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Virology

False positive Herpes Simplex IgM serology in COVID-19 patients correlates with SARS-CoV-2 IgM/IgG seropositivity

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ABSTRACT

Differentiating COVID-19 from other causes of viral pneumonia, like herpes simplex (HSV), can be complicated by shared clinical and laboratory features. Viral pneumonia is mostly diagnosed based on molecular or serological techniques. Serological immunoassay interferences, often attributed to concurrent appearance of heterologous (viral) immunoglobulins, is well-known, but has not been studied in COVID-19 patients. Following false positive HSV immunoglobulin M (IgM) results in our index patient, 25 other COVID-19 patients were tested for HSV-1/2 IgM with the chemiluminescent Liaison assay and Euroimmun enzyme-linked immunosorbent assay. Forty-five percent of COVID-19 patients tested positive for HSV IgM with Liaison. No HSV indices were positive with Euroimmun enzyme-linked immunosorbent assay, suggesting immunoassay interference. Significant correlation between HSV IgM and SARS-CoV-2 IgM/IgG positivity was found. Adding 0.5% polyvinylpyrrolidone, inhibiting non-specific solid-phase adsorption, abolished interference in 22% of false positive cases, suggesting interference caused by solid-phase reactive IgM. Hence, serologic immunoassay results should be interpreted with caution in COVID-19 patients.

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1. Introduction

Immunoassays are commonly used as routine serological laboratory tests to demonstrate the presence of virus-specific immunoglobulins (IgM/IgG) in order to diagnose viral infections and determine course of treatment [1]. However, immunoassays may be subjected to interferences, causing false positive or false negative results. These erroneous serological results can have a major impact on clinical investigation, patient care and public health. Hence, correct identification of known and novel interfering factors in serological assays is important. Frequently reported causes of positive interference are rheumatoid factor (RF) or other autoantibodies, heterophilic antibodies, human anti-animal antibodies, albumin, complement, lysozyme, fibrinogen and paraproteins [2,3]. Either these molecules cross-react by structurally resembling the analyte of interest, either through interaction with the antigen or antibody constituents of the assay, for example RF IgM binds the Fc fragment of human IgG's [4].

In case of serological IgM detection on antigen-coated magnetic microparticles with chemiluminescent immunoassays (CLIA) of the Liaison® XL diagnostics platform (DiaSorin, Italy), interferences have been described due to the concurrent appearance of other immunoglobulins [1,5-7]. During an outbreak of parvovirus B19 in 2009 it was shown that B19 IgM-positive sera had falsely elevated Epstein-Barr (EBV) IgM in 84% (57 out of 68) of cases and herpes simplex (HSV) IgM in 90% (61 out of 68) of cases [6]. Here, interference was attributed to the direct binding of IgM antibodies to the surface-modified polystyrene micro particles of other assays and were thus termed as "solid-phase reactive antibodies" [5].

Currently, humanity is facing the ongoing outbreak and rapid spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), resulting in the global COVID-19 pandemic. As of the beginning of December 2021, more than 264 million cases have been reported in 191 different countries [8]. In analogy to the falsely elevated results in B19 parvovirus IgM-positive sera, serology assays might also be affected in SARS-CoV-2 infected patients, particularly the HSV or EBV IgM CLIA [5,6].

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Table 1
Characteristics of this cohort of COVID-19 affected individuals.

	Total population (n = 26)	SARS-CoV-2 IgM negative (n = 11)	SARS-CoV-2 IgM positive (n = 15)	*P*-value
Female gender (n; %)	12 (46%)	9 (82%)	3 (20%)	$P = 0.004$[a]
Intubation (n; %)	5 (19%)	1 (9%)	4 (27%)	$P = 0.356$[a]
Intensive Care (n; %)	6 (23%)	1 (9%)	5 (33%)	$P = 0.197$[a]
Death (n; %)	3 (12%)	1 (9%)	2 (13%)	$P = 1.000$[a]
Age (years)	70.7 ± 13.7	75.0 ± 15.0	67.6 ± 12.1	$P = 0.177$[b]
Days of hospitalization (days)	24.6 ± 20.4	19.3 ± 13.7	28.5 ± 23.9	$P = 0.261$[b]
Bilirubin total (mg/dL)	1.2 ± 1.4	0.7 ± 0.3	1.5 ± 1.8	$P = 0.216$[b]
Bilirubin conjugated (mg/dL)	0.7 ± 1.1	0.3 ± 0.1	1.1 ± 1.4	$P = 0.154$[b]
Bilirubin unconjugated (mg/dL)	0.4 ± 0.3	0.4 ± 0.2	0.5 ± 0.4	$P = 0.719$[b]
AST (U/L)	43.7 ± 48.7	21.3 ± 8.9	60.1 ± 59.1	$P = 0.025$[b]
ALT (U/L)	55.6 ± 88.4	16.6 ± 9.2	86.0 ± 108.2	$P = 0.027$[b]
GGT (U/L)	150.7 ± 239.7	26.4 ± 7.6	248.4 ± 287.6	$P = 0.013$[b]
ALP (IU/L)	135.4 ± 141.7	73.2 ± 28.6	184.2 ± 174.9	$P = 0.035$[b]
LDH (U/L)	295.5 ± 89.5	267.9 ± 61.0	316.8 ± 103.7	$P = 0.201$[b]
HSV IgM+ Liaison 327002 (n; %)	14 (54%)	2 (18%)	12 (80%)	$P = 0.004$[a]
HSV IgM+ Liaison 327003 mean of 2 experiments (n; %)	9 (35%)	1 (9%)	8 (53%)	$P = 0.036$
HSV IgM+ Euroimmun ELISA positive (n; %)	0 (0%)	0 (0%)	0 (0%)	
HSV IgM Liaison 327002 (index)	2.050 (0.559; 3.501)	0.753 (0.499; 1.060)	3.501 (1.730; 3.501)	$P = 0.0057$[c]
HSV IgM Liaison 327003 mean of 2 experiments	0.767 (0.499; 1.261)	0.499 (0.499; 0.746)	1.110 (0.640; 1.740)	$P = 0.0025$[c]
HSV IgM Euroimmun ELISA (index)	0.580 (0.270; 0.725)	0.280 (0.160; 0.670)	0.650 (0.460; 0.860)	$P = 0.0145$[c]
SARS-CoV-2 IgM + Cypress Diagnostics positive (n; %)	15 (58%)	-	-	-
SARS-CoV-2 IgG + Cypress Diagnostics positive (n; %)	22 (85%)	9 (82%)	13 (87%)	$P = 1.000$[a]

