Asymptomatic COVID-19 infection: diagnosis, transmission, population characteristics

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ABSTRACT
A novel coronavirus first discovered in late December 2019 has spread to many countries around the world. An increasing number of asymptomatic patients have been reported and their ability to spread the virus has been proven. This brings major challenges to the control of the transmission. The discovery and control of asymptomatic patients with COVID-19 are the key issues in future epidemic prevention and recovery. In this narrative review, we summarise the existing knowledge about asymptomatic patients and put forward detection methods that are suitable for finding such patients. Besides, we compared the characteristics and transmissibility of asymptomatic patients in different populations in order to find the best screening, diagnosis and control measures for different populations. Comprehensive preventive advice is also provided to prevent the spread of infection from asymptomatic patients.

INTRODUCTION
COVID-19 is an acute respiratory tract infection that arose in late 2019. The causative pathogen of COVID-19 has been confirmed to be a novel coronavirus. The virus was named SARS-CoV-2 by the WHO.1 As of 14 November 2020, there have been 52852674 confirmed cases of COVID-19, including 1295328 deaths.2 Many potential drugs have been used to treat COVID-19.3 As the pandemic has developed, an increasing number of asymptomatic patients has been found, causing worldwide anxiety. Two conditions must be met for asymptomatic COVID-19 cases: first, the absence of self-perceived or clinically recognisable symptoms; and second, a positive test for SARS-CoV-2. This definition actually includes patients during the latent period and covert infected patients who are asymptomatic from onset to recovery. For some latent cases, although patients do not show any symptoms at the time of testing, they may eventually develop symptoms as the virus replicates in the body. Presymptomatic patients will be used in this review to represent latent cases, thus differentiating between the two when needed. When not specifically stated, this article focuses on cases that do not exhibit symptoms at the time of initial diagnosis of COVID-19. Current studies have shown that the combined proportion of patients who are asymptomatic at initial testing reaches 15.6%, of which approximately 48.9% are presymptomatic infections.4 The proportion of asymptomatic patients varies considerably among different groups (children, pregnant women, elderly, etc). The potential transmission of asymptomatic viruses imposes a significant burden on countries with secondary attack rates (SARs) ranging from 0% to 80%.5 This review aims to summarise the diagnosis, transmission and prevention of asymptomatic patients with COVID-19,

Key messages
What was already known?
- Asymptomatic patients have the potential for transmission.
- Asymptomatic patients are ubiquitous in people of all ages.

What are the new findings?
- The characteristics of asymptomatic patients vary in different groups.
- Prevention of asymptomatic transmission requires context-specific methods.

What is their significance?
- Clinical: Assist in the diagnosis of asymptomatic patients in different populations.
- Research: Suggest the proportion and characteristics of asymptomatic patients.
analyse the differences in the proportion, transmission capacity and characteristics of asymptomatic patients in different populations and finally propose methods for effective surveillance and control of the disease. We have summarized the main findings of this article using an (figure 1), which captures the content of the article for readers at a single glance.

**Detection of asymptomatic patients**

**Clinical testing**

There is no significant change in blood cell count and inflammation indicators in asymptomatic compared with patients with symptomatic COVID-19.\(^6\) Multiple organ dysfunction often occurs in symptomatic patients with little change in organ function in asymptomatic patients.\(^7\) An analysis of the imaging features of an asymptomatic infection showed that nearly half of the asymptomatic infections (47.62%) had lung abnormalities, and among the covert infections, 64.39% had lung abnormalities.\(^8\) Meng et al found that ground-glass opacity on CT scan was the main manifestation of asymptomatic patients (94.8%), with peripheral (75.9%) or unilateral (58.6%) location. The lower lung lobe (68.9% right vs 62.1% left) is more involved than the upper lobe (51.7% right vs 53.4% left).\(^9\) CT detects symptoms of COVID-19 earlier than nucleic acid testing does. About 75% of the patients who were diagnosed negative by reverse transcription-polymerase chain reaction (RT-PCR) had abnormalities in their chest CT and had positive chest CT for COVID-19, but the negative result of RT-PCR turned positive after 5.1±1.5 days. In addition, studies have shown that chest CT (98%) has higher sensitivity than RT-PCR (71%) for detecting patients with COVID-19.\(^10\) A recent study found that fluorodeoxyglucose positron emission tomography/CT can identify possible COVID-19 disease in the absence of or before symptom onset.\(^11\) Care should be taken when considering CT as a screening tool. CT scan has high sensitivity (86%–98%), but its diagnosis of COVID-19 often overlaps with that of other viral pneumonia, resulting in low specificity (about 25%).\(^12\) Moreover, the sensitivity of CT testing is affected by a variety of factors, such as disease severity, the proportion of patients with comorbidities, the proportion of asymptomatic patients and the age of asymptomatic patients.\(^13\) Therefore, we do not recommend the use of CT alone in the initial diagnosis of COVID-19. In low-prevalence populations, CT chest scans may be more effective in detecting asymptomatic cases.\(^14\) It has also been found to be more reliable for early diagnosis in the elderly, but less reliable in young children.\(^15\)\(^16\)

