Short Communication

A novel SARS-CoV-2 IgG line-blot for evaluating discrepant IgG test results — Observations in pre-pandemic and follow-up samples of five patients

Andrea Steiner [a], Mathias Pletz [b], Bettina Löffler [c], Michael Baier [c,*]

[a] Department of Occupational Medicine, Jena University Hospital, Jena, Germany
[b] Centre of Infectious Diseases and Infection Control, Jena University Hospital, Jena, Germany
[c] Institute for Medical Microbiology, Jena University Hospital, Jena, Germany

Abstract To confirm discrepant SARS-CoV-2-IgG results in four standard assays we applied for the first time a prototype of a coronavirus IgG-line-blot which employs antigens from seasonal coronaviruses, SARS-1 and SARS-CoV-2 combined with avidity testing as a confirmatory tool in a follow-up of five cases including pre-pandemic samples.

Introduction

Since the beginning of the COVID-19 pandemic a large number newly developed PCR assays detect viral RNA in respiratory samples with high accuracy while several formats of serological assays are available to find SARS-CoV-2 specific antibodies in blood samples with different reliability.[1–3] Whereas PCR-tests provide a snapshot on the infectiousness, serological tests may predict previous infection and perhaps a certain degree of immune protection in an individual.[4] The latter are also important to reflect and monitor the infection rate in a population and thereby serving as a tool to develop prevention strategies and to implement hygiene measures.

Here we present observations (i) that, depending on the serological assay used, specific SARS-CoV-2 antibodies drop below cut off levels in less than 9 weeks after PCR proven infection, (ii) that false positive results may pretend

* Corresponding author.
E-mail address: michael.baier@med.uni-jena.de (M. Baier).

Figure 1 table:

case (PCR status)	time since onset of symptoms (days)	T1	T2	T3	T4	T5	T1 [S/Co]	T2 (AU/ml)	T3 (AU/ml)	T5 (gray scale value)	229E	NL63	OC43	HKU1	SARS-1	SARS-2	229E	NL63	OC43	HKU1	SARS-1	SARS-2
		qualitative results					quantitative results				line blot (T5) - semiquantitative results						line blot (T5) - avidity					
A (+ till day 19)	-2310	-	-	-	-	-	0,21	4,9	0,30	0,00	(+)	(+)	+	++								
	20	+	+	+		+	2,09	76,5	6,38	3,40	(+)	(+)	++	+++	++	+++		0,75 (high)	0,67 (high)	0 (low)		0,15 (low)
	37	+	+	+	+	+	1,98	108,0	4,99	2,60	(+)	(+)	+	+	+	++			0,38 (low)	0 (low)		0 (low)
	42	+	+	+	+	+	1,65	100,0	3,93	2,10			(+)	+	+	++			1,47 (high)	0,25 (low)		0,24 (low)
	58	+	+	+	+	+	1,63	117,0	3,70	4,60	(+)	(+)	++	+++	+++	+++		0,67 (high)	0,59 (high)	0,38 (low)		0,29 (low)
	63	+	+	+	+	+	1,25	136,0	3,60	4,50	(+)	(+)	+	++	+++	+++			0,78 (high)	0,65 (high)	0,23 (low)	0,34 (low)
B (+ till day 13)	-1304	-	+	-	-	-	0,25	49,8	0,32	0,00	+	(+)		(+)			0,83 (high)					
	-745	-	+	-	-	-	0,28	46,2	0,30	0,00	(+)	(+)										
	18	+	+	-	+	(+)	0,77	59,3	0,49	0,80	(+)	+			(+)	(+)		0,86 (high)				
	56	-	+	-	+	+	0,69	62,8	0,54	2,20	(+)	+		(+)	+	++		0,84 (high)			0 (low)	0 (low)
C (+ till day 20)	-453	-	-	-	-	-	0,26	6,7	0,28	0,00		(+)										
	20	+	+	+	+	+	0,94	52,1	1,32	1,50		(+)	(+)	(+)	+	+					0 (low)	0 (low)
	58	-	+	-	+	+	0,62	98,9	0,89	2,50		(+)			+	++					0 (low)	0,15 (low)
D (+ till day 37)	-181	-	-	-	-	-	0,24	<3,8	0,28	neg	(+)	++	+++	+++				0 (low)	0 (low)	0,2 (low)		
	20	+	+	+		+	1,71	26,9	7,13	3,40	+	+++	+++	+++	+++	+++	0,71 (high)	0,76 (high)	0,77 (high)	0,96 (high)	0,36 (low)	0,33 (low)
	21	+	+	+	+	+	1,67	26,6	4,41	3,10	+	++	+++	+++	++	+++	0,84 (high)	0,75 (high)	0,67 (high)	0,95 (high)	0,3 (low)	0,26 (low)
	58	+	+	+	+	+	1,40	89,4	4,36	1,80		(+)	(+)	+	+	+					0,5 (low)	0,56 (low)
E (all PCR neg)	-593	-	-	-	+	(+)	0,54	<3,8	0,63	0,60	+++	+++	+	+	(+)	(+)	0,81 (high)	0,73 (high)	0,3 (low)	0,69 (high)		
	26	(+)	-	-	-	-	0,49	<3,8	0,31	0,00		(+)										
	40	-	-	-	-	-	0,48	8,4	0,31	0,00	(+)	++		(+)				0,91 (high)				

