# SARS-CoV-2: exposure to high external doses as determinants of higher viral loads and of increased risk for COVID-19. A systematic review of the literature

## SARS-CoV-2: esposizione a dosi esterne elevate quali determinanti di cariche virali più elevate e di rischio aumentato per COVID-19. Una revisione sistematica della letteratura

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## ABSTRACT

The determinants of the risk of becoming infected by SARS-CoV-2, contracting COVID-19, and being affected by the more serious forms of the disease have been generally explored in merely qualitative terms. It seems reasonable to argue that the risk patterns for COVID-19 have to be usefully studied in quantitative terms too, whenever possible applying the same approach to the relationship 'dose of the exposure vs pathological response' commonly used for chemicals and already followed for several biological agents to SARS-CoV-2, too. Such an approach is of particular relevance in the fields of both occupational epidemiology and occupational medicine, where the identification of the sources of a dangerous exposure and of the web of causation of a disease is often questionable and questioned: it is relevant when evaluating the population risk, too. Specific occupational scenarios, basically involving health workers, exhibit important proportions of both subjects simply infected by SARS-CoV-2 and of ill subjects with, respectively, mild, moderate, and severe disease. Similar patterns have been described referring to various circumstances of community exposure, e.g., standing in crowded public places, travelling on crowded means of transport, living in accommodation or care homes, living in the same household as a COVID-19 case. The hypothesis that these findings are a consequence not only of high probabilities of exposure, but also of high doses (as a product of both intensity and duration, with possible autonomous effects of peaks of exposure) deserves to be systematically tested, in order to reconstruct the web of causation of COVID-19 individual and clustered cases and to cope with situations at critical risk for SARS-CoV-2, needing to be identified, mapped, and dealt with at the right time. A limited but consistent set of papers supporting these assumptions has been traced in the literature. Under these premises, the creation of a structured inventory of both values of viral concentrations in the air (in case and if possible, of surface contaminations too) and of viral loads in biological matrixes is proposed, with the subsequent construction of a scenario-exposure matrix. A scenario-exposure matrix for SARS-CoV-2 may represent a useful tool for research and practical risk management purposes, helping to understand the possibly critical circumstances for which no direct exposure measure is available (this is an especially frequent case, in contexts of low socio-economic level) and providing guidance to determine evidence-based public health strategies.

**Keywords:** SARS-CoV-2, quantification, exposure level, viral load, risk, COVID-19

## KEYPOINTS

■ **A systematic review has been performed, evaluating the studies published and indexed in Pub Med related to the relationship between the dose of the exposure to SARS-CoV-2, the viral loads and the risks of subsequent disease.**

■ **Some studies have been identified, respectively presenting a semi-quantitative risk assessment, quantifying the exposure to SARS-CoV-2 (almost entirely referring to the air transmission), analysing the relationship between the presence of SARS-CoV-2 and the viral load in biological matrixes on the one hand, frequency and / or severity of the clinical pattern on the other hand; their results are synthetically exposed.**

■ **The collected evidence supports the conception that the dose of the exposure to SARS-CoV-2 (as the product both of the intensity and the duration, with possible autonomous effects of the exposure peaks) has a positive connection with the viral load as well as the frequency and the severity of the resulting disease.**

■ **It is proposed to implement a structured inventory of the measured levels of exposure to SARS-CoV-2 and to operate this tool for the purpose of constructing a scenario – exposure matrix, suitable for supporting both the research and the practical risk management: i.e. a tool to help the understanding of potentially critical circumstances in respect of which no direct exposure measure exists (a contingency particularly frequent in contexts of low socio-economic level) and to route evidence-based, public health strategies.**