We suggest adopting the method of joint testing. It is

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**Figure 1** Examples of detection, transmission and screening of asymptomatic patients. Existing detection methods for asymptomatic patients include imaging, nucleic acid and serological diagnostics. Joint detection methods are highly efficient, accurate and more suitable for the discovery of asymptomatic patients. These methods are applied to screen out among discharged patients and persons released from isolation. It is also essential to conduct general monitoring and follow-up among the general population. These all will contribute to preventing further transmission of asymptomatic patients in homes, hospitals, and transmission through patient contaminants, thereby reducing the risk of infection in healthy people.
possible to combine both CT and RT-PCR to achieve adequate sensitivity and specificity. Research has shown that CT of the chest has been considered as a very important strategy for supplementary diagnosis.17

**Laboratory testing**

**Nucleic acid detection**

RT-PCR of respiratory specimens is currently the gold standard for diagnosis of COVID-19, with a sensitivity of approximately 96%. Both the virus load of the sampling site and operation of the test influence the accuracy of the results. In the early stages of viral infection, there may be some false-negative results due to insufficient amount of virus on the swab. Therefore, it may be better to perform multiple swab tests from different parts of the body when necessary.18 There are a variety of non-neuronal cell types in the olfactory epithelium of the nasal cavity, expressing two host receptors, angiotensin-converting enzyme 2 and transmembrane protease serine 2. These two receptors are considered to play an essential role in the binding and accumulation of SARS-CoV-2, which may make the nasal epithelium an enhanced binding site for the virus.19 For asymptomatic patients, a nasopharyngeal swab may be a better sampling method than the commonly used sputum and throat swabs. More recently, further studies have shown that nucleic acid testing using saliva samples appears to be more sensitive than nasopharyngeal swabs.20 The sensitivity and specificity of existing PCR methods are highly dependent on the performance of the kits and negatively correlated with the proportion of elderly patients.13 The prevalence of false-negative results suggests that nucleic acid testing is not entirely appropriate for the detection of asymptomatic patients. Any missed infections pose a threat to public safety.

Additionally, the cost of testing is high, it takes a long time, and health professionals are needed to perform the sampling. These factors all limit the large-scale application of RT-PCR for the screening of asymptomatic patients. In response to these problems, LabCorp Laboratories, Inc has developed an RT-PCR test kit for home specimen collection, enabling individuals to collect their own nasal swab samples at home, partially solving the problem of location limitation for nucleic acid testing.18 In addition, loop-received isothermal amplification (LAMP) is an economical and efficient alternative to standard PCR developed for the rapid detection of SARS-CoV-2. At present, a RT-LAMP kit developed by Sherlock Biosciences, Inc has been approved by Food and Drug Administration and European Union Association. According to Zhu et al, the accuracy and specificity of the nanoparticle-based RT-LAMP assay can reach 100%.21

