



OPEN Harmonization of IgG antibody values against the SARS-CoV-2 Spike protein

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The COVID-19 pandemic led to the rapid development of vaccines and diagnostic tests. To evaluate the response of IgG antibodies against the SARS-CoV-2 Spike protein in healthcare workers, a cohort study (2020–2022) was conducted in three Portuguese hospitals. Each hospital used a different assay (Abbott CMIA, Roche Elecsys ECLIA, Siemens ADVIA Centaur), which presents challenges to comparability. This study aimed to harmonize anti-S IgG levels across hospitals using a mathematical approach for combined analysis, and to estimate anti-S IgG levels over time, thus better understanding immunity dynamics. Antibody levels were measured at six time points: before vaccination, after full vaccination, 3, 6, and 12 months after the second dose, and after booster. Various harmonization strategies were tested, including WHO international conversion and quantile interpolation, followed by Deming regression. Quantile interpolation plus regression proved to be more effective than WHO conversion, preserving each hospital's data distribution and aligning the harmonized values with the Abbott CMIA used as the reference scale. After harmonization, antibody titers followed the expected trend: a sharp rise post-vaccination, a gradual decline over 12 months (659.32 BAU/mL before booster), and a notable increase after the booster (25,919.89 BAU/mL). Although cross-laboratory validation was lacking, this approach proved practical, reproducible, and promising for multicenter studies that integrate serological data from diverse platforms, at the European and global levels.

Keywords Harmonization, Healthcare workers, IgG antibody, SARS-CoV-2, Quantile interpolation, Deming regression

In 2019, an outbreak of coronavirus disease (COVID-19) was declared a Public Health Emergency of International Concern¹. To address this public health crisis, various diagnostic tests and vaccines were developed to prevent and mitigate COVID-19². Due to close contact with COVID-19 patients, healthcare workers (HCW) face a higher risk of infection³. Given the close and prolonged contact with patients with COVID-19, healthcare professionals in hospitals are at higher risk of infection compared to the general population³. Vaccinating this group is also essential to protect vulnerable individuals who come into close contact with them, and to reduce transmission, as a considerable proportion of hospitalized COVID-19 patients acquire the infection within the hospital³. As with any other intervention, it is essential to monitor vaccine performance to assess its risks, benefits, and effectiveness against emerging variants, both in the general population and among HCW. Measurement of IgG antibodies against the SARS-CoV-2 Spike protein is one approach to monitoring immunity over time and is particularly useful to characterize the immune response to both natural infection and vaccination⁴. However, the rapid development of laboratory methods to detect SARS-CoV-2 antibodies led to the creation of independent assays by different manufacturers, resulting in outcomes that are not directly comparable⁵.

Recognizing the need for a standardized approach to harmonize serological data, the WHO established an international standard that allows for comparability by converting results into Binding Antibody Units per

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milliliter (BAU/mL)^{6,7}. This conversion involves calibrating serological assays in International Units (IU) and BAU using the WHO International Standard for anti-SARS-CoV-2 immunoglobulin, which serves as a common reference for quantifying antibody binding and neutralizing activity⁸. This calibration reduces interlaboratory variation and facilitates comparison between different methods, enabling a uniform interpretation of serological data⁸. However, several studies have shown that this conversion presents some inaccuracies, highlighting the need for additional methods beyond those recommended by the WHO². Although some studies report good agreement between assays and calibrated values⁷, others indicate significant discrepancies, with certain tests producing results substantially different from the calibrated reference value^{9,10}.

These limitations underscore the importance of developing and applying additional robust harmonization strategies to improve the comparability of serological results across different assays. In this context, our study addresses this gap by rigorously applying and evaluating advanced statistical methodologies to harmonize antibody titers from different hospital centers, ensuring reliable cross-assay comparisons. Given this, the main objective of the present study is to convert the serological data corresponding to anti-S SARS-CoV-2 IgG antibody levels, determine the best harmonization approach, ensure consistency and uniformity among the datasets collected from three hospital centers in Portugal, and assess antibody levels over the different time points.

Materials and methods

Study setting

In 2021, a prospective cohort study was implemented among healthcare professionals in hospital settings to assess the vaccine effectiveness against symptomatic SARS-CoV-2 infection¹¹. Within this study, a total of 3034 healthcare workers (HCW), all eligible for COVID-19 vaccination, were included from a previous cohort study conducted at three Portuguese hospital centers, and systematic serological IgG anti-S SARS-CoV-2 testing was performed. Although the study followed participants prospectively, data regarding the pre-vaccination period (prior to December 2020) were collected retrospectively. As such, the study period was defined as spanning from 2020 to 2022. The study was coordinated by the Department of Epidemiology of the Instituto Nacional de Saúde Doutor Ricardo Jorge (INSA), in collaboration with three hospital centers: Centro Hospitalar de Lisboa Ocidental (CHLO), Centro Hospitalar Tondela-Viseu (CHTV), and Centro Hospitalar e Universitário de Coimbra (CHUC)¹¹. In this context, serological data were collected, specifically IgG antibodies against the SARS-CoV-2 Spike protein (anti-S SARS-CoV-2 IgG), to evaluate the immunity conferred by vaccination. According to the study protocol, and to assess the evolution of the immune response to vaccination, six distinct time points were defined for the collection of serological samples¹¹:

1. Time point 1: Pre-vaccination (CHUC)
2. Time point 2: Post-vaccination (CHUC, CHLO)
3. Time point 3: 3 months after vaccination (CHUC, CHLO)
4. Time point 4: 6 months after vaccination (CHUC, CHLO, CHTV)
5. Time point 5: 12 months – Before booster (CHUC, CHLO)
6. Time point 6: Post-booster (CHUC, CHLO, CHTV)

Antibody levels are expected to follow the pattern described in systematic reviews, which show that they peak within the first three months after vaccination and tend to decline progressively over time¹². Additionally, studies assessing the impact of revaccination on antibody dynamics have confirmed that administering booster doses is an effective strategy to enhance immunity against SARS-CoV-2¹².

For antibody measurement, the three hospital centers adopted different laboratory methodologies, namely:

1. CHUC: CMIA–SARS-CoV-2 IgG II Quant assay (Abbott, AU/mL) method was used to quantify IgG antibodies against SARS-CoV-2. SARS-CoV-2 reagents (Abbott, Rungis, France) were used for the qualitative detection of anti-N IgG antibodies (targeting the SARS-CoV-2 nucleocapsid protein), and the SARS-CoV-2 IgG II Quant (Abbott, Rungis, France) was used for the quantification of anti-S IgG antibodies (targeting the Spike protein of SARS-CoV-2)¹³.
2. CHLO: Elecsys® Anti-SARS-CoV-2 method: The Elecsys® Anti-SARS-CoV-2 method, an automated ECLIA (Electrochemiluminescence Immunoassay), was used for the quantitative detection of total antibodies against SARS-CoV-2 in human plasma¹⁴. The anti-N assay detects IgG, IgA, and IgM antibodies. For anti-RBD antibody detection, the Elecsys® Anti-SARS-CoV-2 assay was used, with additional measurement after 1:50 dilution in samples with high values. Detection of IgG, IgM, and IgA against the Spike protein was performed using a direct ELISA (Enzyme-Linked Immunosorbent Assay) method adapted from Amanat et al.^{15,16}.
3. CHTV: The ADVIA Centaur® SARS-CoV-2 IgG (sCOVG) method, a two-step chemiluminescent immunoassay, was used for the qualitative and semi-quantitative detection of IgG antibodies specific to SARS-CoV-2. The assay targets the receptor-binding domain (RBD) of the S1 subunit of the Spike protein and was performed on human serum or lithium heparin plasma¹⁷.