## RIASSUNTO

I determinanti del rischio di essere infettati da SARS-CoV-2, contrarre COVID-19 ed essere affetti dalle forme più gravi della malattia sono stati generalmente esplorati in termini meramente qualitativi. Pare ragionevole argomentare che i *pattern* di rischio per COVID-19 abbiano da essere utilmente studiati anche in termini quantitativi ogni volta che sia possibile, applicando a SARS-CoV-2 lo stesso approccio alla relazione "dose di esposizione *vs* risposta patologica" comunemente usato per gli agenti chimici e già seguito per altri agenti biologici. Un tale approccio risulta di rilevanza peculiare nei campi dell'epidemiologia occupazionale e della medicina occupazionale, ove l'identificazione delle fonti di un'esposizione pericolosa e della rete di causazione di una malattia è spesso incerta e discussa; esso è rilevante anche quando si valuti il rischio di popolazione. Scenari lavorativi specifici, che coinvolgono fondamentalmente operatori sanitari, mostrano proporzioni importanti tanto di soggetti semplicemente infettati da SARS-CoV-2, quanto di soggetti malati con malattia, rispettivamente, lieve, moderata e grave. Quadri analoghi sono stati descritti in riferimento a variegate circostanze di esposizione di comunità, per esempio, lo stare in luoghi pubblici affollati, il viaggiare all'interno di mezzi di trasporto affollati, l'essere

ospitati all'interno di case di riposo assistite, il vivere nello stesso ambiente familiare di un malato di COVID-19. L'ipotesi che queste risultanze siano una conseguenza non solo di elevate probabilità di esposizione, ma anche di dosi elevate (come prodotto tanto dell'intensità quanto della durata, con possibili effetti autonomi di picchi di esposizione) merita di essere testata in modo sistematico, allo scopo sia di ricostruire la rete di causazione di casi individuali e clusterizzati di COVID-19 sia di fronteggiare situazioni di rischio critico per SARS-CoV-2, che necessitano di essere tempestivamente identificate, mappate e risolte. Un complesso limitato, ma coerente di lavori che supportano tali assunzioni è stato reperito in letteratura. Sotto queste premesse, viene proposta la costruzione di un repertorio strutturato di valori di concentrazioni virali in aria (se del caso e se possibile, anche di contaminazioni di superfici) e di cariche virali in matrici biologiche, con la conseguente costruzione di una matrice scenario-esposizione. Una matrice scenario-esposizione per SARS-CoV-2 potrebbe costituire uno strumento utile per scopi di ricerca e di pratica gestione del rischio, aiutando la comprensione di circostanze possibilmente critiche riguardo alle quali non si disponga di alcuna diretta misura dell'esposizione (un'eventualità particolarmente frequente nei contesti di basso livello socio-economico) e fornendo una guida alla definizione di strategie di sanità pubblica basate su evidenze.

**Parole chiave:** SARS-CoV-2, quantificazione, livello di esposizione, carica virale, rischio, COVID-19

## INTRODUCTION

The worldwide, very heavy impact of the COVID-19 epidemic has induced, within a few months, the publication of a huge amount of scientific papers, part of which centred upon epidemiologic and pathogenetic aspects. In front of rapidly increasing public health necessities, among the most relevant research topics about SARS-CoV-2 and COVID-19, the ascertainment of the web of causation of the disease clearly emerges; any prevention and protection strategy has to be consequently established, the level of the actions to be taken being a function of the spread, and the severity of the risk.

Intuitively, there is not the same risk from being exposed to a pathogenic agent (a biological one such as others of a different nature) for minutes, just on sporadic occasions and remaining at a distance from the source rather than from being exposed for hours, on a daily basis for a long period and staying close to the source.

Many official documents issued by the World Health Organisation (WHO), the European Centre for Disease Prevention and Control (ECDC), and, in Italy, the Ministry of Health and the National Health Institute base the risk assessment in front of COVID-19 upon a clean cut-off line between the individuals who are simply contacts from those who are close contacts of SARS-CoV-2 positive subjects; this dichotomous, very practical and easy-to-handle standard considers, inside a semiquantitative frame, both the timing of the relationship with the SARS-CoV-2 positive subject(s) and the distance from the source(s), further modulating in force of the characteristic of the environmental context and, in the event of, the use of personal protection. Contacts and close contacts are respectively labelled as 'low risk' and 'high risk' subjects; the two categories involve distinctly different patterns of actions to be taken.

It seems reasonable to conjecture developments of this approach, whenever useful and possible, also implementing the risk assessment in quantitative terms and subsequently modulating the operational patterns for COVID-19 prevention and control.

A fully quantitative approach to the risk profiles deriving from the exposure to dangerous agents and their distinguished management, possibly following the individuation of a dose-response relationship and checking for the sources of uncertainty, is ordinary when dealing with the toxic and carcinogenic effects of chemicals and radiations.

