Case Report **IJCR (2021) 5:201**

International Journal of Case Reports (ISSN:2572-8776)

Detection of SARS-CoV-2 by real time Reverse Transcriptase-Polymerase Chain Reaction assay in pleural effusion

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ABSTRACT

SARS-CoV-2 (severe acute respiratory syndrome-coronavirus-2) is a novel coronavirus identified for the first time in Wuhan (China) in 2019, responsible of the current pandemic infection known as Coronavirus-19 disease (COVID-19). Wide range of clinical presentation of COVD -19 has been observed, from asymptomatic carriers to ARDS.

The common signs and symptoms of SARS-CoV-2 infection include fever, fatigue, dry cough, and dyspnoea; the severity of the disease is due to the impairment of the respiratory function.

The radiological findings include a large variety of lesions; bilateral interstitial pneumonia is the most concerning presentation of COVID-19. Pleural involvement has been described in a minority of cases: pleural thickening had been observed in 32% of cases whereas pleural effusion is uncommon being described in only 5%. Furthermore, pleural involvement has been significantly associated with a worse prognosis.

Coronavirus 2 (SARS-CoV-2), beyond the nasopharyngeal swab, has been detected in other samples; up to now, data about RT-PCR specific results in the pleural fluid of patients suffering from coronavirus disease 2019 5 (COVID-19) are very limited.

The current gold standard for diagnosis is nucleic acid detection by real time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) in nasopharyngeal swab.

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How to cite this article:
Gatti E., Ulisciani S., Guglielmo M., Tinivella M., Farina C., Ascenzi C., Righi L., De Renzi G., Ciacco C.. Detection of SARS-CoV-2 by real time Reverse Transcriptase-Polymerase Chain Reaction assay in pleural effusion. International Journal of Case Reports, 2021; 5:201.

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In this report, a case of a positive RT-PCR for Sars-Cov-2 in the pleura fluid and in the nasopharyngeal swab of a patient affected by bilateral interstitial pneumonia and severe respiratory failure is described.

As the presence of SARS-Cov-2 in the pleural fluid seems to be associated to a poor prognosis, physicians should carry out the specific RT-PCR assay both in the nasopharyngeal swab and in the pleural sample also when the fluid amount is very scarce and not recognizable in the chest X ray. Furthermore, the analysis of multiple samples allows to increase the test reliability.

Keywords: COVID-19; SARS-CoV-2; Pleural effusion

Introduction

The diagnosis of 2019 coronavirus disease (COVID-19) is usually based on a positive real-time reverse-transcription-polymerase chain-reaction (RT-PCR) assay for 2019 novel coronavirus (SARS-CoV-2) by using a nasopharyngeal swab test.

Coronavirus disease 2019 (COVID-19), due to the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has become an epidemiological threat and it represents a worldwide concern for the continuing increase of the number of both confirmed and fatal cases [1].

The most frequent signs and symptoms due to Sars-Cov-2 infection [1,2,3] include fever, shortness of breath, cough (either with or without sputum), sore throat, nasal congestion, dizziness, chills, muscle ache, arthralgia, weakness, fatigue or myalgia, and chest tightness. Further symptoms are headache, diarrhoea, abdominal pain, vomiting, chest pain, rhinorrhoea, or pharyngalgia, excessive mucus production with expectoration, haemoptysis, and dyspnoea and olfactory and gustatory impairments.

Approximately 90% of the patients present more than one symptom.

The radiological findings include a wide variety of lesions [1,3]. The majority of patients show bilateral pneumonia whereas only a small percentage of COVID-19 patients show unilateral pneumonia; the lesions are more likely to be localized in the periphery than in the centre of the lungs and they are more patchy than oval.

The most frequent computed tomography (CT) [4,5,6] findings are bilateral patchy shadows and ground-glass opacities (GGO), sometimes associated with the halo sign; multilobe involvement and focal lesions (patches, stripes, or nodules) are also very characteristic. Less characteristic CT findings include centrilobular nodules, tree-in-bud sign, cystic change, pleural effusion, interstitial fibrosis, or lymphadenopathy.