All procedures implemented in this cohort study were based on the Guidance Document “Cohort study to measure COVID-19 VE among health workers in the WHO European Region”¹⁸. All HCWs were invited to participate by an email sent by the occupational health service of each hospital. After accepting to participate and signing a written consent form, each HCW answered an enrolment questionnaire implemented in the REDcap platform¹⁹. Written informed consent was obtained from all study participants after being informed that participation was voluntary and that he/she could withdraw from the study at any time without justification.

or consequences, particularly about the offer of vaccination. All data were anonymised before statistical analysis by the occupational health services of each hospital. The study protocol was approved by the ethics committee of the Instituto Nacional de Saúde Doutor Ricardo Jorge (date of approval: 16 April 2021) and by the ethics committees of the two participating hospitals, namely by the ethics committee of Centro Hospitalar Tondela-Viseu (CHTV) (Ref.06/16/04/2021, date of approval 16 April 2021) and Centro Hospitalar Lisboa Ocidental (CHLO) (Ref. 2141, date of approval 19 May 2021).

Statistical methods

To ensure the most appropriate approach for converting serological data, several techniques were applied. Although the conversion established by the WHO has some inaccuracy, we nonetheless considered its application as one of the approaches during the process. For this purpose for the first approach, we used the WHO Second International Standard for anti-SARS-CoV-2 immunoglobulin, developed by the WHO Expert Committee on Biological Standardization in October 2022 (WHO/BS/2022.2427)²⁰. The alignment coefficients of the international standard, declared by the manufacturers as perfectly linear, are, respectively^{7,21}:

Abbott Architect SARS-CoV-2 IgG II Quant assay: 1 UA/mL = 0.142 BAU/mL

Elecsys® Anti-SARS-CoV-2: 1 U/mL = 1.029 BAU/mL

ADVIA Centaur® SARS-CoV-2 IgG: 1 Index = 21.8 BAU/mL

For the second approach, we propose quantile interpolation, which consists of several phases: first, the linearization and normalization of the data; second, the calculation of the quantiles; third, the interpolation of these quantiles; fourth, the application of Deming regression; and finally, the reversal of the normalization and linearization using the equations obtained from the Deming regression. This method aimed to align the distributions of antibody levels measured by different assays by estimating, for each individual, the corresponding value in a reference method based on shared quantiles.

But before talking about the conversion methods, an important step was accomplished, statistical models were applied to predict the truncated values of each test by center and time point, using as predictor the rank-based index of individuals according to their antibody levels. The models used can be seen in Appendix A, such as a graphical example of a time point, “6 months after vaccination”.

The interpolation phase followed several steps. First, the values of each method were ordered, and then the data were linearized and normalized since the different laboratory methods presented markedly distinct behaviors, which hindered the direct application of regression models. To address this, the bestNormalize package was applied. This package tests multiple transformation techniques, such as Yeo-Johnson, double reverse log, square root transformation, and inverse hyperbolic sine function, and automatically applies the one that best approximates a normal and linear distribution²⁷. The Yeo-Johnson transformation technique can be represented by the Eq. (1)²⁸.

$$g(x; \lambda) = \begin{cases} \frac{(x+1)^\lambda - 1}{\lambda} & \text{se } \lambda \neq 0 \text{ e } x \geq 0, \\ \log(x+1) & \text{se } \lambda = 0 \text{ e } x \geq 0, \\ \frac{(1-x)^{2-\lambda} - 1}{\lambda - 2} & \text{se } \lambda \neq 2 \text{ e } x < 0, \\ -\log(1-x) & \text{se } \lambda = 2 \text{ e } x < 0. \end{cases} \quad (1)$$

Regarding the double reverse log method, this method consists on Eq. (2)²⁸.

$$\log_{10}(\text{maximum} - \text{minimum}) - \log_{10}(\text{maximum} - x), \quad (2)$$

This transformation was performed individually for each hospital center and time point, ensuring that the alignment process was tailored to each context. After the best transformation is selected and applied, the data are standardized by subtracting the mean and dividing by the standard deviation.

A specific example is provided to illustrate how this process was implemented in practice. In this case, we focus on the “6 months after vaccination” time point, as it includes data from the three hospital centers: CHUC, CHLO, and CHTV. Regarding the linearization of CHLO data, this was performed using the double reverse log method. For CHTV data, the Yeo-Johnson transformation was applied. Finally, for CHUC, linearization was performed using the inverse hyperbolic sine function. For all hospital centres, linearization was followed by normalization, that is, subtracting the mean and dividing by the standard deviation. The linearization and normalization process performed for the remaining time points can be consulted in full in Appendix B.

Following the transformation step, quantiles were computed using the rank function, and two interpolation methods were applied: linear interpolation and cubic interpolation. The choice of interpolation method was based on the correlation between the interpolated values and the reference values; the method yielding the highest correlation was selected for each comparison.

Linear interpolation is a technique that assumes the estimated point lies on the line connecting the two nearest adjacent points, one on the left and one on the right²². Considering that the values of x , which represent the quantiles of the reference distribution sorted in ascending order, and given a point x such that $x_i < x < x_{i+1}$, the linear interpolation process consists of determining the position of x between these two points and calculating the corresponding value on the line defined by them²². Here, each x_i and x_{i+1} are quantiles corresponding to observed data points, with y_i and y_{i+1} representing the values of the variable at those quantiles. Thus, the linear interpolation at x is given by Eq. (3)²², where $\hat{y}(x)$ denotes the estimated value at point x . The index i varies along the sorted quantiles such that the interval $[x_i, x_{i+1}]$ contains the point x .

$$\hat{y}(x) = y_i + \frac{(y_{i+1} - y_i)(x - x_i)}{x_{i+1} - x_i} \quad (3)$$

Regarding cubic interpolation, this technique is particularly useful to ensure a smooth and continuous conversion of anti-S SARS-CoV-2 IgG antibody data, maintaining the accuracy of measurements from different laboratory methods²³. This approach, besides fitting the data smoothly while preserving continuity, allows for a higher order of accuracy as the spacing between points decreases, unlike simpler methods such as linear interpolation, which do not guarantee the same smoothness nor continuity of derivatives²³. The formula for cubic interpolation, between two consecutive points x_i and x_{i+1} , is defined by Eq. (4)²³:

$$P(x) = c_1 + c_2(x - x_i) + c_3(x - x_i)^2 + c_4(x - x_i)^3, \quad \text{where } x_i \leq x \leq x_{i+1} \quad (4)$$

