Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Clinical Microbiology and Infection 27 (2021) 808–810

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Letter to the Editor

Re-evaluating positive serum samples for SARS-CoV-2-specific IgA and IgG antibodies using an in-house serological assay

Margherita Cacaci [1, 2, †], Giulia Menchinelli [1, 2, †], Rosalba Ricci [2], Flavio De Maio [1, 2], Melinda Mariotti [1], Riccardo Torelli [2], Grazia Angela Morandotti [2], Francesca Bugli [1, 2], Maurizio Sanguinetti [1, 2, *], Brunella Posteraro [1, 3]

[1)] *Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy*
[2)] *Dipartimento di Scienze di Laboratorio e Infettivologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*
[3)] *Dipartimento di Scienze Mediche e Chirurgiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*

ARTICLE INFO

Article history:
Received 9 September 2020
Received in revised form
11 December 2020
Accepted 15 December 2020
Available online 2 January 2021

Editor: L. Leibovici

To the Editor,

We read the recent article by Caruana et al. exploring the current landscape of diagnostic tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), and signalling interpretive issues of test results [1]. We were particularly interested in serological testing, which may fill the gap between negative results of RT-PCR—the reference standard for SARS-CoV-2 diagnosis [2]—and clinical (and radiological) findings suggestive of COVID-19 [3,4]. Like molecular testing [5], targeting the SARS-CoV-2 spike (S) protein (or the subunit S1 thereof) rather than the nucleocapsid (N) protein with an ELISA to detect virus-specific antibodies in patient serum may be crucial for diagnostic yield [6]. Sensitivity of ELISAs based on the N or S protein varies depending on the infection timing [1]. Additionally, testing for only IgM and IgG [7–9] may be limited in samples taken around symptom onset [10]. In this context, individuals who present within the first week after symptom onset could benefit from IgA testing [11]. In a recent study [11], the S1-based IgA Euroimmun (Lübeck, Germany) assay revealed good sensitivity compared with an S (or S1) -based IgG Wantai test (Beijing, China) or Euroimmnun assays with individuals sampled at early infection times. Consistently, Caruana et al. experienced a 96% sensitivity with samples collected 15–30 days post infection, using an N-based ELISA (Epitope Diagnostics, San Diego, CA, USA) [1]. Finally, mild (non-hospitalized), moderate (hospitalized) or severe (admitted to the intensive care unit) illness may affect antibody responses in individuals with COVID-19 [8,9].

Using in-house ELISA targeting the SARS-CoV-2 N protein [7], we re-evaluated positive results from the Euroimmnun ELISA for SARS-CoV-2-specific IgA and IgG detection for 122 serum samples of individuals admitted to the emergency department of our institution for suspicion of COVID-19. The institutional ethics committee approved the study (no. 27015/20), and informed consent was obtained from all individuals. Except for 105 individuals with RT-PCR-confirmed SARS-CoV-2 infection, COVID-19 diagnosis in 17 RT-PCR-negative individuals was based on both abnormal radiological findings and positive serology results. Initially, reproducibility of in-house ELISA was assessed testing 30 serum samples from individuals with COVID-19 with different levels of IgA or IgG antibodies. We found that the coefficients of variation were 1.38%–32.22% and 2.06%–21.05% for IgA and IgG, respectively, whereas intra-class correlation coefficients were 0.88 and 0.98 for IgA and IgG, respectively.

As shown in Table 1 and depicted in Fig. 1, all samples with positive IgA/IgG results by Euroimmnun ELISA included samples positive for IgA ($n = 119$) and IgG ($n = 113$); of these samples, 110 were positive for both IgA and IgG, nine for only IgA and three for only IgG. In parallel, samples with positive IgA/IgG results by in-house ELISA included samples positive for IgA ($n = 98$) and IgG ($n = 111$); of these samples, 95 were positive for both IgA and IgG, 3 for only IgA and 16 for only IgG. The in-house assay detected 96/119 IgA-positive samples and 109/113 IgG-positive samples, corresponding to a positive per cent agreement of 80.7% (95% CI 72.4%–87.3%) and 96.5% (95% CI 91.2%–99.0%), respectively. Discrepancies