Figure 1. Qualitative and quantitative results of five IgG assays (T1 to T5) in a longitudinal follow up of five cases (A to E). The line blot prototype (T5) offers 6 recombinant nucleocapsid (NC) antigens specific to seasonal coronavirus 229E, NL63, OC43 and HKU1, SARS-1 and SARS-CoV-2. (RC — reaction control, CC — conjugate control, CO—cut off).

immune protection in individuals without PCR proven exposure and (iii) that these limitations can be addressed with a SARS-CoV-2 specific line blot to facilitate final and consistent diagnosis.

Methods

To prevent hospital outbreaks at the Jena University Hospital with 1.300 beds and 3.500 health care workers (HCW) employed, strict protective measures were introduced, e.g. PCR screening of nasopharyngeal swabs was performed in all HCW returning from risk areas and with occupational or private contacts with confirmed SARS-CoV-2 cases. Members of staff tested positive and with COVID19-typical symptoms were enrolled in a follow up program to monitor viral secretion and specific antibody dynamics.[5] PCR was performed using QIAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Hilden, Germany) for extraction and LightMix Modular SARS and Wuhan CoV E-gene (TIBMOLBIOL, Berlin, Germany) for amplification on LightCycler 480 II instrument. Specific IgG-antibodies were detected with five serological assays (EDI Novel Coronavirus COVID-19 IgG ELISA, Epitope Diagnostics, San Diego, USA (T1); Liaison SARS-CoV-2 S1/S2 IgG CLIA, Saluggia, Italy (T2); MAGLUMI 2019-nCoV IgG (CLIA), Snibe, Shenzhen, China (T3)[6]; Cleartest Corona, Seroprax, Wesel, Germany (T4) and recomLine Coronavirus IgG/avidity (RUO), Mikrogen, Neuwied, Germany (T5)). The fifth test (T5) is a prototype of a newly developed commercial line blot format containing recombinant nucleocapsid antigen fragments from all four seasonal coronaviruses (CoV), SARS-1 and SARS-CoV-2. Pre-pandemic blood samples from enrolled HCW were included.

Results

1.736 swabs were screened from 1.344 employees (median age: 37 years, 36% male) between March 11th and May 15th 2020 and 35 HCW (2,6%) were tested positive. Four of five individuals with COVID19-typical symptoms and risk contacts enrolled in the follow up had PCR proven infection, some with prolonged RNA shedding up to day 37 (refer to Fig. 1).

Each of the five serological assays applied showed its own characteristics: (i) IgG-ELISA signals in test 1 (T1, ELISA, antigen: nucleocapsid, a in Fig. 2) decreased over time with two individuals (case B and C) dropping below the cut off level and thereby turning seronegative again eight to nine weeks after PCR proven infection. (ii) In test T2 (IgG CLIA, spike protein, b in Fig. 2) signals gained in intensity almost throughout the entire observation period in three of four cases. In case B two pre-pandemic samples were tested positive with this assay. (iii) In test 3 (T3, IgG CLIA, nucleocapsid and spike antigen, c in Fig. 2) a constant decrease in IgG intensity was observed, but in case B no signal was detectable in none of all four samples. (iv) Qualitative readings from the lateral flow assay (LFA, T4) showed a delayed response in cases A and D and a positive result in a pre-pandemic sample in case E.

