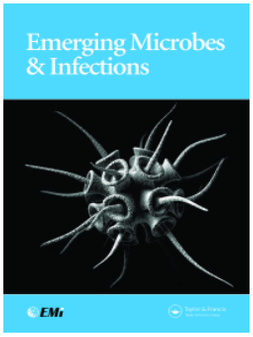

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SARS-CoV-2 mRNA vaccine BNT162b2 triggers a consistent cross-variant humoral and cellular response

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ABSTRACT

As the SARS-CoV-2 pandemic continues to rage world-wide, the emergence of numerous variants of concern (VOC) represents a challenge for the vaccinal protective efficacy and reliability of commercially available high-throughput immunoassays. Our study demonstrates that, the administration of two doses of BNT162b2 vaccine elicited a robust SARS-CoV-2-specific immune response assessed up to three months after full vaccination in a cohort of 37 health care workers (HCWs). SARS-CoV-2 specific antibody response, evaluated by four commercially available chemiluminescence immunoassays (CLIA), was qualitatively consistent with the results provided by the gold-standard in vitro neutralization assay (NTA). However, we could not observe a correlation between the quantity of antibody detected by CLIA assays and their neutralizing activity tested by NTA. Almost all subjects developed a SARS-CoV-2 specific T cell response. Moreover, vaccinated HCWs developed a similarly protective neutralizing antibodies response against the EU (B.1), Alpha (B.1.1.7), Gamma (P.1), and Eta (B.1.525) SARS-CoV-2 variants, while Beta (B.1.351) and Delta (B.1.617.2) strains displayed a consistent partial immune evasion. These results underline the importance of a solid vaccine-elicited immune response and a robust antibody titer. We believe that these relevant results should be taken into consideration in the definition of future vaccinal strategies.

INTRODUCTION

At the end of 2019 a new coronavirus, SARS-CoV-2, was firstly described in Wuhan, China, as being responsible for pneumonia within the scenario of a new disease: coronavirus disease 2019 (COVID-19). To contain COVID-19 spreading, countries worldwide have adopted several long-lasting rigorous restrictions but the dramatic and unpredictable health, social and economic consequences undermine the compliance to further stringent lockdowns. The acquisition of immunity by vaccination represents, therefore, the most promising chance to contain COVID-19 pandemic.

The fine-tuning of COVID-19 vaccines has been extraordinary rapid and timely, and has being demonstrating to be safe and largely proficient in controlling symptomatic SARS-CoV-2 infections; however, the precise kinetic of SARS-CoV-2- specific immune responses, elicited by vaccination, needs to be further addressed. Data produced so far suggest that virus-specific neutralizing antibodies targeting the receptor binding domain (RBD) of the spike (S) protein decay to some extents over a period of several months [1]. Nonetheless, they remain detectable [2] and nearly all vaccinated re-infected or breakthrough infected subjects show mild symptoms, suggesting that vaccine-driven immune memory results in protection from severe COVID-19.

The appearance of SARS-CoV-2 strains displaying mutations in the different spike protein domains, raises some worries on the possibility of evading vaccination-induced neutralizing antibodies. These variants include: the B.1.1.7 (α) strains firstly detected in the United Kingdom and now spread worldwide; the B.1.351 (β) and P.1 (γ) lineages identified in South Africa and Brazil, respectively; the B.1.525 firstly identified in Nigeria (η); and the B.1.617.2 strain recently isolated in India (δ). The Alpha strain developed a non-synonymous mutation at amino acid 501 (N501Y); the Beta and Gamma variants additionally acquired amino acid replacements at 417 and 484 positions (K417N/T, E484K), among others, shared by the Eta variant as well; while the Indian Delta variant overall displays the following mutations: T19R, 156del, 157del, R158G, L452R, T478K, D614G, P681R, D950N [3]. Besides, several mutations raised in the N-terminal domain (NTD) of these lineages, implicating an *in-vivo* selective pressure on the RBD and NTD sites. The appearance of these variants, with potential lowered susceptibility to antibody responses, could challenge the effectiveness of the worldwide vaccinal campaign, as already documented in the scientific literature [4–13].

Likely, alongside humoral immunity, vaccine-induced immunological memory relies also on the induction of cellular immunity. This is driven by $CD4^+$ and $CD8^+$ T cells, which employ different protective strategies contributing to the control of SARS-CoV-2 infection. Studies documented a protective T cell response in COVID-19 patients and the reported presence of SARS-CoV-2-specific T cell reactivity in uninfected subjects raises remarkable queries concerning cross-reactivity due to previous infections with other coronaviruses.

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Wide-ranging researches on the cellular immune response to vaccination and its duration are currently under investigation as well as its role in cross-protection to the new emerging viral variants.

Herein, we assessed humoral immune response against SARS-CoV-2 European strain (lineage B.1) on serum from 37 BNT162b2 mRNA-vaccinated health care workers (HCWs), who were never infected by SARS-CoV-2. For each subject enrolled analyses were performed over a 3-month span of time from the second vaccine dose. At each time point results obtained employing 4 different chemiluminescence immunoassays (CLIA) assays were compared to those derived by gold standard virus neutralization test requiring live pathogen [14]. At T5 (30 days after II dose), collected serum were tested even against Alpha, Beta, Gamma, Delta and Eta variants, while T cell response was assessed by QuantiFERON assay at T6 (90 days after II dose).

MATERIAL AND METHODS

Study design

An observational, longitudinal prospective study was designed to evaluate the development of immune response in infection naïve HCWs induced by BNT162b2 (Comirnaty) anti-SARS-CoV-2 vaccine; vaccine was administered according to the Pfizer vaccination schedule: II dose administered 21 days after I. The primary end-point of the study was to characterize the development of SARS-CoV-2-specific Neutralizing Antibodies, monitoring immunoglobulin kinetic at consecutive time-points: one day before vaccination (T0), 10 days after I dose (T1), 20 days after I dose (T2), 10 days after II dose (T3), 20 days after II dose (T4), 30 days after II dose (T5) and 90 days after II dose (T6); evaluation was conducted analyzing subjects serum samples with 4 different CLIA. Secondary end-points were: i) evaluation of effective neutralization against main relevant SARS-CoV-2 variants; ii) agreement among different CLIA assays and the gold-standard Neutralization Assay (NTA); iii) assessment of specific T-cell response against viral S protein by means of Interferon-γ Release Assay (IGRA) at T6. Study design is summarized in Figure 1.

SARS-CoV-2 Ab measurement

All collected serum samples were analysed using four different CLIA assays: LIAISON® SARS-CoV-2 S1/S2 IgG (311450 - DiaSorin, Saluggia, Italy) to measure antibodies against the SARS-CoV-2 native S1/S2 proteins, while iFlash-2019-nCoV Nab (C86109 - Shenzhen YHLO Biotech Co, Shenzhen, China), LIAISON® SARS-CoV-2 TrimericS IgG (P/N311510 - DiaSorin, Saluggia, Italy) and Elecsys Anti-SARS-CoV-2 S (09 289 267 190 - Roche Diagnostics Rotkreuz, Switzerland) to quantify the specific RBD-binding antibodies. Moreover, samples collected at T0 and T5 were analyzed with iFlash SARS-CoV-2 IgG and IgM (C86095G – C86095M - Shenzhen YHLO Biotech Co, Shenzhen, China) to exclude a possible ongoing asymptomatic infection since that the

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assay targets Nucleocapside and Spike proteins (non-neutralizing antibodies). All the assays characteristics are summarized in Table 1.