Pleural involvement is uncommon and observation of pleura effusion is very rare being observed in about 5% of pneumonia. Lately, thoracic ultrasound has become a very important tool to locate the pleural fluid presence [7].

Coronavirus 2 (SARS-CoV-2) has been detected not only in nasopharyngeal swab but also in sputum, blood, urine, and faeces [8,9]; up to now, there is only limited data on RT-PCR results in the pleural fluid of patients suffering from coronavirus disease 2019 [10] (COVID-19).

The current gold standard for diagnosis is nucleic acid detection by real time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) in nasopharyngeal swabs; however, the sensitivity of testing varies with timing of testing relative to exposure being estimated from 33% up to 80% [1].

Case Report

At the end of November 2020, a 70-year-old Caucasian male patient begun to suffer from fever, dyspnoea and reduction of the oxygen saturation; he was treated by general practitioner with oral steroids and empirical antibiotics, without benefits. Suspecting COVID-19, the patient underwent nasopharyngeal swab which resulted positive for SARS-Cov-2.

Because of the persistence of fever and dyspnoea and worsening of the oxygen desaturation, the patient was admitted to hospital with the diagnosis of SARS-CoV-2 pneumonia.

He was non-smoker, mildly overweight (BMI 27

kg/mq) and he had a medical history of prostatic hypertrophy treated with silodosin and chronic depression treated with venlafaxine and valproic acid.

The physical examination revealed a body temperature of 38°C, blood pressure of 142/89 mmHg, heart frequency of 100 beats per minute with normal ECG, respiratory rate of 26 breaths per minute and oxygen saturation of 75%. The physical examination showed no remarkable alterations. Laboratory findings revealed mild leucocytosis with severe lymphopenia (500/mcL), mild anaemia, elevated levels of lactate dehydrogenase (534 U/l), reactive C protein (22,82 mg/dL), procalcitonin (5,82 ng/mL) and D-dimer (1034 FEU ng/mL).

Results of urinary antigen tests for Legionella pneumophila and Streptococcus pneumoniae were negative. Blood culture was negative as well.

