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SHORT REPORT

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# Serum anti-Spike immunoglobulin G levels in random blood donors in Italy: High-titre convalescent plasma is easier than ever to procure

Daniele Focosi[1] | Silvia Meschi[2] | Sabrina Coen[2] | Maria Carla Iorio[1] | Massimo Franchini[3] | Maria Lanza[1] | Fabrizio Maggi[2]

[1]North-Western Tuscany Blood Bank, Pisa University Hospital, Pisa, Italy

[2]Laboratory of Virology, National Institute for Infectious Diseases, Lazzaro Spallanzani IRCCS, Rome, Italy

[3]Carlo Poma Hospital, Mantua, Italy

**Correspondence**
Daniele Focosi, North-Western Tuscany Blood Bank, Pisa University Hospital, via Paradisa 2, 56124 Pisa, Italy.
Email: daniele.focosi@gmail.com

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## Abstract

**Background and Objectives:** COVID-19 convalescent plasma (CCP) has retained potency and clinical efficacy against SARS-CoV-2 and is currently of utmost value for seronegative immunocompromised patients. Since most of the effect is due to the vaccine boost of infection-elicited antibodies, there is a theoretical concern that the frequency of suitable donors is declining.

**Materials and Methods:** In this single-institution serosurvey, we screened 599 consecutive donors attending our area in two different seasons (300 in November 2022 and 299 in February 2023) using the Abbott Alinity® anti-Spike immunoglobulin G assay.

**Results:** More than 80% of random donors qualify according to the FDA criteria for high-titre CCP (>4350 AU/mL), with a stable trend.

**Conclusion:** Despite reduced anti-Spike vaccine boost deployment in the general population, we have shown here that high-titre CCP units are easier than ever to procure. This finding also has implications for the derivation of standard immunoglobulins, which are finally approaching the potency of hyperimmune serum and could soon represent an alternative to CCP.

## Keywords

blood donors, convalescent plasma, COVID-19, SARS-CoV-2

## Highlights

- COVID-19 convalescent plasma (CCP) is retaining clinical utility for immunocompromised patients.
- More than 80% of random blood donors in Italy qualify as high-titre CCP donors according to their anti-Spike immunoglobulin G content.
- Lots of standard immunoglobulins deriving from current donations are likely to have high anti-Spike antibody content.

## INTRODUCTION

COVID-19 convalescent plasma (CCP) has been the main first-line treatment for the COVID-19 pandemic in spring 2020, based on the expected efficacy of anti-SARS-CoV-2 Spike-neutralizing antibodies (nAb). Most randomized controlled trials (RCTs) have failed when low-titre CCP units were delivered to late-stage hospitalized patients who were seropositive and immunocompetent [1], but it later emerged that, as per any other antiviral, efficacy is maximized when high-titre units are delivered to early outpatients [2]. Given the widespread

Daniele Focosi and Silvia Meschi contributed equally.

  

availability of oral small-molecule antivirals, interest in CCP for outpatients has almost disappeared. There is instead a growing interest in passive immunotherapies for immunocompromised patients who cannot mount a protective serological response after either vaccination or infection, for which full safety [3] and efficacy at reducing mortality have been established in RCTs [4]. These patients often have pharmacokinetic contraindications to small-molecule antivirals [5], and to date, none of the authorized anti-Spike monoclonal antibodies (mAbs) has retained in vitro efficacy [6–8]. Furthermore, mAbs come with a significant risk of treatment-emergent immune escape [9].

As such, there is an interest in both CCP (as an immediately available treatment with preserved efficacy against emerging strains [10, 11]) and standard polyclonal immunoglobulins. The latter is an industrial CCP derivative regularly administered to immunocompromised patients: while it comes with a 6-month delay between collections and marketing, the steady-state heterologous immunity granted by hybrid exposures (vaccinations + infection) provides opportunities for durable efficacy, hence equating hyperimmune serum [12].

In this study, we investigated the prevalence of high anti-Spike immunoglobulin G (IgG) titres (as defined by FDA standards [13] later adopted by the European Commission [14]) using high-throughput serology between two distinct groups of blood donors in two different time frames (end of 2022 and beginning of 2023).

## MATERIALS AND METHODS

### Donors and samples

Residual anonymized serum aliquots from 599 blood donors (300 from November 2022 and 299 from March 2023) attending one of the transfusion services afferents to the North-Western Tuscany Blood Bank (which would otherwise be disposed of after mandatory serological disease screening) were tested for anti-Spike IgG levels.

### Anti-Spike IgG testing

Briefly, 35 µL of serum from each aliquot were tested using the commercial chemiluminescence microparticle antibody assay SARS-CoV-2 IgG II Quant (Alinity I, Abbott Diagnostics, Chicago, IL, USA) according to the manufacturers' instructions. The assay is designed to detect IgG antibodies to the receptor binding domain of the S1 subunit of the Spike protein of SARS-CoV-2. Values ≥50 arbitrary units (AU)/mL were considered positive.

