


ORIGINAL ARTICLE

Comparison of nasopharyngeal samples for SARS-CoV-2 detection in a paediatric cohort

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Aim: The diagnosis of coronavirus disease 2019 (COVID-19) depends on accurate and rapid testing. Choosing an appropriate sample may impact diagnosis. Naso-oro-pharyngeal swabs (NOS) are most frequently used, despite several limitations. Since studies suggest nasopharyngeal aspirate (NPA) as a superior alternative in children, we hypothesised collecting both nasopharyngeal swab and aspirate would improve our diagnostic accuracy.

Methods: Observational, longitudinal, prospective study from 7 March to 7 May in a tertiary paediatric hospital in Lisbon. The objective was to compare the rate of detection of SARS-CoV-2 between NOS and NPA samples collected simultaneously.

Results: A total of 438 samples collected from 85 patients with confirmed COVID-19. There were 47.7% overall positive specimens – 32% (70/219) positive NOS and 63.5% (139/219) positive NPA. The tests were 67.6% concordant ($k = 0.45$). 50.3% had positive NPA with negative NOS, while 1.3% had positive NOS with negative NPA. NPA proved to be more sensitive (98.6% with 95% confidence interval 91.2–99.9% vs. 49.6% with 95% confidence interval 41.1–58.2%, $P < 0.001$). Additionally, the difference between NPA and NOS positive samples was statistically significant across all population groups (age, health condition, clinical presentation, contact with COVID-19 patients or need for hospitalisation), meaning NPA is more sensitive overall.

Conclusions: Nasopharyngeal aspirates had greater sensitivity than naso-oro-pharyngeal swabs in detecting SARS-CoV-2. Our results suggest paediatric patients would benefit from collecting nasopharyngeal aspirates in hospital settings, whenever feasible, to improve diagnosis of COVID-19.

Key words: COVID-19; diagnosis; molecular biology; polymerase chain reaction; SARS-CoV-2.

What is already known on this topic

- 1 COVID-19 diagnosis depends on accurate and rapid testing.
- 2 Choosing the most appropriate sample for detection may have substantial impact.
- 3 Currently, nasopharyngeal swab is the most recommended sample for detection of SARS-CoV-2.

What this paper adds

- 1 Nasopharyngeal aspirate was more sensitive for SARS-CoV-2 detection in our study.
- 2 A diagnostic approach based solely on nasopharyngeal swab samples would have missed 11 (12.9%) out of 85 SARS-CoV-2 infected patients.
- 3 Children would benefit from collecting a nasopharyngeal aspirate to improve diagnostic rate.

Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) is the novel virus responsible for coronavirus disease

2019 (COVID-19),¹ a global pandemic that spread from China in December 2019 and has led to more than 90 million confirmed infections and more than 2 million deaths world-wide to date. The first adult SARS-CoV-2 infection in Portugal was confirmed on 2 March and the first paediatric case on 7 March 2020.

A growing body of literature confirms that children are less likely to become infected than adults, even in countries with low case rates, despite infection across every age group.^{2–4} Disease in children is overall milder and to date, critical disease and death remains very rare.^{2–4} In addition, published studies seem to suggest that children play a minor role in transmission of the disease, with lower attack rates than adults.⁵ Even though this could suggest lower viral shedding, some studies found similar viral loads to adults.^{3,6} As such, fast identification of SARS-CoV-2 in

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This study has been presented as a poster presentation at the ESCMID Conference on Coronavirus Disease (ECCVID) that took place from 23 to 25 September 2020, as well as an oral presentation at the National Congress of the Portuguese Society of Pediatrics that took place from 29 to 30 October 2020, where it won the Grand Jury Prize (1st prize).

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children remains important to understand and curb transmission, especially in hospitals.

