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Seroprevalence of SARS-CoV-2 antibodies in Italy in newborn dried blood spots

Abstract

Obejctives: Serosurveys can be used to monitor COVID-19 seroprevalence and conduct surveillance. Dried blood spot (DBS), used increasingly as a valuable sample to assay severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) antibodies (Ab), has several advantages, particularly in infants, due to the limited amount of blood required and its utility in testing a large number of samples in a limited time-frame. We evaluated SARS-CoV-2 IgG Ab prevalence in newborn DBS in the Trentino region of Italy, during the time period January 2020 – December 2021.
Methods: Anti-SARS-CoV-2 IgG levels were determined in DBS by means of Anti-SARS-CoV-2 QuantiVac IgG ELISA assay (Euroimmun, Lubeck, Germany).
Results: Analyses included 2,400 DBS from newborns (54% M, 46% F), samples being collected 2–3 days after birth. The first DBS that tested positive for anti-SARS-CoV-2 IgG antibodies was found in March 2020 and, up to May 2020, only 4 positive results were detected overall. Starting from June 2020, the positivity thresholds increased according to the epidemiological waves of the COVID-19 pandemic in Italy, with a robust increment in the winters of 2020 and 2021. The percentage of positive DBS rose from 0 to 6% to 10–47%, in 2020 and 2021, respectively.
Conclusions: This study demonstrates DBS is a suitable tool for both epidemiological purposes and surveillance in the SARS-CoV-2 pandemic, particularly in newborns and pregnant women, saving blood waste and sparing patients any discomfort.

Keywords: COVID-19; newborn dried blood spots (DBS); SARS-CoV-2 IgG antibodies; seroprevalence study.

Introduction

Since late December 2019, the COVID-19 outbreak has been a great challenge for public health worldwide, including countries with well-developed health care systems. Despite warnings and the implementation of public health mitigation strategies by health authorities, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) continues to spread (1–5). Specific populations at the greatest risk in the SARS-CoV-2 pandemic include individuals with other comorbidities (e.g., cardiac, pulmonary, or neurologic diseases; diabetes mellitus; obesity or immunosuppression), the elderly, pregnant/postpartum women, and infants [1–3]. The prompt and accurate identification of all infected individuals is of crucial importance in controlling SARS-CoV-2 rapid transmission, enabling the rapid isolation and traceability of contacts, and containing the risk of spread, regardless of the presence or absence of COVID-19 symptoms [4, 5].

In the current diagnostic landscape, nucleic acid amplification tests (NAATs) are the gold standard for diagnosing SARS-CoV-2 infections [6], while serological testing has been successfully used for conducting epidemiological serosurveys, investigating antibody responses mounted against SARS-CoV-2 infection, and assessing the real prevalence of infection in the population [7].

As has been well established, humoral immunity is transmitted from mother to fetus through the placenta.

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Recent studies investigated anti-SARS-CoV-2 antibodies transmission in pregnant women who had active COVID-19 infection or had received the COVID-19 vaccine and found that anti-SARS-CoV-2 IgG, IgM and IgA were transferred to the fetus via the placenta [3, 8, 9]. However, Atyeo and colleagues highlighted that SARS-CoV-2-specific antibody transfer, in third-trimester infection, was significantly reduced with respect to influenza- and pertussis-specific antibodies, and SARS-CoV-2-specific transfer was linked to SARS-CoV-2-antibody glycosylation profile alterations [3].

Dried blood spot (DBS) antibody analysis, an alternative method to venous blood samples antibody testing, has already been used extensively in screening for several viruses including Hepatitis B, Hepatitis C and HIV [10]. Recent studies have demonstrated the feasibility of using DBS for home blood collection for SARS-CoV-2 antibody screening, also in infants [11]. Small-scale feasibility studies have evaluated DBS samples for SARS-CoV-2 antibody detection in high-risk populations, using enzyme immunoassays [12, 13]. More recently a large serosurvey was also conducted in order to monitor population-level dynamics of COVID-19 detecting SARS-CoV-2 nucleocapsid (N) and spike (S) IgG antibodies in DBS from newborns [14].