Regarding biological agents, a general Quantitative Microbial Risk Assessment (QMRA) methodology has been developed, nowadays applied mainly to drinking water and food safety but suitable for evaluating the airborne infections too.[1-6]

It seems reasonable to make an effort to approach SARS-CoV-2, in similar terms, too.

Occupational scenarios involving health workers exhibit relevant proportions of both subjects simply infected from SARS-CoV-2 and of ill subjects with, respectively, mild, moderate, and severe disease.[7] As far as Italy is concerned, corresponding data about health workers has been collected, analysed, and published by the National Institute for the Insurance of Work Accidents and Occupational Diseases (Inail).[8]

Other occupational scenarios show COVID-19 clusters within population groups at work, e.g., in meat and poultry processing facilities.[9]

COVID-19 clusters have been described related to scattered circumstances of community exposure, e.g., standing in public places such as wet markets[10] and shopping malls,[11] or being looked after inside assisted retirement homes,[12] or living in the same household as a COVID-19 case.[13,14]

The risk of SARS-CoV-2 infection associated with travelling on different means of transport has been studied, limited to few scenarios. A seven-fold risk related to "travelling with a case" has been reported.[14] COVID-19 clusters have been signalled related to travelling on cruise ships[15,16] and, anecdotally, aircraft.[17,18] A familiar cluster of SARS-CoV-2 infection associated with a railway journey has been reported.[19] A huge spread of SARS-CoV-2 through the New York subways has been conjectured.[20]

In general, the risk of becoming infected by SARS-CoV-2 appears definitely related to confined, crowded, and poorly ventilated spaces where the virus actually has particu-

lar opportunities to reach high concentrations in the air; an additional role for the conveyance and the diffusion of SARS-CoV-2 via air conditioning systems is plausible for every kind of indoor scenarios.[21] A minor contribution to the risk by the contamination of surfaces is believable.[22]

The hypothesis that these findings are a consequence not only of high probabilities of exposure, but of high doses too (as a product of both intensity and duration, with possible autonomous effects of peaks of exposure) deserves to be systematically tested, in order both to reconstruct the web of causation of COVID-19 individual and clustered cases and to cope with situations at critical risk of SARS-CoV-2 infection which need to be identified, mapped, and dealt with at the right time.

The hypothesis that high doses of exposure to SARS-CoV-2 are related not only to the infection rate, but also to higher viral loads and worse clinical conditions for COVID-19 patients deserves to be organically investigated too.

## OBJECTIVES

This paper considers the studies dealing with SARS-CoV-2 and COVID-19 and providing quantitative or, at least, semiquantitative information about the relationship among the exposure to the virus, the viral load, the frequency of the infection, the frequency and the severity of the disease. The collected evidence has been used to test the reasonableness of a proposal focused on the construction of a structured inventory of exposure measures and of a subsequent scenario-exposure matrix; this is intended as a tool for research and practical risk management purposes, helping to understand the possibly critical circumstances with respect to the available no direct exposure measure (which is an especially frequent case in contexts of low socioeconomic level) and providing guidance to the determination of evidence-based public health strategies.

## MATERIALS AND METHODS

A systematic review has been performed by means of PubMed (last access on 20.08.2020). Both MeSH-terms and any others evaluated as possibly relevant for the subject have been adopted as key words for the research. The MeSH terms SARS-CoV-2* and COVID-19* have been matched with the following others:

■ MeSH terms: viral load risk, infections, occupations, ships, aircraft, railroads, air conditioning;
■ not MeSH terms: exposure, quantification, QMRA, quantitative microbial risk assessment, cluster, workers' health, health workers, transports, means of transport, travelling, airplanes, flight, railways, subways, air sampling, surface contamination.

No time limits have been imposed on the research, for almost all the papers about SARS-CoV-2 and COVID-19 have been published during 2020 (on the date of the last access, just 11 papers dated 2019 resulted indexed in PubMed).

No limits have been imposed on the research regarding the characteristics and the quality of the studies.

The review has included all the studies presenting quantitative data about the levels of the human exposure to SARS-CoV-2 and/or the viral loads in humans, relating the information to the risk of being affected by COVID-19 and/or the severity of the disease.

