Towards an Accurate and Systematic Characterization of Persistently Asymptomatic Infection with SARS-CoV-2

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Summary

People with persistently asymptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection experience no symptoms throughout the course of infection, while pre-symptomatic individuals report symptoms attributable to the virus. Transmission of SARS-CoV-2 from individuals without symptoms contributes to pandemic spread, but the extent of transmission from persistently asymptomatic individuals remains unknown. We describe three methodological issues that hinder attempts to estimate this proportion. First, incomplete symptom assessment likely overestimates the asymptomatic fraction. Second, studies with inadequate follow-up misclassify pre-symptomatic individuals. Third, serological studies may identify people with previously unrecognized infection, but reliance on poorly defined antibody responses and retrospective symptom assessment may result in misclassification. We provide recommendations regarding definitions, detection, documentation and follow-up to improve the identification and evaluation of people with persistently asymptomatic SARS-CoV-2 infection and their contacts. Accurate characterisation of the persistently asymptomatic fraction may shed light on COVID-19 pathogenesis, transmission dynamics, and inform public health responses.

Introduction

Among the immense challenges of the coronavirus disease 2019 (COVID-19) pandemic are mitigating viral spread and understanding the spectrum of illness severity, both of which depend on accurate descriptions of the diverse clinical presentations of SARS-CoV-2 infection. Control of spread in particular has been limited by the variable incubation period,[1] well documented pre-symptomatic transmission[2] with approximately 25-40% of transmission occurring before the onset of symptoms,[3] and heterogeneous transmission dynamics, where clusters and superspreading events play a major role in propagating the pandemic, while many infected individuals lead to no subsequent cases.[4-6] Despite over 75,000 peer-reviewed and preprint publications about SARS-CoV-2 and COVID-19 since January 2020, the size and characteristics of the persistently asymptomatic fraction remain poorly understood.

A person with asymptomatic SARS-CoV-2 infection has laboratory confirmed SARS-CoV-2 with no symptoms at all throughout the duration of infection whereas a symptomatic person reports symptoms attributable to SARS-CoV-2. Defining the proportion of SARS-CoV-2 infection that is truly asymptomatic will help to better characterise the COVID-19 illness severity spectrum, pathogenesis, transmissibility, and immunity, and will inform control policies. Systematic reviews that only include studies with sufficient time to exclude pre-symptomatic infection have estimated the percentage of SARS-CoV-2 infections that remain completely free of symptoms at 20% (95% confidence interval, CI 17-25%) and 14% (95% CI 5-24%).[7,8] The individual studies included in these reviews rarely estimated an asymptomatic fraction greater than 50%. The range of estimates of asymptomatic SARS-CoV-2 reported in studies that used a wider variety of study designs goes from as low as 4% to over 80%, (Table 1).[9,10]

[Table 1]

There are three main reasons for ongoing confusion about the asymptomatic population. First, investigators have not yet developed a consistent case definition, meaning that symptom assessments differ substantially between studies and over time, with minor or atypical symptoms almost certainly missed in the earliest descriptions. Second, cross-sectional studies that assess symptoms at a single time point, or studies with a short follow up period, may incorrectly categorise individuals as asymptomatic when they are actually pre- or post-symptomatic.[21,22] Third, the time course and durability of the SARS-CoV-2 antibody response remain poorly understood, so there may be major limitations when using serological surveys, particularly when they are coupled with retrospective clinical history, to estimate the asymptomatic fraction.

This article summarises these limitations, using examples from studies that have reported on asymptomatic SARS-CoV-2 infections (Table 1), and gives recommendations for future studies that will describe this important subset of individuals.