**Serological testing**

Testing for specific antibodies in patients’ blood is another option for COVID-19 diagnosis. Li et al’s research showed that IgM antibodies and IgG antibodies appeared successively within 1 to 2 weeks after infection. The amount of IgG antibody reaches its highest value a few weeks after the onset of the disease. It will persist for months or even years, but the duration is still unclear.22 Many rapid tests for IgM or IgG have been produced so far. In these tests, using the principle of colloidal gold immunochromatography, results can be obtained within 15 min using 20 μL whole blood sample (or 10 μL of serum/plasma samples).23 This method shortens the detection time. The tests are simple to perform and the results are easy to interpret, which breaks through the limitations of personnel and location of the existing detection methods. Antibody detection makes large-scale screening possible, which is important for the detection of asymptomatic patients. Some people who tested negative for nucleic acid tested positive for antibodies, suggesting that antibody testing can be used as a supplementary method for RT-PCR to improve diagnostic accuracy and reduce missed diagnosis.24 In particular, for patients whose CT scan shows lung lesions but with negative pharyngeal swabs, specific IgM screening is useful for early detection and isolation. IgM–IgG combined antibody detection is considered to be a more reliable detection method, and it has greater specificity and sensitivity compared with single IgM or IgG tests. However, there are also some problems with antibody detection. There are significant individual differences in the IgG response to SARS-CoV-2 in different populations. Virus-specific IgG levels were significantly lower in asymptomatic patients compared with the symptomatic group, and a proportion of patients may not even have a SARS-CoV-2-IgG response.25 Additionally, the tests do not confirm the presence of the virus, but only provide evidence of recent infection. Even after recovery, IgG can persist for a long time.26 Furthermore, antibody test results are susceptible to interference from other viral infections, resulting in some false-positive results.

**Joint testing**

To improve the accuracy of detection and prevent potential virus transmission by asymptomatic patients, joint detection may be the most effective way to fill the gap. Symptoms of COVID-19 pneumonia frequently overlap with those of other viral infections, leading to low specificity of chest CT scans.27 We do not recommend chest CT as the main screening tool. However, the sensitivity of chest CT is higher than that of RT-PCR; thus, it is reasonable to consider combining these two methods to achieve better sensitivity and accuracy.28 Moreover, it is reported that combined use of nucleic acid detection and specific serum detection can help the discovery of asymptomatic patients.29 The combination of serum antibody ELISA and quantitative PCR improves the detection rate of early diagnosis.30
Asymptomatic patients in different populations

Regarding the proportion of asymptomatic patients in the general population, a systematic review study found that the proportion of diagnosed patients who were asymptomatic at initial testing ranged from approximately 20%–75%. After at least 7 days of follow-up of these patients, most reported symptoms within the first week. Only 4.1% remained asymptomatic throughout the disease. However, another study declared that the overall estimate of those who remained asymptomatic throughout the course of infection is 20%.31 Participant selection bias can affect the estimate. The exact proportion of asymptomatic patients needs to be further studied. Studies of viral shedding have found that asymptomatic viral hyperload periods can last up to 9 days. For pre-symptomatic patients, this number was 6 days before the onset of symptoms.32 The viral load of asymptomatic appears similar to that of symptomatic patients.33

Regarding transmission in asymptomatic patients, the SARs in asymptomatic patients in the home setting was 0.7%, which was lower than the 18% in symptomatic cases.34 In another study, SARs and observed reproduction in asymptomatic index cases were found to be about one-seventh of those in symptomatic index cases, while those in presymptomatic cases were two-thirds of those in symptomatic cases.35 Adults showed a higher susceptibility than children.36 Transmission was mainly associated with group activities, such as sharing meals, and not with the disease status of the cases.37

Asymptomatic patients in children

A meta-analysis showed that about 26% of paediatric patients were asymptomatic.37 Compared with adults, children may be more inclined to have mild or asymptomatic infections,38 but they may have higher viral loads and longer latency and duration of viral shedding than adults.39 Viral loads in the nasopharynx/oropharynx of asymptomatic children are lower compared with symptomatic children. However, viral load is often influenced by the duration of infection and cannot be used as evidence of the strength of transmission.40 Some children do not show any symptoms but may develop radiological changes.41 Recently, a small number of children without respiratory symptoms have been found to develop a rare complication of the multisystem inflammatory syndrome, with overlapping features with Kawasaki disease. Also, some children are often accompanied by symptoms such as rhabdomyolysis and acute renal failure.42 These atypical symptoms should be addressed to optimise early identification and hospital management of the child.