The coefficients c_1 , c_2 , c_3 , and c_4 are defined to ensure that the interpolating function behaves smoothly. Specifically:

$$\begin{aligned} c_1 &= f_i, & \text{function value at point } x_i, & \text{constructed from the observed data;} \\ c_2 &= f'_i, & \text{numerical approximation of the derivative at point } x_i; \\ c_3 &= \frac{3S_{i+1/2} - f'_{i+1} - 2f'_i}{\Delta}; \\ c_4 &= \frac{2S_{i+1/2} - f'_{i+1} - f'_i}{(\Delta)^2}, \end{aligned}$$

where $S_{i+1/2}$ represents an estimate of the slope between points x_i and x_{i+1} , and Δ is the difference between these points.

The quantile interpolation was followed by regressions that account for errors in both measurements, specifically the Deming regression, which used the resulting equation to transform the data. Deming regression is preferred when it is known or assumed that the errors in X and Y have different magnitudes²⁴. Accordingly, Deming regression is characterized by the following Eq. (5)²⁵:

$$y = a + b_D x; \quad (a = \bar{y} - b\bar{x}), \quad (5)$$

The formula for b is given in Eq. (6):

$$b_D = \frac{(\hat{\lambda}S_{yy} - S_{xx}) + \sqrt{(S_{xx} - \hat{\lambda}S_{yy})^2 + 4\hat{\lambda}S_{xy}^2}}{2\hat{\lambda}S_{xy}}, \quad (6)$$

where $S_{xy} = \sum (x_i - \bar{x})(y_i - \bar{y})$, $S_{xx} = \sum (x_i - \bar{x})^2$, $S_{yy} = \sum (y_i - \bar{y})^2$ and $\hat{\lambda}$ represents the ratio between the analytical error variances of x and y , calculated based on m pairs of observations (x, y) , as shown in Eq. (7):

$$\hat{\lambda} = \frac{\frac{1}{2m} \sum_{i=1}^n (x_{i1} - x_{i2})^2}{\frac{1}{2m} \sum_{i=1}^n (y_{i1} - y_{i2})^2} \quad (7)$$

The final step of the proposed technique consists of obtaining the result of the Deming regression and converting it back to the original data scale. Beyond the line fitted to the normalized data, it is necessary to derive the equation corresponding to the raw data, that is, without the normalization and linearization transformations previously applied. To achieve this, the transformations must be inverted directly on the fitted equation to recover the original relationship between the data. This way, it becomes possible to apply the equation to the raw values and use it for converting data from one hospital center to another.

After applying the harmonization techniques, the WHO coefficients and the quantile interpolation are followed by the Deming regression, separately, the geometric mean was calculated for each time point using both harmonization methods. The geometric mean of anti-SARS-CoV-2 IgG antibody levels was used instead of the arithmetic mean, to account for the skewed distribution of the data. It can be calculated using the Eq. (8)²⁶:

$$GM = \exp \left(\frac{1}{n} \sum_{i=1}^n \log(x_i) \right) \quad (8)$$

In addition, a boxplot was created to illustrate the distribution of anti-SARS-CoV-2 IgG antibody levels at each time point, allowing for visualization of the median, data dispersion, and the presence of potential outliers.

Finally, to validate the harmonization approaches and determine which one best preserved the original data behavior, comparative plots were created to assess the evolution of values from each hospital center before and after applying both harmonization strategies.

Hospitals	Linear	Cubic
CHLO-CHUC	0.9865099	0.9865281
CHTV-CHUC	0.9867735	0.9867733

Table 1. Correlation between anti-SARS-CoV-2 IgG antibodies from CHLO and CHUC, and between CHTV and CHUC, after linear and cubic interpolation at the “6 months after vaccination” time point.

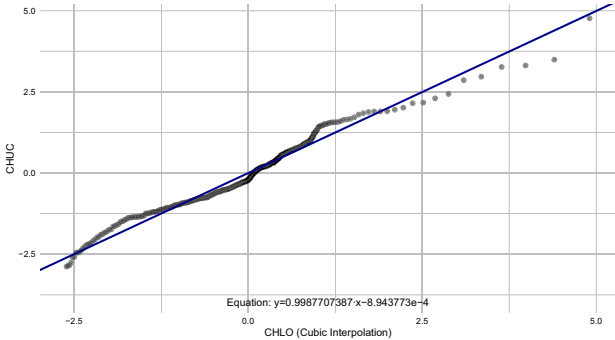


Fig. 1. Deming regression line between CHUC and CHLO at the “6 months after vaccination” time point.

CHUC	CHLO
Mean (a): 7.52165	Logarithm base (c): 10
	Mean (d): 0.399617
Standard deviation (b): 0.9354809	Standard deviation (e): 0.1385818
	Maximum (f): 4.242042
	Minimum (g): 2.799593

Table 2. Normalization values for CHUC and CHLO at the “6 months after vaccination” time point.

Results
Harmonization through quantile interpolation followed by regression analyses: example of Deming regression application

Using the same example mentioned previously, we present below the results of applying the method at the “6 months after vaccination” time point. The application of the two types of interpolation resulted in the correlations shown in Table 1.

Based on the correlation, cubic interpolation was chosen for CHLO and linear interpolation for CHTV. The quantile interpolation resulted in the same sample size for both hospital centers. With the same sample size in both hospital centers and a more linear behavior of the data, it was possible to apply Deming regression to estimate the conversion equation between the centers. For this purpose, the function mcreg was used.

CHLO: The equation obtained for the normalized CHLO data is presented in Eq. (15) and graphically illustrated in Fig. 1.

$$y = 0.9987707387 \cdot x - 8.943773 \times 10^{-4}, \tag{9}$$

where x corresponds to the data from the CHLO hospital center and y represents the values converted to the CHUC hospital center. At all time points, the data was converted to CHUC units, since this hospital center not only presented values with a higher order of magnitude, which facilitates conversion, but also showed consistent behavior aligned with expectations across the different time points, thus ensuring the quality and robustness of its data.