(v) With the coronavirus (CoV) line blot prototype (T5, nucleocapsid from coronaviruses 229E, NL63, OC43, HKU1, SARS-1 and SARS-CoV-2) specific SARS-CoV-2-IgG was detected in all follow up samples from four PCR proven cases (for details refer to Fig. 1). The intensity of the gray scale values of SARS-CoV-2 specific bands showed an increase in three of four cases (d in Fig. 2) and avidity
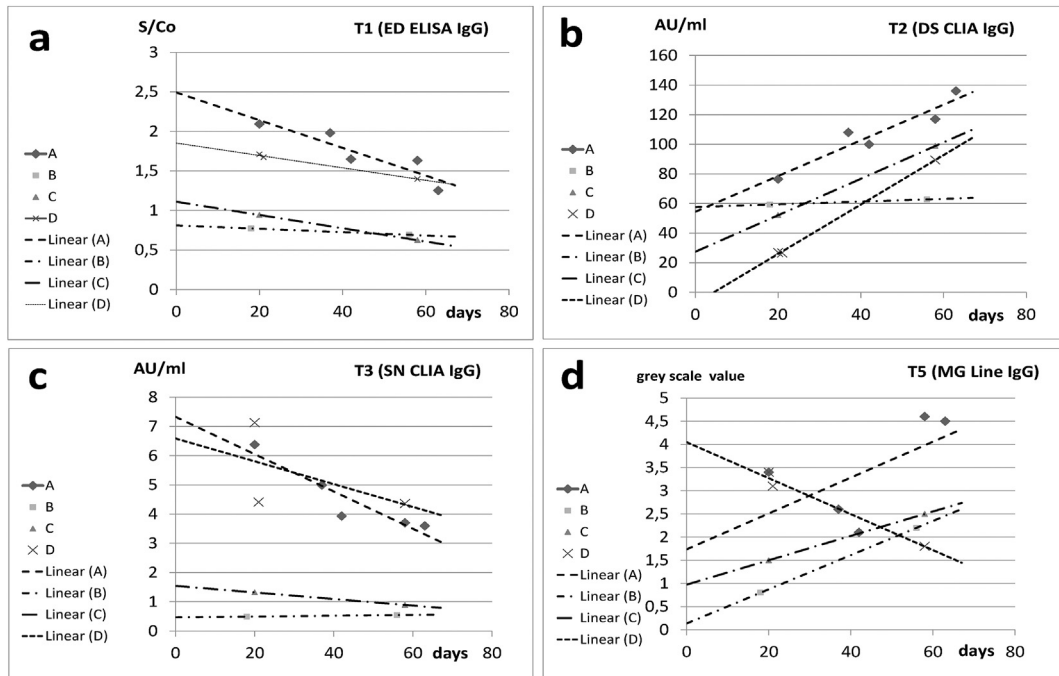
Figure 2. Follow up of quantitative IgG readings of four SARS-CoV-2 specific assays. Linear trends are displayed for each case. (ED - Epitope Diagnostics, DS — DiaSorin, SN — Snibe, MG — Mikrogen).

gradually rises during follow up (case A in Fig. 1). However, the absolute avidity level remained low over the nine weeks observation period. In pre-pandemic samples of four out of five cases (80%) IgG against seasonal coronaviruses were present. In SARS-CoV-2 positive samples from cases with pre-pandemic seasonal CoV signals, the corresponding 229E, NL63, OC43 or HKU1-antigens were always co-reacting. Parallel reactivity was more prominent for beta-CoV (OC43, HKU1, SARS-1) than for alpha-CoV (229E, NL63). SARS-1-signals accompany SARS-CoV-2 in all cases, albeit in reduced intensity, but both were never seen in pre pandemic specimen.

Discussion

We followed up five cases after suspected COVID19-infection with five different SARS-CoV-2 IgG assays including a recently developed line blot prototype for the first time. In all four cases with PCR proven infection we observe IgG sero-conversion in at least three out of four tests at day 20. One case with no positive PCR results turned out to remain seronegative.

With the applied standard tests we note a number of inconsistencies: While early loss of IgG is evident with the ELISA test at day 56 and 58 (T1, case B and C) other assay formats produce delayed (T4, case A), false negative (T3, case B) or false positive results (T2, pre-pandemic samples case B).[3,7] The capture antigen involved seems to play a role, but other factors may contribute as well, since assay T3, containing both nucleocapsid and spike antigen (the first considered to be more sensitive than the latter) was unable to identify specific antibodies in all samples in case B and one in case C.[1,2]

The coronavirus line blot in the present prototype edition is equipped with recombinant nucleocapsid antigens of a representative variety of coronaviruses: along with SARS-CoV-2 four seasonal alpha and beta CoV (229E, NL63, OC43, HKU1) and SARS-1. This multivalent approach offers the opportunity to (i) differentiate between specific and cross reacting anti-bodies for SARS-CoV-2-infection, and (ii) provides additional information on IgG status for the less pathogenic CoV and SARS-1 (refer to case series A, B and D).[2,8] In our panel, we observed cross reactivity of SARS-CoV-2 with seasonal CoV (more prominent in beta-CoV) but far less in case of seasonal CoV with SARS-CoV-2 (case E, negative for SARS-CoV-2) pointing to a higher specificity of SARS-CoV-2 nucleocapsid antigen in this assay. However, SARS-1- and SARS-CoV-2-antigens do cross react in this line format prototype to a high degree. The completion of the line blot with additional CoV-specific antigens e.g. spike protein domains would certainly increase the diagnostic value of the assay.

Avidity testing ad on allows specifying early and late infection phase. This follow up, however, was too short to reach the state of full avidity maturation, but this marker could be a valuable tool in future.

Is there a place for line blots in routine serology diag-nostic for SARS-CoV-2? The introduction of a two-step test strategy for e.g. HIV, lyme disease and syphilis using line or westernblot blot systems has largely improved sensitivity and specificity of antibody detection.[9] Now, a similar approach may help to overcome at least in a subset of not conclusive cases some limitation of the available test for-mats, which do not allow simultaneously assessment of reactivities against other CoV.

In this follow up with PCR proven SARS-CoV-2 infection over nine weeks we observed inconsistencies in SARS-CoV-2 IgG dynamics when different test assays were deployed.

Our data demonstrate that a coronavirus line blot format containing specific antigens from different CoV and recruited after a first screening step as a confirmatory tool can help to increase specificity, to distinguish infection with other e.g. seasonal CoV and to define the phase of infection, as IgG avidity can be measured.

Acknowledgements

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