1. Lack of consistent reporting of symptoms

Our understanding of the possible clinical presentations of SARS-CoV-2 infection has evolved since the beginning of the pandemic and many studies that report an asymptomatic proportion of patients have not completely described or assessed COVID-19 symptoms based on what we know now. The first large descriptive studies of hospitalized patients with COVID-19 from China in January 2020, used information extracted from medical records and reported that the

most common symptoms were fever, cough, fatigue and myalgia.[23,24] Gastrointestinal symptoms were uncommon in those case series, though now they are more widely recognised and some reports suggest they may occur in up to half of individuals.[25,26] Anosmia and dysgeusia were first documented in March 2020, may be more prevalent in milder cases,[27,28] and are strongly associated with SARS-CoV-2 infection.[19] A large study using a symptom tracking smartphone application found that it became more common for individuals with COVID-19 to report anosmia or dysgeusia in the UK after the association of these symptoms with infection was reported widely in the media.[19,27]

Many studies have used an unclear or uncomprehensive method of symptom ascertainment, making it hard to interpret the reported frequency of symptoms. Information extracted retrospectively from medical records or reports that rely upon spontaneous reporting by study participants will likely underestimate the frequency of mild or atypical symptoms. In a cohort of 147 individuals diagnosed with SARS-CoV-2 infection at homeless shelters in Boston, 88% (129/147) were classified as asymptomatic when asked only about a narrow range of symptoms that included a "*history of cough and shortness of breath*." They were also given "*the option to report other symptoms*," a strategy that does not reliably capture a complete clinical picture.[15] A large study of infections in Iceland considered only the following symptoms compatible with COVID-19, "*cough, fever, aches, and shortness of breath.*"[16] A report of individuals infected on the *Diamond Princess* cruise ship omitted commonly reported symptoms, including anosmia and gastrointestinal complaints, which might have led to an overestimated asymptomatic rate of 44% (311/712).[11] Additionally, it is not clear how the language barrier was addressed, since symptom assessment occurred in Japan from a presumably multinational and multilingual cohort. Other

studies of the Diamond Princess outbreak have estimated different rates of asymptomatic infections, including a modeling study that estimated 18% (credible interval 16-20%) and another study of the early phase of the outbreak that reported 14% (24/172), but only tested "suspected cases" (defined as those with fever or respiratory symptoms) which might have biased the outcome.[29,30]

Two detailed investigations of outbreaks at nursing facilities – one in Washington State and one in Illinois – from March 2020 did not include assessment for changes in smell or taste, since these symptoms were not widely recognised at that time.[12,17,31] A study of an outbreak associated with a call centre in South Korea found that just 4% of nearly 100 cases were persistently asymptomatic, though the list of symptoms enquired about is not described in the report.[9] Details about case definition and manner of symptom assessment are required to interpret study results and incomplete symptom assessment risks overestimate of the asymptomatic fraction.

In describing the experience with the virus in the town of Vo, Italy, investigators reported a persistently asymptomatic fraction of 43%.[13] Study participants were tested for SARS-CoV-2 by nasopharyngeal swab and received a survey with symptom assessment on February 24 and again on March 7, an interval of 12 days. Symptomatic cases were defined as those who *"required hospitalization and/or reported fever (yes/no or a temperature above 37 degrees Celsius) and/or cough and/or at least two of the following symptoms: sore throat, headache, diarrhea, vomit, asthenia, muscle pain, joint pain, loss of taste or smell, shortness of breath."* While reported symptom assessment was systematic and comprehensive, requiring at least two minor symptoms

be present in cases confirmed by reverse transcriptase polymerase chain reaction (RT-PCR) may have led to misclassification of some individuals with mild symptoms as asymptomatic.

2. Inadequate follow up time

An absence of symptoms at the time of a positive RT-PCR test is insufficient to determine whether an individual has persistently asymptomatic infection because an RT-PCR test result can be positive before symptom onset.[3,32,33] Cross-sectional studies can therefore assess the proportion of people with and without symptoms at the time of testing but cannot distinguish pre-symptomatic from asymptomatic infection.

The duration of follow up needed to capture pre-symptomatic individuals is the maximum duration of the incubation period, and over 95% of infected individuals who develop symptoms will do so within 14 days, making this a reasonable length of follow up to rule out the vast majority of pre-symptomatic cases.[1] Two examples show the importance of follow-up time in studies with different contexts and inclusion criteria. Among residents of a nursing home in the USA who were tested after a health care worker was found to be infected, 48 tested positive for SARS-CoV-2, of whom 21 had symptoms and 27 were asymptomatic at the time of testing. Over the next seven days, 24 of the initial 27 without symptoms developed symptoms and were therefore pre-symptomatic at the time of testing.[17] In South Korea, 110 of 303 individuals were initially asymptomatic at a clinical treatment centre, a setting designed for individuals with mild or no symptoms, and 21 eventually developed symptoms indicating a persistently asymptomatic fraction in this cohort of 29%.[34]