The equation obtained from the Deming regression on normalized data was back-transformed to the original scale to allow direct conversion of raw values between hospital centers. The normalization values are specified in Table 2.

$$\frac{\operatorname{asinh}(y') - a}{b} = 0.9987707387 \times \frac{(\log_{10}(f - g) - \log_{10}(f - x')) - d}{e} - 0.0008943773 \tag{10}$$

$$y' = \sinh \left(\left(0.9987707387 \times \frac{(\log_{10}(f - g) - \log_{10}(f - x')) - d}{e} - 0.0008943773 \right) b + a \right) \tag{11}$$

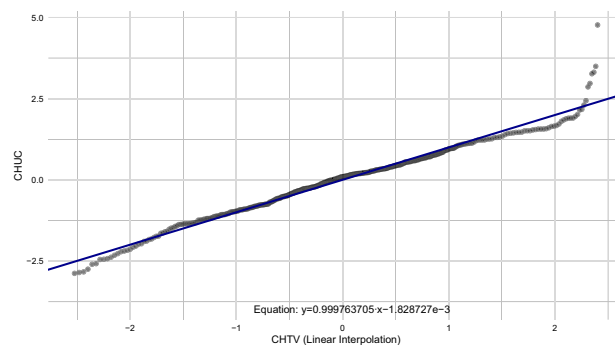


Fig. 2. Deming regression line based on normalized data between CHUC and CHTV at the “6 months after vaccination” time point.

CHUC	CHTV
Mean (a): 7.52165	Lambda (c): -0.2873793
Standard deviation (b): 0.9354809	Mean (d): 1.645739
	Standard deviation (e): 0.4443264

Table 3. Normalization values for CHUC and CHTV at the “6 months after vaccination” time point.

The values of x correspond to the normalized CHLO data, and the values of y represent the respective conversion to the original scale, as expressed in Eqs. (10) and (11).

CHTV: For CHTV, the equation was obtained based on the normalized data and is graphically represented in Fig. 2.

$$y = 0.999763705 \cdot x - 1.828727 \times 10^{-3}, \quad (12)$$

where x represents the CHTV data and y the values after conversion.

After obtaining the equation based on the normalized data, it is necessary to convert it back to the original scale and for this the normalization values are specified in Table 3.

$$\frac{\operatorname{asinh}(y') - a}{b} = 0.999763705 \times \frac{(x' + 1)^c - 1}{e} - d - 0.001828727 \quad (13)$$

$$y' = \sinh \left(\left(0.999763705 \times \frac{(x' + 1)^c - 1}{e} - d - 0.001828727 \right) b + a \right) \quad (14)$$

As shown in Eqs. (13) and (14), the CHTV data are represented by x , while y corresponds to the values after conversion to the original scale.

This procedure was repeated for all time points and hospital centers. The main differences between cases arose during the normalization and linearization steps, where different transformations were applied. A detailed description of the specific procedure applied to each of the other time points is available in Appendix C.

Comparison of anti-S SARS-CoV-2 IgG antibodies at various time points using harmonized data

Two different harmonization methods were applied to the anti-SARS-CoV-2 IgG antibody titers obtained from the participating hospital centers. The first method used the conversion equations established by the WHO, which involve specific conversion factors for each analytical method depending on the hospital of origin. The second method used interpolation through Deming regression, and after obtaining all the equations, all results were converted to the units of CHUC, followed by the calculation of the geometric mean for each time point.

Table 4 presents the geometric mean values for each evaluated time point according to both harmonization approaches.

When comparing the results obtained through the two harmonization methods, differences were observed in the longitudinal patterns of anti-SARS-CoV-2 IgG antibody levels. Using the WHO conversion factors, the increase in antibody levels after the initial vaccination was less pronounced, and the temporal evolution was less consistent. Specifically, a decrease was observed at 3 months, followed by an unexpected increase at 6 months, and a subsequent decline at 12 months before the booster dose.

In contrast, the harmonization based on Deming regression revealed a sharp increase in antibody titers after vaccination, followed by a progressive decline at 3, 6, and 12 months. After the booster dose, both methods

Time Point	WHO (BAU/mL)	Deming regression (UA/mL)
Pre-vaccination	45.72	321.96
Post-vaccination	80.78	10,335.49
3 months after vaccination	31.50	2,049.75
6 months after vaccination	53.66	798.05
12 months – Before booster	18.07	659.32
Post-booster	653.84	25,919.89

Table 4. Geometric mean of anti-SARS-CoV-2 IgG antibody levels at different time points after harmonization using WHO coefficients and Deming regression.

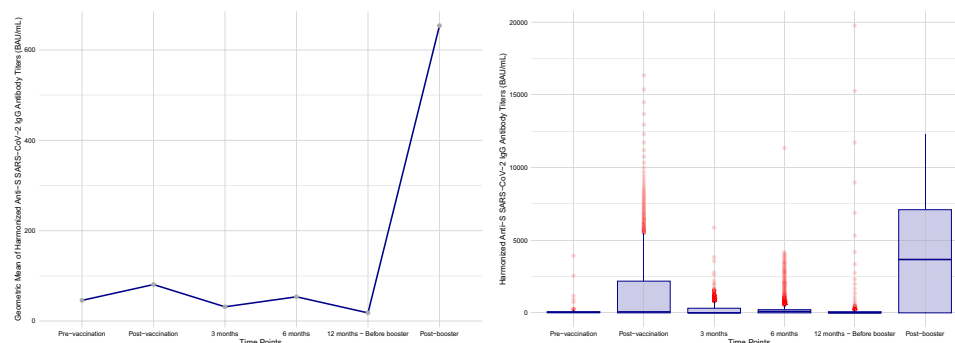


Fig. 3. Geometric mean and distribution of anti-SARS-CoV-2 IgG antibody levels at different time points after harmonization using WHO coefficients.

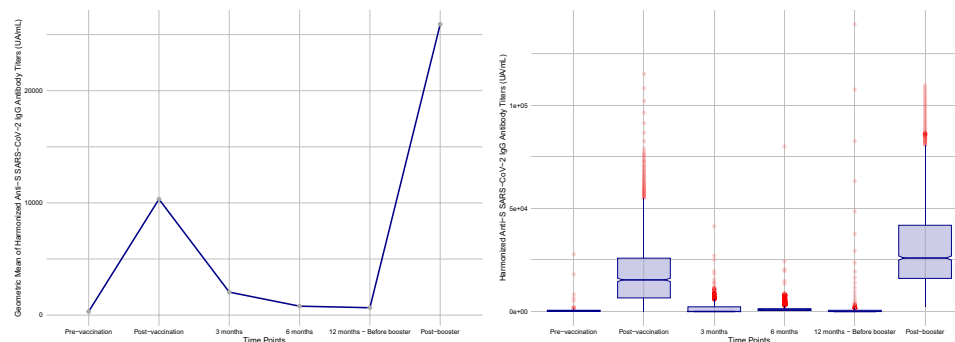


Fig. 4. Geometric mean and distribution of anti-SARS-CoV-2 IgG antibody levels at different time points after harmonization using Deming regression.

showed a marked rise in antibody levels, with Deming regression indicating values even higher than those recorded after the initial vaccination schedule.

Figures 3 and 4 illustrate the variation and distribution of antibody levels at each time point for both harmonization methods.

The results of the comparative plots, which assess the evolution of values from each hospital center before and after applying both harmonization strategies, are shown in Figs. 5, 6, and 7.