The report follows the scheme of the PRISMA statement checklist,[23] whenever its items result applicable.

## RESULTS

The huge amount of scientific papers tumultuously published, starting from the last part of 2019, about SARS-CoV-2 (more than 23,000 in PubMed at 20.08.2020) and COVID-19 (more than 43,000 in PubMed at 20.08.2020) mainly follow qualitative and descriptive approaches, except for a limited pattern dealing with quantitative or, at least, semiquantitative aspects of the exposure to SARS-CoV-2 related to infection frequency, disease frequency, and disease severity. All the relevant items have been selected and their results are summarized as follows.

### SEMIQUANTITATIVE RISK ASSESSMENT (1 PAPER)

Maltezou et al. [24] studied a cohort of 2,833 healthcare workers with a history of dealing with COVID-19 patients; a risk classification was performed for each of them, following the characteristics of the contact (distance and duration), the performance (or not) of aerosolized-generating procedures (e.g., intubation), the use (or not) of personal protection equipment, and the use (or not) of a surgical mask by the COVID-19 patients. 1,599 health workers (47.1%) were classified as low-risk, 765 (22.5%) as moderate-risk, and 1,031 (30.4%) as high risk. The high-risk healthcare workers showed the highest risk of contracting the disease.

### EXPOSURE ASSESSMENTS (6 PAPERS)

By means of vacuum pumps at a flow rate of 1.5 L/min, Faridi et al. [25] performed 10 indoor air samplings for SARS-CoV-2 inside intensive care rooms with COVID-19 patients at the main Iranian hospital in Tehran. The samplers were placed at a distance ranging from 2 to 5 metres from the patients' beds. Real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assays targeting RNA-dependent RNA polymerase (RdRp) and Envelop (E) genes were used. All the collected samples resulted negative.

Chia et al. [26] studied the air and surface contamination by SARS-CoV-2 inside 3 Airborne Infection Isolation Rooms (AIIRs) with COVID-19 patients at the National Centre for Infectious Diseases in Singapore; these rooms had 12 air changes per hour. Both air and surface samples were analysed by means of RT-PCR assays targeting the E genes. The air samples were collected by means of NIOSH BC 251 bioaerosol samplers and the results were different relating to the size of the collected particles. The

samples from 2 of the 3 AIIRs tested positive in particles with sizes >4 μm and 1-4 μm in diameter while the samples from the fractionated size <1 μm were all negative, as were all the non-size-fractionated filter cassette samples. Total SARS-CoV-2 concentrations in positive samples ranged $1.84 \times 10^3$ to $3.38 \times 10^3$ viral copies/m³. Surface samples (8 to 20 for each room) were collected using sterile swabs and qualitatively classified as "positive" or "negative"; positive detection was recorded as long as amplification was observed in at least one assay. The rooms with viral RNA detected in air also had surface contamination detected; the median cycle threshold (Ct) values of the clinical specimens for patients with and without environmental surface contamination were 25.69 (IQR 20.37-34.48) and 33.04 (28.45-35.66), respectively.

Liu et al.[27] evaluated the occurrence of airborne SARS-CoV-2 at 19 sites in two hospitals exclusively dedicated to COVID-19 patients and at 11 public sites in Wuhan, China: the air samples were collected by means of vacuum pumps at a flow rate of 5.0 L/min and analysed by means of a droplet-digital-PCR-based detection method (ddPCR) assay. The viral RNA concentration ranged 0 to 113 viral copies/m³ (18/25 positive samples) in the hospital sites and 0 to 11 viral copies/m³ (2/11 positive samples) in the public sites.

By means of the median cycle threshold (Ct) values, Ong et al.[28] quantified the contamination of air, environmental surfaces, and personal protective equipment surfaces deriving from 3 (A, B, C) COVID-19 patients hospitalized in AIIRs (12 air changes per hour) in Singapore. The air samples were collected by means of vacuum pumps at a flow rate of 6.0 m³/h and analysed by means of RT-PCR assays targeting the E genes; all of them resulted negative. The surface samples were collected using sterile swabs. All the surface samples related to A and B patients resulted negative; 16/20 surface samples referred to the patient C (who presented a median Ct value of 25.69 in nasopharyngeal samples compared with 31.31 and 35.33 for the patients A and B) resulted positive.