Three publications about pregnant women in New York City show the importance of accurate reporting of symptoms and adequate follow-up.[14,35,36] The first report, stating that "29 of the 33 patients who were positive for SARS-CoV-2 at admission (87.9%) had no symptoms of COVID-19 at presentation," had a median follow up time of two days post-partum, an insufficient period to exclude pre-symptomatic infection.[14] In fact, two subsequent publications with an overlapping cohort of obstetric patients with longer follow up, found that the asymptomatic fraction was much lower, including one study where just 46/158 (29%) remained asymptomatic throughout follow up (63 were asymptomatic at diagnosis) and another study with at least two weeks of follow up time for patients where 4/43 (9%) remained asymptomatic (12 were asymptomatic at diagnosis).[35,36]

Several other cross-sectional studies in different contexts have at times been interpreted inappropriately as reporting the asymptomatic fraction, including a study at Boston homeless shelters, a report of an outbreak on a cruise ship off South America, and a study of infections in Iceland, among others.[10,15,16,37] Additionally, an RT-PCR test may remain positive after the period of infectiousness since the median duration of nasopharyngeal swab shedding is 22 days.[22] It is therefore also important to assess for prior symptoms if the timing of infection is unknown.

3. Issues with assessment of symptom status in seroprevalence studies:

Antibody test characteristics are defined by comparison with RT-PCR as a reference standard and we have insufficient understanding of their performance for RT-PCR-negative (or untested) individuals with prior asymptomatic infection. Antibody durability in these cases is another

concern, with one study finding that among previously RT-PCR-positive individuals, 40% (12/30) of asymptomatic, but only 13% (4/31) of symptomatic individuals, became seronegative after about 8 weeks.[38]

A large seroprevalence study in Spain reported that nearly a third of people with SARS-CoV-2 antibodies were asymptomatic.[20] Symptom assessment was comprehensive and systematic and although there was no follow up period, those with positive IgG titres would have been out of the pre-symptomatic period.[39] In the study, IgG antibodies were found in 8.0% (95% CI 6.0-10.6%) of participants with a prior negative RT-PCR test and in 4.2% (95% CI 3.8-4.5%) of those who never had an RT-PCR test. The authors suggest that those with a prior negative RT-PCR test might have received late RT-PCR testing in the setting of a compatible syndrome, but provide no evidence for this and this was not assessed in the study.[20] To interpret these results properly, it would be important to understand the study population better; were these individuals tested because they indeed had a compatible syndrome and/or a close contact? In that case, they are likely true positives. However, it is important to consider the possibility that some or many of these individuals might be false positives.

Why understanding the persistently asymptomatic fraction is important

Gaps in our understanding limit development of optimal public health strategies to control the pandemic. For instance, we do not know whether people with persistently asymptomatic SARS-CoV-2 infection have demographic, clinical, immunological or virologic characteristics that differ from those who develop symptoms, or how their transmission potential differs. Studies reporting on asymptomatic individuals with SARS-CoV-2 infection often include small numbers

of study participants, without detailed descriptions of baseline characteristics or comparison with participants with symptoms. This evidence gap precludes analyses of how asymptomatic individuals might differ from those who develop symptoms. More detailed descriptions would allow for a richer understanding of differences between these populations and pooled analyses would be possible if individual patient data were available. In future research studies, meticulous description of methods used to enrol participants and assess the persistently asymptomatic fraction will also make it easier to investigate study heterogeneity in systematic reviews of this topic,[7] and better inform modelling studies that make assumptions about viral transmission dynamics based on estimates of the persistently asymptomatic fraction.[40] This information will improve pandemic control strategies.

Detailed follow-up of people with persistently asymptomatic SARS-CoV-2 infection will also allow a definitive understanding of viral dynamics and antibody responses in these individuals, which could help determine whether they develop a sufficiently robust and durable antibody response after infection and how they will respond to vaccines. Furthermore, the characteristics of this group may help explain the wide spectrum of illness severity and COVID-19 pathogenesis. Lastly, with a growing understanding that mild symptoms may be associated with SARS-CoV-2 infection coupled with lower barriers to diagnostic testing, more cases could be readily identified and help reduce community transmission.