Discussion

This study aimed to test an alternative method for harmonizing anti-SARS-CoV-2 IgG antibody titers, and the results indicate its feasibility and robustness. The application of regression-based methods, particularly Deming regression combined with quantile interpolation, appears to be a suitable approach for the harmonization of serological data. This approach enabled the integration of results obtained from different laboratory methodologies. Importantly, this methodological framework not only ensures the reliability of the findings but also supports the reproducibility of the study and its potential extension to other population cohorts, including large-scale international multicenter studies.

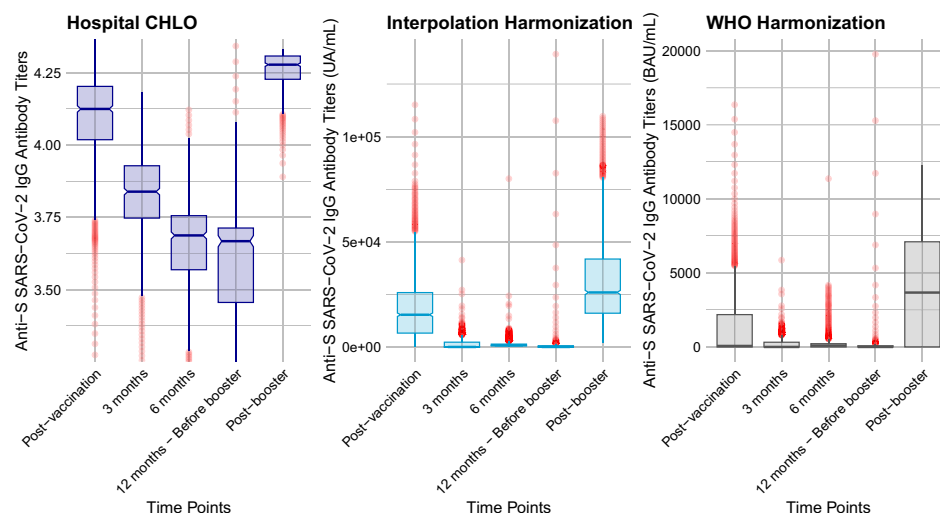


Fig. 5. Comparison of the original anti-SARS-CoV-2 IgG antibody titres from CHLO with the results obtained after both harmonization methods.

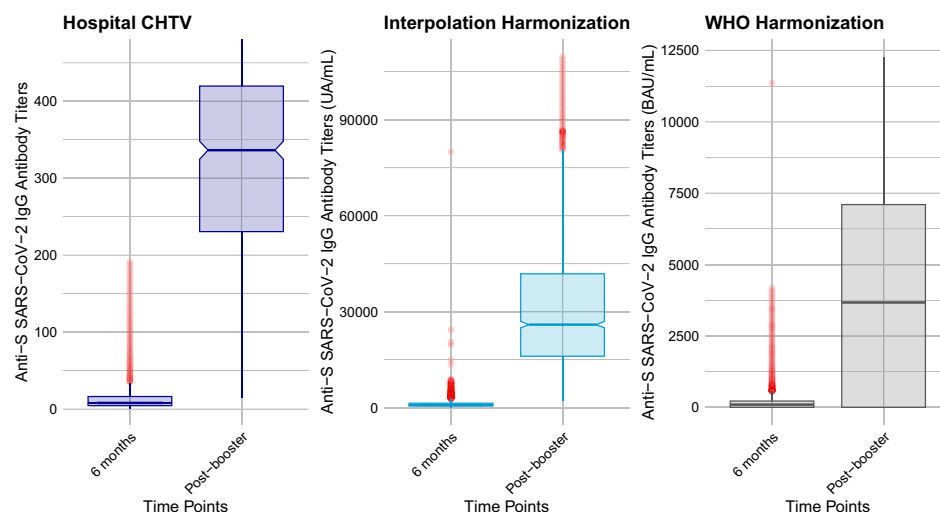


Fig. 6. Comparison of the original anti-SARS-CoV-2 IgG antibody titres from CHTV with the results obtained after both harmonization methods.

The level of anti-SARS-CoV-2 IgG antibodies observed after the third dose was significantly higher than after the second dose, demonstrating that the administration of the booster had a substantial impact on increasing antibody levels. These results suggest that the booster dose enhances the immune response by substantially increasing anti-SARS-CoV-2 IgG antibody titres. This pattern has been described in other studies as well, which also reported a significant increase in antibody levels following booster administration^{29–31}. Moreover, a study specifically focused on the antibody response to COVID-19 vaccination, also conducted among HCW, reported results similar to those of the present study, with a geometric mean of approximately 600 UA/mL prior to the booster and around 24,000–26,000 UA/mL after the booster dose. This aligns closely with our findings, where we observed 659.32 UA/mL before and 25,919.89 UA/mL after the booster³².

Regarding the behavior of the data after harmonization, graphical visualizations allowed us to observe, albeit cautiously, that harmonization based on quantile interpolation more faithfully preserves the original behavior of the data when compared with the harmonization obtained through the WHO-established coefficients. Additionally, the analysis of the geometric mean behavior following the latter approach revealed unexpected patterns: the increase in antibody titres after vaccination was less pronounced than anticipated, and, unpredictably, an increase was observed at six months, which is not consistent with the immunological pattern described in the literature^{29–31}. Furthermore, to ensure that harmonization did not introduce artificial changes in antibody levels, analyses were conducted separately for each hospital center, confirming that the

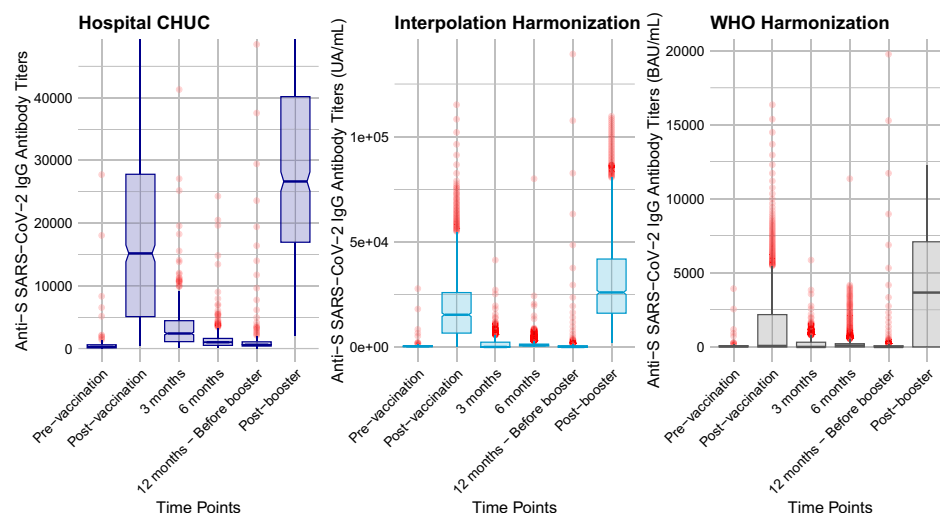


Fig. 7. Comparison of the original anti-SARS-CoV-2 IgG antibody titres from CHUC with the results obtained after both harmonization methods.

temporal patterns remained consistent. These results suggest, with due caution, that the harmonization method based on quantile interpolation followed by Deming regression may provide a more appropriate and effective harmonization, ensuring greater fidelity to the real behavior of the data over time.