Rahmani et al.[29] published a synthetic but relevant review about the technical methods for sampling and detecting Coronaviruses in the air, defining the boundary conditions for the sampling, the sampling procedure, the sampling time and flow rates, the sampling culture medium, the preparation, storage and transfer conditions, the identification techniques.

Santarpia et al.[30] collected and analysed surface samples, high-volume (50 L/min) environmental air samples, and low-volume (4 L/min) personal air samples inside rooms where COVID-19 patients were hospitalized in Nebraska (USA). Surface and aerosol samples were analysed by RT-PCR targeting the E gene of SARS-CoV-2. Overall, 70.6% of the personal samples were positive for SARS-CoV-2; the level of air contamination was expressed in terms of copies/μL. The highest airborne concentrations were recorded by means of personal samplers while a patient was receiving oxygen through a nasal cannula (19.17 and 48.22 copies/L). A variability in the degree of the environmental contamination (as measured by the percentage of positive samples) was observed from room to room and from day to day.

A synopsis of the results of the 6 above discussed papers is presented in the annexed table 1.

| FIRST AUTHOR BIBLIOGRAPHIC REFERENCE | KIND OF THE STUDY | KIND OF THE SAMPLINGS | RESULTS | NOTES |
|---|---|---|---|---|
| Faridi et al.[25] | Evaluation of air contamination | Environmental active air samplings | All the collected samples negative | Intensive care unit rooms were investigated |
| Chia et al.[26] | Evaluation of air contamination | Environmental active air samplings | Part of the samples positive, $1.84 \times 10^3$ to $3.38 \times 10^3$ viral copies/m³ | Intensive care unit rooms were investigated |
| Chia et al.[26] | Evaluation of environmental surfaces contamination | Surface samplings by the means of sterile swabs | Part of the samples positive | Intensive care unit rooms were investigated |
| Liu et al.[27] | Evaluation of air contamination | Environmental active air samplings | 0 to 113 viral copies/m3 (18/25 positive samples) in the hospital sites and 0 to 11 copies/m³ (2/11 positive samples) in the public sites | Both hospital sites and public sites were investigated |
| Ong et al.[28] | Evaluation of air contamination | Environmental active air samplings | All the collected samples negative | Intensive care unit rooms were investigated |
| Ong et al.[28] | Evaluation of environmental surfaces contamination and of contamination of protective equipment surfaces | Surface samplings by the means of sterile swabs | Part of the samples positive (16/20) in one out of three evaluated rooms (none in the two others | Intensive care unit rooms were investigated |
| Rahmani et al.[29] | Review about the technical methods for sampling and detection of Coronaviruses in air | / | / | / |
| Santarpia et al.[30] | Evaluation of air contamination | Environmental active air samplings | Some positive samples with values up to 48.22 copies/L | Hospital rooms were investigated |
| Santarpia et al.[30] | Evaluation of environmental surfaces contamination | Surface samplings by the means of sterile swabs | Positive samples up to 19.17 and 48.22 copies/L | Hospital rooms were investigated |

**Table 1.** Exposure assessment (6 papers).
**Tabella 1.** Valutazioni dell'esposizione (6 studi).

## POSITIVITY VS NEGATIVITY FOR SARS-COV-2 IN BIOLOGICAL SAMPLES AND FREQUENCY OF SYMPTOMS (1 PAPER)

Following the dichotomous approach "positivity vs negativity for SARS-CoV-2 in biologicals samples", regardless of the positivity level, Lombardi et al.[31] studied a population of 1,573 healthcare workers who underwent nasopharyngeal swab testing for SARS-CoV-2. There were 139 positive tests (8.8%); a marked difference emerged between symptomatic (122/503, 24.2%) and asymptomatic (17/1,070, 1.6%) subjects (p<0.001). Physicians were the group with the highest frequency of positive tests (61/582, 10.5%), whereas administrative staff and technicians had the lowest frequency (5/137, 3.6%).

## VIRAL LOADS AND CLINICAL FIGURES (12 PAPERS)

The viral load resulted determined alternatively by means of the Ct of the real-time polymerase chain reaction (RT-PCR) for SARS-CoV-2 genes and the number of viral copies per volume unit of collected body fluids.

