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Absence of SARS-CoV-2 in the effluent of peritoneal dialysis patients

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NOTE: Please do not leave any blank spaces. Appropriate wording can be found in the ***Peritoneal Dialysis International*** **declaration policy document**

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Abstract:

The pandemic of respiratory disease (Covid-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is life-threatening in peritoneal dialysis (PD) patients. In PD patients with systemic viral infections, peritoneal effluent may be theoretically contaminated. We searched for the presence of SARS-CoV-2 genetic material by real-time reverse transcriptase–polymerase chain reaction (qRT-PCR) assays in serial PD effluents of three PD infected patients. Nasopharyngeal swabs obtained at admission showed high viral load in all three patients whereas none of the PD effluent specimen tested positive, even after dialysate concentration. Those results support at most a very low SARS-CoV-2 dissemination risk by the peritoneal effluent of PD patients. Imposing special disposal procedures, such as the instillation of hypochlorite in the drainage bags to prevent viral spread to healthcare workers, are probably not required.

Introduction:

The pandemic of respiratory disease (Covid-19) caused by a novel human coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is particularly life-threatening in the elderly and comorbid populations including patients with kidney failure (1). Approximately 300,000 patients receive peritoneal dialysis (PD) worldwide (2). The peritoneal effluent has been previously identified as a potential source of contamination in patients with systemic viral infections secondary to hepatitis C virus or human immunodeficiency virus (3)(4). Although SARS-CoV-2 has recently been detected in some body fluids (5), it is unknown whether the peritoneal effluent from patients with Covid-19 also represents a potential source of infection and how it should be handled during the current pandemic (6)(7). To address this concern, we searched for the presence of SARS-CoV-2 genetic material by quantitative real-time reverse transcriptase–polymerase chain reaction (qRT-PCR) assays in serial PD effluents of three PD infected patients hospitalized in our unit.

Methods:

Serial samples of nasopharyngeal swab, blood and PD effluent from three consecutive PD patients were obtained throughout the active phase of the disease. All experiments were performed in two certificated independent laboratories (Namur and Brussels, Belgium). We collected a 50 ml peritoneal effluent sample after a 12-hours dwell time and spun it at 3000 rpm, 20 min at 4°C or not, respectively. For centrifugated samples, supernatant was discarded and 1mL of pellet was used. RNA was extracted from 550µL of clinical specimen using Abbott m2000sp instrument, according to manufacturer's magnetic microparticle-based protocol (Abbott molecular, IL, USA) in Brussels and from 100 µl of clinical specimens following guanidinium thiocyanate and phase separation method in Namur. Total RNA was further analyzed by qRT-PC assays targeting SARS-CoV-2 RdRp and E genes respectively by using published primers and probes published in ref (8). qRT-PCR cycle threshold (CT) values were

collected. The cycle threshold value correlates with the number of copies of the virus in an inversely proportional and exponential manner. RNA was considered undetectable if CT value > 45; the lower limit of detection of the qRT-PCR assay targeting SARS-CoV-2 E gene used was 6.6 copies of RNA per reaction equating to 0.66 copies/µl, and 4.6 copies of RNA per reaction equating to 0.58 copies/µl in the original sample for RdRp gene. Internal control (IC) RNA incorporated into the different biological matrices were used to deliver reliable results in case of negative testing. To consider a negative result as valid, IC value of the corresponding sample must be < 35.

Finally, to ensure detectability of SARS-CoV-2 RNA in PD dialysate, serial dilutions were performed from the positive control of the Coronavirus (COVID-19) CE IVD genesig® kit in 2 different sterile matrix (Physioneal® 1,36% (Baxter Healthcare Corporation) and Extraneal® (Baxter Healthcare Corporation)). These serial dilutions both showed linear decrease in CT values ($y = -3.362x+34.401$ $R^2= 0.9995$ and $y = -3.164x + 34.017$ $R^2 = 0.996$), respectively after dilution from 1/10 to 1/100 000 (supplementary figure 1 A and B).

Written informed consent was obtained from all 3 patients.

Results:

Serial samples were collected from three mild and moderate SARS-CoV-2 infected PD patients hospitalized in our unit. Clinical features are summarized in Table 1. All three were admitted for dry cough, but fever was present in one patient only. None presented with severe acute respiratory distress syndrome nor required immediate supplemental oxygen, but one patient need higher-level oxygen support measures and non-invasive ventilation to correct hypoxemia. Two patients developed intestinal symptoms. All three had lymphopenia ($<1.2x10^9$/L) and, moderate acute phase response. On admission, bilateral glass-ground opacities abnormalities in chest computed tomography scan were detected among all patients, one patient had extended involvement.