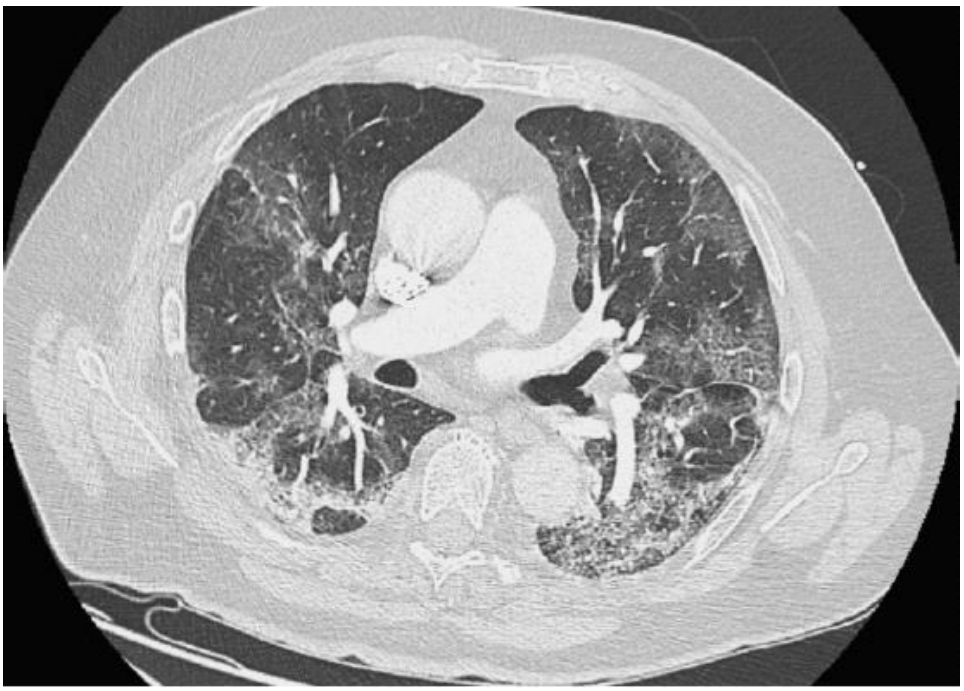


Fig.1: CT scan showing bilateral, multilobar ground glass opacities and crazy-paving consolidation without evidence of both pulmonary embolism and pleural effusion.

At day 1, a contrast enhanced chest CT showed bilateral and multilobar ground glass opacities and crazy-paving consolidations; no pulmonary embolism was observed (Fig. 1). TUS examination performed with both convex and linear probes showed regular lung sliding, B-lines and bilateral lung consolidation in the lower chest fields. Pleural effusion was not found.

The patient was treated with endovenous steroids, empiric antimicrobial therapy (piperacillina/tazobactam), low molecular weight heparin at prophylactic dosage (LWMH) and non-invasive ventilation with Continuous Positive Airways Pressure by helmet.

According to current recommendations of the Italian regulatory authority of drugs about the treatment of Sars-Cov-2 pneumonia neither hydroxychloroquine nor antiprotease drugs nor remdesivir, whose administration is approved in only patients not requiring ventilation, were used [8].

The baseline PaO2 and the ratio between the arterial oxygen partial pressure and the fraction of oxygen inhaled (P/F ratio) showed very low levels (PaO2: 37.1 mmHg and P/F ratio less than 200); therefore, a non-invasive respiratory support by helmet continuous positive airway pressure (CPAP) with 8 cm H2O Positive End Expiratory Pressure (PEEP) and 60% FiO2 was started.

Due to the worsening of gas exchanges, at day 7, the patient underwent both chest X ray and thoracic ultrasound (TUS); chest X ray showed a progression of the bilateral infiltrative lesions of

the lung without evidence of pleural effusion (Fig 2), whereas TUS revealed mild pleural effusion at the basis of left side with a transversal diameter of only 10 mm (Fig. 3A).