Recommendations

We make six recommendations to allow for accurate ascertainment of asymptomatic infection status and eventually define the asymptomatic fraction.

1. Define persistently asymptomatic infection clearly

The term "persistently asymptomatic SARS-CoV-2 infection" should be reserved for people who have no known COVID-19 symptoms, including no atypical or mild symptoms, throughout the course of infection. Cross-sectional studies should report proportions without symptoms as "asymptomatic at the time of testing."

2. Use a standard, broad symptom definition

There are numerous clinical case definitions with emphasis on different symptoms from various groups including the World Health Organization, the European Centre for Disease Prevention and Control, the United States Centers for Disease Control and Prevention, and the Canadian Ministry of Health and Long-term Care, (Table 2). We recommend standardisation of clinical definitions and favour the symptom list in the Canadian case definition at this time, which is the most comprehensive. This definition allows documentation of the most common symptoms, and characterisation of cases as typical, atypical, mildly symptomatic, or persistently asymptomatic.

[Table 2]

3. Assess symptoms prospectively and retrospectively for the minimum appropriate follow up period

A minimum follow-up period of 14 days from last possible exposure (or first positive test if exposure is unknown) will differentiate pre-symptomatic from persistently asymptomatic individuals. Investigators should report the follow-up period, together with baseline

characteristics of individuals with all clinical presentations, including age, gender and ethnic group as a minimum.

An investigation of non-hospitalised household contacts of individuals with SARS-CoV-2 infection in Wisconsin and Utah performed an assessment consistent with our recommendations, including with systematic, detailed symptom assessment and adequate follow up period, and may be a model for similar studies moving forward.[41]

4. Clearly report testing protocols used for SARS-CoV-2 detection

Details of testing, including timing, site, and test platform are necessary to interpret results from studies reporting on asymptomatic cases. Timing of testing should reflect the SARS-CoV-2 viral load dynamics and incubation period and not be done prior to day five after exposure for those without symptoms.[42] The optimal site of testing is actively being studied but most clinical experience to date is with nasopharyngeal or oropharyngeal testing. Salivary testing might be less sensitive and may have other handling constraints (i.e. rapid time to processing) that require further study.[43] Poor sampling may yield false negative results. This was suggested in the report of four symptomatic individuals from Italy who initially had negative nasopharyngeal RT-PCR tests which were positive when a repeat sample was obtained by an otolaryngologist 6-72 hours later.[44] In another study, suspected false negative RT-PCR tests had significantly lower amounts of human DNA compared with other samples.[45] While RT-PCR based platforms are most commonly used now, less sensitive rapid antigen testing is likely to become much more common.[46] The sensitivity of antigen tests for persistently asymptomatic cases is unknown at this time.

5. Detailed reporting of serologic studies to understand for asymptomatic infection

Serologic testing could become a helpful adjunct to define the persistently asymptomatic fraction. To interpret results, researchers should clearly report the time window between suspected infection and antibody testing. Symptom recall bias may be worse with longer delays. In a follow up of the Iceland study, researchers clearly reported the timing of exposures and antibody testing.[16,47] They found 10% (142/1421) of those quarantined after a COVID-19 exposure had detectable antibodies without prior symptoms and without reported PCR testing.[47] The pretest probability for infection is higher in quarantined individuals compared with a random population sample and, though this study did not estimate the population wide asymptomatic fraction, it improves on prior serologic studies assessment of asymptomatic cases. Serial testing can help define antibody decay trajectories, an important variable for estimating the asymptomatic fraction from serological studies.

6. Design studies to minimise biases that affect ascertainment of the asymptomatic fraction

Research studies to measure the persistently asymptomatic fraction of SARS-CoV-2 infection need to be designed so that the absence or presence of symptoms does not affect selection into the study. The ideal study design would screen a population and follow those infected with SARS-CoV-2 prospectively. Clinical and demographic data would be collected at baseline, with frequent (even daily) comprehensive symptom assessments, serial RT-PCR testing from multiple body sites and intermittent measurements of antibody titres and immune response. Detailed contact tracing studies in unbiased populations should also be done so that secondary attack rates

can be compared between people with persistently asymptomatic and symptomatic infection, and the duration of their period of infectiousness can be determined.