This study highlights the importance of harmonizing the data collected from different hospital centers and potentially from different countries. This ensures not only the comparability of the results but also the robustness of the conclusions. A consistent analysis of the dynamics of specific antibody responses after primary vaccination and booster administration is essential to guide public health strategies, particularly in a global context marked by the progressive decline in anti-SARS-CoV-2 antibodies and the ongoing emergence of new variants²⁹. It is therefore crucial to evaluate whether a booster dose can rapidly reactivate immunological memory and significantly enhance the protective response, and whether this immunological pattern is sustained with subsequent booster doses. The findings of this study support this key question²⁹.

However, an important limitation that should be highlighted is the absence of a direct evaluation of technical variability and the degree of agreement between the different laboratory methods, beyond regression-based statistical harmonization. Without this direct assessment, confidence in the equivalence of measurements across methods can be compromised, as potential biases or residual uncertainties might not be fully captured by the statistical approaches used. Different commercial immunoassays vary considerably in antigen composition, coating density, signal generation, calibration, reference materials, and analytical protocols³³. As a result, reported antibody levels generally reflect assay-specific signals rather than absolute biological concentrations. Additionally, variations in sample dilution and the limited linear range of each assay may prevent the expected proportional relationship between dilution and measured concentration, particularly at high antibody levels where signal saturation can occur. Statistical harmonization across assays can help improve comparability at the population level. However, it should be interpreted with caution, as it does not imply analytical or biological equivalence and may not accurately reflect individual or absolute antibody concentrations in the absence of direct cross-assay validation using shared reference samples. Furthermore, the selection of the Abbott CMIA assay as the reference scale represents an additional methodological limitation. As no analytical gold standard was available, the choice of a reference assay was necessarily pragmatic and may influence the numerical scale of the harmonized values. Nevertheless, because the analyses focused on longitudinal patterns rather than absolute antibody concentrations, this limitation is unlikely to affect the main conclusions substantially. To address this gap, future studies should include the analysis of the same samples in the three participating hospital centers, allowing direct validation of the consistency of the transformations applied in the harmonization process, a procedure already adopted in other studies². This approach will strengthen the robustness of the harmonization method applied in this study, ensuring greater reliability and comparability of the results obtained using the different laboratory methods. Until such evaluation is performed, conclusions based on harmonized data should be interpreted with caution, as unquantified measurement discrepancies may persist.

In addition to this structural limitation, there were also practical challenges in the conversion and harmonization of data from the three hospital centers. The limited literature on robust methodologies for this type of harmonization made it challenging to clearly define the most appropriate method. However, this challenge also represents one of the innovative aspects of our work, as it led to the careful and rigorous application of harmonization techniques that demonstrated consistent performance and ensured comparability between the different datasets. Furthermore, post-harmonization analysis was essential to explore the immune response to vaccination in detail.

Finally, although the chosen methods proved to be effective, it is important to reflect on their specific strengths and limitations. Quantile interpolation, especially when combined with Deming regression, preserves

Time point	Hospital	Main model	Other approaches
Post-vaccination	CHLO	lm(y ~ bs(index, knots = seq(20, 240, by = 20)))	glm(y ~ log(index), family = gaussian(link = "log"))
	CHUC	lm(y ~ bs(index, knots = seq(200, 700, by = 100)))	glm(y ~ I(index ³), family = gaussian(link = "log"))
3 months	CHLO	lm(y ~ poly(index, 10))	Polynomial regression with lowest BIC among degrees 5 to 20
6 months	CHLO	lm(y ~ poly(index, 10))	Polynomial regression with lowest BIC among degrees 5 to 20
	CHTV	lm(y ~ bs(index, knots = seq(600, 800, by = 200)))	glm(y ~ index, family = gaussian(link = "log"))
12 months	CHLO	lm(y ~ poly(index, 12))	Polynomial regression with lowest BIC among degrees 5 to 20
	CHUC	lm(y ~ bs(index, knots = seq(390, 420, by = 5)))	glm(y ~ index, family = gaussian(link = "log"))
Post-booster	CHLO	glm(y ~ log(index), family = gaussian(link = "log"))	-
	CHUC	lm(y ~ poly(index, 10))	Polynomial regression with lowest BIC among degrees 5 to 20
	CHTV	glm(y ~ log(index), family = gaussian(link = "log"))	glm(y ~ index, family = gaussian(link = "identity"))

Table 5. Models used for prediction of maximum values and other approaches considered.

the original distributional features of the data and accounts for measurement errors in both laboratory methods, providing an advantage over simple linear regression. A limitation of the quantile interpolation method is that the estimation of quantiles is influenced by the smallest available sample size across hospital centers and time points. This may reduce statistical precision for some quantiles, particularly in imbalanced datasets. Despite this, overall trends and relative comparisons across centers and time points are preserved. Furthermore, its validity depends on the continued support from laboratory assay comparisons and technical validation.

Conclusion

The interpolation of quantiles followed by regression enabled effective harmonization of serological data across different hospital centers, allowing for the joint comparison and analysis of anti-SARS-CoV-2 IgG antibody titres obtained through distinct laboratory methods. This approach preserved the expected temporal pattern of the IgG antibody response to SARS-CoV-2, lending robustness to the longitudinal results and enabling more accurate identification of the effect of vaccination over time.

The results demonstrated that the administration of the booster dose had a significant impact on increasing anti-SARS-CoV-2 IgG antibody levels, with substantially higher values than those observed after the second dose. This evidence reinforces the importance of the booster as a key strategy in controlling the COVID-19 pandemic. However, further studies encompassing subsequent boosters in 2023, 2024 and 2025 will be necessary to assess whether this trend persists over time.

Despite the absence of laboratory validation through cross-quantification of the same individuals across different laboratories, a crucial step to fully confirm the adequacy of the method, the methodology presented here appears promising and highly applicable. In particular, this approach proves useful for European studies that perform joint analyses of data from multiple countries and laboratories, where harmonization is essential to ensure the comparability and reliability of results, as well as for national investigations involving multiple laboratories.