## RESULTS

Figure 1 reports the distribution of anti-Spike IgG levels in the 599 donors. Briefly, among the 300 donors from the November 2022 cohort, 8 (2.7%) were seronegative, 66 (22%) had levels >40,000 AU/mL and 11 (3.7%) had levels ≥80,000 AU/mL. Among the 299 donors from the March 2023 cohort 5 (1.7%) were seronegative, 48 (16.1%) had levels >40,000 AU/mL and 4 (1.3%) had levels ≥80,000 AU/mL. None of these parameters was statistically different ($p > 0.05$) between the two cohorts. Importantly, in the November 2022 cohort, 242 (80.7%) had levels >4350 AU/mL (a threshold shown to correlate with nAb titres >1:160 against wild-type SARS-CoV-2 and used by the FDA to define high-titre CCP), which remained stable at 249 (83.3%) in March 2023. Globally, 491 of 599 (82%) of random donors then would have qualified as high-titre CCP donors without any past medical history screening.

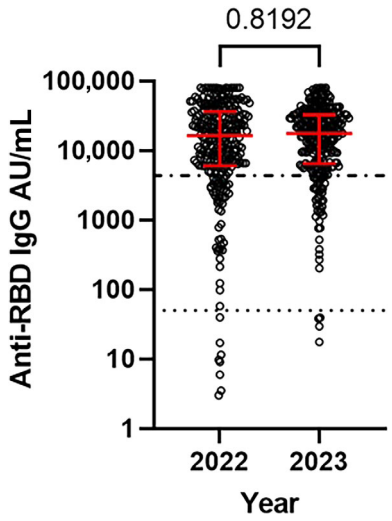


**FIGURE 1** Distribution of anti-Spike immunoglobulin G (IgG) levels in the two cohorts of blood donors.

## DISCUSSION

The recruitment of CCP donors is currently at very low volumes (mostly on-demand collections when infectious diseases wards have to manage some refractory immunocompromised COVID-19 patients). Identification of suitable donors is typically based on the collection of past medical histories, with most centres mandating documented exposures to both infection and vaccination, with at least one such event in the past 6 months. Since vaccine programmes are massively declining after most of the population has gotten the second boost (third dose), there are concerns that recruitment of qualified CCP donors could become more and more difficult over time.

We have shown here that the prevalence of high-titre CCP donors is still exceedingly high and steady. The main contributor to this evidence is likely the high-level circulation of SARS-CoV-2 after the removal of non-pharmaceutical interventions such as face masks or social distancing mandates: this translates into asymptomatic or pauci-symptomatic diseases that remain largely undiagnosed. This finding that more than 80% of plasma collections qualify as high-titre CCP has major consequences for CCP procurement and intravenous immunoglobulin (IVIG) qualification, respectively.

First, the criterion of identifying suitable CCP donors based on past medical history comes with the risk of excluding many suitable donors who had an asymptomatic and hence undocumented SARS-CoV-2 infection in the last 6 months. We then suggest relaxation of recruitment rules and avoidance of selection at all. An easier workflow would be to test random fresh plasma units that have not been initially intentionally collected as CCP, and relabel them as CCP whenever high-throughput serology satisfies the threshold mandated by local regulatory authorities. At the current stage, this approach seems cost-effective. An addendum to this consequence is that most hospitalized patients receiving random fresh plasma units for coagulation purposes are actually also receiving short-lasting COVID-19 prophylaxis, which is a welcome extra benefit.

Second, IVIG batches released from plasma manufacturers (made from pools of plasma units collected 6 months earlier) are currently approaching the anti-Spike IgG content of hyperimmune serum [12]: the latter product is much more complex to achieve, requiring dedicated manufacturing chains that no plasma manufacturer is currently willing to implement. Then the widespread availability of high-titre IVIG could represent a cheap, safe and effective pre-exposure prophylaxis (PEP) for immunocompromised patients, given the recent deauthorization of the Evusheld® mAb cocktail because of circulating resistant variants of concern [15, 16]. In summary, IVIG could soon represent a broad-spectrum PEP solution, embedding SARS-CoV-2, and saving money and time for both patients and the healthcare system. Despite RCTs of IVIG failing to show clinical benefit in hospitalized patients in the pre-vaccine era of the pandemic [17, 18], the heterologous immunity induced by hybrid exposure [10, 11] (vaccine + infection) has the potential to provide clinical benefit.

That said, it is important to note that the FDA threshold used here was based on correlation studies with viral neutralization assays employing the wild-type (Wuhan) SARS-CoV-2 lineage. Given reports of reduced neutralization by CCP against recent XBB.* sublineages compared with wild-type SARS-CoV-2 [10, 11], it is urgent for regulatory authorities to reassess high-throughput serology cut-offs correlating with high nAb titres specifically against XBB.*, or alternatively to rely on high-throughput serology employing XBB.* Spike antigens. With the recent WHO recommendation to remove wild-type SARS-CoV-2 and introduce XBB.* Spike into next COVID-19 vaccines, commercial development and marketing of XBB.* Spike-based high-throughput serology have become more likely to come.

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D.F. conceived the study and wrote the first draft; M.C.I. and M.L. provided donor samples; S.C. and S.M. performed laboratory tests; M.F. and F.M. revised the manuscript.

## CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflict of interest related to this manuscript.

## DATA AVAILABILITY STATEMENT

An anonymized dataset is available from the corresponding author upon reasonable request.

## ORCID

*Massimo Franchini* https://orcid.org/0000-0002-8795-0580

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