NTA was used to quantify the titer of neutralizing antibody for all samples at each time point using the SARS-CoV-2 lineage B.1 (EU): results were considered positive if higher or equal to 1:10 serum titre [15,16].

Furthermore, the serum samples collected at one month after II dose (T5) were used to evaluate the *in vitro* neutralizing response against the different isolated variants. Further details are reported in the Supporting Information.

SARS-CoV-2 variants

SARS-CoV-2 variants, including the lineage B.1 (EU) (accession number: EPI_ISL_412973), assumed as comparator virus, as well as Alpha (lineage B.1.1.7) (accession number: EPI_ISL_1909218), Beta (lineage B.1.351) (accession number: EPI_ISL_1578464), Gamma (lineage P.1) (accession number: EPI_ISL_1578455), Delta (lineage B.1.617.2) (accession number: EPI_ISL_1970729) and Eta (lineage B.1.525) (accession number: EPI_ISL_1649798) were isolated from positive nasopharyngeal swabs (NPS). Viruses were isolated, propagate and titrated to prepare virus for neutralization assay using the permissive cell line VERO C1008 (Vero 76, clone E6, Vero E6; ATCC1CRL-1586TM). All the strains were identified by means of whole genome sequencing and the sequences were submitted to GISAID.

T-cells response evaluation

The T-cell response induced by vaccination was evaluated at T6 by a whole blood Interferon-Gamma Release immuno Assay (IGRA) using QuantiFERON® (QNF) ELISA SARS-CoV-2 assay (QIAGEN, Hilden, Germany).

Briefly, venous blood was collected in four dedicated Quantiferon® tubes, namely: Ag1 for $CD4^+$ T cell stimulation, Ag2 for $CD4^+$ plus $CD8^+$ T cell stimulation, Mitogen as positive control and Nil as negative control. Both Ag1 and Ag2 were coated with mixture of S protein peptides to induce IFN-γ production. After a 16-24 hours incubation at 37° C, tubes were centrifuged and the separated plasma was analyzed by a microplate of enzyme-linked immunosorbent assay (ELISA) for IFN-γ dosage: a value of 0.15 IU/ml was used as positive cut-off. The QNF ELISA is for research use only.

Twenty unvaccinated healthy controls with no nucleocapside and spike antibodies were used as controls (HCs).

Statistical analysis

In descriptive statistics, data mean and standard deviation (sd) and median and range were used for quantitative variables with Gaussian and non-Gaussian distribution, respectively; as well as numbers and

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percentages for qualitative variables. Ratios between NTA and NTA of variants were described as geometric mean. Quantitative variables association was evaluated through Pearson correlation coefficient r. Intraclass correlation coefficient (ICC) among NTA and Ab measurements (or positivity) was calculated after fitting mixed models (with patients and time as random factors) to take into account the clustered nature of the data. Assay's sensitivity and specificity were calculated using NTA as gold-standard. Statistical analyses were perfor med by means of STATA software (StataCorp LLC, Texas, USA) and MedCalc (MedCalc Software Ltd, Ostend, Belgium). Differences were considered significant when P value < 0.05.

RESULTS

A total of 37 SARS-CoV-2 infection naïve HCWs vaccinated between December 28, 2020, and February 23, 2021 was enrolled. Among them, 9 were males and 28 females with a media age of 40 (range 24 - 65) years. All the enrolled HCWs were tested for the presence of non-neutralizing anti-SARS-CoV-2 N and S Subunits (NNAs), in order to check for previous SARS-CoV-2-infection. None of them resulted to be SARS-CoV-2 N and S antigens experienced neither at T0, nor T5 (supporting information).

Antibody response in SARS-CoV-2 vaccinated HCWs

Results of the kinetics of systemic humoral response elicited by vaccine showed that a weak production of SARS-CoV-2 specific antibodies (Fig. 2A) was detectable in nearly 50% of the enrolled HCWs since 10 days post the first vaccine dose (T1) (Fig. 2B). The percentage of subjects resulting positive to the antibodies increased at T2 above 90% and stabilized at 100% for the following timepoints (Fig. 2B). The antibody titer reached the climax 10 days after the second dose (T3), then to gradually decrease over time (Fig. 2A).

A similar trend was observed for neutralizing antibodies analysed by NTA: 12/37 (32.4%) HCWs developed detectable SARS-CoV-2 NT antibodies at T1 (mean±sd: 5.1±13.46), and neutralizing antibody titers increased to 81.1% at T2 (mean±sd: 30.3±38.19), becoming detectable in all but 7 subjects (Fig. 2B). However, neutralization antibody peak production was registered slightly later, at T4 (mean±sd: 339.5±337.75) (Fig. 2A). Overall, there was good qualitative agreement between IgG quantification by CLIA assays and NTA (Diasorin S1/S2: ICC=0.85; i-Flash: ICC =0.78; Roche Elecsys S: ICC =0.81; Diasorin Trimeric: ICC =0.81), but quantitative correlation was almost absent taking into account all the timepoints (Diasorin S1/S2: ICC =0.51; i-Flash: ICC=0.18; Roche Elecsys S: ICC =0.13; Diasorin Trimeric: ICC=0.11). No correlation with sex or age was detected with antibody production quantified by different techniques at any timepoint.

T-cell response in SARS-CoV-2 vaccinated HCWs

QuantiFERON assay was used for detecting $CD4^+$ and $CD4^+$ plus $CD8^+$ SARS-CoV-2 specific T-cell-mediated response in vaccinated subjects at T6.

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All but 4 SARS-CoV-2 vaccinated subjects (33/37, 89.2%) showed SARS-CoV-2 specific $CD4^+$ T cell specific response at T6. Whereas $CD4^+$ plus $CD8^+$ SARS-CoV-2 specific cell-mediated immunity was observed in 36/37 (97.3%) HCWs (Fig. 3A). Overall, after the complete vaccination schedule, the whole percentage of full responders (i.e. subjects developing both SARS-CoV-2 specific cellular and humoral response) was 86.5% (32/37). However, 100% of the subjects displayed at least one of the T-cell mediated cellular response (i.e. either $CD4^+$ or $CD4^+$ plus $CD8^+$) and the humoral response. As expected, none of the twenty HCs enrolled showed SARS-CoV-2 specific T-cell response (supporting information). No significant correlation was observed between age/sex and SARS-CoV-2 specific T-cell response.

Correlation between SARS-CoV-2 specific neutralizing antibodies and T-cell response

Neutralizing antibody level measured by NTA were soundly correlated with both SARS-CoV-2 specific $CD4^+$ ($p=0.0001$) (Fig. 3B) and SARS-CoV-2 specific $CD4^+$ plus $CD8^+$ (Fig. 3C) T-cell response ($p=0.0005$) at T6. Conversely, no significant correlation was observed between SARS-CoV-2 specific T-cell response and antibody quantification measured by any of the CLIA assays, as representatively depicted for i-Flash assay in figure 3D and 3E and in Supplementary figure 1 (Fig. S1) for all the other CLIA tests.