* Corresponding author: Maurizio Sanguinetti, Dipartimento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy.
E-mail address: maurizio.sanguinetti@unicatt.it (M. Sanguinetti).
† Margherita Cacaci and Giulia Menchinelli contributed equally to this letter, and both should be considered first author.

https://doi.org/10.1016/j.cmi.2020.12.014

Table 1
Summary of serological SARS-CoV-2 antibody testing results for 122 symptomatic COVID-19 patients sampled at different days from the emergency department admission

Patient group (no. of tested)	No. (%) of samples with positive results for:			
	Immunoglobulin A detected with:		Immunoglobulin G detected with:	
	N-based in-house assay	S-based Euroimmun assay	N-based in-house assay	S-based Euroimmun assay
SARS-CoV-2 infection[a]				
Confirmed ($n = 105$)	88 (83.8)	104 (99.0)	101 (96.2)	100 (95.2)
Unconfirmed ($n = 17$)	10 (58.8)	15 (88.2)	10 (58.8)	13 (76.5)
Severity on admission[b]				
Mild ($n = 31$)	19 (61.3)	30 (96.8)	26 (83.9)	27 (87.1)
Moderate ($n = 86$)	74 (86.1)	84 (97.7)	80 (93.0)	81 (94.2)
Severe ($n = 5$)[c]	5 (100.0)	5 (100.0)	5 (100.0)	5 (100.0)
Testing from admission, days				
0–5 ($n = 32$)	23 (71.9)	30 (93.8)	25 (78.1)	26 (81.3)
6–20 ($n = 8$)	7 (87.5)	8 (100.0)	6 (75.0)	7 (87.5)
21–40 ($n = 26$)	21 (80.8)	25 (96.2)	24 (92.3)	25 (96.2)
>40 ($n = 56$)	47 (83.9)	56 (100.0)	56 (100.0)	55 (98.2)

Abbreviations: COVID-19, coronavirus disease 2019; N, nucleocapsid; S, spike; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.
[a] According to positive (confirmed) or negative (unconfirmed) results for SARS-CoV-2 RNA detection by RT-PCR. Except for 105 patients with confirmed SARS-CoV-2 infection, diagnosis of SARS-CoV-2 infection in 17 individuals with negative RT-PCR results was based on both clinical/radiological presentation and positive serology (by Euroimmun assay) findings.
[b] According to the individuals' requirement for non-hospitalization (mild), hospitalization (moderate) or intensive care (severe).
[c] Samples from these individuals also tested positive for IgM by the indicated N-based in-house assay. However, IgM results for all the 122 samples included in the study were not reported because these results were beyond the comparison purposes between in-house and Euroimmun assays.