In the present study, the newborn DBS sampling strategy was employed to investigate the maternal-fetal seroprevalence of anti-SARS-CoV-2 antibodies (Abs) using the Euroimmun Anti-SARS-CoV-2 QuantiVac ELISA assay (IgG).

Materials and methods

Specimens

From January 2020 through December 2021, DBS samples were collected 2–3 days after birth from infants born in the Trentino Alto Adige region, Italy. After collection, DBS were stored in a dedicated room under a controlled temperature (18 ℃), punched (4.76 mm) into Eppendorf tubes and stored at 2–8 ℃ until tested. Since all were leftover samples from Screening Programs of Metabolic Disorders, they were completely anonymous, available data including only gender, and no further information (e.g., name, date of birth, demographic characteristics, vaccine characteristics etc.). The study was conducted in accordance with the Declaration of Helsinki, and the Institutional Review Board of the University of Padova (protocol no. 27444).

Quantification of anti-SARS-CoV-2 IgG

One hundred blinded DBS disks/months were included in the quantification study. IgG antibodies against the S1 portion of the SARS-CoV-2 spike protein were measured by Anti-SARS-CoV-2 QuantiVac ELISA (IgG) immunoassays, using the platform for automation Euroimmun

Analyzer I (Euroimmun, Lübeck, Germany), following the manufacturer's instructions (IFU EI_2606-10G_A_IT_C05, v Nov 30, 2021) [15, 16]. According to the manufacturer, ELISA immunoassay is calibrated against the WHO IS and gives results in relative units (UR) per milliliter and binding antibody units (BAU) per milliliter. The manufacturer's cut-off is: ≤25.6 kBAU/L (negative), ≥25.6 kBAU/L and ≤35.2 kBAU/L borderline; ≥35.2 kBAU/L positive. In this study, we considered values above 25.6 kBAU/L as reactive.

Evaluation of protein stability of DBS

Five DBS disks/month were taken to evaluate the stability of protein distributions across the analysis time-frame, by quantification of total IgG (mg/L) and albumin (g/L) with the immunonephelometric method on Dimension Vista 1,500, BN II instruments (Siemens Healthineers), following the manufacturer's instructions.
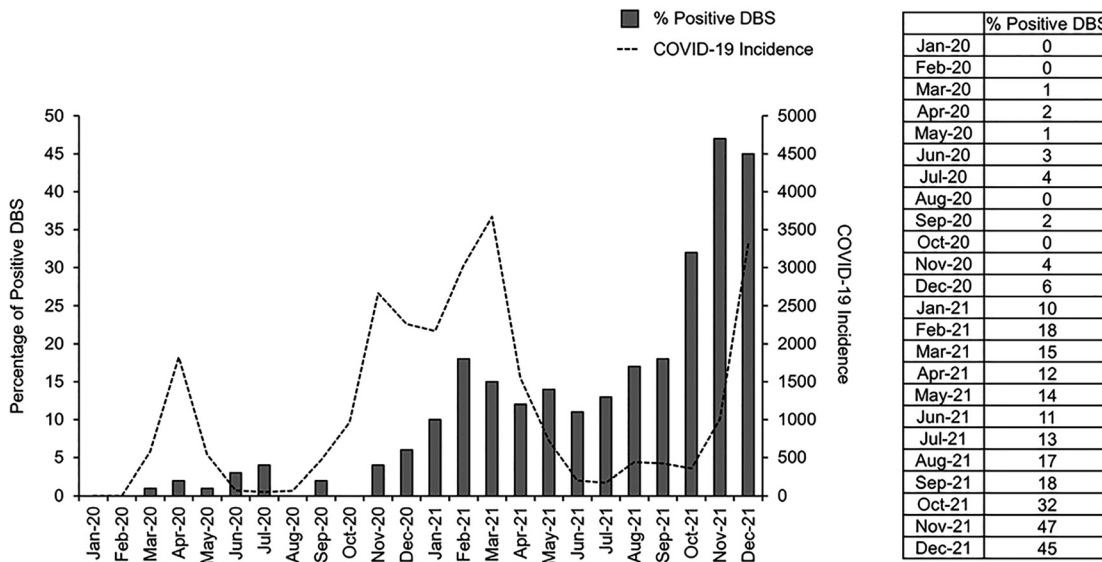
Statistical analysis