Neutralizing antibody response against SARS-CoV-2 variants

Sera collected at T5 from all vaccinated HCWs were tested by NTA against the lineage B.1 (EU), assumed as reference virus, as well as Alpha (lineage B.1.1.7), Beta (lineage B.1.351), Gamma (lineage P.1), Delta (lineage B.1.617.2) and Eta (lineage B.1.525) variants (Fig. 4A). Mean values ± sd were: 172.4±124.31 for EU strain (Fig 4), 175.7±113.2 for Alpha (Fig 4B), and 114.1±93.76 and 137.6±93.36 for Gamma (Fig. 4D) and Eta (Fig 4F) strains, respectively. Conversely, a reduction of approximatively 55.6% and 86.3% of neutralizing antibody titers was observed from EU to Delta and Beta strains, respectively (Fig. 4E and 4C) (mean values ± sd: Delta, 76.5±52.08; Beta 23.5±19.89). Specifically, there was a 7.3-fold reduction in the neutralization titers against the Beta variant, whereas the fold decline was 2.4 against the Delta strain (Figure 4). Although at lower titers, neutralizing antibodies against the Beta and Delta variants were still detectable in 35/37 (94.5%) and 36/37 (97.2%) subjects, respectively. As a whole, the neutralization ability of Pfizer/BioNTech vaccine immune sera against the EU variant was maintained to that against the Alpha ($p=0.0005$), Gamma ($p=0.0031$) and Eta ($p=0.0043$) variants, but significantly declined in comparison to that against the Beta ($p=0.148$) and Delta ($p=0.051$) variants (Fig. 5). All the other NTA correlation between variants are reported in Supplementary figure 2 (Fig. S2).

Correlation between SARS-CoV-2 specific antibodies titer (CLIA) and variant neutralization ability (NTA)

Analyses of correlation between antibody titers measured with different CLIA assays and NTA performed in SARS-CoV-2 variants revealed that none of the CLIA techniques is significantly correlated with the NTA of the whole variants panel, as representatively reported in Figure 6 for i-Flash assay and in supplementary

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figure 3 (Fig. S3) for all the other CLIA test. On the other hand, the Delta and the Eta variants NTA significantly correlated with all the CLIA tests employed (Fig. 6 and Fig. S3).

DISCUSSION

In this study, we report a longitudinal prospective and exhaustive estimation of the development of antibody triggered by the mRNA vaccine BNT162b2 for SARS-CoV-2, assessed by the use of different techniques.

Results show that antibody level was feeble or undetectable after one vaccine dose (T2) in SARS-CoV-2 naïve enrolled HCWs, mirroring previous reports investigating antibody responses in SARS-CoV-2 uninfected subjects after a single dose of SARS-CoV-2 mRNA Vaccine [17,18]. Therefore, the administration of only one dose could be not enough to prompt a broad immune defense in uninfected subjects mainly considering that a suboptimal immune response could favor the emergence of viral resistance or escape variants.

Conversely, the administration of two doses of BNT162b2 vaccine elicited a robust SARS-CoV-2-specific immune response, consistently with how previously observed [18], which was detectable both as antibody production and T cell-driven immune activation in all the enrolled subjects. The antibody titer was measured, at each time point, employing four different CLIA methodologies and tested in a NTA assay to investigate their functional neutralizing properties. Seric antibody titration is the method of choice for determining the SARS-CoV-2 serostatus, on which depend clinical diagnoses, patients' management and determination of seroprevalence within the population for epidemiologic purposes. Overall, results obtained with different techniques were all consistent among one another from a qualitative point of view, confirming that the clinical detection of antibodies targeting the neutralizing viral epitopes reliably represent the actual neutralization capacity [16]. Neutralizing antibodies evolve over time, improving the antigen binding affinity [19]. Consequently, this affinity maturation could explain the apparent discrepancy between the timing of the peak of antibody amount measured by CLIA and the neutralization capability measured by NTA. Moreover, we could not observe a correlation between the quantity of antibody detected by CLIA assays and their neutralizing activity tested by NTA. This may suggest that a NTA, relying on the employment of an actual virus, may elicit a broader antibody array binding sites outside those commonly recognized by commercial CLIA, which could contribute to virus neutralization. On the other hand, the use of completely different cut-off values and quantification ranges of *in-vitro* assays and automated tests could be ascribed for the lack of quantitative correlation of the compared assays.

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Together with the antibody titer, we evaluated the SARS-CoV-2-specific T cell response. At T6, an enhanced T cell activation was detected in all the enrolled subjects compared to uninfected non-vaccinated ones, as shown by QuantiFERON test. The intensity of such immune activation significantly correlated with the neutralization titer assessed by NTA, but oddly, we could not detect a positive correlation with the quantity of antibodies detected by CLIA. The immune response is a complex multi-faced process, in which many components partake. Although harder to evaluate in a high-throughput manner, the pivotal role that T cells cover in SARS-CoV-2-elicited immune response it is now well established, to the point that it is not uncommon to detect a low antibody titer or neutralization ability associated nonetheless with a protective response able to avoid disease progression [20–23].

In our study, antibody quantification, NTA and T-cell responses were not affected by age at any time point. However, considering that the natural involution of immune systems occurs nearby 60s [24] and only 3/37 of the enrolled subjects were above this threshold, further studies performed on larger cohort with a wider distribution of ages are necessary to confirm this data. Together with age, a wider cohort would be necessary to confirm gender-related conclusions.

A challenge we are facing at present is the emergence of new SARS-CoV-2 variants that could impair the efficacy of current vaccines as many of the mutations reside in the antigenic supersite in the NTD [25] or in the ACE2-binding site, which is a major target of potent virus-neutralizing antibodies [11,13,26,27]. We tested by neutralization assay many of the variants of concern (VOC), as defined by the CDC [3], together with the EU SARS-CoV-2. In order to have a deeper insight, we chose not to employ pseudoviruses, but rather the actual authentic isolated. Indeed, the use of lentiviral particles could provide discrepant results compared to natural strains, likely because the mock pseudoviruses cannot completely portray the biology of natural isolates.

Our results indicate that vaccinated HCWs developed a similarly protective NT antibodies response against the EU, Alpha, Gamma and Eta variant. Conversely, the Beta and the Delta strains displayed the highest immune escape, as demonstrated by the lower neutralization titer. Yet, serum from the majority of vaccinated subjects maintained a neutralizing activity against both Beta (94.5%) and Delta (97.2%) strains that could be active in avoiding the onset of severe COVID-19 symptoms. Unexpectedly, we cannot extrapolate a fully conclusive picture about the correlation between the amount of detected antibodies and the level of protection, as seen by the four CLIA methods employed and the NTA performed on variants, respectively. Indeed, the positive correlation between any of the CLIA employed and the neutralization of the EU, Alpha, Beta and Delta variants was maintained in almost any case. However, the same could not be assessed for all the Gamma and the Eta variants. Moreover, in this study we did not investigate SARS-CoV-2 specific T cell response against the different VOC. However, results reported in previous studies suggest that though the efficacy of the antibody response may be somewhat decreased by some mutations

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naturally acquired by the viral strains, the SARS-CoV-2 specific T-cell response is still preserved, thus granting for protection [23].