Abbreviations: RF = rheumatoid factor; HSV IgM = herpes simplex virus immunoglobulin M; CLIA = chemiluminescence immunoassay; ELISA = enzyme-linked immunosorbent assay; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyl transferase; ALP = alkaline phosphatase; LDH = lactate dehydrogenase; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; IgG = immunoglobulin G.
[a] Fisher exact test.
[b] Student's *t* test.
[c] Mann-Whitney U test.

Interpretation of potentially false positive HSV IgM serology results in COVID-19 patients can be complicated, since COVID-19 and HSV may present with similar clinical manifestations and laboratory results. Especially in immunocompromised hosts, disseminated HSV infections have been reported, presenting with meningitis, encephalitis, hepatitis and/or pneumonia. Cases of viral pneumonia due to HSV have also been described in immunocompetent hosts [9]. Differential diagnosis is further complicated by similar findings in HSV and SARS-CoV-2 associated pneumonia on high resolution Computed Tomography (CT) consisting of diffuse ground-glass attenuation and/or consolidations [10,11]. Moreover, confirmation of true positive HSV serology and HSV-associated pneumonia with other diagnostic tools is complicated since debate continues as to whether HSV nucleic acid detection in lower respiratory tract specimens, for example bronchoalveolar lavage fluid (BALF), with reverse transcription-polymerase chain reaction (RT-PCR) represents true clinical infection rather than innocuous viral shedding [12]. Besides sensitivity issues, collection of the necessary BALF specimen with bronchoscopy is relatively contra-indicated in patients with suspected COVID-19 and should only be considered in certain diagnostic or therapeutic settings [13–15].

Up till now, non-specific binding to the solid phase of infectious-disease immunoassays remains elusive in COVID-19 patients. We hereby demonstrate false positive herpes simplex IgM serology in COVID-19 patients, and consistently link this interference to SARS-CoV-2 IgM/IgG seropositive samples. Furthermore, this paper shows the importance of interference elimination studies, providing clinical laboratories with answers to this interference during the ongoing COVID-19 crisis and potential new outbreaks in the future.

2. Material and methods

2.1. Patient inclusion and ethics statement

A cohort of COVID-19 affected individuals (N = 26, 12 females, 14 males), requiring hospitalization at the GZA hospital group between March and May 2020, were included in this study. Diagnosis of COVID-19 was confirmed with an in-house developed SARS-CoV-2 polymerase chain reaction (PCR) performed on nasopharyngeal swab or bronchoalveolar lavage fluid in all patients. Serum samples were collected for routine diagnostic purposes using standard procedures. All study participants consented to the use of their sera and clinical data for medical and research purposes, according to GZA institutional review board requirements.

2.2. SARS-CoV-2 rRT-PCR

Viral RNA was extracted with the QIAsymphony DSP Virus/Pathogen Mini kit (Qiagen®; Hilden, Germany, 937036) according to the manufacturer's protocol on a QIAsymphony SP instrument. rRT-qPCR was performed using the TaqMan® Fast Virus 1-Step Master Mix (Applied Biosystems®; Foster City, CA, 4444436) and oligonucleotide primers with TaqMan® 5' FAM / 3' Black Hole Quencher® probes from the 2019-nCoV CDC EUA Kit (Integrated DNA Technologies®; Coralville, IA, 500 rxn, cat. nr. 10006606), amplifying the nucleocapsid gene. A positive control plasmid was used within each run, containing the full nucleocapsid gene (2019-nCoV_N_Positive Control, Integrated DNA Technologies®; Coralville, IA, cat. nr. 10006625). The rRT-PCR reaction was performed with a QuantStudio™ 7 Real-Time PCR cycler.