GraphPad Prism version 9.1 for Windows (GraphPad Software, LLC) was used for descriptive statistics, and for non-parametric tests (Kruskal–Wallis test and Spearman's correlation analysis). Stata 16.1 (Statacorp, Lakeway Drive, TX, USA) was used for Fisher's exact test, and χ^2 for trend analyses.

Results

A total of 2,520 DBS samples were collected. The quantification study cohort comprised 2,400 DBS spots collected from infants 2–3 days after birth during the period January 1, 2020 – December 31, 2021. Of the 2,400 DBS, 1,301 (54%) were from males and 1,099 (46%), from females. On investigating the anti-SARS-CoV-2 IgG measured in the first month (Jan 2020), the mean (±SD) level was 13.02 kBAU/L (±3.59 kBAU/L), and all results (n=100) were below the manufacturer's suggested negative threshold (25.6 kBAU/L). Therefore, in this study, we preferred to choose a threshold of 25.6 kBAU/L for reactivity, since the mean + 3 times the standard deviation of the series of DBS results from January 2020 was 23.8 kBAU/L. Tukey's box plots of measured anti-SARS-CoV-2 IgG levels are reported in Figure 1. No significant difference was found between males and females for anti-SARS-CoV-2 IgG (χ^2=0.002, p=0.968). All results in the first two months of 2020 were below the reactivity threshold. The first DBS that tested positive for anti-SARS-CoV-2 IgG antibodies was found in March 2020. In March, April and May 2020, only four positive results were detected overall, whilst starting from June 2020, the positivity thresholds increased according to the epidemiological waves of the COVID-19 pandemic in Italy, with few cases in spring-summer 2020 and increments in the winters of 2020 and 2021 [17] (Figure 2).
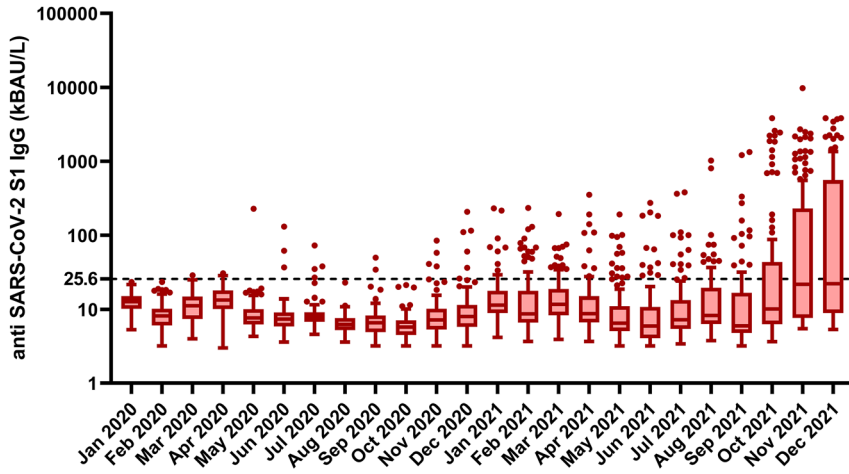
Figure 1: Tukey's box plots of anti-SARS-CoV-2 S1 IgG levels (n=2,400, 100/month). Box represent medians and IQRs. The dotted line corresponds to the assay threshold (25.6 kBAU/L) for discriminating positive from negative samples.