The distinction between asymptomatic and pre-symptomatic individuals should not distract from the overwhelming evidence that individuals without symptoms can transmit the virus , usually when they are pre-symptomatic, emphasising the need to continue implementing non-pharmaceutical interventions such as physical distancing, universal masking and handwashing.[2] In addition, testing policy in outbreak settings and high-risk environments such as long-term health care facilities needs to reflect this critical fact: individuals without symptoms in close contact with an index case will need to be tested as part of the outbreak investigation to identify cases and allow for effective control measures.

To date, absence of comprehensive understanding about asymptomatic SARS-CoV-2 infection makes it difficult to inform public health strategies on the best way to control the pandemic. Uncertainty about the existence, characteristics, prognosis and role of asymptomatic SARS-CoV-2 infection in this pandemic will continue unless we have systematically and accurately collected data.

Setting [Ref]	Asymptomatic % reported	Follow-up period	Symptom assessment	Notes
1. Incomplete symptom reporting or restrictive symptom assessment				
Diamond Princess cruise ship [11]	311/712 (44%)	Adequate	"cough, dyspnoea, chest pain, sore throat, nasal discharge"	Symptoms prospectively assessed
Skilled nursing facility in the US [12]	13/33 (39%)	Adequate	"typical (fever, cough, shortness of breath, hypoxia) and atypical (sore throat, nasal congestion, diarrhoea, decreased appetite, chills, myalgias, headaches, new onset confusion) symptoms"	Authors note that memory impairment may have resulted in an overestimate in asymptomatic rate
Call centre in South Korea [9]	4/97 (4.1%)	Adequate	Not defined	Face-to-face interviews for symptom assessment
Vo, Italy [13]	42.5% (95% CI 31.5% - 54.6%) with a total of 81 cases	12 days	Reportedly comprehensive	Mix of prospective and retrospective symptom assessment
Pregnant women presenting for delivery in New York City [14]	26-29/33 (78.9-87.9%)	Inadequate	"Fever or other symptoms of COVID-19"	Symptom screen on admission; unclear how symptoms assessed during follow up period
2. Cross-sectional studies or inadequate follow up				
Boston homeless shelters [15]	129/147 (87.8%)	Inadequate	Cough, shortness of breath, other symptoms optional	Single time point symptom screen
Iceland [16]	525/1221 (43%)	Inadequate	"cough, fever, aches, and shortness of breath"	Single time point symptom screen
US nursing home [17]	3/48 (6.3%)	7-day prospective follow up	Comprehensive	Nurse-administered symptom assessments on day 1 and day 7
Cruise ship [10]	104/128 (81%)	Inadequate	Not described	Mechanism of symptom assessment not clear
Long-term care facilities in US [18]	257/631 (40.7%)	Inadequate	Comprehensive	Symptom assessments by case reports
USS Theodore Roosevelt [19]	44/238 (18.5%)	Not well defined	Comprehensive	Convenience sample; retrospective symptom assessment
3. Serological study				
Spain [20]	21.9 (95% CI 19.1 – 24.9) to 35.8 (95% CI 33.1 – 38.5) out of >61,000 participants screened	Single time point but serological survey	"fever, chills, severe tiredness, sore throat, cough, shortness of breath, headache, anosmia or ageusia"	Antibody responses of asymptomatic cases currently poorly defined