Nasopharyngeal swabs obtained at admission showed high viral load in the three patients (mean CT value < 30 for specific RdRp and gene E assay). None of the PD effluent specimen tested positive, even after dialysate concentration. Blood sample was positive in only one patient (Table 2).

Discussion:

In this report we used highly sensitive qRT-PCR to demonstrate the absence of SARS-CoV-2 genetic material in serial PD effluent samples from patients with mild to moderate active Covid-19, whereas concomitant nasopharyngeal swabs showed high viral loads. Those results support at most a very low SARS-CoV-2 dissemination risk by the peritoneal effluent of PD patients. Such data are in line with the absence of SARS RNA detection in PD effluents from PD patients with SARS infection in 2003 (9).

SARS-CoV-2 is an RNA virus belonging to the broad family of coronaviruses. Transmission occurs primarily via respiratory droplets from coughs and sneezes. Unlike HCV or HIV characterized by parenteral or sexual transmission, coronaviruses have been inconstantly found in body secretions and excretions. SARS-CoV-2 RNA has been detected in stools in one third to one half of infected patients (5), but in blood (5) and in the pleural fluid (10) only in very few severely ill patients with severe broncho-alveolar injury, suggesting potential local viral translocation. It has not been found in the urine (5) nor in the amniotic fluid (11) of infected patients. It is therefore not surprising that SARS-CoV-2 RNA was not found in the PD effluent of our patients. Still, hematogenous diffusion within the peritoneal cavity or viral translocation across the peritoneal membrane barrier in patients with acute colitis might theoretically occur. Also, intra- or peri-luminal viral transmission via touch contaminations are possible.

PD samples were tested here for the presence of RNA by qRT-PCR, a sensitive amplification assay capable of detecting extremely small amounts of SARS-CoV-2 RNA in

fluid, and using primers based on two recognized nucleotide sequences of two independent regions (highly specific RdRp gene and E gene) in an attempt of maximizing the chance to amplify and identify SARS-CoV-2. All tests on PD effluent were negative.

The present data must however be taken cautiously since they rely on PD effluent samples analysis from a small number of patients, and our observation requires replication in a larger cohort. Independently of the awaited confirmation of those results, in-hospital PD exchanges for Covid-19 infected patients still should be performed with waterproof disposable gown, gloves, face shield and FFP2 face mask and with special care when handling the PD catheter as SARS-CoV-2 may remain viable on plastic for up to 3 days (12).

In conclusion, we did not find SARS-CoV-2 genetic material in PD effluents of three covid-19 infected patients. These results suggest that imposing special disposal procedures, such as the instillation of hypochlorite in the drainage bags to prevent viral spread to healthcare workers, are probably not required, but it seems prudent to drain spent PD effluent into a toilet that needs to be disinfected thereafter.

Table 1. Clinical Features of PD Patients Infected With SARS-CoV-2

	Patient 1	Patient 2	Patient 3
	Baseline		
Clinical characteristics			
Age (years)	60	53	68
Gender	Male	Female	Male
Major comorbidity	Ischemic heart disease	None	Stroke
Cause of ESKD	Diabetes mellitus	ADPKD	Glomerulonephritis
PD duration (months)	56	1	11
Mode of dialysis	CAPD	CAPD	CAPD
Icodextrin 12h-dwell	+	+	-
Mean 12h-dwell ultrafiltration (ml)	350	500	200
Previous PD peritonitis	No	No	No
SARS-CoV-2 history			
Source of SARS-CoV-2	Contact with infected person	Contact with infected person	Contact with infected person
Other family members affected	Yes (wife)	Yes (husband)	No
Duration from illness onset to admission (days)	2	3	1
Signs and symptoms at presentation			
Fever	+	-	-
Dry cough	+	+	+
Shortness of breath	+	-	+
Rhinorrhea	-	+	-
Diarrhea	+	-	+
Vital signs at presentation			
Temperature	38.5°C	37.6°C	37.2°C
Respiratory rate (breaths/min)	20	18	19
Pulse oximeter O2 saturation	95%	97%	92%
Blood pressure, mmHg	138/90	122/95	152/101
Heart rate, bpm	95	105	92
Baseline blood investigations			
WBCs, $x10^9$/L	6.75	5.4	5.02
Platelets, $x10^9$/L	181	104	173
Lymphocytes, $x10^9$/L	0.72	0.74	0.84
C-reactive protein, mg/L	8.6	8.8	22.3
LDH, U/L	372	211	425
Chest CT-scan	Right and left-sided patchy consolidation and multiple bilateral ground-glass opacities. Extension: 10-25%	Subpleural patchy consolidation in the right lung and multiple bilateral ground-glass opacities. Extension: 10%	Multiple bilateral ground-glass opacities with crazy paving. Extension: 25-50%
	Clinical course		
Day 7 blood investigation			
WBCs, $x10^9$/L	8.79	5.42	6.39