2.3. Serology assays

SARS-CoV-2 virus-specific antibodies were determined qualitatively with the immunochromatographic COVID-19 IgM/IgG Rapid Test (Cypress Diagnostics®; Hulshout, Belgium, 360) and anti-SARS-CoV-2 IgG was determined quantitatively on the Alinity I platform with the SARS-CoV-2 IgG II Quant assay (Abbott® 06S6122, lot 30307FN00). A chemiluminescent immunoassay-based serology assay for herpes simplex (HSV-1/2 IgM/IgG, 310820 and 310800) was performed on the Liaison platform (Liaison® XL analyzer, DiaSorin; Sallugia, Italy), with Diasorin cutoffs (positive index ≥1.1; equivocal

index 0.9−1.1; negative index <0.9). For HSV-1/2 IgM, all samples were tested on two different reagent lots (327002 and 327003) and for HSV-1/2 IgG on reagent lot 325001.

2.4. Interference confirmation

Common cross-reacting interferences known to create false positive serological results were excluded, that is rheumatoid factor was determined for each patient (Alinity c, Abbott®; Chicago, IL) and routine serology testing was performed on the Liaison® XL analyzer, DiaSorin and Alinity I, Abbott® for heterologous virus IgM's. On all 26 samples anti-HSV-1/2 IgM and HSV-1 (glycoprotein C1)/HSV-2 (glycoprotein G2)IgG were redetermined with an independent enzyme-linked immunosorbent assay (ELISA) method (Euroimmun®; Lübeck, Germany, 2531-9601-1 M and 2531-9601-2 G). Semiquantitative ratios were calculated dividing the extinction of each sample by the extinction of the provided calibrator with HSV IgM positive ratio ≥1.1; borderline ratio 0.8 to 1.0; negative ratio <0.8 and HSV-1/2 IgG positive cutoff ≥22 RU/mL; borderline cutoff 16 to 21 RU/mL; negative cutoff <16 RU/mL. Furthermore, the reference standard method (Real-time PCR) for HSV diagnostics was performed on the available BALF-samples by an external laboratory.

2.5. Interference elimination

The presence of solid phase reactive antibodies was tested, by modifying the Liaison® HSV-1/2 IgM kit. Here, 0.1% and 0.5% polyvinylpyrrolidone (Sigma®; Saint Louis, MO, PVP-360) was added to the HSV-1/2 IgM dilution buffer (buffer A) as previously described [5−7]. This blocking agent is known to inhibit non-specific IgM reactivity through sterically hindering non-specific binding sites on the solid phase (i.e., magnetic particles). The HSV IgM results after both treatments were compared with original indices and statistically analyzed. Standard quality control was performed after each assay modification.

2.6. Statistical analysis

Statistical analyses were performed using IBM SPSS statistics v. 26 (IBM Analytics) and Prism 5.0 (GraphPad Software). Linear regression analysis with logarithmic transformation was performed with MedCalc software. *P*-values of <0.05 were considered statistically significant. Significant differences between patients with and without SARS-CoV-2 virus specific antibodies were continuously compared with the Student's *t* test (in case of Gaussian distribution) and the Mann-Whitney U test (in case normality could not be assumed). Categorical variables were compared using the Fisher exact test. Results were presented as mean ± standard deviation or median (interquartile range), where appropriate.

3. Results

3.1. Index case and clinical characteristics of patient cohort

A 44 year-old healthcare worker presented to the emergency department with fever, dry cough, thoracic pain and exertional dyspnea after working during several weeks in a nursing home with confirmed COVID-19 cases. SARS-CoV-2 RT-PCR test on nasopharyngeal swab at admission was negative. CT scan of the thorax showed diffuse peripheral alveolar consolidations, compatible with a viral pneumonia (Fig. 1). Due to a high clinical and radiographic suspicion of COVID-19, the patient was placed in isolation. SARS-CoV-2 PCR was repeated 48 hours after admission on nasopharyngeal and rectal swab. Both tests remained negative. Serology for hepatotropic viruses, performed because of liver enzyme abnormalities at initial blood examination, showed elevated HSV IgM with an index of 2.150 (positive index ≥1.1) and HSV IgG above 30.0 (positive index ≥1.1) using a chemiluminescent immunoassay (Liaison®) (Supplementary Table S1).