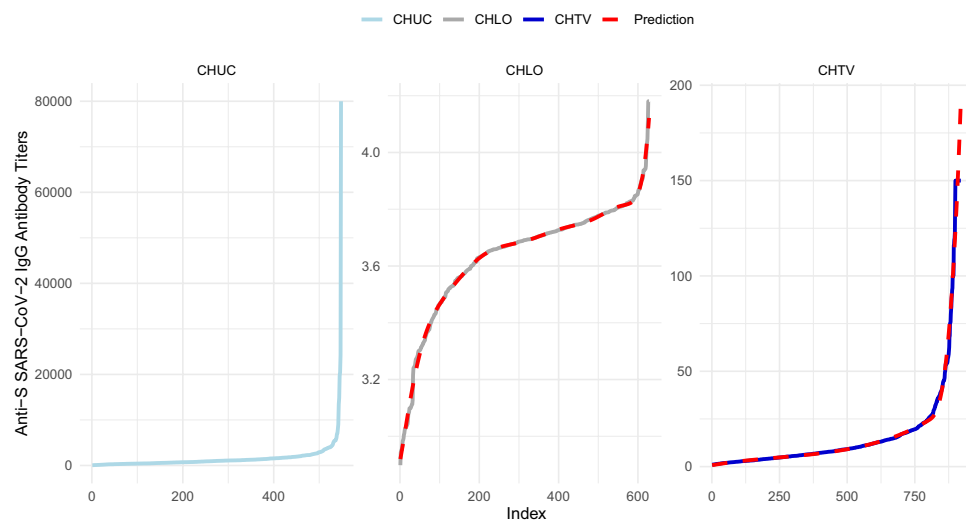


Fig. 8. Prediction of anti-SARS-CoV-2 IgG antibody values at the “6 months after vaccination” time point for CHLO and CHTV.

Time point	CHLO	CHUC	CHTV
Post-vaccination	Double reverse log	Square root	—
3 months after vaccination	Double reverse log	Yeo-Johnson	—
12 months - Before booster	Exponential	Yeo-Johnson	—
Post-booster	Double reverse log	asinh	Double reverse log

Table 6. Linearization methods applied by hospital and time point.

Hospitals	Linear	Cubic
CHLO-CHUC	0.9719804	0.9719808

Table 7. Correlation between anti-SARS-CoV-2 IgG antibodies from CHLO and CHUC after linear and cubic interpolation at the “Post-vaccination” time point.

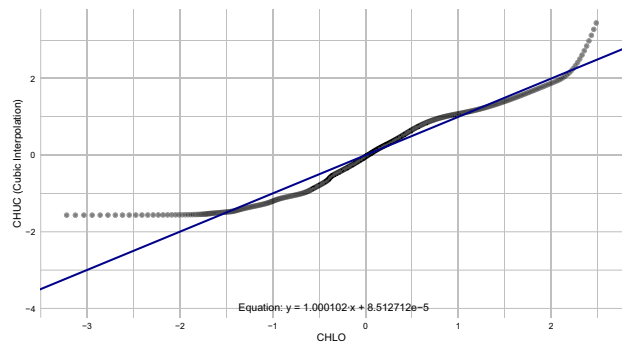


Fig. 9. Deming regression line for the normalized data between CHUC and CHLO at the “Post-vaccination” time point.

CHUC	CHLO
Mean (a): 121.3719	Logarithm base (c): 10
Standard deviation (b): 63.23817	Mean (d): 0.625 537 1
	Standard deviation (e): 0.1825038
	Maximum (f): 4.537207
	Minimum (g): 2.763963

Table 8. Normalization values for CHUC and CHLO at the “Post-vaccination” time point.

Hospitals	Linear	Cubic
CHLO–CHUC	0.9577056	0.957717

Table 9. Correlation between anti-SARS-CoV-2 IgG antibodies from CHLO and CHUC after linear and cubic interpolation at the “3 months” time point.

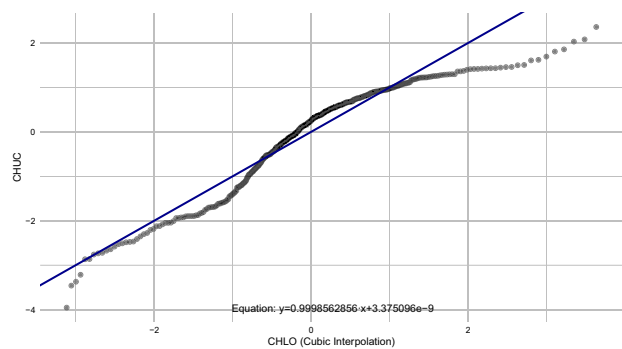


Fig. 10. Deming regression line for the normalized data between CHUC and CHLO at the “3 months” time point.

CHUC	CHLO
Lambda (a): -0.1092117	Logarithm base (d): 10
Mean (b): 5.14768	Mean (e): 0.519122
Standard deviation (c): 0.4843323	Standard deviation (f): 0.1541969
	Maximum (g): 4.308853
	Minimum (h): 2.779793

Table 10. Normalization values for CHUC and CHLO at the “3 months” time point.

Hospitals	Linear	Cubic
CHLO-CHUC	0.903993	0.9040296

Table 11. Correlation between anti-SARS-CoV-2 IgG antibodies from CHLO and CHUC after linear and cubic interpolation at the “12 months” time point.

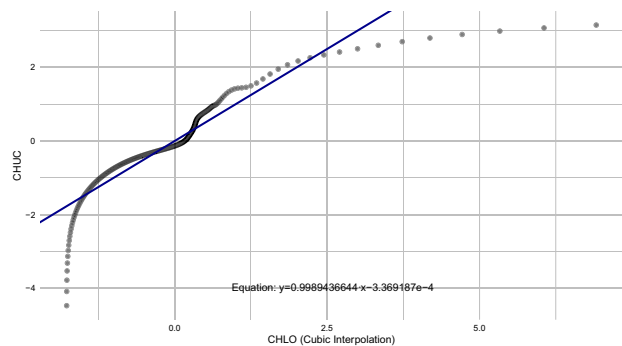


Fig. 11. Deming regression line on normalized data between CHUC and CHLO at the time point “12 months - Before booster”.

Hospitals	Linear	Cubic
CHUC-CHLO	0.9895989	0.989627
CHTV-CHLO	0.964782	0.964779

Table 12. Correlation between anti-SARS-CoV-2 IgG antibodies of CHLO and CHUC, and between CHTV and CHUC, after linear and cubic interpolation at the “Post-booster” time point.

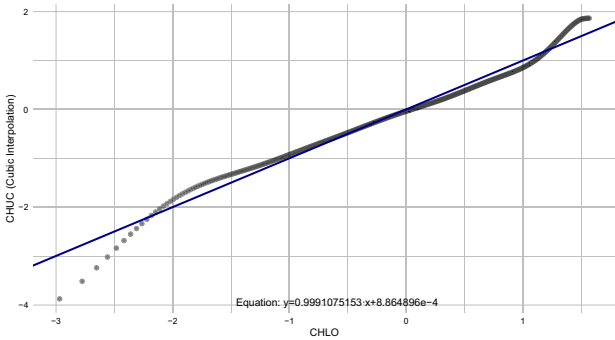


Fig. 12. Deming regression line between CHUC and CHLO at the “Post-booster” time point.

