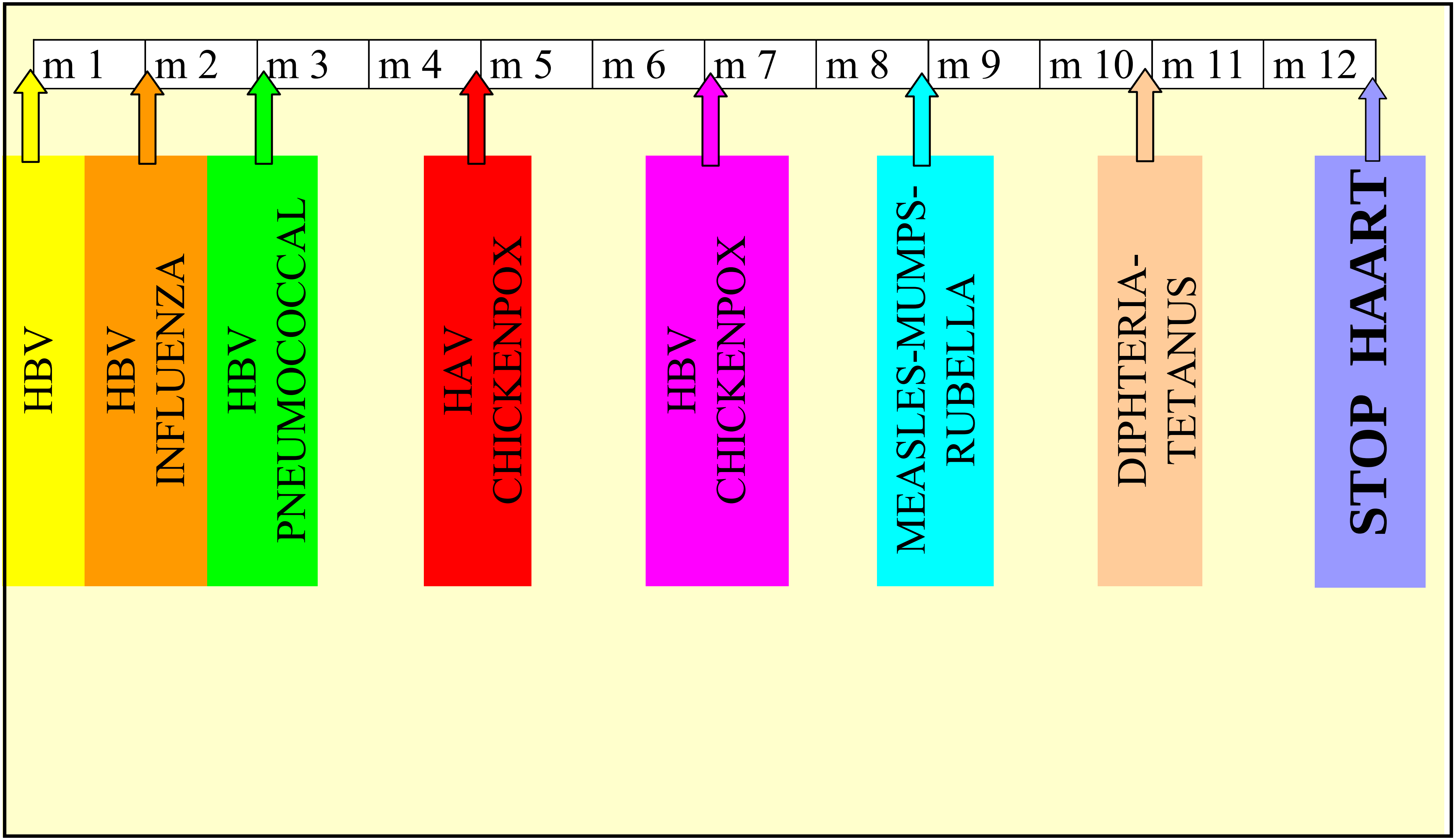


TABLE 1: Inclusion criteria
Age ≥18 years.
Asymptomatic HIV infection
CD4>500/mm^3 ≥6 months prior to inclusion
CD4 nadir >300/mm^3
VL<200 copies/mL ≥ 6 months prior to inclusion
VL previous to treatment >5000 copies/mL
HAART ≥ 1 year prior to inclusion
Informed consent

TABLE 2: Exclusion criteria
Pregnant women
Basal creatinine >2.5 mg/dL
Allergy to either a vaccine or a ingredient of it
Chronic hepatitis B
GOT/GPT > 250 IU/L

SUBJECTS AND METHODS (II)

- Patients were on HAART up to 2 months after the last immunization (month 12). Afterwards, HAART was discontinued (**Figure 1**) for at least 6 months (month 18).

- Evolution of immune responses to vaccination agents after HAART interruption were analysed on the whole cohort and compared between groups (VG and PG).