|	% Positive DBS
Jan-20	0
Feb-20	0
Mar-20	1
Apr-20	2
May-20	1
Jun-20	3
Jul-20	4
Aug-20	0
Sep-20	2
Oct-20	0
Nov-20	4
Dec-20	6
Jan-21	10
Feb-21	18
Mar-21	15
Apr-21	12
May-21	14
Jun-21	11
Jul-21	13
Aug-21	17
Sep-21	18
Oct-21	32
Nov-21	47
Dec-21	45

Figure 2: Percentage of DBS tested positive for anti-SARS-CoV-2 IgG antibodies (chart and table) in comparison with the COVID-19 incidence in Trentino Alto Adige region (dotted line). A significant increase in average of 1.58% (SE=0.094%) was found (χ^2 for trend=285, p<0.001).

The percentage of positivity ranged from 0 to 6% in 2020 (23 total positive cases in 2020) to 10–47%, with 252 positive newborn DBS. The percentage of positive DBS significantly increased across the studied month, with a statistically significant increase in average of 1.58% (standard error=0.094%) (χ^2 for trend = 285; p≤0.001). Positive DBS in females and males did not differ across all studied months (Fisher's exact tests, p-value>0.161). To evaluate the stability of DBS samples, the distribution levels of total IgG and albumin, across the analyzed months, were determined for a total of 120 DBS, 5 disks/month. As expected, total IgG and albumin contents between 2020 and 2021 were comparable. The overall value ranges were 29.05–116 mg/L in 2020 and 27.3–92 mg/L in 2021 for total IgG (Kruskal-Wallis test p=n.s.); 0.108–

0.343 g/L in 2020 and 0.093–0.277 g/L in 2021 for albumin (Kruskal-Wallis test p=n.s.) (Figure 3).

Discussion

The first case of COVID-19 in Italy was officially detected on February 21st, 2020. From that time on, SARS-CoV-2 viral spread caused five pandemic waves that posed a great challenge for national public health systems worldwide. Different studies have modeled epidemic waves, both before and after vaccination strategies, correlating viral spread with pollution, the areas of quarantine and intermittent open-close strategies [18, 19]. These studies, in addition to serosurvey studies, have proven to be essential
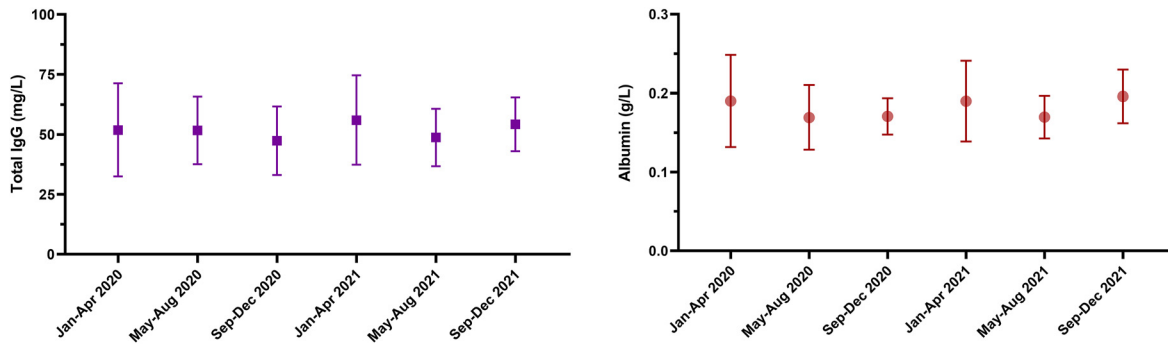
Figure 3: Levels of total IgG (left chart) and albumin (right chart), across the analyzed months (n=120, 5/month). Total IgG and albumin contents were comparable between 2020 and 2021 (Kruskal-Wallis test p=n.s.). Dots and Lines and represent medians and IQRs.

tools for seroprevalence assessments and the prediction of immunity in populations, especially those at high-risk [7].