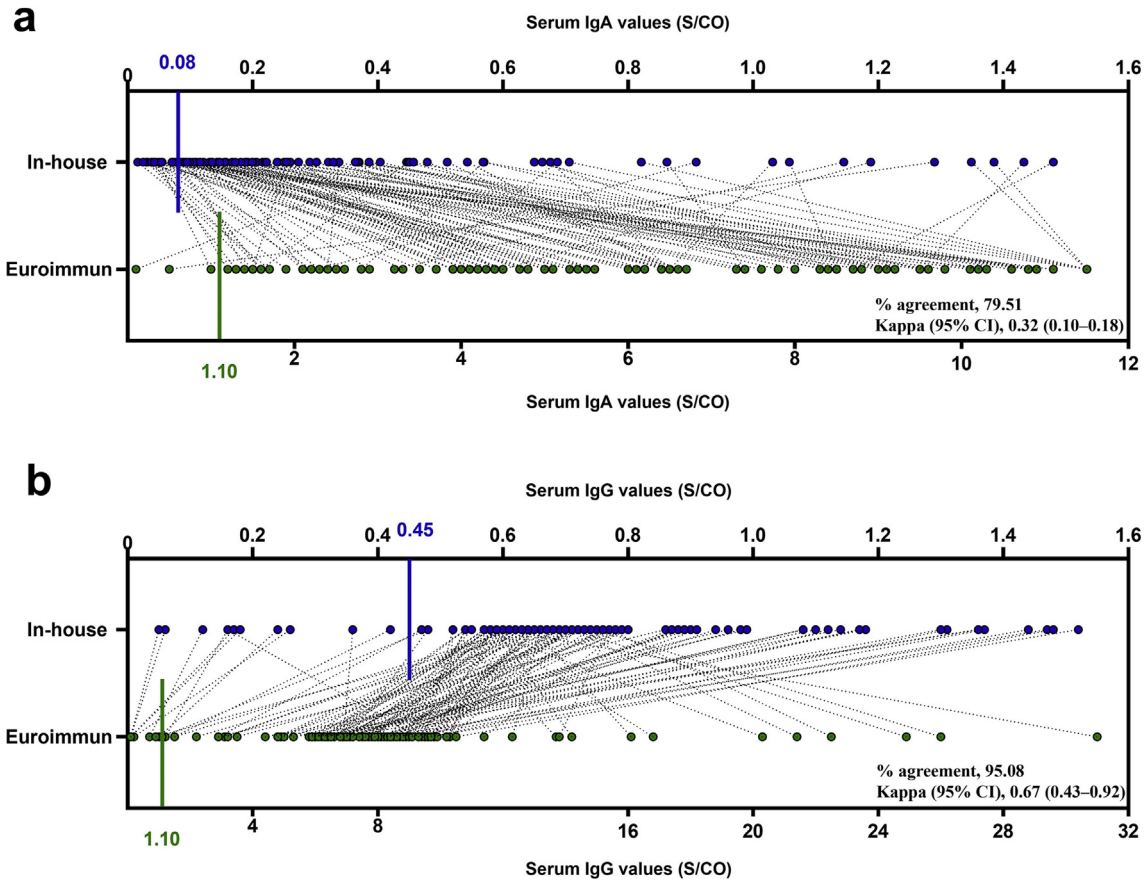

Fig. 1. Agreement of results for 122 serum samples obtained with the Euroimmun and the in-house ELISA tests. Unlike the commercial Euroimmun assay, the in-house assay for IgA and IgG detection was developed based on the use of a recombinant nucleocapsid protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as described elsewhere [7]. For both assays, the antibody levels are shown expressed as spectrometrically measured values divided by the cut-off (S/CO), as are the percentage between-assay agreement values calculated for IgA and IgG antibodies, respectively. The cut-offs for IgA (0.08 and 1.10) and IgG (0.45 and 1.10) antibodies in both assays are marked with vertical blue (in-house assay) or green (Euroimmun assay) lines. The Cohen's κ values indicate fair (range 0.21–0.40) or substantial (range 0.61–0.80) agreement for IgA and IgG results, respectively. Among five samples that tested positive with the in-house assay but negative with the Euroimmnun assay, two were positive for IgA antibodies and three for IgG antibodies, respectively.

between the two assays mainly involved samples that tested negative for IgA by the in-house assay (Table 1). These samples were from individuals with mild (11/30 samples) or moderate (12/62 samples) disease, as well as those collected within the first 5 days (9/30 samples) or after 40 days (9/56 samples) of admission. Although N-based serological correlates of protection from SARS-

CoV-2 infection are not fully understood [12], similar to us, other investigators emphasized the role of anti-SARS-CoV-2 IgA in the current serodiagnostic arsenal for SARS-CoV-2 [13,14], especially in the early phase of infection [15].

We also determined the specificity of N-based serological testing using sera from 85 healthy blood donors or from 15 individuals with non-SARS-CoV-2 respiratory infection and we found that no sera were positive with the N-specific IgA (and IgG) assay. Furthermore, we observed that IgG antibodies detected in two individuals who tested positive—one with the in-house ELISA only and one with both the in-house and Euroimmun ELISAs—were able to neutralize the Vero E6 cell-cultured SARS-CoV-2 (titres were 1 : 80 in both individuals). Likewise, IgA antibodies detected in two other individuals who tested positive—one with the in-house ELISA only and one with both in-house and Euroimmun ELISAs—were able to neutralize the Vero E6 cell-cultured SARS-CoV-2 (titres were 1 : 20 and 1 : 640, respectively). Although these observations are consistent with recently published data [16,17], for reasons of comparability, we did not include data regarding the detection of IgM antibodies by the assay.