Table 1: Assessment of selected studies reporting the asymptomatic fraction

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Source	Symptom	Citation
World Health Organization	Fever AND cough OR Three or more of the following: fever, cough, general weakness/fatigue, headache, myalgia, sore throat, coryza, dyspnea, anorexia/nausea/vomiting, diarrhea, altered mental status OR Recent onset anosmia or ageusia without another explanation	https://www.who.int/publications/i/item/WHO-2019-nCoV-Surveillance_Case_Definition-2020.1 (accessed September 7, 2020)
European Centre for Disease Prevention and Control	At least one of the following symptoms: cough, fever, shortness of breath, or sudden onset anosmia, ageusia or dysgeusia	https://www.ecdc.europa.eu/en/covid-19/surveillance/case-definition (accessed September 7, 2020)
Centers for Disease Control of the USA	At least two of the following symptoms: fever, chills, rigors, myalgia, headache, sore throat, nausea or vomiting, diarrhea, fatigue, congestion or runny nose OR Any one of the following: cough, shortness of breath, difficulty breathing, new olfactory disorder, new taste disorder	https://wwwn.cdc.gov/nndss/conditions/coronavirus-disease-2019-covid-19/case-definition/2020/08/05/ (accessed September 7, 2020)
Health Canada; Ontario Ministry of Health and Long-Term Care	Any of the following: Common symptoms: fever, new or worsening cough, shortness of breath Other symptoms: sore throat, difficulty swallowing, new olfactory disorder, nausea/vomiting, diarrhea, abdominal pain, runny nose or nasal congestion (in the absence of underlying reason for these symptoms such as seasonal allergies, postnasal drip, etc.) Atypical symptoms: unexplained fatigue/malaise, myalgias, delirium, unexplained or increased number of falls, acute functional decline, exacerbation of chronic conditions, chills, headaches, croup, conjunctivitis	https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/symptoms.html and http://www.health.gov.on.ca/en/pro/programs/publichealth/coronavirus/2019_guidance.aspx#case and http://www.health.gov.on.ca/en/pro/programs/publichealth/coronavirus/docs/2019_reference_doc_symptoms.pdf (accessed September 7, 2020)

Table 2: Symptoms considered consistent with COVID-19 from various case definitions

Contributors

EAM, AR, and MC conceptualised the manuscript and wrote the first draft. IIB and NL contributed significantly to the methods, and reviewed and edited the manuscript. All authors contributed significantly to the writing and editing of the final submission.

Conflicts of Interest

IIB has consulted for BlueDot, a social benefit corporation that tracks the spread of emerging infectious diseases. EAM, AR, NL and MC report no conflicts of interest.

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References

1. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. *Annals of Internal Medicine* 2020; **172**(9): 577-582. doi: 10.7326/M20-0504.

2. Qiu X, Nergiz AI, Maraolo AE, Bogoch II, Low N, Cevik M. Defining the role of asymptomatic SARS-CoV-2 transmission: a living systematic review. *medRxiv* 2020: 2020.09.01.20135194.

3. He X, Lau EHY, Wu P, et al. Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nature Medicine* 2020; **26**(5): 672-5.

4. Adam DC, Wu P, Wong JY, et al. Clustering and superspreading potential of SARS-CoV-2 infections in Hong Kong. *Nat Med* 2020. https://doi.org/10.1038/s41591-020-1092-0

5. Bi Q, Wu Y, Mei S, et al. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. *The Lancet Infectious diseases* 2020; **20**(8): 911-9.

6. Laxminarayan R, Wahl B, Dudala SR, et al. Epidemiology and transmission dynamics of COVID-19 in two Indian states. *Science* 2020: eabd7672. DOI: 10.1126/science.abd7672

7. Buitrago-Garcia D, Egli-Gany D, Counotte MJ, et al. Occurrence and transmission potential of asymptomatic and presymptomatic SARS-CoV-2 infections: A living systematic review and meta-analysis. *PLoS Med* 2020; **17**(9): e1003346.

8. Byambasuren O, Cardona M, Bell K, Clark J, McLaws M-L, Glasziou P. Estimating the extent of asymptomatic COVID-19 and its potential for community transmission: systematic review and meta-analysis. *medRxiv* 2020: 2020.05.10.20097543.

9. Shin Young P, Young-Man K, Seonju Y, et al. Coronavirus Disease Outbreak in Call Center, South Korea. *Emerging Infectious Disease journal* 2020; **26**(8).