As SARS-CoV-2 PCR was repeatedly negative, an acute invasive HSV infection with viral pneumonia and viral hepatitis was withheld as a probable diagnosis. Bronchoscopy with BALF collection was performed for HSV 1/2 and SARS-CoV-2 PCR testing 3 days after admission. Intravenous (IV) therapy with acyclovir was initiated. However, 12 hours after the first dose our patient developed acute kidney

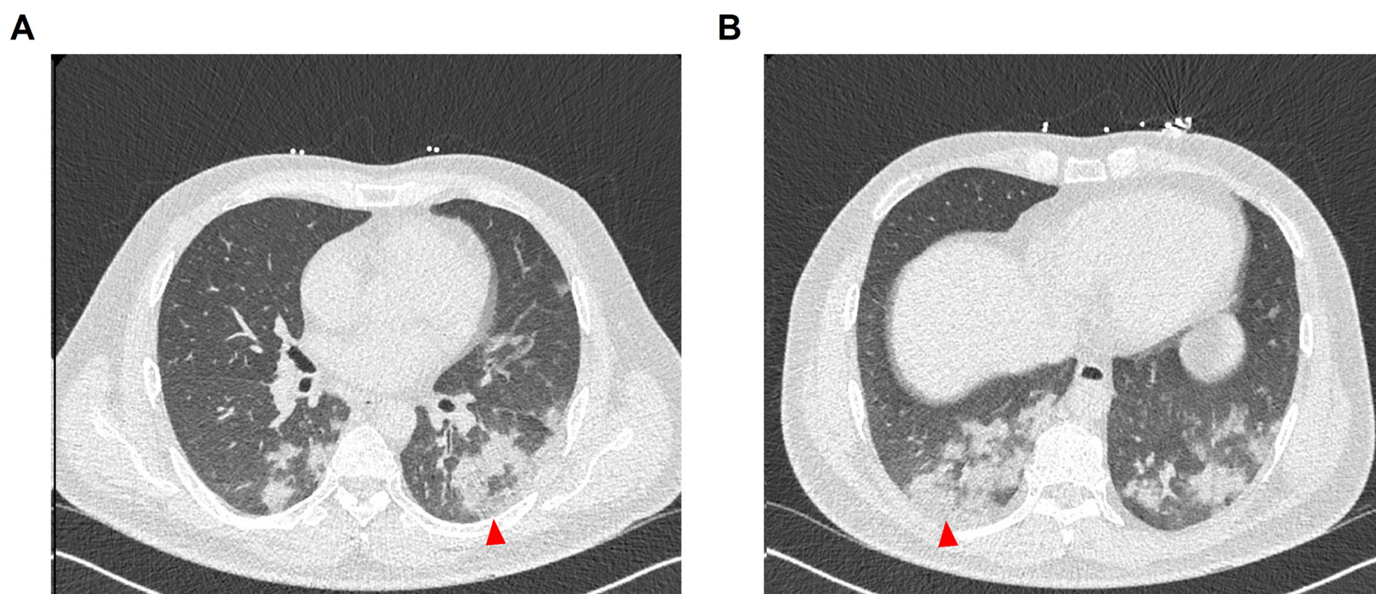

Fig. 1. CT-imaging of the thorax without contrast in the index case. (A-B) show multiple peripheral alveolar consolidations in both lower lobes and left upper lobe of the lung (red arrowhead), compatible with a viral pneumonia. No axillar adenopathies, a few subcentimetric mediastinal lymph nodes, sometimes calcified, no pathologic pericard fluid and normal lining of trachea were observed.

injury due to intratubular precipitation of crystals, a known complication of IV administration of acyclovir [16]. After cessation of IV acyclovir and start of IV fluid therapy, kidney function normalized. Following recovery of his respiratory complaints, the patient was discharged from the hospital after 12 days. The HSV IgM levels fell over time, following resolution of his respiratory complaints. Results of PCR on BALF came back positive for SARS-CoV-2 (CT-value = 26.23) and negative for HSV-1/2, which confirmed the COVID-19 diagnosis and raised the possibility of a false positive HSV serology in our patient. SARS-CoV-2 serology was also performed to confirm former SARS-CoV-2 infection in our patient and was positive for SARS-CoV-2 IgM and IgG. Hence, the cohort was expanded with 25 additional hospitalized RT-PCR confirmed COVID-19 patients with clinical characteristics, routine biochemical lab analysis, and HSV and SARS-CoV-2 serology summarized in Table 1 and Supplementary Table S1. No patients in the additional cohort were treated with antiviral drugs as none of the patients were initially suspected to have an HSV infection.

3.2. HSV IgM in SARS-CoV-2 patients

Similar to our index case, 14 (54 %) of the 26 PCR-confirmed COVID-19 patients' sera were positive for HSV IgM using the Liaison® HSV-1/2 IgM kit (lot 327002) (Fig. 2**A** and Supplementary Table S1, column 2). Results were repeated using a second reagent lot (327003) of the HSV-1/2 IgM kit, still showing an average of 35% (n = 9; mean index result per patient in two experiments) of COVID-19 patients with positive HSV IgM index (Fig. 2**B** and Supplementary Table S1, column 3). In total 9 (35%) patients' sera were positive for HSV IgM on both reagent lots (lot 327002, 327003) and 5 (19%) extra patients were positive using one reagent lot (lot 327002) but negative using the other (lot 327003). None of the HSV IgM positive patients had clinical signs suggestive of herpes simplex reactivation nor used immunosuppressive drugs, which made reactivation of a latent herpes simplex infection driven either directly or indirectly by SARS-CoV-2 infection, less likely. Interestingly, when testing our cohort with an independent anti-HSV-1/2 IgM ELISA (Euroimmun®) none of the patients' sera were positive for HSV IgM (Fig. 2**C** and Supplementary Table S1, column 5), Furthermore, all 26 SARS-CoV-2 patients had positive indices of HSV IgG with the Liaison® HSV-1/2 IgG assay (lot 325001) (Fig. 2**D** and Supplementary Table S1, column 4), but 3 patients tested negative or borderline for HSV-1 and HSV-2 IgG with the Euroimmun® ELISA (Fig. 2**E,F** and Supplementary Table S1, column 6-7).