In conclusion, we suggest that serology targeting the SARS-CoV-2 S protein, such as the Euroimmun ELISA, should be preferable. We recorded the highest sample positivity rates with S-based testing for IgA antibodies in individuals tested early or in individuals with mild COVID-19 (not requiring hospitalization) on admission (Table 1). Hence, we propose considering IgA testing in all situations where serology is the solely practicable diagnostic strategy for SARS-CoV-2 infection. Future studies will help to decide on the deployment of serological assays for specific contexts in COVID-19 diagnostics.

Transparency declaration

The authors declare that they have no conflicts of interest. No external funding was received for this study.

Acknowledgements

We wish to thank Franziska Lohmeyer for her English language assistance.

References

[1] Caruana G, Croxatto A, Coste AT, Opota O, Lamoth F, Jaton K, et al. Diagnostic strategies for SARS-CoV-2 infection and interpretation of microbiological results. Clin Microbiol Infect 2020;26:1178–82.
[2] WHO.int. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance. World Health Organization; 2020. Available from: https://apps.who.int/iris/handle/10665/331329. [Accessed 8 September 2020].
[3] Rodriguez-Morales AJ, Cardona-Ospina JA, Gutiérrez-Ocampo E, Villamizar-Peña R, Holguin-Rivera Y, Escalera-Antezana JP, et al. Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. Travel Med Infect Dis 2020;34:101623.
[4] Cheng MP, Papenburg J, Desjardins M, Kanjilal S, Quach C, Libman M, et al. Diagnostic testing for severe acute respiratory syndrome-related coronavirus 2: a narrative review. Ann Intern Med 2020;172:726–34.
[5] Tang YW, Schmitz JE, Persing DH, Stratton CW. Laboratory diagnosis of COVID-19: current issues and challenges. J Clin Microbiol 2020;58:e00512–20.
[6] Zheng M, Song L. Novel antibody epitopes dominate the antigenicity of spike glycoprotein in SARS-CoV-2 compared to SARS-CoV. Cell Mol Immunol 2020;17:536–8.
[7] Guo L, Ren L, Yang S, Xiao M, Chang D, Yang F, et al. Profiling early humoral response to diagnose novel coronavirus disease (COVID-19). Clin Infect Dis 2020;71:778–85.
[8] Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis 2020. Epub ahead of print.
[9] Long QX, Liu BZ, Deng HJ, Wu GC, Deng K, Chen YK, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. Nat Med 2020;26:845–8.
[10] Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. Cochrane Database Syst Rev 2020;6:CD013652.
[11] GeurtsvanKessel CH, Okba NMA, Igloi Z, Bogers S, Embregts CWE, Laksono BM, et al. An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment. Nat Commun 2020;11:3436.
[12] Cheng MP, Yansouni CP, Basta NE, Desjardins M, Kanjilal S, Paquette K, et al. Serodiagnostics for severe acute respiratory syndrome-related coronavirus 2: a narrative review. Ann Intern Med 2020;173:450–60.
[13] La Rosa Fabián C, Urquizo Briceño L. Anti-SARS-Cov-2 IgA in current scenario of IgM and IgG rapid test: a new alternative for the diagnostic of covid-19. Sep 26 SN Compr Clin Med 2020:1–3. Epub ahead of print.
[14] Orth-Höller D, Eigentler A, Stiasny K, Weseslindtner L, Möst J. Kinetics of SARS-CoV-2 specific antibodies (IgM, IgA, IgG) in non-hospitalized patients four months following infection. Sep 19 J Infect 2020;S0163–4453. 30593-4. Epub ahead of print.
[15] Pieri M, Ciotti M, Carlozzi N, Frassanito ML, Meloni A, Cistera A, et al. SARS-CoV-2 infection serology validation of different methods: usefulness of IgA in the early phase of infection. Clin Chim Acta 2020;511:28–32.
[16] Grzelak L, Temmam S, Planchais C, Demeret C, Tondeur L, Huon C, et al. A comparison of four serological assays for detecting anti-SARS-CoV-2 antibodies in human serum samples from different populations. Sci Transl Med 2020;12:eabc3103.
[17] Chen Y, Tong X, Li Y, Gu B, Yan J, Liu Y, et al. A comprehensive, longitudinal analysis of humoral responses specific to four recombinant antigens of SARS-CoV-2 in severe and non-severe COVID-19 patients. PLoS Pathog 2020;16:e1008796.