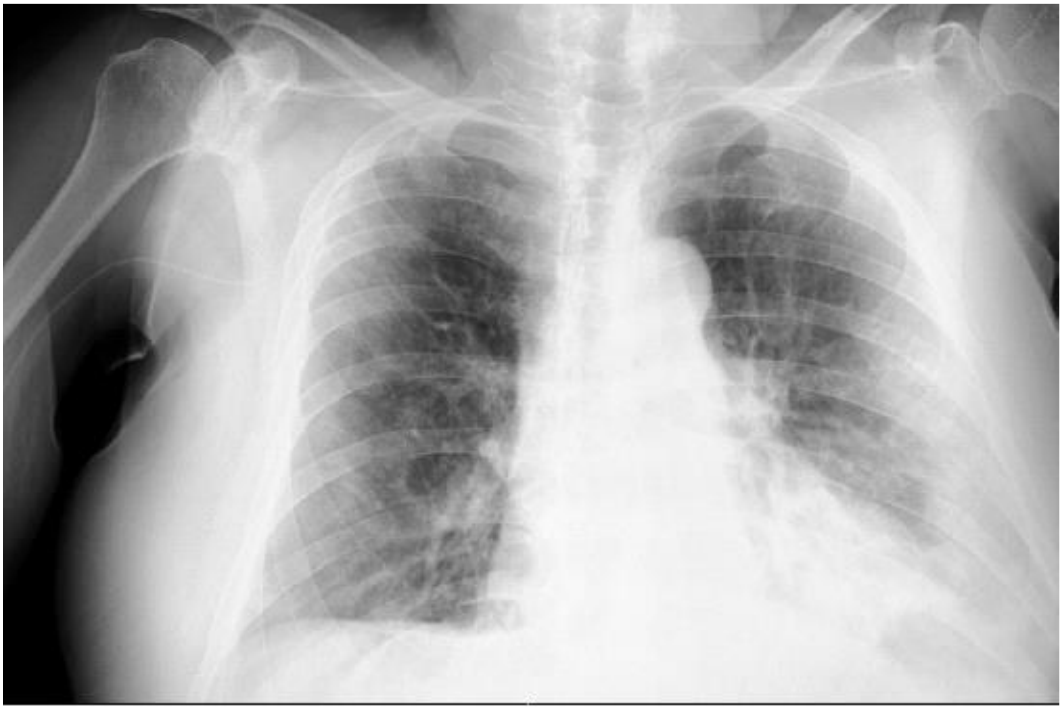


Fig.2: bilateral infiltrative lesions of the lung without evidence of pleural effusion.