METHODS:

- Blood samples were taken monthly from month 12 to 18. There were analysed:

SUBJECTS AND METHODS (III)

- Humoral responses with ELISA commercial assays (months 12 and 18). Results were obtained as:
  - ✓Qualitative (seropositive or seronegative): VVZ, measles, mumps.
  - ✓Quantitative (antibody (Ab) titration): HAV, HBV, *S. pneumoniae*, rubella, tetanus and diphteria. They were also transformed in qualitative results.

- Cellular responses with ELISPOT and AMPLISPOT assays (months 12, 15 and 18): HAV, HBV, influenza, *S. pneumoniae*, VVZ, measles, mumps and tetanus.

SUBJECTS AND METHODS (IV)

STATISTICAL ANALYSIS

- A SPSS 10.0 software was used. The analysis between groups was done either with t test or Mann-Whitney test when necessary. To compare into groups, we used either t test or Wilcoxon test when necessary.

RESULTS (I)

GENERAL CHARACTERISTICS

• After stopping HAART 25 patients were evaluated (12 in PG and 13 in VG). There were not significant differences in CD4-T cell count and viral load either at inclusion or at month 12 (**Table 3**).

• All patients in VG received the 11 programmed immunizations. No adverse events were registered. Humoral responses in VG were correct and significantly higher than in PG. Cellular responses were not different between groups[11].

• Therefore in month 12 VG have higher (but not all significant) humoral responses to vaccines than PG, and there were no differences in cellular responses between groups (**Table 4**).

RESULTS (II)

TABLE 3: Characteristics of patients

	Placebo-group	Vaccines-group	p
Age (years) (mean ± SD)	43.1 ± 8.9	36.5 ± 8	0.06
Sex (men/women)	10/3	11/2	1
CD4 (cells/mm^3) (mean ± SD)			
Month 0	898.4 ± 306.65	880.69 ± 247.65	0.87
Month 12	773.36 ± 222.36	751.86 ± 415.94	0.87
Nadir CD4 (cells/mm^3)(mean ± SD)	456.4 ± 119.8	449.1 ± 104.2	0.87
Peak PVL (log copies RNA/mL)	4.8 ± 0.7	4.7 ± 0.9	0.64
Viral load < 200 copies/mL month 12 (%)	100	100	1

RESULTS (III)

TABLE 4: Humoral responses (qualitative) at month 12

Seropositive patients, %	Placebo-group (n=12)	Vaccines-group (n=13)	p
Rubella	100	100	1
S. Pneumoniae	15.4	38.5	0.378
Tetanus	76.9	84.6	0.22
Diphteria	38.5	61.5	0.412
Hepatitis A	76.9	100	0.22
Hepatitis B	38.5	84.6	0.041
Mumps	84.6	100	0.48
Measles	92.3	100	1
Varicella	92.3	100	1

RESULTS (IV)

COMPARISON BETWEEN GROUPS

- There were not significant differences in the evolution of humoral and cellular responses between PG and VG after interrupting HAART in any of the administered vaccines (**Figures 2** to **5**)

FIGURE 2: Humoral responses in PG and VG (quantitative)