Efforts to control the pandemic depend on accurate and rapid diagnostic testing.⁷ Appropriate specimen selection is important for the diagnosis of respiratory viral infections such as COVID-19.⁷ Despite showing better accuracy, collecting samples from the lower respiratory tract in paediatric patients with mostly mild disease is controversial, given the invasiveness and potential risks.^{8,9} From the upper respiratory tract, oropharyngeal and nasopharyngeal swabs (NOS) are the most frequently used samples, in spite of lower sensitivity in detecting early infection, inconsistency for serial viral load monitoring and a significant rate of false-negative results, probably due to suboptimal quality of sample collection^{7,9,10} Other alternatives have been studied, namely sputum,¹¹ saliva¹² or nasopharyngeal aspirate (NPA).^{13,14} Published literature suggests that NPA is considered superior to other types of samples for the detection of respiratory viruses.^{13–17} Sputum and saliva collection may prove difficult to obtain in children, since many are unable to produce a sample with enough quality.^{11,12} In addition, variability in the collection method, sample preparation and processing may impact the performance of saliva testing, not to mention the range in sensitivity (25–71.4%) and viral load detection in several published studies in children so far.^{12,18} Therefore, we hypothesised collecting both NOS and aspirate would improve our diagnostic accuracy. The objective of this study was to compare the sensitivity of SARS-CoV-2 detection between the two specimens.

Methods

Since the beginning of the COVID-19 pandemic, Hospital Dona Estefânia has been the reference paediatric hospital in southern Portugal. Here, we performed an observational, longitudinal, prospective, cohort study from 7 March to 7 May (2 months). We included all paediatric patients with a positive real-time reverse transcriptase polymerase chain reaction (rRT-PCR) test for SARS-CoV-2 by either sample (NOS and/or NPA). For clinical purpose, we considered every positive to be a true positive for the virus. Additional data collected from medical records included sex, age, past medical history, SARS-CoV-2 exposure history, clinical features and PCR results. Local ethics committee approval for the study was granted. Informed consent was obtained from a parent or guardian before performing the collection.

Diagnosis of COVID-19 was based on the Direção Geral de Saúde (Directorate General for Health, DGS) guidelines, which were built from World Health Organization and European Center for Disease Prevention and Control interim guidance.

Sample collection always followed a particular order according to our standard protocol. First, for NOS, we collected a nasopharyngeal exudate by introducing a flocked swab through one nostril until reaching the nasopharynx, rotating several times and then leaving it in place during 10 s to allow fluid absorption. Oropharyngeal specimen was collected next by swabbing the posterior pharynx with a dacron swab, avoiding the tongue. Both swabs were inserted in a single tube of viral transport medium. For NPA, we instilled 2 mL of sterile nasal saline solution in both nostrils and performed an aspirate with a mucus extractor. All materials for collection were approved and handled as

recommended by international guidelines. All samples from our unit were simultaneously sent to testing.

Molecular detection of SARS-CoV-2 viral RNA was performed by rRT-PCR. Nucleic acid was extracted using the automated acid extraction platform EasyMAG and molecular detection was conducted using a RT-PCR commercial kit specific for SARS-CoV-2 detection approved by the National Institute of Health Doutor Ricardo Jorge. Three target genes (E, N and RdRp) were simultaneously detected during the assays. The cycle threshold (Ct) values and cycle quantification (Cq) values of rRT-PCR were indirectly associated to the number of SARS-CoV-2 RNA copies/mL in specimens.¹⁹ A cut-off Ct value equal or less than 35 was defined as a positive test result. Our samples were processed in a level II biosafety Molecular Biology Laboratory. The National Institute of Health Doutor Ricardo Jorge serves as an independent entity for external quality evaluation and performs routine confirmation testing of our samples to ensure the veracity of the result.

Sample size was estimated to detect a sensitivity of 95% with a confidence interval (CI) of 95%. Values are expressed as percentages for qualitative variables or as means or medians and standard deviations or interquartile ranges (IQR) for continuous variables. Continuous variables were compared using student's *t*-test or Mann–Whitney *U*-test and categorical variables with Pearson χ^2 test or Fisher exact test for independent samples or McNemar test for paired samples. The target significance level was 0.05. Statistical analysis was performed with IBM SPSS Statistics 25 software (IBM Corp., Armonk, NY, USA).