Clementi et al.[32] related the RT-PCR amplification profile of a set of 200 nasopharyngeal swabs collected from COVID-19 patients (not furtherly specified) during an acute phase of the 2020 COVID-19 epidemic in Italy (100 in April, 100 in May); the mean Ct values of the positive samples collected in May were significantly higher than the ones collected during the previous month (ORF1a/b gene: 31.85 ± 0.32 vs 28.37 ± 0.5, p<0.001; E gene: 33.76 ± 0.38 vs 29.79 ± 0.63, p<0.001), indicating a decreasing time trend of the viral loads.

To et al.[33] followed a cohort of 23 COVID-19 patients hospitalized in Hong Kong; viral load was ascertained by reverse transcriptase quantitative PCR (RT-qPCR). The median viral load determined in posterior oropharyngeal saliva and other respiratory samples at presentation was 5.2 $\log_{10}$ copies per mL. Salivary viral load was highest during the first week after symptom onset and subsequently declined with time (slope -0.15, 95%CI -0.19;-0,11). In one patient, viral RNA was detected 25 days after symptom onset. Older age was correlated with higher viral load (Spearman's=0.48, 95%CI 0.074; 0.75; p=0.020).

Liu et al[34] studied the SARS-CoV-2 loads, expressed by means of the Ct value, in swabs and bronchoalveolar lavage fluid (BALF) samples from 12 COVID-19 patients hospitalised in Shenzhen, China. The RT-qPCR was targeted on the ORF 1a/b and N viral genes; the results were assumed positive when the Ct value was ≤37.0 The viral load (ranging 20-35) resulted crucial in predicting the disease severity, being strongly correlated with a clinical lung injury score (r -0.765; p<0.01).

Pan et al.[35] studied the SARS-CoV-2 loads, expressed by the means of the Ct value, in serial samples (throat swabs, sputum, urine, and stool), collected at different times from the onset of symptoms, from 2 COVID-19 pa-

tients hospitalized in Beijing, China. The samples were examined by means of an RT-qPCR assay targeted on the N gene. The viral loads in throat swabs and sputum samples peaked at around 5–6 days after the onset of symptoms, ranging from around $10^4$ to $10^7$ copies per mL during this time.

Pujadas et al.[36] evaluated nasopharyngeal swab samples for SARS-CoV-2 collected from 1,145 COVID-19 patients hospitalized at Mount Sinai, New York, USA. The samples were examined by means of RT-qPCR (unspecified genes). The overall mean $\log_{10}$ viral load was 5.6 copies per mL (SD 3.0), and median $\log_{10}$ viral load was 6.2 copies per mL (IQR 3.0-8.0). Mean $\log_{10}$ viral loads significantly differed between the patients who were alive (No. 807; mean $\log_{10}$ viral load 5.2 copies per mL) versus those who had died (No. 338; 6.4 copies per mL) by the end of the study period. After checking for possible confounders, the authors concluded that an independent relationship between high viral load and mortality exists, proposing that a transition from qualitative testing into quantitative measurements of viral loads will assist clinicians in risk stratification, choosing among alternative therapies and trials, and defining the isolation measures on the basis of infectivity. Kelleni[37] challenged the clinical significance of these findings affirming that a paper by Walsh et al.[38] found little to no difference of the viral load values, in samples from the upper respiratory tract, between presymptomatic, asymptomatic and symptomatic patients. Walsh et al.[38] reviewed 113 studies about SARS-CoV-2 in different body fluids and actually concluded that a "relatively consistent trajectory of SARS-CoV-2 viral load over the course of COVID-19" exists, the viral load detected from upper respiratory tract samples peaking around the onset of symptoms or a few days thereafter and generally becoming undetectable about two weeks after the onset of symptoms; they signalled a prolonged virus detectability in stool samples too, with an unclear clinical significance.

Shi et al.[39] studied the association between viral loads (determined by the means of copy number of the SARS-CoV-2 N gene in pharyngeal swabs) and values of a set of serum biomarkers (IgM anti-SARS-CoV-2, CRP and SAA) in 114 COVID-19 patients hospitalized in Jiangsu, China. Viral loads showed different trends among cases with different levels of severity, viral loads of IgM-negative patients tending to increase over time after onset. As the disease worsened, the positive rates of CRP and SAA also showed trends of increase. Different CRP/SAA type showed positive associations with viral loads in patients with different levels of severity of the disease and at different times after onset.