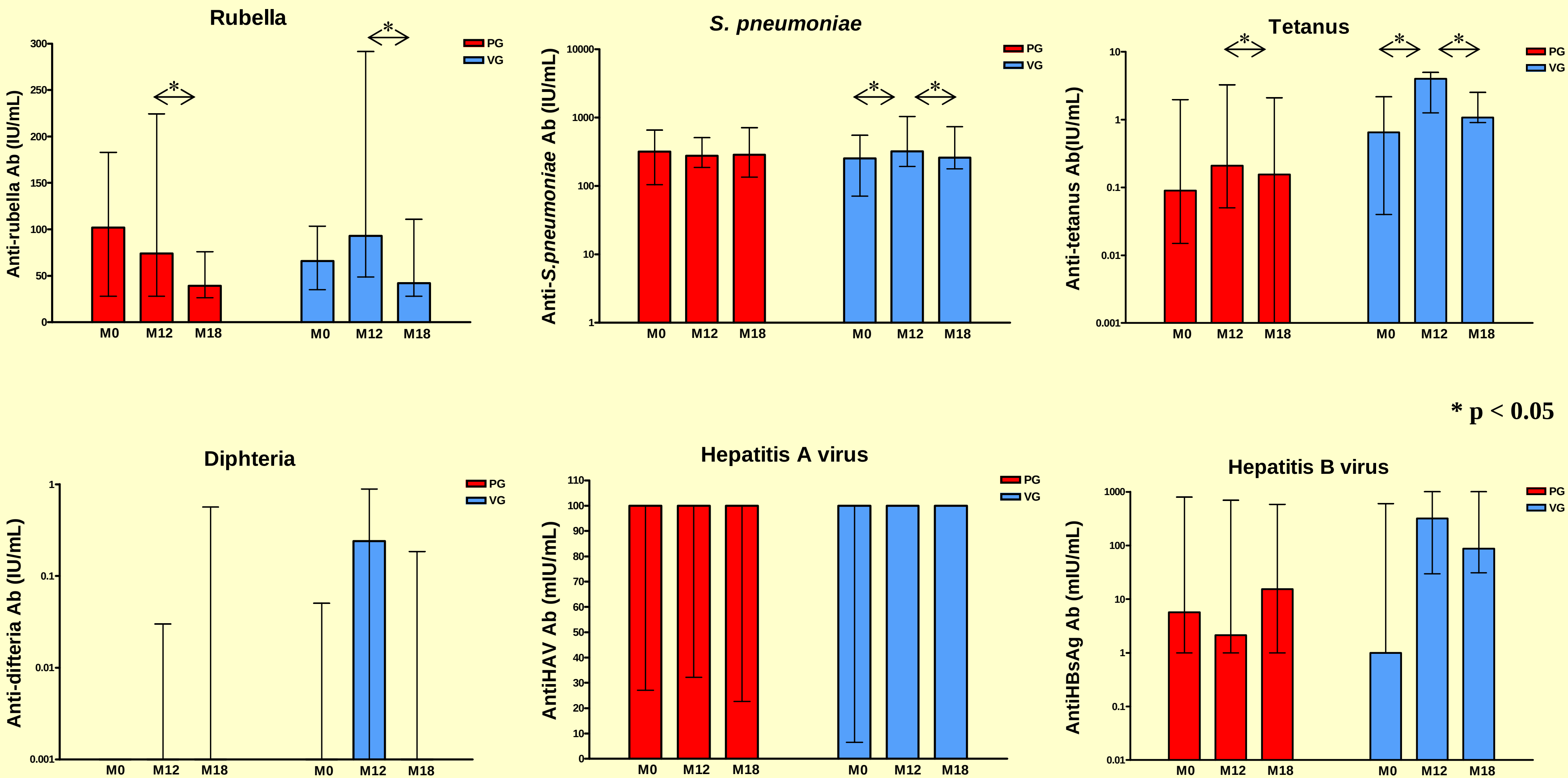


FIGURE 3: Humoral responses in PG and VG (qualitative)

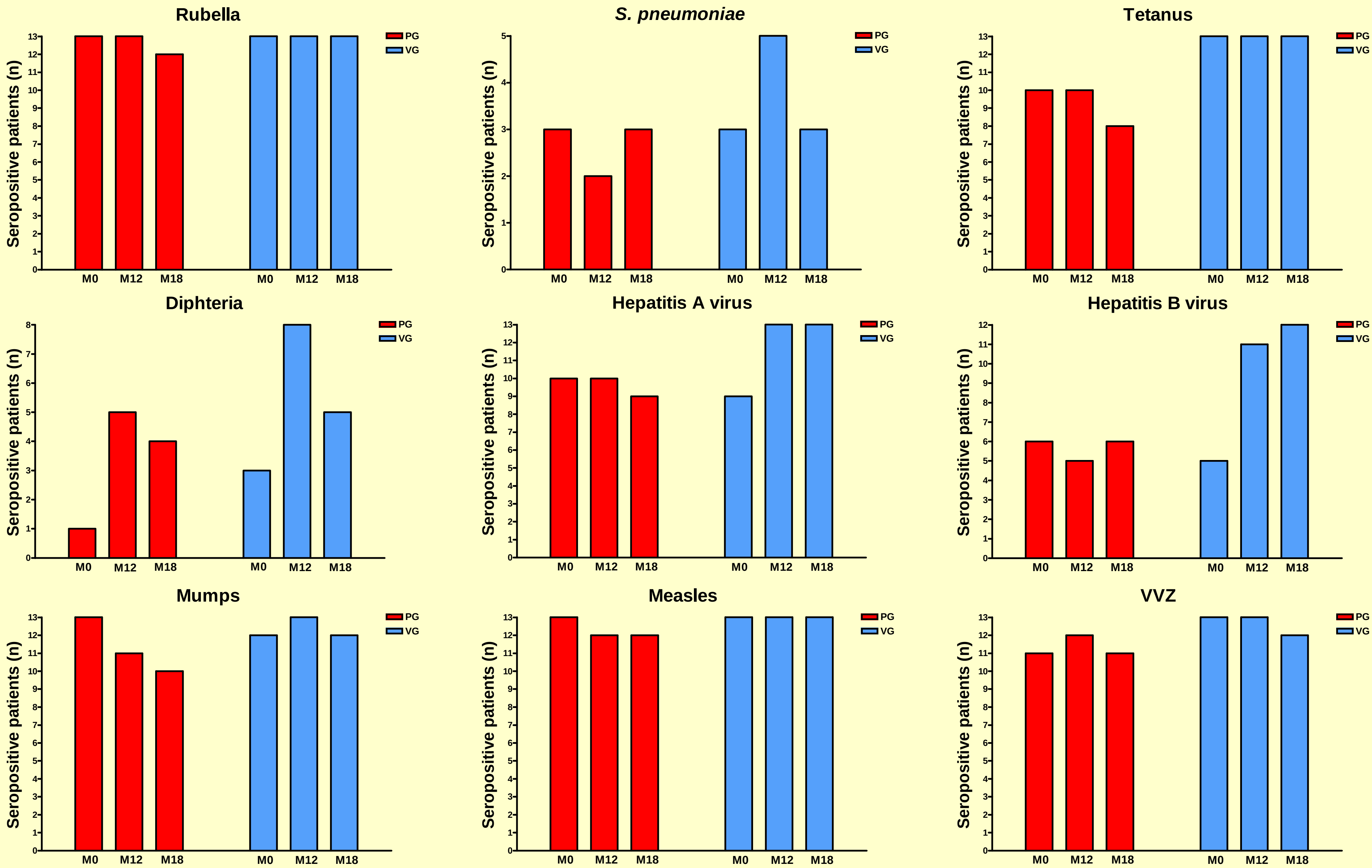


FIGURE 4: ELISPOT cellular responses in PG and VG (quantitative)