Newborn DBS has already been validated for the measurement of SARS-CoV-2 antibodies, and, since they contain maternal IgG Abs, they might be useful also for evaluating maternal seroprevalence [14]. Pregnant/postpartum women and infants represent specific populations at the greatest risk in the SARS-CoV-2 pandemic due to reduced residual respiratory capacity, decreased viral immune response and increased risk of thromboembolic events [8–10].

In this study, we evaluated anti-SARS-CoV-2 IgG, measured in a series of 2,400 DBS, collected from infants 2–3 days after birth. The levels of anti-SARS-CoV-2 IgG towards the S1 portion of the Spike (S) protein were determined by CE-IVD, an enzyme-linked immunosorbent assay (ELISA).

The reactivity rates, determined month-by-month from January 2020 through December 2021, were compared with COVID-19 incidence, geographically matched with the area of DBS collection. As shown in Figure 2, from January 2020 through December 2020, the peak in incidence during the pandemic wave foreran the seroprevalence peak by two months (e.g., April 2020 and June 2020 incidence and prevalence peaks, respectively). That this trend seemed to disappear after January 2021 might be due to the cumulative increase in prevalence in reproductive-aged females with SARS-CoV-2 infection prior to the vaccination period. In Italy, the vaccination campaign started in January 2021 for healthcare workers and fragile patients and later, in March–April 2021, for reproductive-aged subjects, whilst pregnant women received vaccines lately, from September 2021, due to the required enhanced safety stringency. However, since antibodies usually persist for a long time, with our data it is not possible to discriminate if the recorded prevalence was due only to antibodies induced by the COVID-19 vaccine or by SARS-CoV-2

infection. The highest value for prevalence, obtained during the first wave (Mar–Sept 2020), was 4%. This finding is in agreement with other results reported in serosurveys in neighboring geographical areas and in a similar time-period. In a study, evaluating 2,602 participants of the Vo' municipality in the Veneto region, a prevalence of 3.5% was registered [20]. A comparable prevalence was found in a further study evaluating 8,285 healthcare workers from the same region [7]. Differently, in two studies considering the Lombardy region, which was a highly hit area in Italy, prevalence ranging from 3 to 43% was found (23,24). Another study from the Lombardy region, reported a prevalence of 11.3% from September 2020 to March 2021 [21].

Since DBS are not usually stored frozen, we performed some stability studies, measuring total IgG and Albumin in a series of 120 samples collected during the entire time period. Results showed an absence of any statistically significant trend or deviations for both measurands during the study period. These findings are in agreement with results reported by Bjorkesten et al. thus supporting the evidence that drying of blood drops does not compromise the detection of proteins and immunoglobulins, when samples are stored at specific temperature conditions [22].

Some limitations of the study should be mentioned. The first is that we did not perform a verification study of the analytical method; however, linearity and clinical performances were previously verified elsewhere by other groups [15, 16]. Furthermore, as DBS were residual material, no other clinical information regarding both infants and mothers was available. For the same reason, it lacked the opportunity to quantify additional types of anti-SARS-CoV-2 antibodies (e.g. Abs against nucleocapsid protein, detectable only after infection) advantageous for the discrimination between vaccine or SARS-CoV-2 infection.

On the other hand, one strength of the study is its inclusion of samples obtained from a vast (Trentino Alto Adige) region of Italy and the high number of DBS analyzed.

Conclusions

The findings made in the present study demonstrate that DBS is a suitable tool for both epidemiological purposes and surveillance in the SARS-CoV-2 pandemic, particularly in newborns, children, and mothers, obviating blood waste and sparing patients' pain.

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Informed consent: Not applicable.
Ethical approval: The study was conducted in accordance with the Declaration of Helsinki, and the Institutional Review Board of the University of Padova (protocol no. 27444).

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