In a broader picture, although the Beta variant resulted to have a better immune escape ability, as demonstrated by our NTA assays, it is not the predominant variant right now. Indeed, the Delta variant, although displayed an intermediate immune escape, is by far more concerning, given the higher viral loads, the higher prevalence and the fast-paced spreading.

These results underline the importance of a solid vaccine-elicited immune response and a robust antibody titer. In turn, this let us speculate about the suitability of repeated vaccine boost doses to achieve and maintain the adequate antibody titer over-time. Although further and broader studies should be performed and no firm conclusions can be drawn due to the limited number of analyzed cases, this should be taken into consideration in the definition of vaccinal strategies. Our findings deliver an important message that should not be underestimated. Therefore, we encourage the scientific and medical communities to deeply consider which guidelines should be more appropriate.

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AUTHORS’ CONTRIBUTIONS

DM conceived the presented idea. AL further developed the project with the help of MB and CF. DM, AM, performed subject enrolment as well as samples collection. DM, AL and AM conceived, planned the experiments, under the supervision of MG. MC, GG, AG, AR, MB, FDP, MC, IB, ML, SGR, CP, SG, VM performed and analysed the experiments. ADS performed statistic analyses. GR and MRG helped with stimulating discussion. MB, CF and DM discussed the data and wrote the manuscript. MC, IC and PV critically reviewed

COMPETING INTERESTS

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The authors declare no competing interests.

ETHICS DECLARATION

The VeNA study was approved by institution Ethics Committee (Comitato Etico Milano Area 1 – N° 0028053). All the subjects signed an informed consent. We obtained informed written consent from all the subjects to perform the procedure and analysis, according to CARE guidelines and in compliance with the Declaration of Helsinki principles.

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FIGURE LEGENDS

Figure 1. Synoptic representation of the study design with timing and type of analyses.

Figure 2. Humoral response. Panel A) Anti-SARS-CoV-2 specific antibody titer overtime, quantified by four different CLIA methods and by neutraliziation assay (NTA). Panel B) Percentage of subjects detected positive to anti-SARS-CoV-2 specific antibodies overtime, quantified by four different CLIA methods and by neutralization assay (NTA). Time of vaccinations is represented by vertical dashed lines.

Figure 3. Cellular response. Panel A) T-cell mediated respone measured by QuantiFERON assay related to CD4+ T-cell only (black) or CD4+ and CD8+ T-cells together (gray). Data refer to T6 and are expressed as international unit per milliliter (IU/ml); the cut-off is 0.15 IU/ml. Panel B and C) correlation between the neutralitization assay (NTA) and cellular CD4+ and CD4++CD8+ T-cells response, respectively. Panel D and E) correlation between anti-SARS-CoV-2 specific antibodies measured by YHLO i-Flash and cellular CD4+ and CD4++CD8+ T-cells response, respectively. The correlation with the quantification of anti-SARS-CoV-2 specific antibodies measured by the other methods are depicted in figure S2.

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Figure 4. SARS-CoV-2 variants of concern (VOC). Panel A) Neutralization assay (NTA) performed at T5 on the SARS-CoV-2 lineage B.1 (EU) and 5 VOCs, α-η. Panels B-F) Comparison between the EU variant and the VOCs α-η, respectively. Lines connect the NTAs of each individual subject.

Figure 5. Neutralization assays correlations. Panel A-E) NTA correlation between the EU variant and the VOCs, respectively, performed at T5. The other correlations between variants are depicted in figure S3.

Figure 6. Correlation between anti-SARS-CoV-2 specific antibodies and neutralization assays (NTA). Panel A-F) correlation between anti-SARS-CoV-2 specific antibodies measured by YHLO i-Flash and the NTA performed on the EU variants and the VOCs, respectively, performed at T5. The correlation with the quantification of anti-SARS-CoV-2 specific antibodies measured by the other methods are depicted in figure S3

Figure S1. Correlation between the quantification of anti-SARS-CoV-2 antibodies measured by the four different methods and the CD4+ T-cell response (panels A-D) or the CD4++CD8+ T-cells response (panels E-H).

Figure S2. Correlations between the NTAs of all the different considered variants.

Figure S3. Correlations between the NTAs of all the different considered variants and the quantification of anti-SARS-CoV-2 antibodies measured by the four different methods at T5 (panels A-D, respectively).

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SUPPLEMENTARY INFORMATION

Neutralization Assay

Neutralizing antibody titres against SARS-CoV-2 obtained by Neutralization assay (NTA) was defined as follow.

Briefly, 50 µL of each serum, starting from a 1:10 dilution followed by serial two-fold series, were transferred in two wells of 96-weel microtitre plates (COSTAR, Corning Incorporated, NY 14831, USA) and mixed with 50 µL of tissue culture infecting dose 50 ($TCID_{50}$) of SARS-CoV-2. All dilutions were made in DMEM with addition of 1% penicillin and streptomycin. After 1 h incubation at 37 °C and 5% CO_2, 50 µL of $2x10^4$ Vero E6 (VERO C1008 ATCC® CRL-1586™) cells were added to each well. After 72 h of incubation at 37°C and 5% CO_2, wells were stained with 0.1% crystal violet solution (Merck KGaA, 64271 Damstadt, Germany) plus 5% formaldehyde 40% m/v (Carlo ErbaSpA, Arese (MI), Italy) for 30 min. Microtiter plates were then washed in running water. Wells were scored to evaluate the degree of cytopatic effect (CPE) compared to the virus control. Blue staining of wells indicated the presence of neutralizing antibodies. Neutralizing titre was the maximum dilution with the reduction of 90% of CPE. A positive titre was equal or greater than 1:10. Positive and negative controls were included in all test run.

Every test included serum control (1:10 dilution), cells control (Vero E6 cells alone) and viral control (three-fold series dilution).

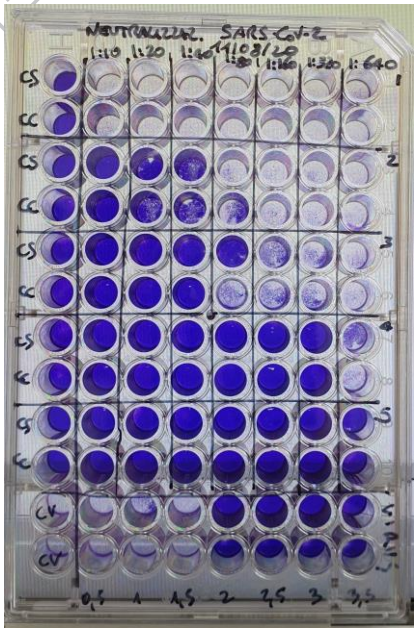

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Figure: A picture of exemplificative neutralization plate

All the samples are tested in duplicate in two-fold scalar dilution indicated on the top of the plate (1:10, 1:20, 1:40, 1:80, 1:160, 1:320 and 1:640). Blue staining of wells indicated the presence of neutralizing antibodies; CS, serum control; CC, cell control; CV, virus control; 1-digit numbers on the right of the plate are pts identification numbers; 11/08/20 on the top of the plate indicate the assay date (August,11, 2020).

iFlash SARS-CoV-2 IgG and IgM

All samples collected at T0 and T5 were analyzed with iFlash SARS-CoV-2 IgG and IgM (Shenzhen YHLO Biotech Co, Shenzhen, China) to exclude a possible ongoing asymptomatic infection since that the assay targets antibodies anti Nucleocapside and Spike proteins (non-neutralizing antibodies). Table 1 reports the assay results.