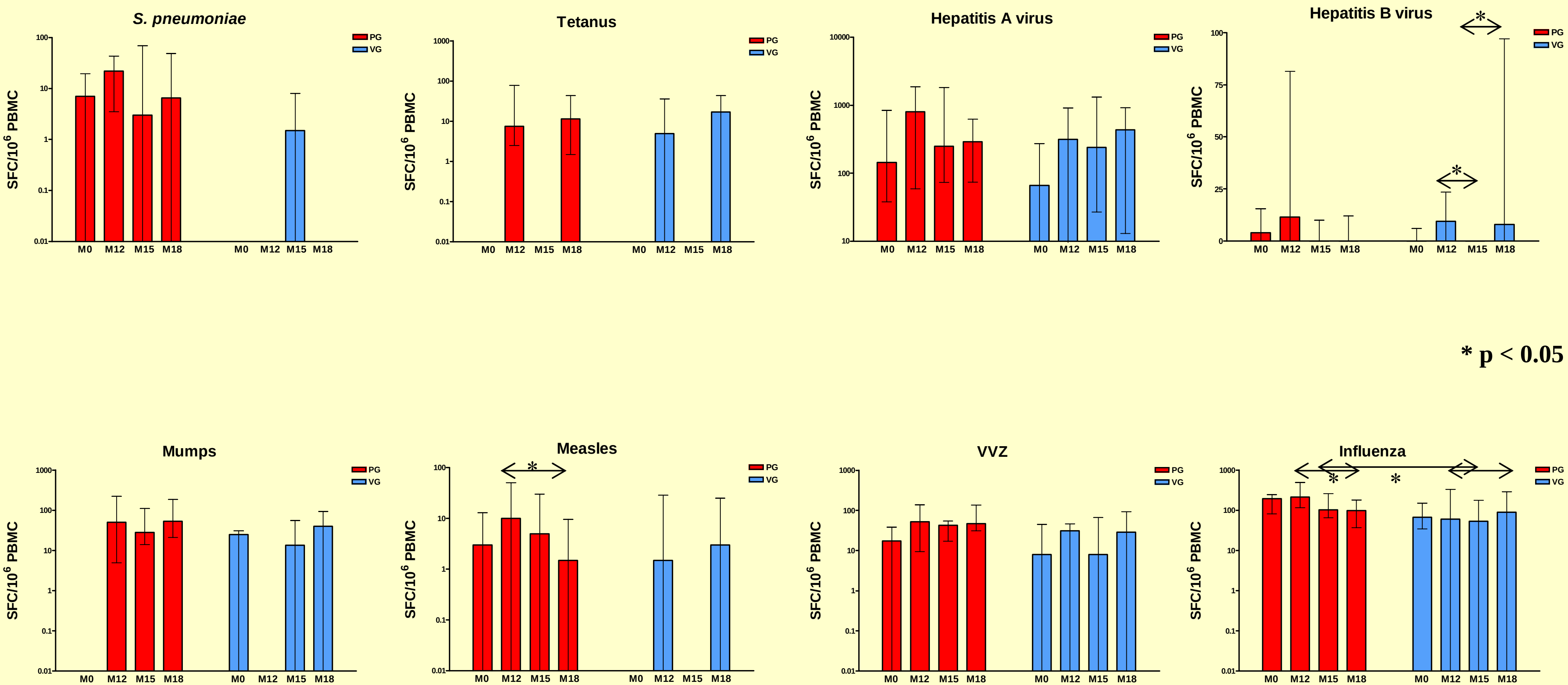
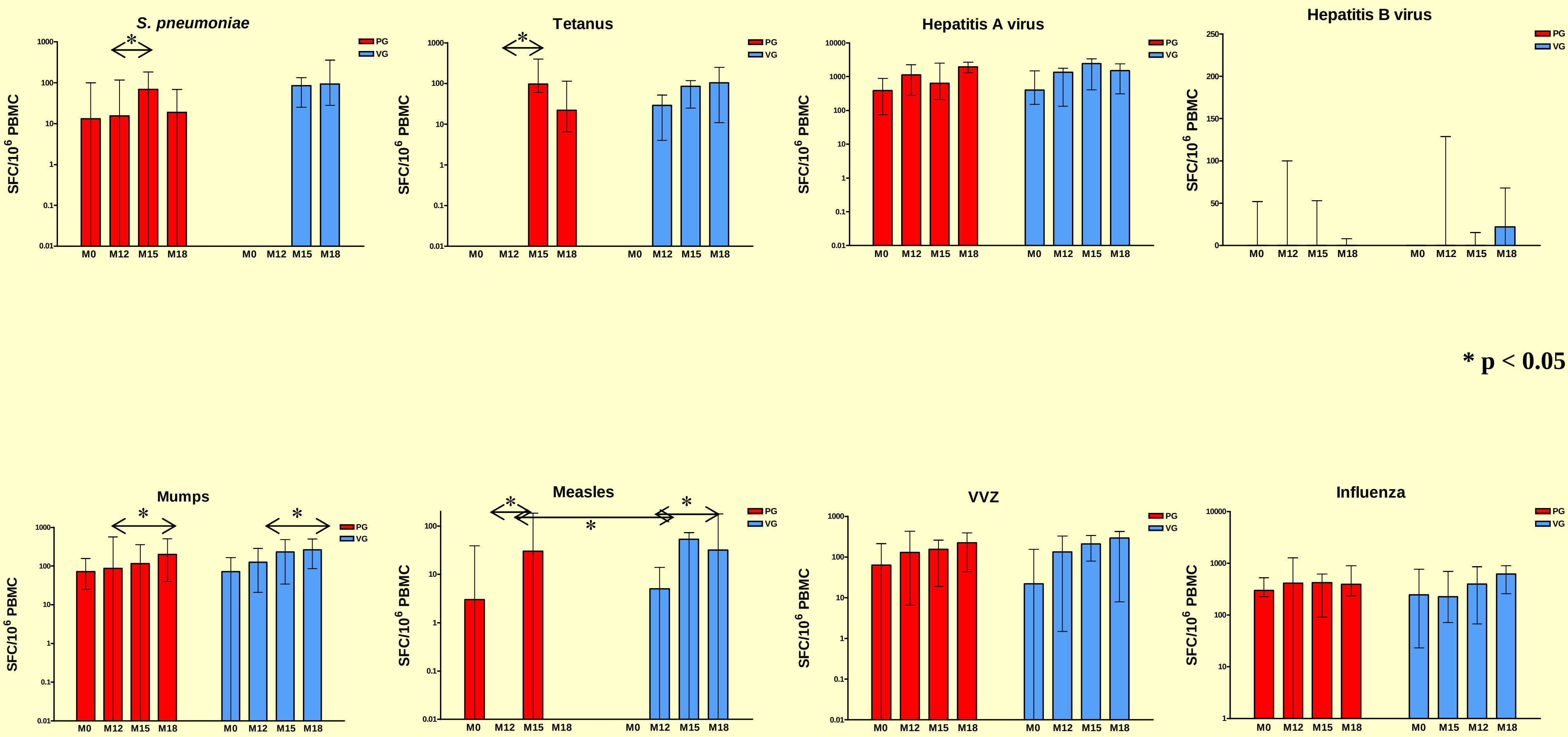