10. Ing AJ, Cocks C, Green JP. COVID-19: in the footsteps of Ernest Shackleton. *Thorax* 2020: thoraxjnl-2020-215091.

11. Sakurai A, Sasaki T, Kato S, et al. Natural History of Asymptomatic SARS-CoV-2 Infection. *New England Journal of Medicine* 2020; **383**(9): 885-886 DOI: 10.1056/NEJMc2013020

12. Patel MC, Chaisson LH, Borgetti S, et al. Asymptomatic SARS-CoV-2 Infection and COVID-19 Mortality During an Outbreak Investigation in a Skilled Nursing Facility. *Clinical Infectious Diseases* 2020. https://doi.org/10.1093/cid/ciaa763

13. Lavezzo E, Franchin E, Ciavarella C, et al. Suppression of a SARS-CoV-2 outbreak in the Italian municipality of Vo'. *Nature* 2020. https://doi.org/10.1038/s41586-020-2488-1

14. Sutton D, Fuchs K, D'Alton M, Goffman D. Universal Screening for SARS-CoV-2 in Women Admitted for Delivery. *New England Journal of Medicine* 2020; **382**(22): 2163-4.

15. Baggett TP, Keyes H, Sporn N, Gaeta JM. Prevalence of SARS-CoV-2 Infection in Residents of a Large Homeless Shelter in Boston. *JAMA* 2020; **323**(21): 2191-2.

16. Gudbjartsson DF, Helgason A, Jonsson H, et al. Spread of SARS-CoV-2 in the Icelandic Population. *New England Journal of Medicine* 2020; **382**(24): 2302-15.

17. Arons MM, Hatfield KM, Reddy SC, et al. Presymptomatic SARS-CoV-2 Infections and Transmission in a Skilled Nursing Facility. *New England Journal of Medicine* 2020; **382**(22): 2081-2090. doi: 10.1056/NEJMoa2008457.

18. Feaster M, Goh Y-Y. High Proportion of Asymptomatic SARS-CoV-2 Infections in 9 Long-Term Care Facilities, Pasadena, California, USA, April 2020. *Emerging Infectious Disease journal* 2020; **26**(10).

19. Payne DC, Smith-Jeffcoat SE, Nowak G, et al. SARS-CoV-2 Infections and Serologic Responses from a Sample of U.S. Navy Service Members - USS Theodore Roosevelt, April 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**(23): 714-21.

20. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *The Lancet*. https://doi.org/10.1016/S0140-6736(20)31483-5

21. Wang Y, Liu Y, Liu L, Wang X, Luo N, Ling L. Clinical outcome of 55 asymptomatic cases at the time of hospital admission infected with SARS-Coronavirus-2 in Shenzhen, China. *The Journal of Infectious Diseases* 2020. https://doi.org/10.1093/infdis/jiaa119

22. Sun J, Xiao J, Sun R, et al. Prolonged Persistence of SARS-CoV-2 RNA in Body Fluids. *Emerg Infect Dis* 2020; **26**(8): 1834-8.

23. Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet*. https://doi.org/10.1016/S0140-6736(20)30566-3

24. Guan W-j, Ni Z-y, Hu Y, et al. Clinical Characteristics of Coronavirus Disease 2019 in China. *New England Journal of Medicine* 2020; **382**(18): 1708-1720. doi: 10.1056/NEJMoa2002032.

25. Pan L, Mu M, Yang P, et al. Clinical Characteristics of COVID-19 Patients With Digestive Symptoms in Hubei, China: A Descriptive, Cross-Sectional, Multicenter Study. *American Journal of Gastroenterology* 2020; **115**(5): 766-73.

26. Burke RM, Killerby ME, Newton S, et al. Symptom Profiles of a Convenience Sample of Patients with COVID-19 - United States, January-April 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**(28): 904-8.

27. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict potential COVID-19. *Nature Medicine* 2020; **26**(7): 1037-1040. doi: 10.1038/s41591-020-0916-2.

28. Romero-Sánchez CM, Díaz-Maroto I, Fernández-Díaz E, et al. Neurologic manifestations in hospitalized patients with COVID-19: The ALBACOVID registry. *Neurology* 2020: 10.1212/WNL.0000000000009937.

29. Yamagishi T, Kamiya H, Kakimoto K, Suzuki M, Wakita T. Descriptive study of COVID-19 outbreak among passengers and crew on Diamond Princess cruise ship, Yokohama Port, Japan, 20 January to 9 February 2020. *Euro Surveill* 2020; **25**(23).