After confirmation of the HSV IgM immunoassay interference, further analysis of our cohort was performed to determine causality. No significant correlation was found between RF positivity (n = 2) and HSV IgM positivity within our total cohort (P = 0.231). Presence of IgM's against other heterologous viruses (cytomegalovirus, EBV, HIV, Hepatitis A/B/C/E) and Toxoplasma was ruled out when routine serology testing was available.

3.3. SARS-CoV-2 serology

In our cohort, 57% of all PCR confirmed COVID-19 patients were found to be SARS-CoV-2 IgM positive and 85% (qualitative assay) to 92% (quantitative assay) IgG positive (Table 1 and Supplementary Table S1, column 8-10). Fig. 2 shows a highly significant correlation between HSV and SARS-CoV-2 IgM positivity. With the Liaison® HSV-1/2 IgM kit (lot 327002) 80% of the HSV IgM positive patients (12 out of 14) were also positive for SARS-CoV-2 IgM (P = 0.0057; Fig. 2**A**). When using the second reagent lot, 89% (8 out of 9) of patients belonged to the SARS-CoV-2 IgM positive subgroup (P = 0.0025, mean of two independent experiments; Fig. 2**B**). Moreover, a significant difference in HSV IgM titers was detected between SARS-CoV-2 IgM negative patients versus SARS-CoV-2 IgM positive patients even with the Euroimmun ELISA (P = 0.0145), although all samples were scored HSV IgM negative (n = 21) or equivocal (n = 5) with the ELISA (Fig. 2**C**, Supplementary Table S1). Hence, the HSV IgM interference seems to be linked to the acute phase of SARS-CoV-2 disease and presence of SARS-CoV-2 IgM. Moreover, regression analysis showed significant correlation between Liaison® HSV-1/2 IgM positivity and the quantitative titer of anti-SARS-CoV-2 IgG (Fig. 2**G**; Coefficient of determination R^2 = 0,5279; $P < 0.0001$), but no correlation between Liaison® HSV-1/2 IgG positivity and the quantitative titer of anti-SARS-CoV-2 IgG (Fig. 2**D,** P = 0.2758 **and** Fig. 2**H**; Coefficient of determination R^2 = 0.02774; P = 0.4161). Hence, HSV IgM positivity does not only correlate with SARS-CoV-2 IgM positivity but also with SARS-CoV-2 IgG positivity.

3.4. Interference elimination

Since the presence of SARS-CoV-2 IgM and IgG seemed to correlate with false positive HSV IgM serology, interference elimination studies were performed on the available HSV reagent lot 327003 as previously described for the Liaison platform [5–7]. To test whether non-specific IgM's directly bind to the beads of the HSV-1/2 IgM kit, in analogy with the previously described interference on EBV IgM in acute parvovirus infections, polyvinylpyrrolidone was added to the dilution buffer in different concentrations (0.1% and 0.5%) to minimize non-specific adsorption of proteins to the solid phase. Although the average of HSV IgM indices decreased with increasing PVP treatment, no significant differences could be obtained in the total cohort, nor in the SARS-CoV-2 IgM negative or positive subpopulation (Fig. 3). However, individual data analysis showed markedly decreased HSV IgM results with the modified PVP-HSV IgM kit. Indeed, 33% (3 out of 9) of positive HSV IgM indices in SARS-CoV-2 IgM positive patients became equivocal when 0.1% PVP was added and 22% (2 out of 9) even became HSV IgM negative when 0.5% PVP was added. As there was a downtrend in HSV IgM indices with increasing concentrations of PVP, even higher PVP concentrations might be needed for complete removal of interference. Noteworthy, indices of positive and negative internal quality control were not significantly affected with modified PVP-HSV IgM kit, though true positive RT-PCR confirmed HSV cases were not available for testing.