FIGURE 5: AMPLISPOT cellular responses in PG and VG (quantitative)



* p < 0.05

RESULTS (V)

EVOLUTION IN THE WHOLE COHORT: HUMORAL RESPONSES

- **Quantitative analysis:** Antibodies against rubella, *S. pneumoniae* and tetanus presented a significant decrease ($p \leq 0.001$), meanwhile titers against diphteria also decreased but not significantly (**Figure 6**).

- **Qualitative analysis:** Some patients presented a negativization of their serology: 1/24 for varicella, 3/24 for mumps, 2/7 for *S. Pneumoniae*, 2/23 for tetanus and 4/13 for diphteria (**Figure 7**).

FIGURE 6: Humoral responses in the whole cohort (quantitative)

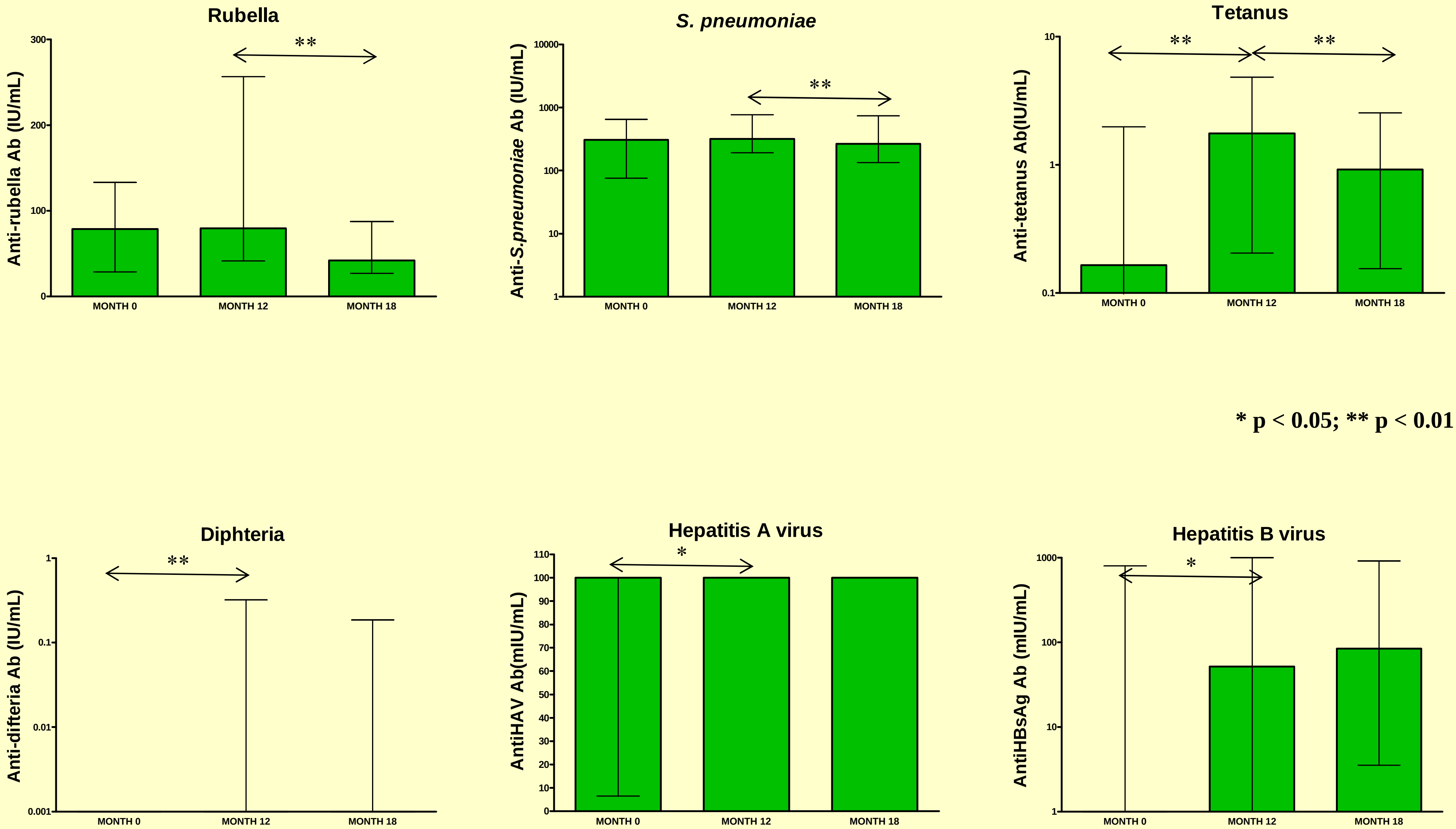
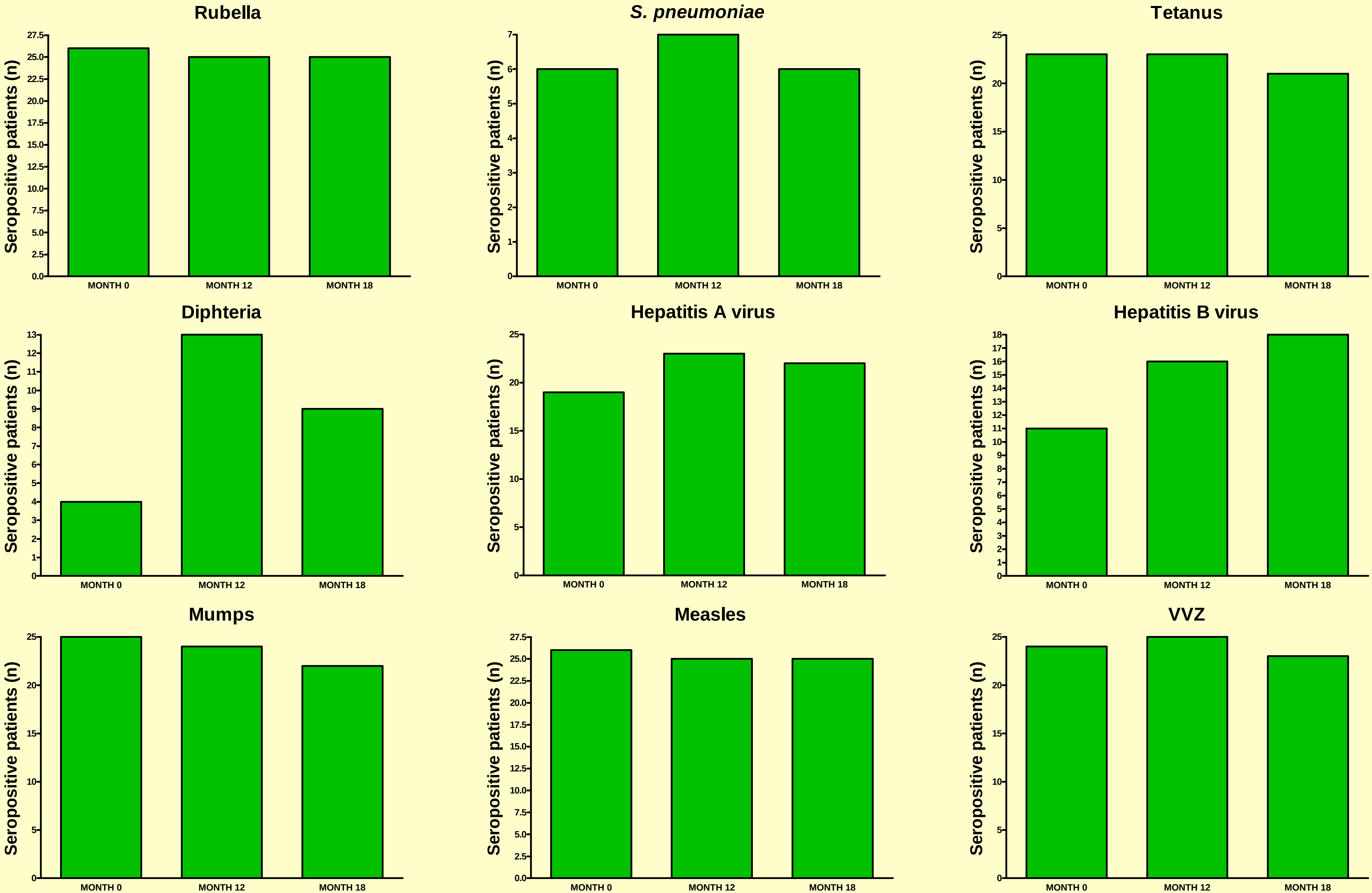