CHUC	CHLO
Mean (a): 10.855 77	Logarithm base (c): 10
Standard deviation (b): 0.646 645 5	Mean (d): 0.719 105 7
	Standard Deviation (e): 0.229 469 9
	Maximum (f): 4.373 191
	Minimum (g): 3.846 343

Table 13. Normalization values for CHUC and CHLO at the “Post-booster” time point.

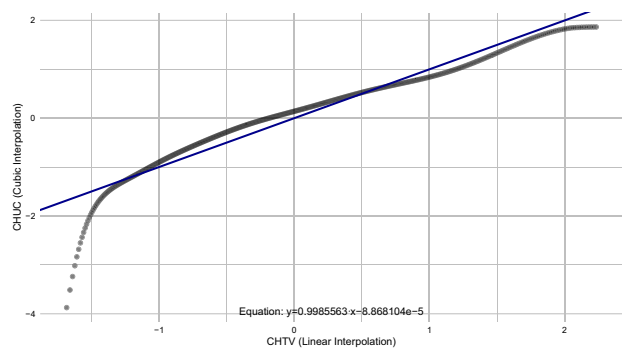


Fig. 13. Deming regression line on normalized data between CHUC and CHTV at the “Post-booster” time point.

CHUC	CHTV
Mean (a): 10.85577	Logarithm base (c): 10
Standard deviation (b): 0.6466455	Mean (d): 0.4864979
	Standard Deviation (e): 0.2652552
	Maximum (f): 538.8488
	Minimum (g): -33.55599

Table 14. Normalization values for CHUC and CHTV at the “Post-booster” time point.

Data availability

The data supporting the findings of this study are funded by the European Centre for Disease Prevention and Control, through a service contract with Epiconcept. Therefore, the availability of these data is subject to restrictions. The data are, however, available upon request.

A Predicted truncated values

To predict the maximum values of the methods, several models were considered in order to select the one providing the best predictions. The models used, as well as the alternative options, are presented in Table 5.

In the case of the polynomial linear regression models, a loop was performed to determine the polynomial degree that resulted in the lowest BIC, considering that an increase in degree would only be deemed advantageous if it led to a reduction of at least 10 units in the BIC³⁴. In Fig. 8, we present an example of the application of the aforementioned models, focusing on the specific time point “6 months after vaccination”.

B Data linearization and normalization

A summary of the methods applied for each time point and hospital center is presented in Table 6.

The application of these methods was always followed by mean subtraction and division by the standard deviation.

C Harmonization through quantile interpolation followed by regression for the other time points

C. 1 Post-vaccination

The correlations obtained after linear and cubic interpolation are presented in Table 7.

Cubic interpolation was chosen due to its slightly higher correlation. The result of the cubic interpolation led to the same sample size for both hospital centers. With equal sample sizes in both centers, it was possible to apply Deming regression. The equation obtained for the normalized data is presented in Eq. (15) and graphically illustrated in Fig. 9.

$$y = 1.000102 \cdot x + 8.512712 \times 10^{-5}$$

(15)

The inverse of the linearization and normalization transformations was applied directly to the fitted equation

to recover the original relationship between the data. The normalization values are presented in Table 8.

$$\frac{\sqrt{y'} - a}{b} = 1.000102 \times \frac{(\log_{10}(f - g) - \log_{10}(f - x')) - d}{e} + 8.512712 \times 10^{-5} \quad (16)$$

$$y' = \left(\left(1.000102 \times \frac{(\log_{10}(f - g) - \log_{10}(f - x')) - d}{e} + 8.512712 \times 10^{-5} \right) b + a \right)^2 \quad (17)$$

C.2 3 months after vaccination

Table 9 presents the results of the interpolation.

Following cubic interpolation, the Deming regression result is shown in Eq. (18) and illustrated graphically in Fig. 10.

$$y = 0.9998562856 \cdot x + 3.375096 \times 10^{-9} \quad (18)$$

However, as previously mentioned, it is essential to determine the equation to apply it directly to the original data. For this, the normalization values to be applied in the equation are provided in Table 10.

$$\frac{\left(\frac{(y'+1)^a - 1}{a} \right) - b}{c} = 0.9998562856 \times \frac{(\log_{10}(g - h) - \log_{10}(g - x')) - e}{f} - 0.0003375096 \quad (19)$$

$$y' = \left(\left(\left(0.9998562856 \times \frac{(\log_{10}(g - h) - \log_{10}(g - x')) - e}{f} - 0.0003375096 \right) c + b \right) a + 1 \right)^{\frac{1}{a}} - 1 \quad (20)$$

12 months – before booster

The correlation results of anti-SARS-CoV-2 IgG antibodies after linear and cubic interpolation are presented in Table 11.

Deming regression was applied to the interpolated data, with the resulting model presented in Eq. (21) and illustrated in Fig. 11.

$$y = 0.9989436644 \cdot x - 3.369187 \times 10^{-4} \quad (21)$$

C.4 Post-booster

Calculation of correlations between the hospitals after applying both interpolation methods, with the results shown in Table 12.

Considering the correlation values, cubic interpolation was used for CHUC and linear interpolation for CHTV. **CHLO:** For this particular hospital, Eq. (22) was obtained from the application of Deming regression, along with the corresponding Fig. 12.

$$y = 0.9991075153 \cdot x + 8.864896 \times 10^{-4} \quad (22)$$

The conversion of the normalized equation to the corresponding one on the original data scale was then performed. The values required for this conversion are presented in Table 13.

$$\frac{\operatorname{asinh}(y') - a}{b} = 0.9991075153 \times \frac{(\log_{10}(f - g) - \log_{10}(f - x')) - d}{e} + 0.0008864896 \quad (23)$$

$$y' = \sinh \left(\left(0.9991075153 \times \frac{(\log_{10}(f - g) - \log_{10}(f - x')) - d}{e} + 0.0008864896 \right) b + a \right) \quad (24)$$

CHTV: For the CHTV hospital, Eq. (25) was obtained based on the normalized data, with the graphical representation shown in Fig. 13.

$$y = 0.9985563 \cdot x - 8.868104 \times 10^{-5} \quad (25)$$

Once the equation with normalized data has been determined, it becomes necessary to convert it so that it can be applied to the original data. The normalization values are summarized in Table 14.

$$\frac{\operatorname{asinh}(y') - a}{b} = 0.9985563 \times \frac{(\log_{10}(f - g) - \log_{10}(f - x')) - d}{e} - 8.868104 \times 10^{-5} \quad (26)$$

$$y' = \sinh \left(\left(0.9985563 \times \frac{(\log_{10}(f - g) - \log_{10}(f - x')) - d}{e} - 8.868104 \times 10^{-5} \right) b + a \right) \quad (27)$$

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Author contributions

A.L.S. contributed to the conception, design, analysis of the study, and wrote the main manuscript text. V.A., A.M., and V.G. contributed to the conception and design of the study, supervised the project, and revised the manuscript. P.A., J.F.V., I.A., A.P., L.A.G., and L.A. collected the data and revised the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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