4. Discussion

Since its outbreak in December 2019 in Wuhan, China, the COVID-19 pandemic has become a global public health crisis. The most common complication that develops in COVID-19 cases is bilateral pneumonia, with risk of progression to acute respiratory distress syndrome [17]. The most common finding on CT thorax are bilateral ground glass opacities with or without consolidation, with a predominant peripheral distribution. However, these findings are also common in other respiratory viral illnesses, such as adenovirus, varicella zoster virus, Epstein-Barr virus (EBV), respiratory syncytial virus and cytomegalovirus [18]. HSV can also cause respiratory disease such as viral pneumonia, mainly in immunocompromised hosts, although immunocompetent hosts can be affected as well [9]. The differential diagnosis between different kinds of viral pneumonia can be complicated by false negative and false positive test results. Especially viral serological tests, which are frequently used in the diagnostic workup for viral illnesses, are known to cause false positive or negative results, with occasionally serious clinical repercussions as a result. Interference in serologic immunoassays can be caused by heterologous viral or non-specific immunoglobulins. This has not been sufficiently studied in COVID-19 cases, though SARS-CoV-2 infection has been reported to produce false positive reactions in a dengue serologic immunoassay [19].

In this study, the presence of a false positive HSV IgM serology in our index patient with suspicion of COVID-19 but with negative

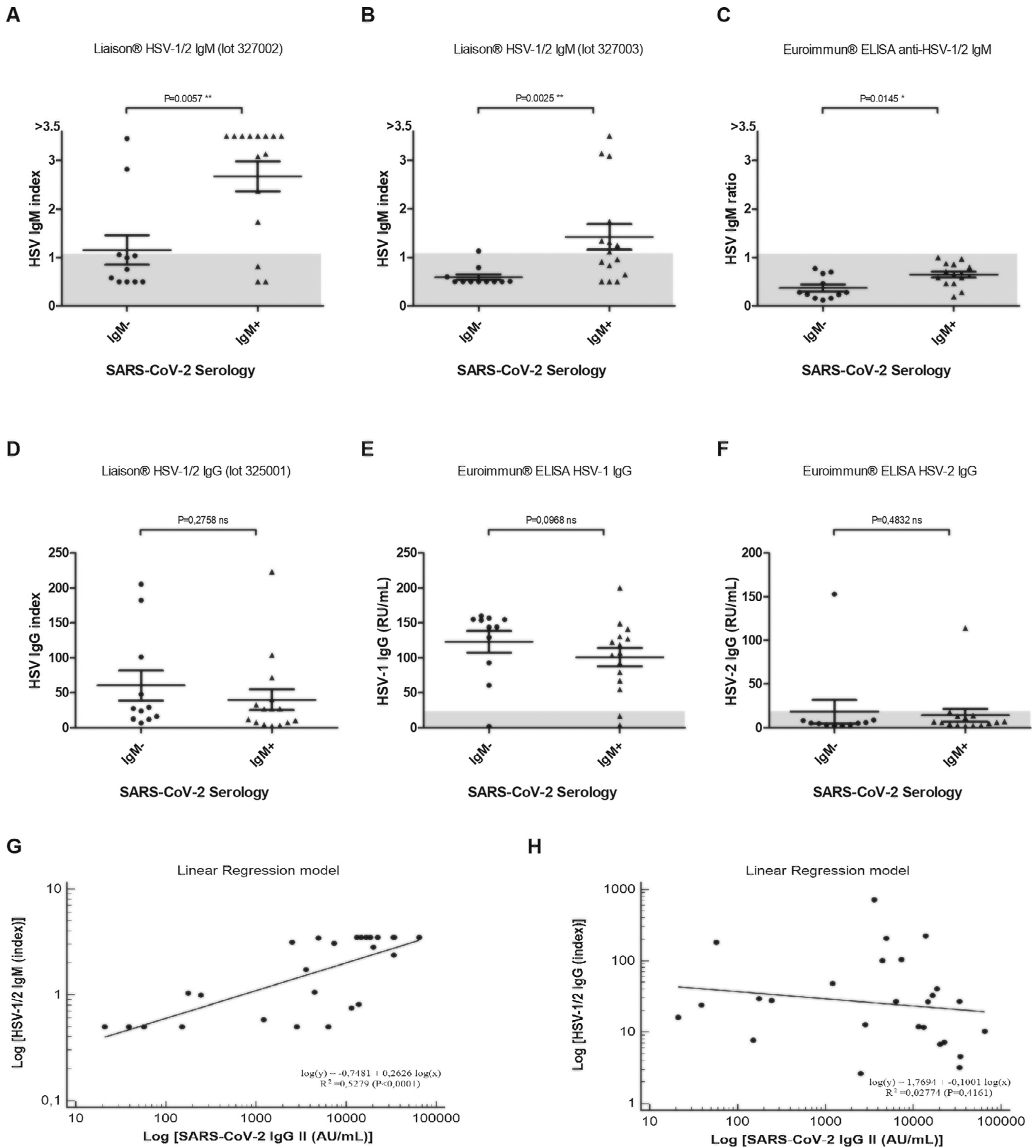

Fig. 2. Herpes simplex virus IgM and IgG serology in SARS-CoV-2 patients. HSV IgM and IgG indices in 26 RT-PCR confirmed COVID-19 patients. HSV IgM was determined with the Liaison® HSV-1/2 IgM kit (lot 327002) (A), with the Liaison® HSV-1/2 IgM kit (lot 327003; average index of two independent tests (B), and with the Euroimmun® ELISA kit (C) The grey area marks the cut-off index or ratio for positivity (≥1.1). HSV IgG was determined with the Liaison® HSV-1/2 IgG kit (lot 325001) (D), and with the Euroimmun® ELISA kit for HSV1 IgG (E) and HSV2 IgG (F). In all cases SARS-CoV-2 IgM was determined with the COVID-19 IgM/IgG Rapid Test (Cypress Diagnostics®) and HSV IgM index was plotted in function of SARS-CoV-2 serology. *P*-values were calculated with the two-tailed Mann-Whitney U test. ELISA = enzyme-linked immunosorbent assay; HSV IgM = herpes simplex virus immunoglobulin M; IgG = immunoglobulin G; RT-PCR = reverse transcription-polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