FIGURE 7: Humoral responses in the whole cohort (qualitative)



RESULTS (VI)

EVOLUTION IN THE WHOLE COHORT: CELLULAR RESPONSES

- Cellular responses presented a general tendency (not significant) to increase after HAART interruption, either measured by ELISPOT or AMPLISPOT, either in a quantitative or qualitative analysis, except for Hepatitis B (**Figures 8** and **9**)

FIGURE 8: ELISPOT cellular responses in the whole cohort (quantitative)

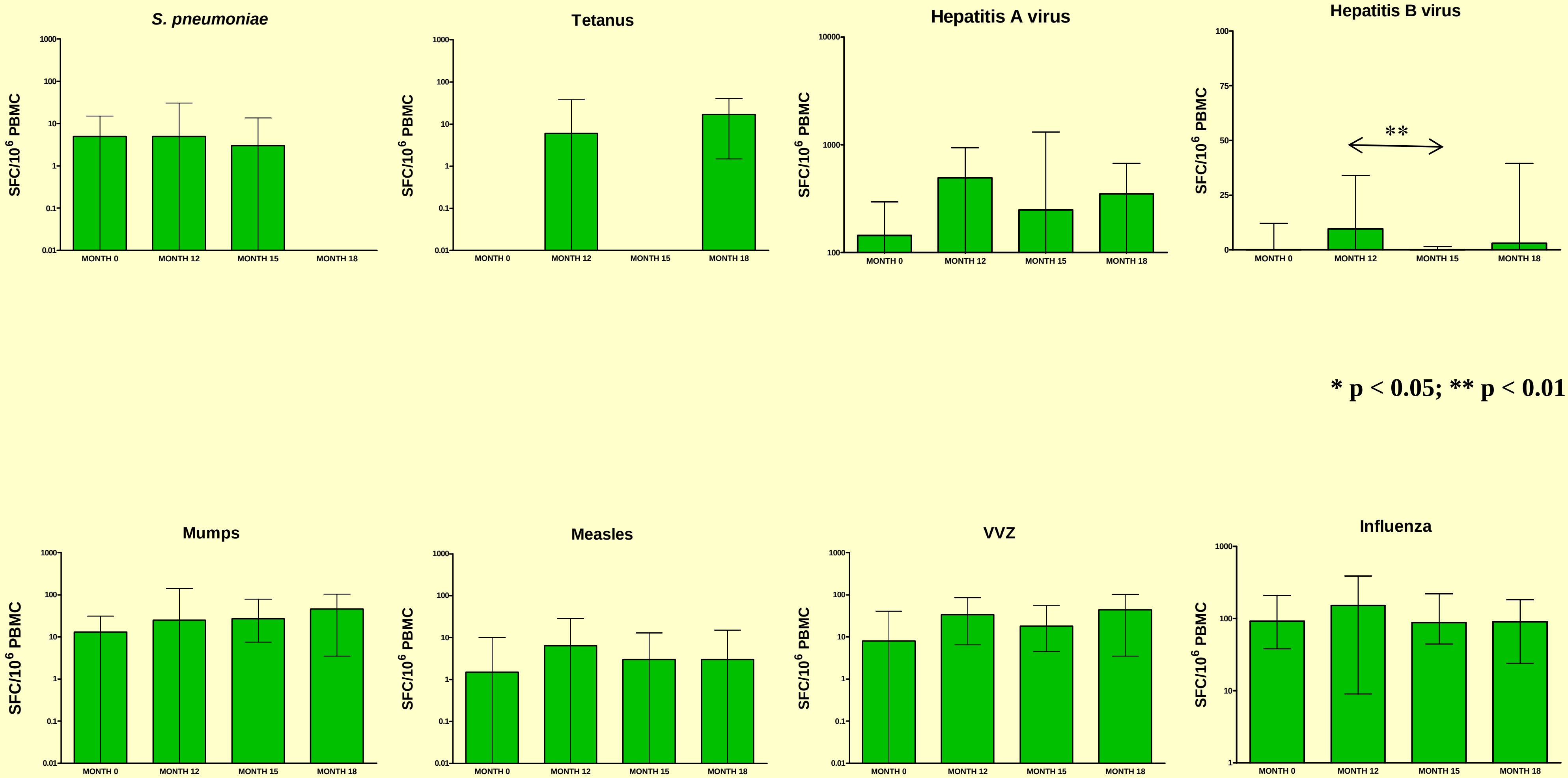
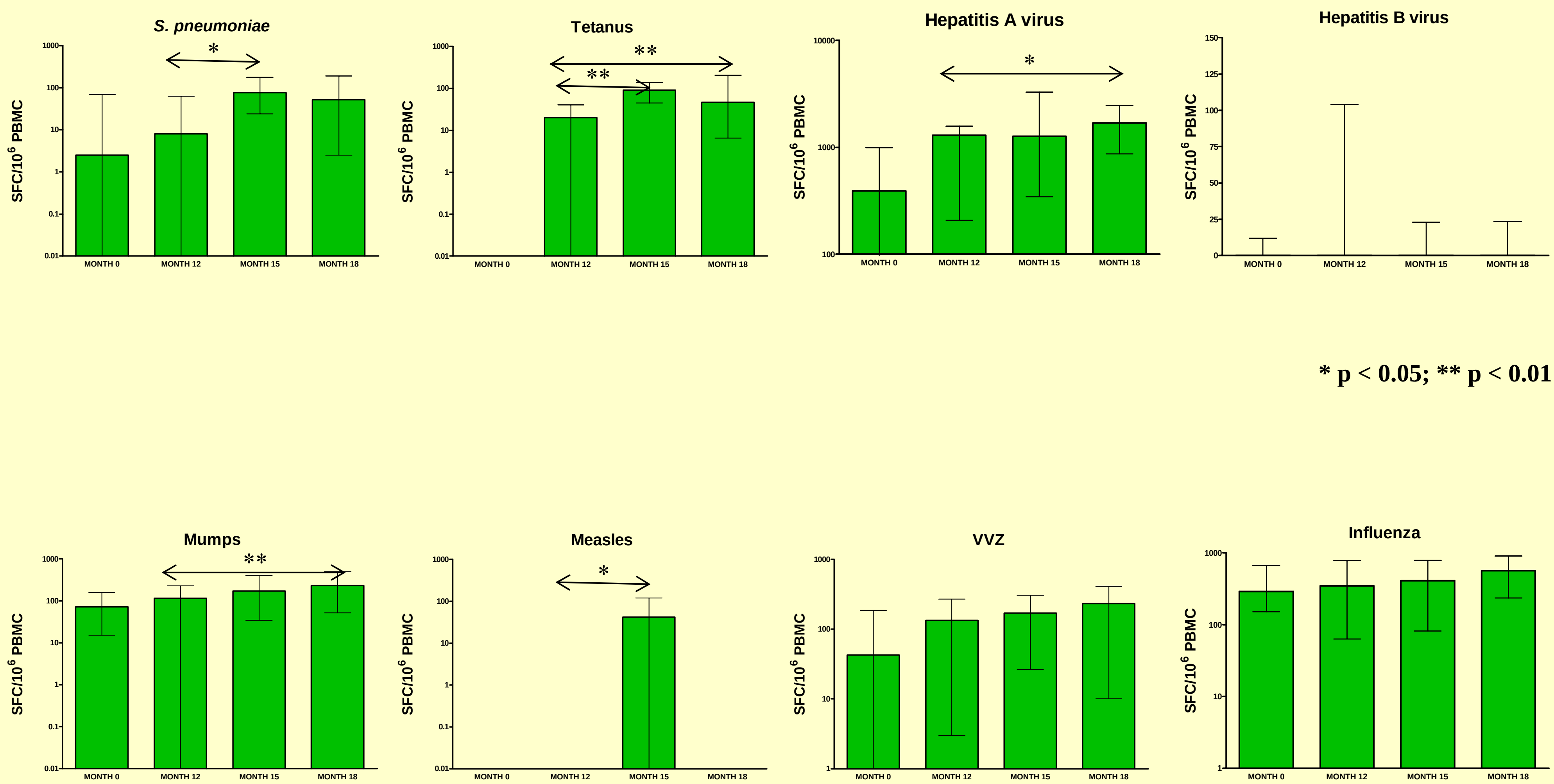


FIGURE 9: AMPLISPOT cellular responses in the whole cohort (quantitative)



CONCLUSIONS

- Interrupting HAART may cause dysfunction in acquired humoral responses to vaccines, even decreasing antibody titers to “unprotective” levels in some patients.

- Clinical consequences of these results should be evaluated in large randomized studies.

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