Asymptomatic patients among pregnant women

The proportion of obstetric patients presenting to the hospital with initial testing asymptomatic was 95.1%, and the proportion remaining asymptomatic throughout the course of the infection was approximately 58.8%. SARS-CoV-2 infection in pregnant women usually seems to be asymptomatic.33 Vertical transmission of SARS-CoV-2 to the fetus has been demonstrated in cases, but the risk of vertical transmission is low, at approximately 2%.44 However, infants whose mothers are infected with COVID-19 are more likely to experience adverse effects such as fetal distress, premature birth, respiratory distress and even death.45 Ventilation support and earlier infection time of pregnant women seem to be the main determinants of adverse fetal outcomes.46 Normal IgM and elevated IgG antibodies in newborns born to asymptomatic mothers have been reported.47 Notably, Molina et al found that pregnant women may experience prolonged viral shedding of SARS-CoV RNA.48 During a pandemic, healthcare workers should be cautious about delivering high-risk pregnant women and universal testing for COVID-19 should be considered in certain populations.49

Asymptomatic patients among the elderly

The proportion of COVID-19 confirmed residents in nursing homes who were asymptomatic at initial testing was approximately 53.6%, and the proportion who remained asymptomatic throughout the follow-up period was approximately 27.9%.5 Compared with younger patients, elderly asymptomatic patients are more likely to develop symptoms during the course of the disease, which is consistent with the finding that disease severity is higher in older patients. Additionally, older patients often have a much longer latency period than younger patients.50 Furthermore, Hitoshi et al found that the duration of illness in the asymptomatic group was positively correlated with patient age. Comorbidities in elderly patients, particularly hypertension, may be predictive of symptom development in initially asymptomatic carriers.51 It was also found in a nursing home report that elderly asymptomatic patients with COVID-19 often exhibit certain symptoms of the geriatric syndrome, such as falls, loss of appetite and altered consciousness.52 We may need to be vigilant for older people with such symptoms.

Transmission and prevention

According to reports, there is no significant difference in the viral load of respiratory samples from asymptomatic and symptomatic patients with COVID-19 as defined by RT-PCR.53 The duration of virus shedding in the asymptomatic group was 19 days, obviously longer than the symptomatic group.54 Asymptomatic patients are thought to make an essential contribution to virus transmission. There is growing evidence that SARS-CoV-2 can be transmitted by droplets, aerosol and conjunctiva. Faecal–oral transmission is also a possibility, whereas vertical mother-to-child transmission has not been confirmed.55 The rate of secondary episodes of asymptomatic infection seems to be lower
than that of symptomatic infection, indicating that asymptomatic patients may be less infectious than symptomatic ones. Chen et al recently claimed that asymptomatic cases are more likely to produce asymptomatic cases, but this study contains limitations and warrants further research. Due to the concealment of the infection, asymptomatic patients still pose a serious threat to public health. The exact proportion of asymptomatic patients needs to be further evaluated, which will affect the effectiveness of subsequent epidemic prevention. At present, to control the further spread of SARS-CoV-2, understanding the mode of transmission in different places is important for timely detection and isolation of asymptomatic patients.

Intrafamily transmission
SARS-CoV-2 mainly spreads among family members. A study in the UK reported that nearly 70% of cases were transmitted within the family after extensive community control measures were taken. It is worth noting that both children and adults are susceptible to SARS-CoV-2 infection. Bi et al showed that children and the general population have a similar risk of infection (7.4% in children aged <10 years vs population average of 6.6%). In families, susceptible individuals have more time and opportunity for exposure to the virus; therefore, the probability of transmission in the family is higher than that in the community. Considering the difficulty in identifying asymptomatic patients, once a case occurs in the family, the health of the whole family is in jeopardy. In Du et al’s article, they reported 14 children with mild or conventional types of COVID-19; all of which were transmitted within the family. Other studies have demonstrated that intrafamily transmission may be the main route of SARS-CoV-2 infection in children. It is estimated that about 75% of paediatric patients have a history of family exposure. Moreover, Xiao et al have detected the presence of SARS-CoV-2 in contaminated household environments, and the virus remains viable and infectious on surfaces for up to 7 days. The risk of transmission increases in contaminated household environments. Maintaining a clean environment, ventilation for more than 2 hours and avoiding nonbiological surface transmission are key to avoiding home transmission.