SARS-CoV-2 PCR on nasopharyngeal and rectal swab, prompted the initiation of nephrotoxic IV acyclovir therapy with acute kidney injury as a result. Only after rRT-PCR on BALF, the diagnosis of COVID-19 was confirmed and HSV pneumonia ruled out. Indeed, frequent false negative results have been reported for RT-PCR testing of SARS-CoV-2 and most reviews estimate a diagnostic sensitivity of 70% [20]. This case substantiates the occurrence of false negatives in upper respiratory tract specimens and suggests higher sensitivity

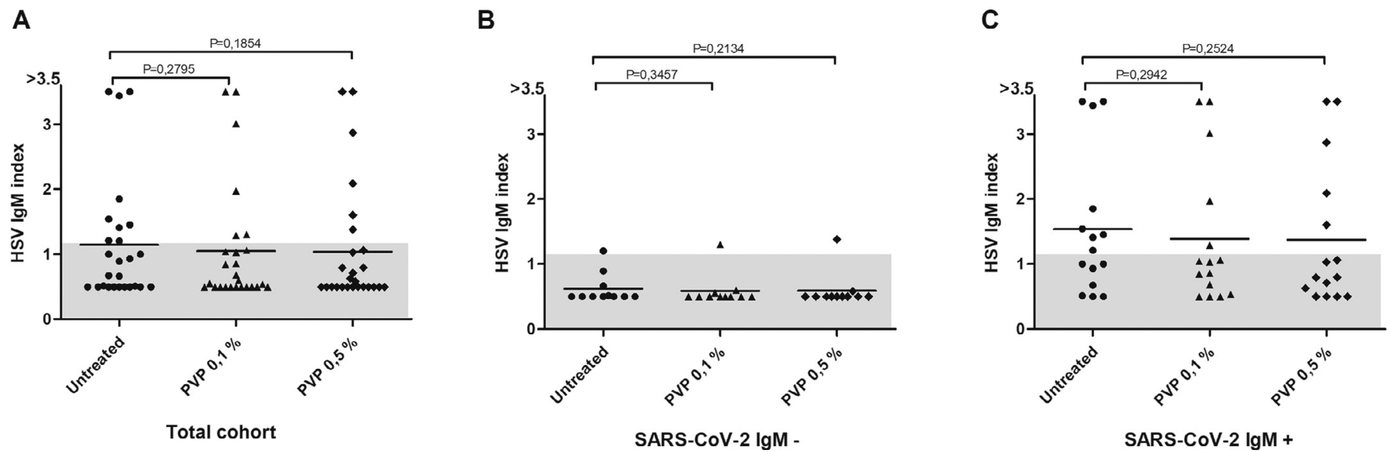

Fig. 3. Box-and-whisker plots comparing the different sample pretreatment methods. HSV IgM index determined with the Liaison® HSV-1/2 IgM kit (lot 327003) without treatment of the sample, after adding 0.1% or 0.5 % PVP to buffer A of the Liaison® HSV-1/2 IgM in 26 RT-PCR confirmed COVID-19 patients (A), in the 11 SARS-CoV IgM negative sera (B) and in the 15 SARS-CoV IgM positive sera (C). The grey area marks the cut-off index or ratio for positivity (≥1.1). *P*-values were calculated for each treatment versus the untreated samples with a one tailed Mann-Whitney U test and box-and whisker plots were visualized according to Tukey's method. HSV IgM = herpes simplex virus immunoglobulin M; PVP = polyvinylpyrrolidone; RT-PCR = reverse transcription-polymerase chain reaction; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