Yu et al. (a) studied 92 COVID-19 patients hospitalized in Zhejiang, China, ascertaining that SARS-CoV-2 viral load in sputum correlates with the risk of COVID-19 progression; severe patients had significantly lower Ct values

than the ones suffering from mild or moderate disease on admission (25 vs 28; p=0.017); the authors argued that these findings point to a higher viral load in the lower respiratory tract.[40]

Yu et al. (b) analysed the results of a set of 400 respiratory samples (nasal swabs, throat swabs, sputum) from 127 COVID-19 patients hospitalized in Beijing, China, relating the results to clinical and imaging data obtained for clinical staging. Both RT-qPCR and Droplet Digital Polymerase Chain Reaction (ddPCR) were performed. The results showed that sputum samples may better reflect the level of virus replication in vivo, the viral load in the sputum samples during the early and progressive stages of the disease being significantly higher than that in the recovery stage (46,800 ± 17,272 vs 1,252 ± 1,027 copies/test; p<0.001).[41]

Zheng et al. evaluated the viral loads in about 3,500 samples (668 sputum, 1,178 saliva, 629 serum, 842 stool, and 180 urine) collected from 96 COVID-19 patients hospitalized in Zhejiang, China, and analysed both the viral loads temporal trends and the correlation between viral loads in different kind of samples and severity of the disease. RT-qPCR was performed for the ORFab1 gene, the samples with Ct values of ≤38.0 being considered positive for SARS-CoV-2. The median virus detectability time in the respiratory samples was 18 days. Referring to the respiratory samples, the patients with severe disease had significantly higher viral loads than patients with mild disease. Viral loads in stool and serum samples showed no significant difference between patients with mild disease and patients with severe disease. SARS-CoV-2 was sporadically found in urine samples.[42]

Zou et al. monitored SARS-CoV-2 loads in the upper respiratory tract by means of 72 nasal swabs and 72 throat swabs from 18 COVID-19 patients with different degrees of disease in Zhuhai, Guangdong, China. The mean Ct values for the Orf1b gene in nasal and throat swabs obtained from the patients with severe disease were lower by 2.8 (95%CI -2.4;8.0) and 2.5 (95% CI -0.8;5.7), respectively, than the values in swabs obtained from patients with mild-to-moderate disease. In general, Ct values resulted inversely related to viral RNA copy numbers, with Ct values of 30.76, 27.67, 24.56, and 21.48 corresponding to $1.5 \times 10^4$, $1.5 \times 10^5$, $1.5 \times 10^6$, and $1.5 \times 10^7$ copies per mL.[43]

A synopsis of the results of the 10 above discussed papers presenting original results is presented in the annexed table 2.

| FIRST AUTHOR BIBLIOGRAPHIC REFERENCE | OBJECT OF THE STUDY | KIND OF THE SAMPLINGS AND THE ANALYSES | RESULTS | NOTES |
|---|---|---|---|---|
| Clementi et al.[32] | COVID-19 patients (not furtherly specified) | •Nasopharyngeal swabs •RT-PCR • Ct values | Significant difference in the means Ct values in two different periods of the epidemic | Italy |
| To et al.[33] | Hospitalized COVID-19 patients | •Several respiratory samples •RT-qPCR •Ct values | Viral loads declining with time after the onset of the diseases | Hong Kong |
| Liu et al.[34] | Hospitalized COVID-19 patients | •Nasopharyngeal swabs and BALFs •RT-PCR •Ct values | Viral loads crucial in predicting the disease severity | China |
| Pan et al.[35] | Hospitalized COVID-19 patients | •Throat swabs, sputum, urine, and stool •RT-qPCR •Copies per mL | The viral loads in throat swabs and sputum samples peaked at around 5–6 days after the symptoms onset | China |
| Pujadas et al.[36] | Hospitalized COVID-19 patients | •Nasopharyngeal swabs •RT-qPCR •Copies per mL | Independent relationship between high viral load and mortality | USA |
| Kelleni.[37] | / | / | / | Challenge of the clinical significance of findings by Pujadas et al.[36] |
| Walsh et al.[38] | / | / | / | Review of 113 studies about SARS-CoV-2 in different body fluids |
| Shi et al.[39] | Hospitalized COVID-19 patients | •Pharyngeal swabs •RT-qPCR •Copies per mL | Positive relation between viral loads and serum biomarkers | China |
| Yu et al.[40] | Hospitalized COVID-19 patients | •Sputum •RT-qPCR •Ct values | Severe patients had significantly lower Ct values | China |
| Yu et al.[41] | Hospitalized COVID-19 patients | •Nasal swabs, throat swabs, sputum •RT-qPCR and Droplet Digital Polymerase Chain Reaction (ddPCR) •Copies/test | Sputum samples may better reflect the level of virus replication in vivo | China |
| Zheng et al.[42] | Hospitalized COVID-19 patients | •Sputum, saliva, serum, stool, and urine •RT-qPCR •Ct values | Patients with severe disease had significantly higher viral loads in respiratory samples | China |
| Zou et al.[43] | COVID-19 patients with different degrees of disease | •Nasal swabs and throat swabs •RT-qPCR •Ct values + copies per mL | Ct values resulted inversely related to viral RNA copy numbers | China |