Results

We collected a total of 438 specimens from 85 patients with confirmed SARS-CoV-2 infection (median of three paired samples per patient, tested at least with a 48-h interval). These accounted for 6.6% of Portuguese cases below 19 years old on 7 May 2020. Our population was 50.6% male, with a median age of 8.7 years (IQR 1.5–13.4 years). Fifteen (17.6%) cases were among infants below 12 months, 31 (36.5%) between 1 and 10 years and 39 (45.9%) were above 10 years old. Sixty-four patients (75.2%) were a close contact of a confirmed COVID-19 patient, while seven patients (8.2%) had contact with someone with acute respiratory tract infection that did not test positive for COVID-19.

Fourteen (16.4%) patients were asymptomatic at diagnosis. The most common symptoms at admission were cough (56.5%), fever (48.2%) and upper respiratory tract symptoms (30.6%). Dyspnoea was present in only 10.6% of cases. Pre-existing medical conditions were present in 19 patients (22.4%). Hospital-acquired infection occurred in five patients (5.9%) which shared a room in another department. All the accompanying parents tested negative.

The median time to diagnosis from the development of symptoms was 2 days (IQR 1–4 days), with no significant statistical difference between age brackets. Fifteen patients (17.6%) tested positive for additional pathogens beside SARS-CoV-2, rhinoviruses (33.3%) being the most common co-infection. Documented viral clearance took an average of 29.3 ± 20.7 days in our population.

There were 47.7% (209/438) overall positive specimens; 32% (70/219) were positive for NOS and 63.5% (139/219) were

Table 1 Comparison of naso-oropharyngeal swabs (NOS) and nasopharyngeal aspirate (NPA) paired samples

	NOS negative, n (%)	95% CI	NOS positive, n (%)	95% CI	Total pairs, n (%)	P value†
NPA positive	70 (50.3)	41.8–58.9	69 (49.6)	41.1–58.2	139 (100)	<0.001†
NPA negative	79 (98.8)	92.3–99.9	1 (1.3)	0.1–7.7	80 (100)	
Total pairs	149 (68)		70 (32)		219 (100)	

†Comparison of paired samples through McNemar test.

Table 2 Comparison of positive nasopharyngeal aspirate (NPA) and positive naso-oropharyngeal swabs (NOS) samples

	Positive NPA, n (%)	Positive NOS, n (%)	P value†
Children <1 year old	20/34 (58.8)	12/34 (35.2)	0.008
Children <5 years old	41/75 (54.7)	19/75 (25.3)	<0.001
Children ≥5 years old	98/144 (68.1)	51/144 (35.4)	<0.001
Children ≥10 years old	72/110 (65.5)	36/110 (32.7)	<0.001
Contact with COVID-19 patient	121/174 (69.5)	58/174 (33.3)	<0.001
No pre-existing condition	114/163 (69.9)	62/163 (38.0)	<0.001
With pre-existing condition	25/56 (44.6)	8/56 (14.3)	<0.001
Symptomatic presentation	101/160 (63.1)	58/160 (36.3)	<0.001
Asymptomatic presentation	38/59 (64.4)	12/59 (20.3)	<0.001
Hospitalisation	94/158 (59.5)	51/158 (32.3)	<0.001
Outpatient management	45/61 (73.8)	19/61 (31.1)	<0.001

The frequency values reflect the number of positives of each type of sample, independently from each other (i.e. positive NPA value encompasses the number of individuals with both positive samples and solely NPA positive samples, and vice-versa).

†Comparison of paired samples through McNemar test.

positive for NPA. Concordance occurred in 148 cases, which translates to a naïve concordance of 67.6% and moderate agreement according to Kappa's coefficient (0.45). Among the paired specimens whose NPA was positive, 50.3% had negative NOS. When NPA resulted negative, only 1.3% had positive NOS (Table 1). Considering NOS the gold-standard, NPA proved to be more sensitive (98.6% with 95% CI 91.2–99.9% vs. 49.6% with 95% CI 41.1–58.2%, $P < 0.001$).