when lower respiratory tract specimens are used, as previously indicated [21]. This index case triggered the testing of HSV serology in 25 other PCR-confirmed COVID-19 patients requiring hospitalization. In this cohort of patients, we observed a very high frequency of false positive HSV IgM serology using two different reagent lots of the Liaison® HSV-1/2 IgM kit. During period of testing, no increase in prevalence of HSV IgM positivity was observed in non-COVID-19 patients. Though BALF-specimens were only available for the index case, false positive HSV serology was confirmed when all 26 sera were found to be HSV IgM negative or equivocal with the Euroimmun® ELISA kit. Interestingly, the interference occurred in one-third (35%) to half (54%) of COVID-19 patients, depending on which Liaison® HSV IgM reagent lot was used. The observed lot-to-lot variability can be ascribed to incomplete antigen coating on the beads, leading to variable exposed bead surface. This potential explanation was further substantiated when chemical blocking with PVP, a polymer competing with non-specific adsorption of proteins to the solid phase, reduced the percentage of HSV IgM false positive sera. Furthermore, presence of false positive HSV IgM serology was significantly more frequent in the group with positive SARS-CoV-2 IgM serology (80 to 89% of all HSV IgM+ sera depending on which reagent lot was used) and correlated with the titer of anti-SARS-CoV-2 IgG. Together, these data suggest that interference is caused, at least in some part, by solid-phase reactive IgM antibodies associated with acute phase COVID-19 and warrants laboratories to use PVP blocking as a quick and inexpensive modification during the ongoing COVID-19 pandemic. Moreover, HSV IgM indices were not only significantly elevated with the chemiluminescent technique, but also with the ELISA, though here all indices remained below ratio of positivity. Furthermore, we showed that three SARS-CoV-2 patients tested false positive on the Liaison® HSV-1/2 IgG assay. Thus, many other immunoassays might be affected in SARS-CoV-2 IgM/IgG positive sera. It is important to mention that two SARS-CoV-2 IgM negative, but IgG positive patients tested HSV IgM positive with Liaison® HSV reagent lot 327002 and one of these two patients with lot 327003. Together with the observed correlation between SARS-CoV-2 IgG and HSV IgM positivity, this indicates that interfering factors are also present during convalescent phase of the disease. However, SARS-CoV-2 IgM could have also been present in these patients but not detected with the assay due to suboptimal sensitivity of the COVID-19 IgM Rapid Test (65%). For both patients, analyzed sera were collected 14 days post-positive SARS-CoV-2 PCR, suggesting that these patients indeed should have COVID-19 IgM antibodies, though the exact onset of illness was based on patient anamnesis and IgM levels might have already declined during seroconversion [22]. Indeed, this well-known IgM decline during seroconversion, together with test-sensitivity and the fact that not all patient's sera were collected 14 days post-positive SARS-CoV-2 PCR, explains why only 57% of all PCR confirmed COVID-19 patients were SARS-CoV-2 IgM positive and 85% IgG positive.

Adding 0.5% PVP to the HSV IgM kit inhibited interference in 22% of all HSV IgM positive COVID-19 sera, but it would be interesting to see whether complete elimination is possible with increased PVP concentrations or a combination with 0.5% PVP and 0.005% PVA (polyvinyl alcohol), as earlier described [7]. Unfortunately, interference elimination studies could only be performed on the more recent HSV reagent lot 327003, whereas interference frequency was much higher with lot 327002, suggesting that PVP treatment would have been even more beneficial when using this kit.

The high percentage of false positive HSV IgM serology in SARS-CoV-2 IgM/IgG positive patients and the earlier published work of Yan et al. showing false positive dengue serology in COVID-19 patients, raises the question as to whether serological assays detecting other viral immunoglobulins could also be affected. In patients for whom routine serology testing was available in our cohort, no other viral IgM assay was found to be positive. However, it would be interesting to test this hypothesis in a larger population.

Lastly, all patients in this cohort were SARS-CoV-2 infected and RT-PCR confirmed between March and April 2020, when the main circulating variant of SARS-CoV-2 in Europe was the D614G variant [23]. It would be of interest to test whether sera of patients infected with other SARS-CoV-2 variants with different antigenicity and different antibody titers, exhibit the same amount of non-specific IgM binding and immunoassay interference.

5. Conclusion

To conclude, this study highlights the pitfalls of using serological assays for diagnosing viral infections due to the presence of non-specific antibodies, in this case in SARS-CoV-2 patients, and confirms the importance of using interference elimination studies if test results seem improbable given the clinical context. Although immunoassays are useful in diagnostic work-up to determine the presence of viral antibodies, clinicians dealing with COVID-19 should be extremely cautious with their interpretation, in particular for HSV serology. Confirmation of the HSV IgM result should be performed with an independent immunoassay and, if possible, with HSV qPCR or viral culture, though these techniques also come with limitations and

should always be utilized in conjunction with a strong clinical suspicion.

Authors' contributions

LV, EVM and TV conceptualized the study, LV performed laboratory experiments, EVM and TV clinically evaluated patients, and LV, EVM and TV analyzed the acquired data and wrote the manuscript. VN, PA and CS contributed to the enrollment and clinical evaluation of patients, review and interpretation of data and revising with final approval of the article. SS, AM and JVS supervised laboratory experiments and contributed to the review and interpretation of data and revising with final approval of the article. All authors agree to be accountable for all aspects of this study in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of GZA (Date. 07/05/2020 /No. 200504RETRO).

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2022.115653.

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Further reading

[1] https://mendel.bii.a-star.edu.sg/METHODS/corona/current/MUTATIONS/hCoV-19_Human_2019_WuhanWIV04/hCoV-19_Spike_new_mutations_table.html. Accessed November 29, 2021.