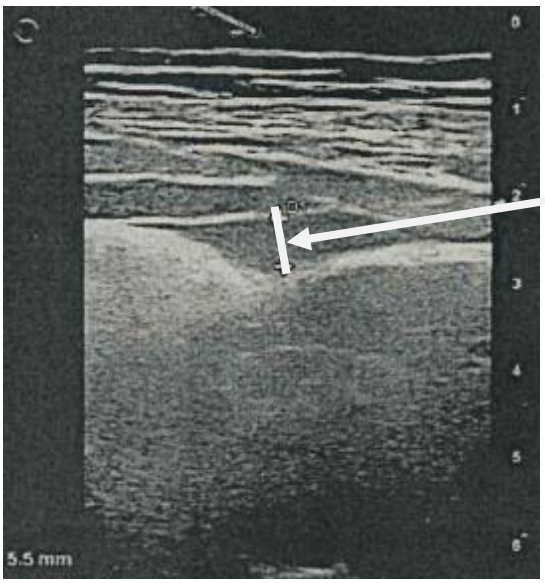


Fig. 3A

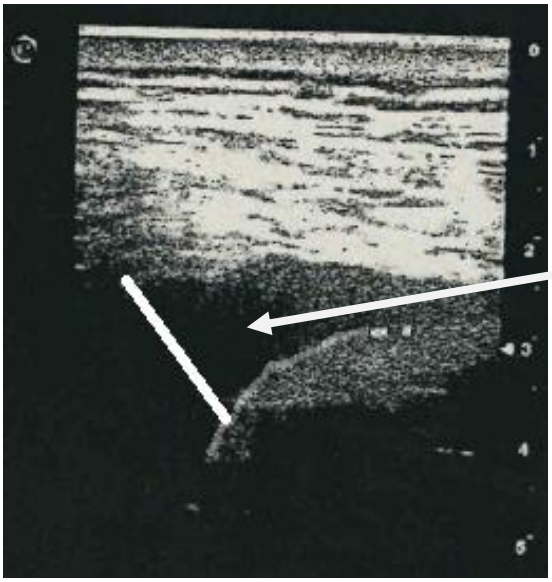


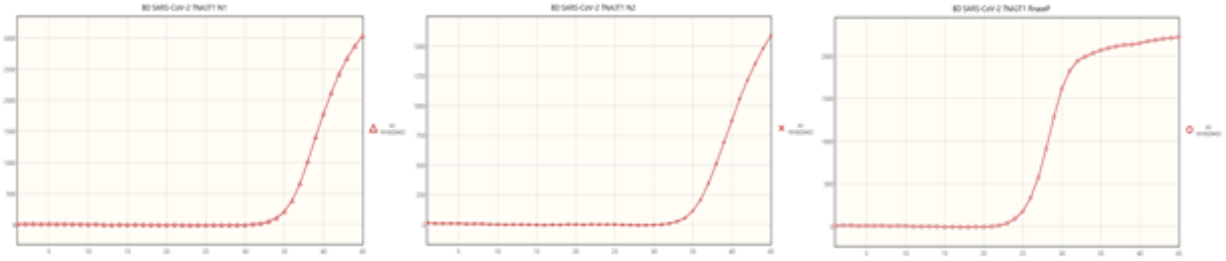
Fig. 3B

Fig. 3A: linear probe TUS (day 7).
The arrow indicates the pleural fluid located at the basis of left hemithorax (width: 10 mm).
Fig. 3B: linear probe TUS 6 days after the previous TUS (day 13).
Mild increase of the pleural effusion is indicated by the arrow (width: 18 mm).

TUS guided thoracentesis was performed and only 20 mL of serum-sanguineous pleural fluid was withdrawn. Because of the small sample of pleural fluid, only cytological analysis and Sars-CoV-2 test were performed.

The cytological examination performed on formalin fixed and paraffin embedded cell-block demonstrated an inflammatory finding. Immuno-chemistry reaction for pancytokeratin (PanCK), calretinin (Calret), CEA, myeloperoxidase

(MPO), CD20, CD3, CD5, CD4, CD8 and CD68 showed the majority of histiocytes (75%) with neutrophilic granulocytes (10%); T lymphocytes (10%, 60% CD8+ and 40% CD4+) and a minority of mesothelial cells (5%). The cytological diagnosis was pleural inflammation characterized by histiocyte prevalence.



A B C

Fig. 4 A, B, C:

Fig 4A and 4B show the amplification of the Sars-Cov-2 N1 and N2 regions corresponding to the phosphoprotein of the nucleocapsids. The Ct (Cycle threshold) score is 33.4 for N1 and 33.1 for N2.

Fig. 4C shows the positive control of the human gene RnasiP (on the x and y axis the Ct and the fluorescence intensity are respectively reported).

Sars-Cov-2 RT PCR in pleural fluid resulted positive and it was tested through the same procedure for the analysis of nasopharyngeal samples (Reagents BD SARS-CoV-2 for BD MAX system) (Fig. 4).

At day 13, Tus was repeated with evidence of mild increase of left pleural effusion reaching a width of 18 mm.

The haematologic data showed a persistent mild anaemia with increase of Reactive C Protein, LDH, and D-Dimer (Tab. 1) whereas values of P/F level remained very low for a long period of time. (Tab. 2)

Tab. 1: Laboratory values at baseline and in the course of hospitalization.

	Reference range	Day 1	Day 7	Day 34	Day 44	Day 50
Erythrocyte (10^6/µl)	4.4-5.6 x 10^6	4.59	4.69	3.59	3.58	4.24
Haemoglobin (g/dl)	13.0-17.0	10.4	10.5	8.3	8.5	9.9
Haematocrit (%)	40.0-52.0	31.8	32.9	26.1	26	31.1
Thrombocytes (10^3/µl)	150-450 x 10^3	214	387	340	303	363
Leukocyte (10^3/µl)	4.5.11 x 10^3	12.6	7.09	7.71	5.31	6.83
Neutrophils	1.90-8.00 x10^3	11.85 (93.9%)	6.49 (91.7%)	5.69 (73.8%)	2.36 (44.5%)	2.38 (34.9%)
Lymphocytes	0,90-5.20x10^3	0.5 (4%)	0.37 (5.2%)	1.20 (15.6%)	2.19 41.2%)	3.64 (43.4%)
Monocytes	0.16-1.00x 10^3	0.27 (2.1%)	0.22 (3.1%)	0.72 (9.4%)	0.60 (11.4&)	0.68 (10.1%)
Eosinophils	0.00-0.80x10^3	0	0	0.06 (0.8%)	0.15 (2.9%)	0.10 (1.5%)
Basophils	0.00-0.20x10^3	0	0	0.02 (0,4%)	0	0
C-reactive protein (mg/dl)	<0,80	23.22	2.40	10.8	2.53	1.45
PCT (ng/ml)	<0.5	6.22	0.17	0.08	0.08	0.06
Creatinine (mg/dl)	0.60-1.30	1.27		0.73	0.80	0.96
MDRD-GFR (ml/min)		56		106	95	77
Potassium (mmol/l)	3.50-5.00	4.57		3.15		4.64
Sodium (mmol/l)	135-145	136		138.7		142.3
Aspartate transaminase (IU/l) AST	10-42	52				17