Hospital transmission
Hospitals are high-risk areas. Effective measures must be taken to protect medical staff and prevent nosocomial infections. Examinations in dermatology, ophthalmology, head and neck surgery are considered to be high-risk procedures for transmission of SARS-CoV-2 from asymptomatic patients. Screening of patients and hospital staff for atypical symptoms is recommended. Laboratory tests should also be conducted when necessary. Frequent testing and rapid reporting of results are particularly important to reduce hospital-acquired infections. For patients with any suspected symptoms or histories of contact with suspected cases, medical personnel should wear full personal protective equipment to evaluate whether patients should be referred for treatment. Unnecessary surgery should be postponed. For essential surgery with a high risk of infection, operating room personnel should study the surgical pandemic guidelines and take special precautions to prevent intraoperative aerosols.

Blood safety is also a factor worth considering in blood transfusion. SARS-CoV and Middle East respiratory syndrome virus RNA has been found in blood samples. Even in respiratory diseases, viral shedding in plasma and serum is common. For this problem, blood centres should conduct rigorous testing and detailed epidemiological investigations of blood donors to rule out the risk of asymptomatic infections. Blood samples obtained from suspected patients with COVID-19 should be recalled. Blood samples can be subjected to pathogen inactivation with photochemical treatment (PCT), if necessary; however, the use of PCT is limited because of its damage to blood components and high cost.

Due to the unique physical condition of pregnant women, testing strategies based on currently known symptoms may miss pregnant women infected with COVID-19, posing a risk to hospitals. Timely detection of pregnant women with COVID-19 and timely preventive measures are important for the protection of pregnant women, parents, newborns, other patients and hospital staff. There is currently no evidence that SARS-CoV-2 can use breast milk as a medium. However, the shedding of the virus in asymptomatic women may also affect the treatment of newborns. Women who are asymptomatic or have mild symptoms after delivery are recommended to breast feed directly under strict infection control measures.

Transmission of pollutants from patients
Current statistics show that the positive rate of SARS-CoV-2 nucleic acid test for nasopharyngeal swabs is 38.13%. Due to the existence of false-negative results, a negative test does not mean that there is no viral on the swab. Given this, biological specimen handling is essential for the prevention of laboratory-related infections. Besides, even after the results of respiratory samples in patients have turned negative, faecal samples remain positive. A meta-analysis indicated that SARS-CoV-2 RNA was detected in the stool samples of 48.1% of patients. Even after the nasopharyngeal test is negative, the test result may still be positive. Virus shedding from the digestive system may be more severe and last longer than from the respiratory tract. Especially in asymptomatic children, this phenomenon can last up to 54 days after admission or 30 days after discharge. Although experts believe that a positive SARS-CoV-2 nucleic acid test for nasopharyngeal swabs indicates infection, confirming that SARS-CoV-2 RNA is also detected in faecal samples. For bronchial disease, the diagnostic threshold for Criterion II of the Beijing Experts Consultation Group is 400 copies/mL, but the upper limit set by the Chinese Association of Medical Science, the Beijing Expert Consultation Group, and the Chinese Medical Association is 9,000 copies/mL.
acid test from a rectal swab does not indicate the presence of live viruses, faecal–oral transmission may still occur. Long-term excretion of viruses through the intestine has been proved in children, reminding us to focus on the management of faeces of patients with COVID-19, especially children. Therefore, special disinfection of used swabs and patient excreta is necessary. Additionally, since it is arduous to determine the presence of asymptomatic patients before laboratory testing, masks used by the public should also be disinfected daily. Global guidelines for the handling of biological samples and contaminants should also be drawn up as soon as possible so that effective disposal can be carried out safely and securely while protecting handlers.

The mutation of SARS-CoV-2

SARS-COV-2 has a certain mutation frequency as RNA viruses since the epidemic of COVID-19. Data published by the WHO indicate that there are four major mutations currently. The D614G mutation in the SARS-COV-2 has become the main type and transmitted globally by June 2020. The ‘Cluster 5’ variant does not appear to be widely disseminated. The VOC 202012/01 variant is more transmissible, but the severity of disease (as assessed by length of hospitalisation and 28-day mortality) and reinfection have not changed. Preliminary studies suggest that the 501Y.V2 variant is associated with a higher viral load and may increase infectivity, but there is no evidence to prove that it causes more severe disease yet. So further investigation is needed.