	One day before vaccination (T0)		Thirty days after II dose (T5)	
	iFlash SARS-CoV-2 IgG and IgM (YHLO)			
n° subjects	Neg < 10 AU/mL Pos > 10 AU/mL	Neg < 10 AU/mL Pos > 10 AU/mL	Neg < 10 AU/mL Pos > 10 AU/mL	Neg < 10 AU/mL Pos > 10 AU/mL
	IgG	IgM	IgG	IgM
1	0.1	0.2	0.2	0.5
2	0.2	0.3	0.6	1.2
3	0.1	0.2	0.5	0.5
4	0.1	0.2	0.2	0.4
5	0.1	0.5	0.3	2.2
6	0.1	0.2	0.5	0.6
7	0.1	0.2	0.2	0.6
8	0.1	0.2	0.4	0.6
9	0.1	0.2	0.3	0.4
10	0.1	0.1	0.2	0.5
11	0.2	0.2	0.7	0.4
12	0.2	0.2	0.3	0.6
13	0.1	0.1	0.4	0.4
14	0.1	0.1	0.2	0.4

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15	0.1	0.1	0.4	0.4
16	0.1	0.2	0.3	0.5
17	0.1	0.2	0.2	0.7
18	0.1	0.2	0.3	0.5
19	0.1	0.2	0.3	0.5
20	0.1	0.2	0.3	0.5
21	0.3	0.7	0.2	0.6
22	0.1	0.2	0.2	1.2
23	0.4	0.7	0.5	0.7
24	0.5	0.6	0.5	1.0
25	0.1	0.2	0.2	0.8
26	0.2	0.2	0.3	0.4
27	0.2	0.2	0.3	0.3
28	0.1	0.1	0.5	1.7
29	0.1	0.2	0.4	0.8
30	0.8	3.9	0.3	0.4
31	0.3	0.4	0.3	0.4
32	0.1	0.2	0.3	0.5
33	0.1	0.2	0.6	0.5
34	0.2	0.4	0.3	1.5
35	0.1	0.3	0.7	0.8
36	0.2	0.2	0.3	0.4
37	0.1	0.2	0.3	0.4

Table 1: iFlash SARS-CoV-2 IgG and IgM assay results are reported in table. Negative and Positive cut-off is 10 AU/mL for both.

QuantiFERON® (QNF) SARS-CoV-2 (QIAGEN, Hilden, Germany)

To evaluate the $CD4^+$ and $CD8^+$ T cell stimulation after vaccination we quantified the IFN-γ by means of QuantiFERON® (QNF) SARS-CoV-2.

Twenty unvaccinated healthy controls with no nucleocapside and spike antibodies were used as controls (HCs); the results are reported in table 2.

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The T cell response is calculated by subtracting the Nil tube value from the Ag tube value (Ag−Nil). A value of 0.15 IU/ml was used as positive cut-off.

QuantiFERON® SARS-CoV-2			
n° unvaccinated subjects	**Target**	**Result (IU/mL)**	**Ag-Nil**
1	**Nil**	0.06	
	Ag1	0.04	**-0.02**
	Ag2	0.05	**-0.01**
	Mitogen	> 10	
2	**Nil**	0.11	
	Ag1	0.09	**-0.02**
	Ag2	0.1	**-0.01**
	Mitogen	8.89	
3	**Nil**	0.09	
	Ag1	0.07	**-0.02**
	Ag2	0.06	**-0.03**
	Mitogen	> 10	
4	**Nil**	0.08	
	Ag1	0.06	**-0.02**
	Ag2	0.07	**-0.01**
	Mitogen	> 10	
5	**Nil**	0.07	
	Ag1	0.04	**-0.03**
	Ag2	0.08	**0.01**
	Mitogen	> 10	
6	**Nil**	0.11	
	Ag1	0.07	**-0.04**
	Ag2	0.1	**-0.01**
	Mitogen	> 10	
7	**Nil**	0.09	

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	Ag1	0.06	**-0.03**
	Ag2	0.1	**0.01**
	Mitogen	> 10	
8	**Nil**	0.1	
	Ag1	0.07	**-0.03**
	Ag2	0.1	**0**
	Mitogen	> 10	
9	**Nil**	0.08	
	Ag1	0.07	**-0.01**
	Ag2	0.09	**0.1**
	Mitogen	> 10	
10	**Nil**	0.11	
	Ag1	0.07	**-0.04**
	Ag2	0.1	**-0.01**
	Mitogen	> 10	
11	**Nil**	0.11	
	Ag1	0.06	**-0.05**
	Ag2	0.1	**-0.01**
	Mitogen	> 10	
12	**Nil**	0.07	
	Ag1	0.05	**-0.02**
	Ag2	0.1	**0.03**
	Mitogen	> 10	
13	**Nil**	0.06	
	Ag1	0.03	**-0.03**
	Ag2	0.05	**-0.01**
	Mitogen	> 10	
14	**Nil**	0.08	
	Ag1	0.05	**-0.03**
	Ag2	0.09	**0.01**
	Mitogen	> 10	
15	**Nil**	0.1	
	Ag1	0.07	**-0.03**

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	Ag2	0.1	**0**
	Mitogen	> 10	
16	**Nil**	0.07	
	Ag1	0.07	**0**
	Ag2	0.08	**0.01**
	Mitogen	> 10	
17	**Nil**	0.06	
	Ag1	0.04	**-0.02**
	Ag2	0.08	**0.02**
	Mitogen	> 10	
18	**Nil**	0.11	
	Ag1	0.08	**-0.03**
	Ag2	0.11	**0**
	Mitogen	> 10	
19	**Nil**	0.1	
	Ag1	0.07	**-0.03**
	Ag2	0.11	**0.01**
	Mitogen	> 10	
20	**Nil**	0.08	
	Ag1	0.06	**-0.02**
	Ag2	0.09	**0.01**
	Mitogen	> 10	

Table 2 : Table shows QuantiFERON® results of twenty unvaccinated healthy subjects