Glutamic-pyruvic transaminase (IU/l) ALT	10-50	13				6
Gamma-glutamyl transferase (IU/l)	10-50	16				13
Lactate dehydrogenase (IU/l)	125.243	534			192	219
Alkaline phosphatase (IU/l)	30-120	38				50
D-Dimer (ng/ml FEU)	<800	1034	5453	2522		456
Fibrinogen (mg/dl)	150-450	711	/	340		333
PT/INR	0.80-1.20	1:43			1.29	1.23
aPTT (sec)	22-34	33.3				30.90

Tab. 2: arterial hemogasanalysis values at baseline and in the course of hospitalization.

Arterial Hemogasanalysis	Day 1	Day 2	Day 3	Day7	Day 20	Day 40	Day 48
Interface	//	CPAP	CPAP	CPAP	CPAP	VM***	NC**
FiO2	21%	55%	60%	60%	60%	40%	2L/min
P/F*	177	140	113	121	211	195	206
pH	7.55	7.47	7.43	7.44	7.49	7.47	7494
pCO2	25.1	32.1	39.4	39.6	37.8	44.6	44.7
pO2	37.1	77.0	67.7	73.8	126	77.9	82.4
HCO3-	22.1	23.4	25.7	26.8	28.9	33	34

* P/F: arterial partial pressure/fraction of oxygen inhaled
** NC: nasal cannula
*** VM: Venturi mask

CPAP treatment was continued until day 20, rotating the patient both to the supine and prone position when the ratio between the partial oxygen pressure and oxygen flow exceeded the critical value of 200 mmHg/L/min and the minimal saturation value was 94%; consequently, a de-escalation plan was begun by using only supplemental oxygen therapy through Venturi face mask.

At day 37, SARS-Cov-2 RT-PCR of the nasopharyngeal swab resulted negative but the clinical condition remained poor for the presence of exertional dyspnoea with desaturation, fatigue, hyporexia, weight loss and need for supplemental oxygen therapy by nasal cannulae or Venturi face mask.

In the following day the clinical conditions got better, TUS showed the disappearance of pleural fluid and the chest X ray revealed the regression of the parenchymal lesions with the persistence of only some interlobular and septal thickening.

The patient was discharged at day 52 with the instructions to perform long-term oxygen therapy by nasal cannulae at 2 L/min flow.

Conclusion

The spread of the virus by respiratory and extra respiratory routes may help explain the rapid worsening of disease. In addition, testing of specimens from multiple sites may improve the sensitivity and reduce false-negative test results. Indeed, the test reliability varies with timing of testing relative to the virus exposure; according to different studies, it can go from 33% up to 80%. In this case, pleural involvement in the course of bilateral and diffuse pneumonia with respiratory failure was associated with a long-term hospitalization due to a very low regression of both functional and radiological findings.

Since the presence of SARS-Cov-2 in the pleural effusion seems to be associated to a poor prognosis, physicians should be alerted to perform TUS and carry out the specific RT-PCR assay in pleural sample also when the fluid amount is very scarce and not recognisable in the chest X ray.

In the pleural fluid of the described case neither the presence of histiocytes nor the lymphocyte subsites typing are related to the diagnoses of Sars-Cov-2 pleuritis, being only expression of an non-specific inflammatory response.

Acknowledgements

None.