**Table 2.** Viral loads and clinical figures (12 papers).
**Tabella 2.** Cariche virali e quadri clinici (12 studi).

## DISCUSSION AND CONCLUSIONS

The review of the indexed literature at 20 August 2020 reveals a limited number of relevant papers, certainly insufficient to depict conclusive figures, but allowing the identification of some evidence, suitable for a transfer from the context of origin to similar scenarios.

It is definitely possible to determine and quantify airborne SARS-CoV-2, applying diffused techniques for active air samplings, likewise it is possible e.g. for influenza[44] and other respiratory viruses.[45] The SARS-CoV-2 concentration in air looms as a promising appraiser of the risk of falling affected by COVID-19, at different levels of severity. The evaluated studies show heterogeneities in the results regarding both the frequency of SARS-CoV-2 detection in air and the viral concentrations; it is worth noting that SARS-CoV-2 results often undetectable in air, even inside rooms where COVID-19 patients are present. More research is needed to identify the determinants of these results (e.g. patients wearing respiratory masks or not, kinds and frequency of aerosols generating clinical manoeuvres, higher or lower air changing rate, different levels and modalities for surface hygiene), and their different weights in different contexts.

It is definitely possible to study the surface contamination itself (a possible determinant of an exposure both directly, by hand touch, and indirectly, by resuspension in air of settled particles) by collecting SARS-CoV-2 by means of sterile swabs; this parameter too looms promising as a risk appraiser.

It is definitely possible to determine the presence of SARS-CoV-2 in biological fluids and to quantify the viral load, evaluating both the cycle threshold (Ct) of the polymerase chain reaction (PCR) for different SARS-CoV-2 genes, and the number of viral copies per volume unit of collected body fluids.

It is subsequently possible to approach the COVID-19 epidemic modelling sets of personal SARS-CoV-2 exposure profiles and transferring the experimental results to different population groups and exposure scenarios, aiming at a reliable exposure assessment.[46]

There is sporadic but significant scientific evidence for a positive relationship between high doses of exposure to SARS-CoV-2, higher viral loads, and worse clinical conditions and outcomes in COVID-19 patients. The hypothesis of a dose-response relationship deserves to be closely investigated; more research is certainly needed respecting this too.

The possibility to apply a dose-response approach to SARS-CoV-2 configures a peculiar interest, both when *a posteriori* the determinants of COVID-19 cases have to be ascertained and when *a priori* the most dangerous situations have to be identified, mapped and dealt with for public health necessities.

Under this perspective, a wide spreading of good quality environmental and biological samplings for SARS-CoV-2 and the subsequent construction of a structured inventory of SARS-CoV-2 levels in air, on surfaces, and in body fluids, is proposed.

A scenario-exposure matrix describing, at least, the most relevant occupational and community contexts and including robust information about the determinants of the values' dispersion could provide a useful tool for the risk assessment process and the definition of public health strategies, resulting particularly useful in contexts of low socio-economic levels, lacking sufficient resources for their own samplings and analyses.

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