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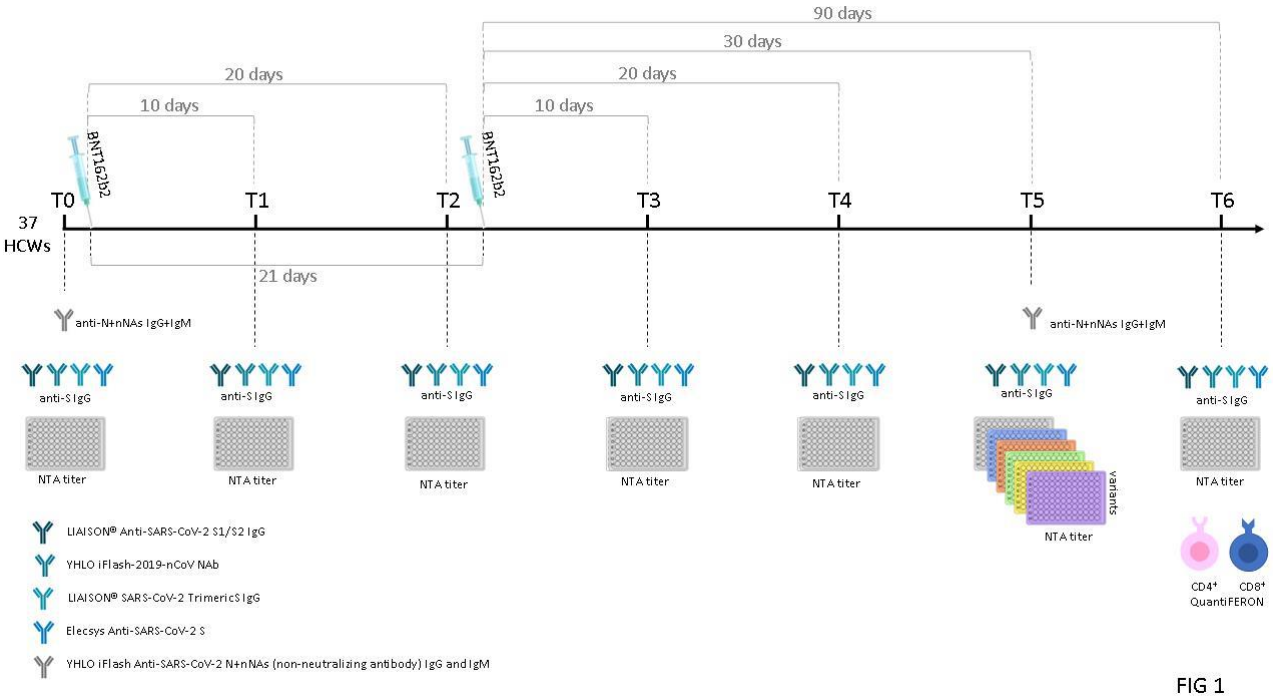

FIG 1

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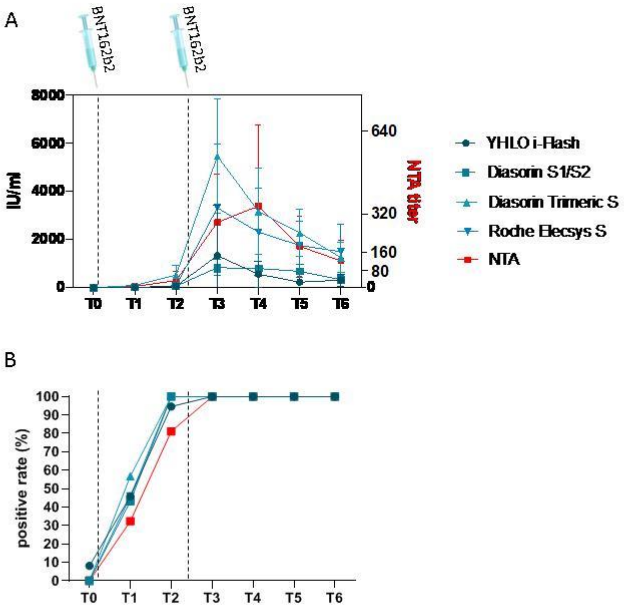

FIG 2

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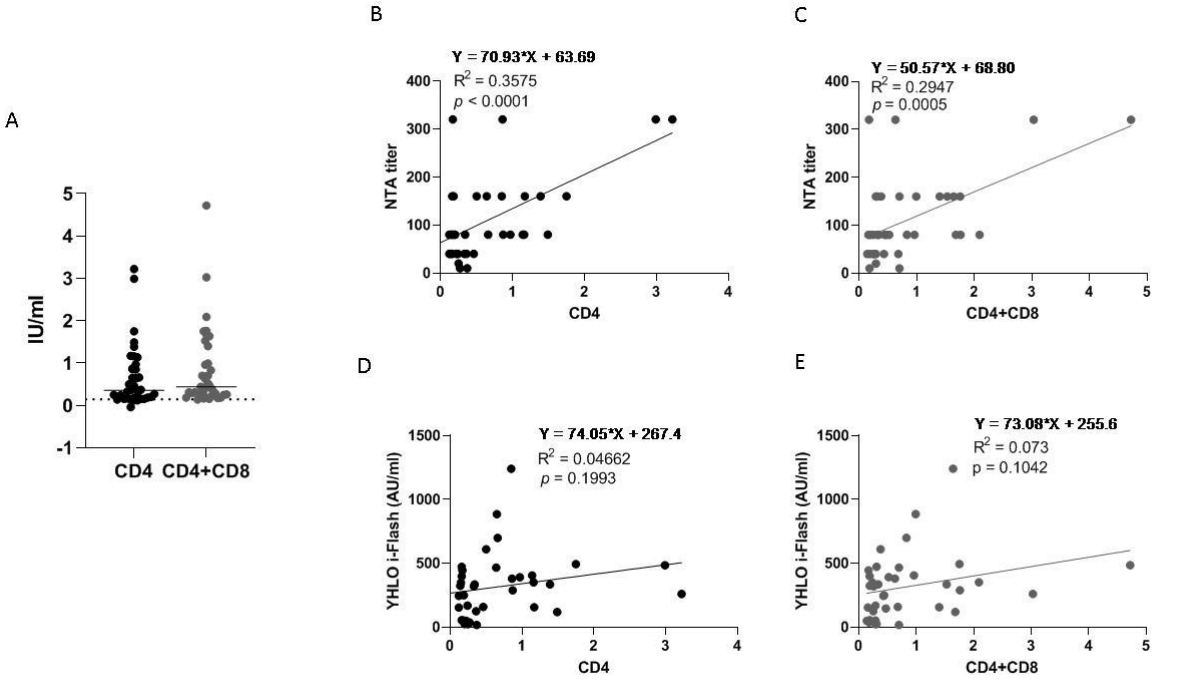