Conflicts of interest and funding statement

This research did not receive any specific grant from funding agencies from the public, commer-

cial, or not-for-profit sectors.

Ethical Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants are following the ethical standards of the institutional and national research committee(s) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

References

[1] W. Joost Wiersinga, MD, PhD; Andrew Rhodes, MD, PhD; Allen C. Cheng, MD, PhD; Sharon J. Peacock, PhD; Hallie C. Prescott, MD, Msc. Pathophysiology, Transmission, Diagnosis, and Treatment of Coronavirus Disease 2019 (COVID-19). A Review Published Online: July 10,2020; doi:10.1001/jama.2020.12839

[2] Jacek Baj, Hanna Karakula-Jucnowicz, Grzegorz Teresinski, Grzegorg Buszewicz, Marzanna Ciesielka, Elzbieta Sitarz, Alicjia Forma, Kaja Karakula, Wojciech Flieger, Piero Portincasa, Ryszard Maciejewski. COVID-19: Specific and Non-Specific Clinical Manifestations and Symptoms: The Current State of Knowledge. J Clin Med. 2020; Jun 5;9 (6): 1753.

[3] Guan WJ, Ni ZY, Hu Y, et al; China Medical Treatment Expert Group for Covid-19. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020; 382(18):1708-1720. doi: 10.1056/NEJMoa2002032

[4] Rodriguez-Morales AJ, Cardona-Ospina JA, Gutierrez-Ocampo E, et al; Latin American Network of Coronavirus Disease 2019-COVID-19 Research (LANCOVID-19) Clinical, laboratory and imaging features of COVID-19: a systematic review and meta-analysis. Travel Med Infect Dis. 2020; 34:101623. doi:10.1016/j.tmaid.2020.101623

[5] Bernheim A, Mei X, HuangM, et al. Chest CT findings in coronavirus disease-19 (COVID-19): relationship to duration of infection. *Radiology*. 2020;295(3):200463. doi:10.1148/radiol. 2020200463

[6] Song, F.; Shi, N.; Shan, F.; Zhang, Z.; Shen, J.; Lu, H.; Ling, Y.; Jiang, Y.; Shi, Y.Emerging 2019 Novel Coronavirus (2019-nCoV) Pneumonia. Radiology, 2020; 295, 210–217.

[7] Nilam J. Soni, MD, Ricardo Franco, MD, Maria I. Velez, MD, Daniel Schnobrich, MD, Ria Dancel, MD, Marcos I. Restrepo, MD, MS, and Paul H. Mayo, MD. Ultrasound in the Diagnosis and Management of Pleural Effusion. J Hosp Med, 2015; December,10(12): 811–816.

[8] Wang W, Xu Y, Gao R, et al. Detection of SARS-CoV-2 in different types of clinical specimens. JAMA,2020; 323 (18): 1843.

[9] Sun J, Xiao J, Sun R, et al. Prolonged persistence of SARS-CoV-2 RNA in body fluids. Emerg Infect Dis. Published online May 8, 2020. doi:10.3201/eid2608.201097

[10] Federico Mei, MD; Martina Bonifazi, MD; Stefano Menzo, MD; Alessandro Di Marco Berardino, MD; Michele Sediari, MD; Luca Paolini, MD; Antonina Re, MD; Francesca Gonnelli, MD; Claudia Duranti, MD; Martina Grilli, MD; Giacomo Spurio Vennarucci, MD; Maria Agnese Latini, MD; Lina Zuccatosta, MD; and Stefano Gasparini, MD. First Detection of SARS-CoV-2 by RealTime Reverse Transcriptase-Polymerase Chain Reaction Assay in Pleural Fluid. CHEST, 2020; 158(4): e143-e146.

[11] https://www.aifa.gov.it/documents/20142/1269602/SOCospedaliera09.12.2020.pdf/021a4ffe-7a80-32ed-ee9c-65a383ff1b47