Platelets, x10^9/L	374	271	301
Lymphocytes, x10^9/L	0.95	0.69	0.71
C-reactive protein, mg/L	52.9	54.3	48.7
LDH, U/L	528	306	419
Respiratory features			
Maximal oxygen requirement (l/min)	10	1	3
Noninvasive ventilation	+	-	-
Invasive ventilation	-	-	-
Length of stay (days)	25	12	11
Clinical outcome	Discharge Home	Discharge Home	Discharge Rehabilitation Unit

ESKD: End stage kidney disease; PD: Peritoneal dialysis; ADPKD: autosomal dominant polycystic kidney disease; CAPD: Continuous ambulatory peritoneal dialysis; APD: Automated peritoneal dialysis; WBCs: White blood cells; LDH: Lactate dehydrogenase; CRP: C-reactive protein; CT-scan: computerized tomography scanner.

Table 2: qRT-PCR results for SARS-Cov-2 detection.

	Patient 1			Patient 2			Patient 3		
Day from diagnosis	**Sample type**	**ARN copies** /ml	**IC** CT value	**Sample type**	**ARN copies** /ml	**IC** CT value	**Sample type**	**ARN copies** /ml	**IC** CT value
0	Nasopharynx	$1.05x10^6$	27.2	Nasopharynx	$2.02x10^5$	27.9	Nasopharynx	$1.3x10^4$	27.5
				PD effluent	Undetectable	26.5	PD effluent	Undetectable	26.6
				PD effluent*	Undetectable	28.3	PD effluent*	Undetectable	26.7
3	Nasopharynx[$]	$3.51x10^8$	31.4	Nasopharynx[$]	$1.15x10^8$	30.1	Nasopharynx[$]	$2.05x10^6$	30.0
	Blood[$]	Undetectable	33.8	Blood[$]	Undetectable	34.4	Blood[$]	$1.06x10^4$	32.7
	PD effluent[$]	Undetectable	33.2	PD effluent[$]	Undetectable	32.9	PD effluent[$]	Undetectable	30.4
	PD effluent*[$]	Undetectable	31.8	PD effluent*[$]	Undetectable	30.7	PD effluent*[$]	Undetectable	30.3
4	Nasopharynx	$3.23x10^5$	31.4	Nasopharynx	$3.7x10^4$	27.3	Nasopharynx	$3.9x10^4$	26.9
	PD effluent	Undetectable	27.8	PD effluent	Undetectable	27.1	PD effluent	Undetectable	27.2
	PD effluent*	Undetectable	28.0	PD effluent*	Undetectable	26.9	PD effluent*	Undetectable	27.2
7	Nasopharynx	84	27.5	Nasopharynx	$3.3x10^3$	26.8	Nasopharynx[$]	Undetectable	29.3
	PD effluent	Undetectable	28.3	PD effluent	Undetectable	26.9	Blood[$]	Undetectable	30.6
				PD effluent*	Undetectable	27.2	PD effluent[$]	Undetectable	28.8
Dialysate cells count									
Day 1		11			7			94	
Day 7		4			73			ND	
Dialysate Culture		sterile			sterile			sterile	

SARS-Cov-2 RNA was considered undetectable if qRT-PCR CT value >45. To consider a negative result as valid, IC value of the corresponding sample must be < 35.

qRT-PCR: Quantitative real-time reverse transcriptase–polymerase chain reaction; CT: Cycle Threshold; IC: internal control; *: concentrated peritoneal effluent, $: qRT-PCR assays targeting E genes. ND: not done

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