FIG 3

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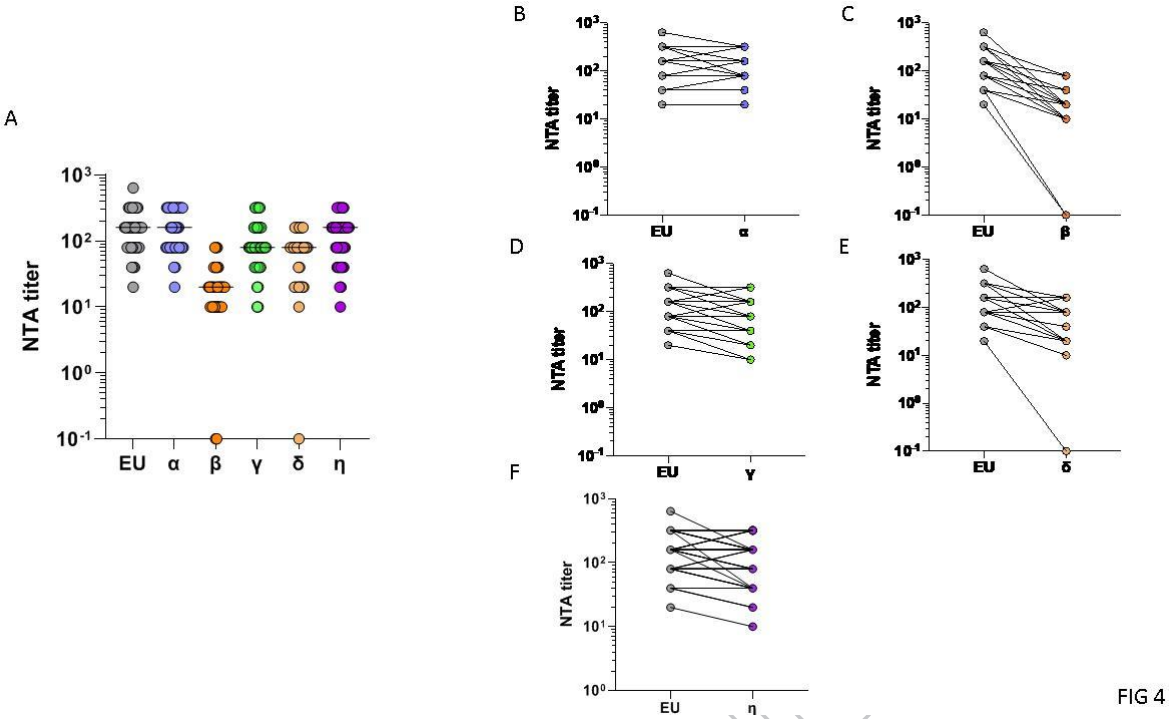

FIG 4

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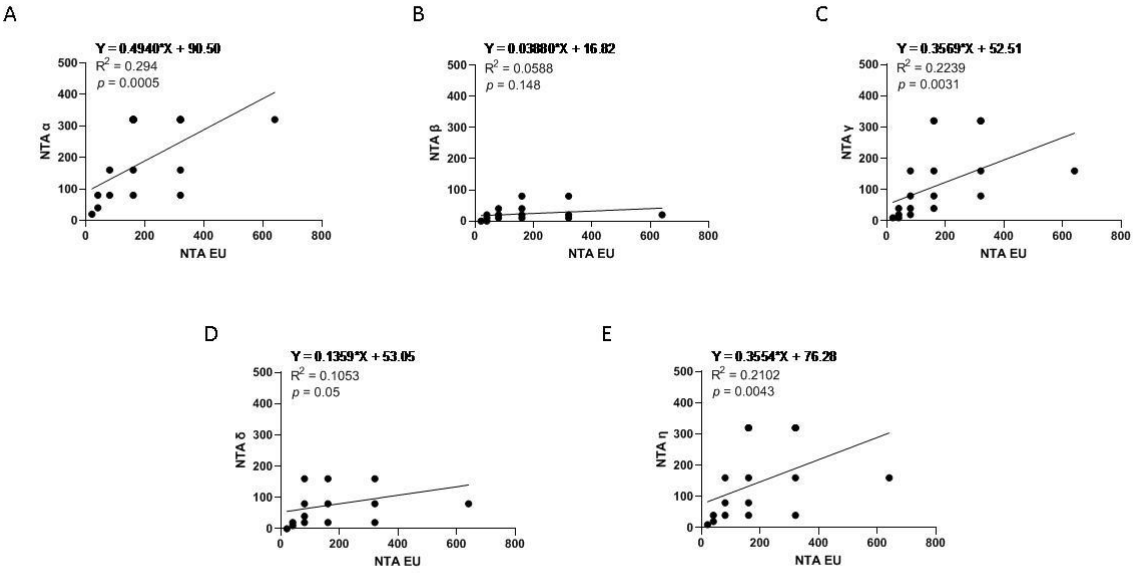

FIG 5

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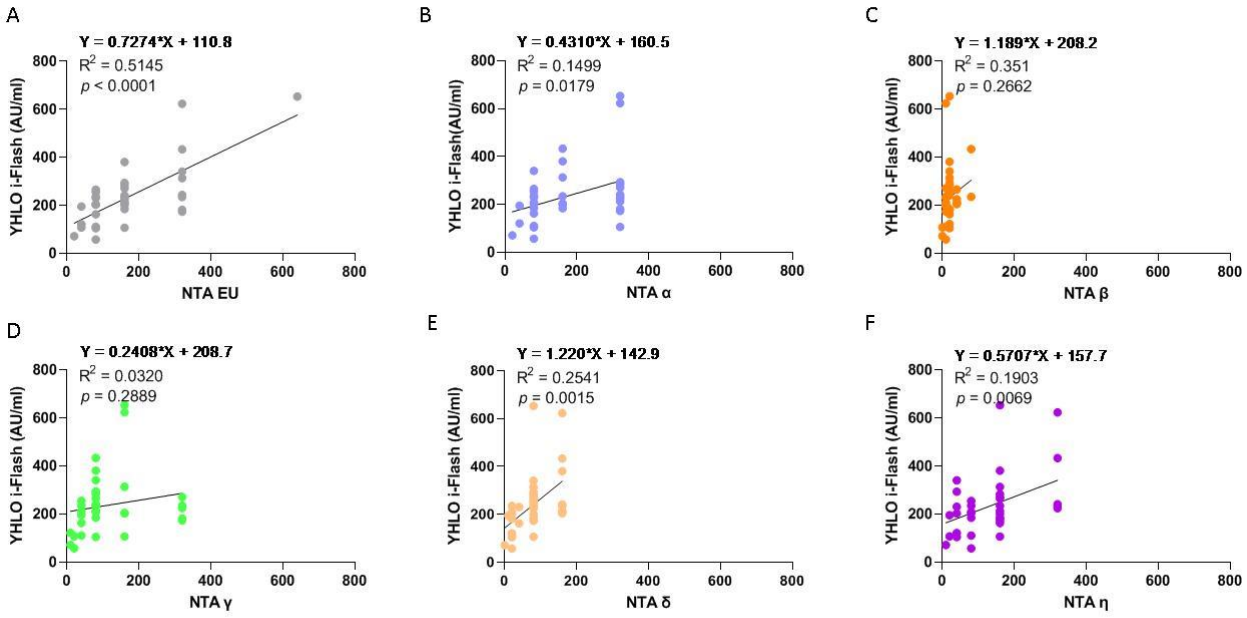