30. Mizumoto K, Kagaya K, Zarebski A, Chowell G. Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. *Euro Surveill* 2020; **25**(10).

31. Kimball A, Hatfield KM, Arons M, et al. Asymptomatic and Presymptomatic SARS-CoV-2 Infections in Residents of a Long-Term Care Skilled Nursing Facility - King County, Washington, March 2020. *MMWR Morbidity and mortality weekly report* 2020; **69**(13): 377-81.

32. To KK-W, Tsang OT-Y, Leung W-S, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *The Lancet Infectious Diseases* 2020; **20**(5): 565-74.

33. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV-1 and MERS-CoV viral load dynamics, duration of viral shedding and infectiousness: a living systematic review and meta-analysis. *medRxiv* 2020: 2020.07.25.20162107.

34. Lee S, Kim T, Lee E, et al. Clinical Course and Molecular Viral Shedding Among Asymptomatic and Symptomatic Patients With SARS-CoV-2 Infection in a Community Treatment Center in the Republic of Korea. *JAMA Internal Medicine* 2020. doi:10.1001/jamainternmed.2020.3862

35. Andrikopoulou M, Madden N, Wen T, et al. Symptoms and Critical Illness Among Obstetric Patients With Coronavirus Disease 2019 (COVID-19) Infection. *Obstetrics & Gynecology* 9000; **Publish Ahead of Print**. doi: 10.1097/AOG.0000000000003996

36. Breslin N, Baptiste C, Gyamfi-Bannerman C, et al. COVID-19 infection among asymptomatic and symptomatic pregnant women: Two weeks of confirmed presentations to an affiliated pair of New York City hospitals. *American journal of obstetrics & gynecology MFM* 2020; **2**(2): 100118-.

37. COVID-19 Outbreak Among College Students After a Spring Break Trip to Mexico - Austin, Texas, March 26-April 5, 2020. *MMWR Morb Mortal Wkly Rep* 2020; **69**(26): 830-5.

38. Long Q-X, Tang X-J, Shi Q-L, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nature Medicine* 2020; **26**(8): 1200-1204. doi: 10.1038/s41591-020-0965-6.

39. Xu X, Sun J, Nie S, et al. Seroprevalence of immunoglobulin M and G antibodies against SARS-CoV-2 in China. *Nature Medicine* 2020; **26**(8): 1193-1195. doi: 10.1038/s41591-020-0949-6.

40. Ferretti L, Wymant C, Kendall M, et al. Quantifying SARS-CoV-2 transmission suggests epidemic control with digital contact tracing. *Science* 2020: eabb6936. DOI: 10.1126/science.abb6936

41. Yousaf AR, Duca LM, Chu V, et al. A prospective cohort study in non-hospitalized household contacts with SARS-CoV-2 infection: symptom profiles and symptom change over time. *Clinical Infectious Diseases* 2020. https://doi.org/10.1093/cid/ciaa1072

42. Kucirka LM, Lauer SA, Laeyendecker O, Boon D, Lessler J. Variation in False-Negative Rate of Reverse Transcriptase Polymerase Chain Reaction-Based SARS-CoV-2 Tests by Time Since Exposure. *Ann Intern Med* 2020; **173**(4): 262-7.

43. Caulley L, Corsten M, Eapen L, et al. Salivary Detection of COVID-19. *Ann Intern Med* 2020. https://doi.org/10.7326/M20-4738

44. Piras A, Rizzo D, Uzzau S, De Riu G, Rubino S, Bussu F. Inappropriate Nasopharyngeal Sampling for SARS-CoV-2 Detection Is a Relevant Cause of False-Negative Reports. *Otolaryngol Head Neck Surg* 2020; **163**(3): 459-61.

45. Kinloch NN, Ritchie G, Brumme CJ, et al. Suboptimal Biological Sampling as a Probable Cause of False-Negative COVID-19 Diagnostic Test Results. *J Infect Dis* 2020; **222**(6): 899-902.

46. Mak GC, Cheng PK, Lau SS, et al. Evaluation of rapid antigen test for detection of SARS-CoV-2 virus. *J Clin Virol* 2020; **129**: 104500.

47. Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. *N Engl J Med* 2020. DOI: 10.1056/NEJMoa2026116