A diagnostic approach based solely on the appraisal of NOS samples would have missed 11 (12.9%) SARS-CoV-2 infected patients.

In addition, by dividing our cohort and performing a bivariate analysis (Table 2) using age, contact with SARS-CoV-2 infected patients, presence of comorbidities, clinical presentation and hospitalisation as covariates, we also found that the difference observed between NPA and NOS positive samples is statistically significant across all population groups, meaning NPA is more sensitive overall.

Discussion

The choice of specimen is important for the diagnosis of respiratory viral infections such as COVID-19.⁷ Nasopharyngeal swab is, at present, the most recommended sample for detection of SARS-CoV-2, since it is assumed to have better sensitivity than nasal oropharyngeal samples.^{8–11} On the other hand, studies so far show poor sensitivity in early stages of disease and an elevated

false-negative rate, probably related to suboptimal quality of sample collection, which is common in children.^{9–11}

Since lower respiratory tract samples are not easily manageable in children, studies are being conducted on several alternatives, such as sputum, saliva, gargled oropharyngeal fluid, nasal and mid-turbinate swab.¹¹ Published studies have so far established that NPA is superior to other samples for the detection of respiratory viruses because of the larger number of epithelial cells aspirated during collection of the sample.¹⁵ It is also a familiar technique to hospitals world-wide. Additionally, dealing with smaller upper airways as in infants requires swab sizes that are not available world-wide, not to mention containment measures, which makes performing NPA easier. In our experience, despite the apparent invasiveness of the procedure, performing NPA was simple, safe and better tolerated by younger children. Risk of aerosolisation from the procedure seems low.^{20–22} However, in our study, given the limited available evidence, all health-care workers performed sample collection in a negative-pressure chamber while wearing personal protective equipment (N95 filtering facepiece respirators, gown, gloves and eye protection).

Since the difference observed between NPA and NOS positive samples is statistically significant across all population groups in our study (regardless of age bracket, health condition, clinical presentation, contact with SARS-CoV-2 infected patients or need for hospitalisation) this may suggest a potential negative impact of performing exclusively NOS in paediatric populations, leading to underdiagnosis.

We are aware that NPA is considered unpractical for outpatient clinical settings. However, we believe NPA should be taken into consideration in specific settings, such as cases with epidemiological link or hospitalised patients with negative test from NOS sample, particularly if community transmission is high.

Although we could hypothesise that NPA may detect lower viral load than NOS, we did not perform an analysis between viral load or Cq/Ct values to infer infectivity. Literature shows that there is a wide variation on limit detection through rRT-PCR and that what is actually measured is the genome presence, not its quantity.²³ This means that identifying the virus on a specimen does not necessarily correlate with infectivity.²⁴ Lambert *et al.*¹⁶ also suggested that the more invasive the respiratory specimen, the more likely a positive PCR result represents viral persistence, rather than an acute infection. In fact, in our study, positive NPA persists longer than NOS in most patients, which could very well relate to viral persistence, as well as to the paucity of symptoms. NPA could simply be more sensitive because the catheter collects deeper cells than through swabbing. On the other hand, NOS has the advantage of being collected first, which may or may not impact sensitivity for either sample. Furthermore, we do not know if NPA would enhance amplification of other viral RNA compared to NOS in our study. More studies are needed to address this question.

Our study has some limitations. First, we present a single centre experience with a small sample size, which mainly resulted from the low number of paediatric COVID-19 patients. Second, it was hospital-based, which may have skewed our sample towards more symptomatic patients on admission, which were probably more infectious and with probable higher viral load. Third, we did not perform a direct analysis between viral load or Cq/Ct values and infectivity.

Conclusions

NPA has higher sensitivity in detecting SARS-CoV-2 when compared to NOS. Our results suggest paediatric patients would benefit from collecting NPA whenever possible to improve test sensitivity and increase the accuracy of diagnosis and confirmation of viral clearance of SARS-CoV-2. Larger prospective studies are required to confirm our conclusions. However, we consider that these are important findings and should be taken into account in clinical practice, especially in the winter months to come.

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