A number of countries have seen the recurrence of cured cases or rapid spread of the disease recently. It may indicate the spread of the SARS-COV-2 variant to some extent with the involvement of asymptomatic carriers. Recently, a variant of SARS-CoV-2, SARS-CoV-2 GZ69, was isolated from an asymptomatic healthcare worker. It was found that the mutation of residues 203–204 in SARS-CoV-2 GZ69 may reduce the anti-proliferation ability of N protein, which may explain the virus persistence in asymptomatic patients. Fabrizio Maggi reported an asymptomatic traveller from Brazil who tested positive for the SARS-CoV-2 P.1 variant in a screening nasopharyngeal swab sample. The results suggest that this may lead to a wider circulation of SARS-CoV-2 variant P.1 in areas other than the Brazilian state of Amazonas. We recommend that strict surveillance measures are necessary, because asymptomatic patients may lead to further transmission of SARS-CoV-2 variant. Moreover, designing specific primer sets for viral variants is useful to assist in large-scale screening.

Screening of discharged patients and persons released from isolation

According to the Regulations for the Administration of Asymptomatic Infected Patients of New Coronavirus issued in China, patients who are asymptomatic and their close contacts should be quarantined and observed for 14 days. Only after 14 days and two consecutive negative nucleic acid tests (sampling interval ≥24 hours), can medical observation be discontinued. For convenience and non-invasiveness, throat swab samples are generally collected for nucleic acid detection. However, Xu et al. found that after nasopharyngeal swabs turn negative, rectal swabs may still show a positive result. The timing of viral nucleic acid shedding differs among tissue types. A meta-analysis proved that PCR using sputum samples to diagnose COVID-19 is more sensitive than stool samples. Rectal swabs are not routinely used in the diagnosis of COVID-19 in many countries. The current clinical application cases are insufficient. Further studies are still needed to confirm its feasibility. Besides, rehabilitation patients may also experience fever screening has major challenges. The proportion of asymptomatic patients is a key factor for further study, which can seriously affect the effectiveness of screening. Rapid identification, registration, reporting, supervising and epidemiological tracking of asymptomatic patients are significant. Special attention should be paid to screening the population who need to return to work and production to prevent ‘import cases’ between regions. The use of thermal scanners and other symptom detection equipment may improve the efficiency and reliability of screening. Setting up multiple sites to filter people multiple times is also worth considering. Moreover, recent studies have shown that nucleic acid testing using saliva samples is highly sensitive, especially for recognising patients in the early stages of the disease. The ease of saliva collection makes self-collection of saliva samples a potentially effective method for large-scale screening of asymptomatic populations. Developing nucleic acid or antibody detection devices that are more suitable for screening at these sites remains the best option for improving the accuracy and sensitivity of the tests. In addition to traveller screening, some countries, such as South Korea, Iceland and Germany have also conducted universal nucleic acid
testing in the population to detect patients and assess the proportion of asymptomatic patients. The risk of transmission of asymptomatic infections is multifaceted. The concealment of transmission makes it difficult to prevent. It seems that the most reliable method of prevention currently is to identify patients with symptoms and track their movements in real time. Many new internet-based technologies have been used to enable the detection of close contacts. Self-reporting based on smartphones and continuous tracking with the code can achieve inexpensive and extensive mass tracking. The establishment of the rapid suspicious case notification platform is also conducive to the self-detection. These measures will contribute to the discovery of asymptomatic patients.

CONCLUSION

The emergence of asymptomatic patients with COVID-19 poses new challenges for infection prevention and control. More work is needed to clarify the exact proportion of asymptomatic patients among the overall total. Available data found significant differences in the proportion of asymptomatic patients diagnosed in different populations. It has been found that asymptomatic patients have the potential for transmission. For the discovery of asymptomatic carriers, the main existing detection methods include imaging, nucleic acid and serological diagnostics. Joint detection methods can improve the accuracy of early diagnosis. To slow down or stop the spread of SARS-CoV-2, our top priority is to take more effective prevention and control measures at home, in hospitals and elsewhere, and find more appropriate methods for population screening. For different groups of people such as children, pregnant women and the elderly, different prevention and treatment measures should be taken according to their specific symptoms and different transmission capabilities. For the public, measures such as wearing face masks, maintaining physical distance, washing hands frequently and not gathering are still the most desirable protective measures.

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