FIG 6

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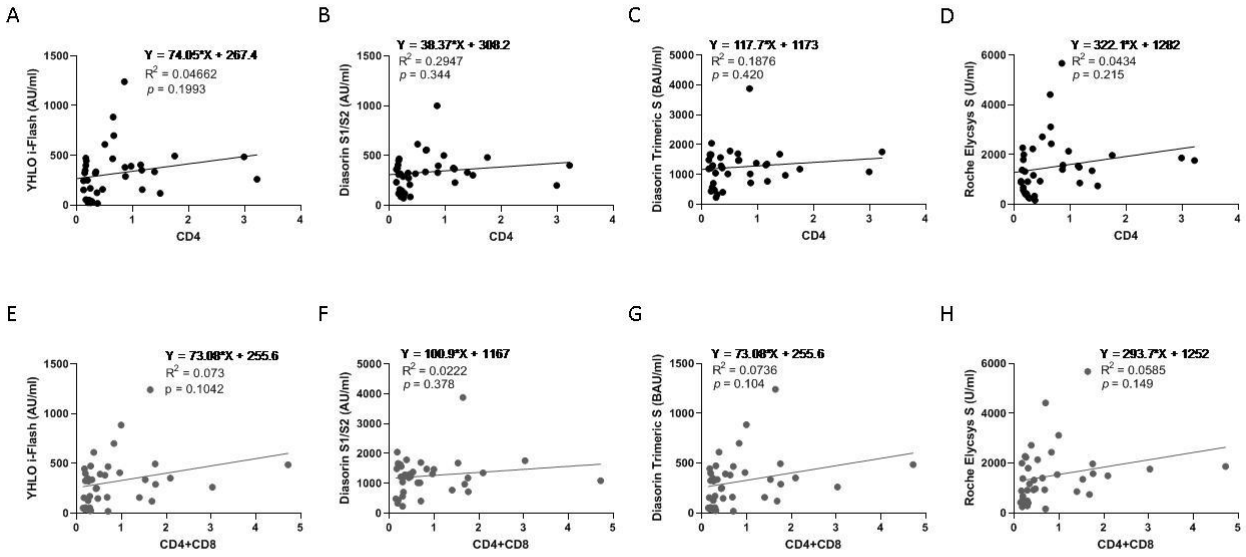

FIG S1

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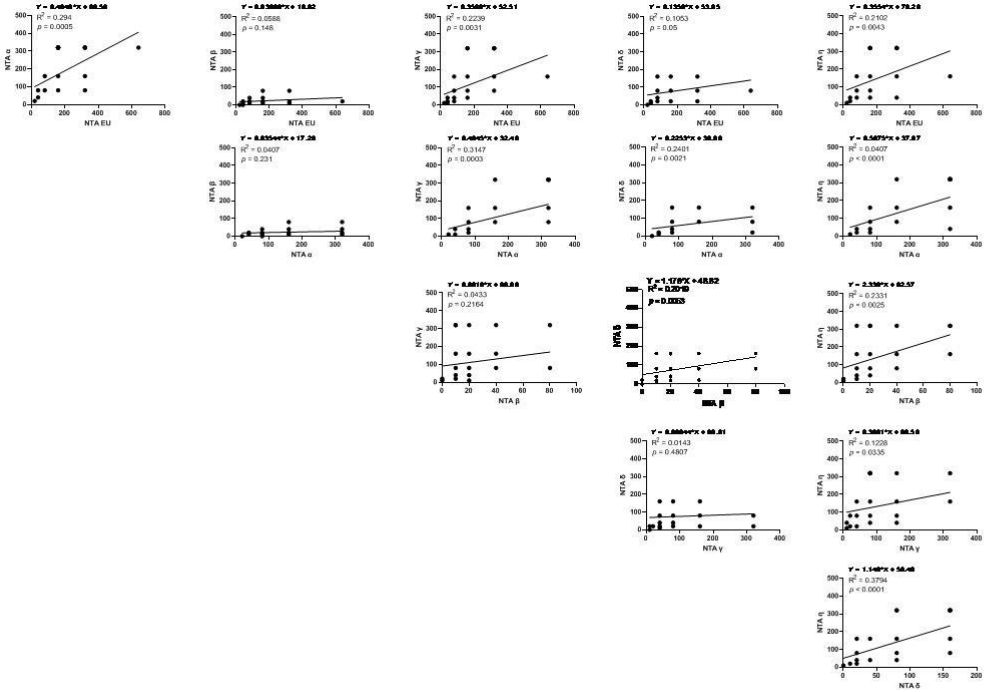

FIG S2

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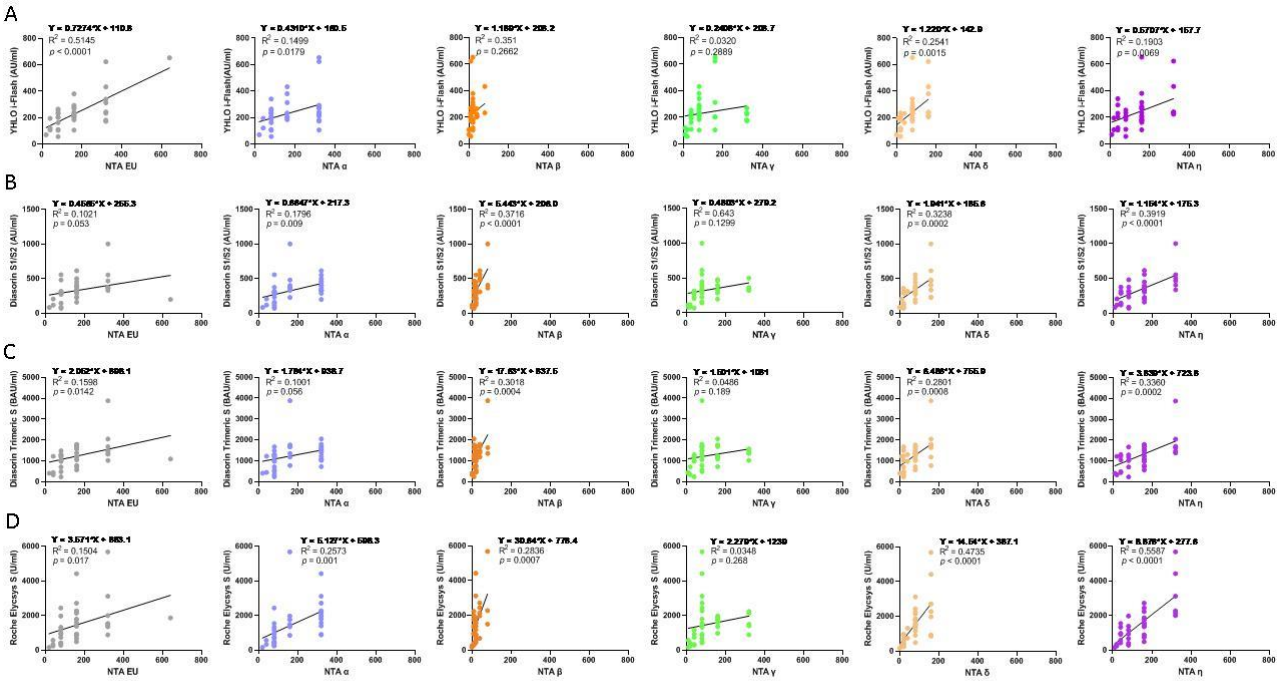

FIG S3

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