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Are antigenic tests useful for detecting SARS-CoV-2 infections in patients accessing to emergency departments? Results from a North-West Italy hospital

Dear Editor,

In the article "Clinical application of a rapid antigen test for the detection of SARS-CoV-2 infection in symptomatic and asymptomatic patients evaluated in the emergency department: A preliminary report.",[1] Turcato et al. presented a study on the use of rapid antigenic tests (Ag-RDTs) instead of the usual real time reverse transcription polymerase chain reaction (RT-PCR) assay to de-

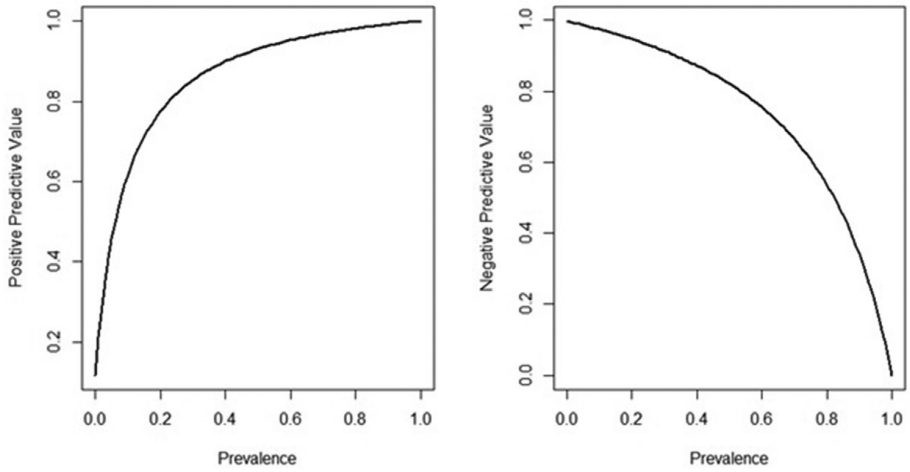

Fig. 1. Positive and negative predictive value estimates in relation to prevalence, using the sensitivity and specificity of the test found in our population (sensitivity = 0.800; specificity = 0.939).

tect the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in the context of Emergency Departments (ED). They observed a general good sensitivity and specificity, lower in the subgroup of asymptomatic patients. Their conclusion is in favour of the use of Ag-RDTs in EDs as an additional tool to address the challenge of containing the SARS-CoV-2 pandemic.

We agree with the authors that the development of reliable but cheaper and faster point-of-care diagnostic tests was expected to be useful either for population-screening or as first aid tests in the emergency room.[2,3] Data on the sensitivity and specificity of currently available Ag-RDTs derive from studies that vary in design, setting, population and type of specimen, thus strongly limiting the comparability and ability to make general inferences. Sensitivity appears to be highly variable, ranging from 29 to 94% compared to the RT-PCR test, but specificity is consistently high (>97%).[4–7] Ag-RDTs were found to perform better in patients with high viral loads (Ct values ≤25 or >106 genomic virus copies/mL)[5,7,8] which usually happens in the pre-symptomatic (0.5–3 days before symptom onset) and early symptomatic phases of the illness (within the first week from symptom onset) but limited data are available about other possible individual modifiers of the accuracy of the assay. A recent Cochrane review highlighted that patients' characteristics were not available or poorly detailed in many studies, with only three out of 22 studies coming from an ED setting.[8]

Between October 26th and November 10th 2020, 455 patients accessed the ED of San Luigi Gonzaga University Hospital in Orbassano (Turin, Italy) and 324 underwent both RT-PCR and Ag-RDT testing. This period corresponds to the first two weeks of the second pandemic wave, with a weekly incidence of SARS-CoV-2 infection in the Region of about 500 confirmed cases/100,000 inhabitants. Data were obtained as part of an observational study described elsewhere[9] and a detailed presentation of methods is available in supplementary material.

The prevalence of SARS-CoV-2 infection in this cohort was 65% measured using RT-PCR as a gold standard. Supplementary Table 1 reports test results: 275 (84%) patients showed concordant results (168 positive and 107 negative), while 49 (15%) showed discordant results (42 patients had a positive RT-PCR and a negative Ag-RDT and 7 vice versa). Cohen's Kappa Statistics ($k = 0.68$ – 95% CI 0.61–0.77) highlighted substantial agreement. Specificity and sensitivity of Ag-RDT were 0.939 (95% CI: 0.895–0.983) and 0.800 (95% CI: 0.746–0.854), respectively, taking RT-PCR as the reference. Overall, the Ag-RDT positive predictive value was 0.960 (95% CI 0.931–0.989), and the negative predictive value was 0.718 (95% CI: 0.646–0.790). The variation of positive and negative predictive values due to difference in prevalence can be observed in Supplementary Table 2 and Fig. 1. Positive predictive value could vary from 0.12, when the prevalence of the disease is 0.01, to 0.77 when the prevalence is 0.20. The negative predictive value could vary from about 1, considering a low prevalence (0.01) to 0.95, considering a higher prevalence (0.20).

No difference in patients' characteristics between true positive and false negative tests was observed (Supplementary Table 3). On the contrary, false negative patients were significantly younger and they were tested significantly later after symptoms onset compared with true negative patients (Table 1). Moreover, fever (64.3% vs 19.6%, $p < 0.0001$) and cough (42.9% vs 15.0%, $p = 0.0003$) were significantly more frequent in false than true negatives, while chronic obstructive pulmonary disease was more frequent in true than false negatives, with a borderline significance (16.5% vs 4.8%, $p = 0.06$). Few true negative patients had bilateral pneumonia ($n = 10$, 9.4%), that was highly present in false negative patients ($n = 25$, 61.0%, p-value for difference < 0.0001) and multivariable analysis confirm these results, suggesting that wrong group allocation for negative patients occurred more frequently in patients with fever, cough, and pneumonia, while it was less likely in patients with COPD.

The infection prevalence and the clinical context where the test is used affect the effectiveness of the test itself[10]: the ideal test in a crowded ED context should help in identifying asymptomatic patients arriving to the ED for reasons other than COVID-19, who are concurrently found COVID-19 positive.

Our results suggest that a negative Ag-RDT test should not exclude COVID-19 in patients that clinically have symptoms that are strongly suggestive of COVID-19. Ag-RDTs alone had a low negative predictive value (we cannot trust a negative result of the test), thus they need to be evaluated in association with clinical judgement. A high level of suspicion should be maintained in patients with fever, cough or pneumonia notwithstanding a negative Ag-RDT. Since the predictive value is strictly related to the prevalence of disease, and then to the pre-test odds, Ag-RDTs are not really useful in settings where the prevalence of disease is high or in patients with high pre-test odds. On the contrary, in periods with low prevalence of the disease or in patients with a low pre-test odds (asymptomatic) or with symptoms probably related to a known COPD, Ag-RDTs can be used alone and we can trust a negative result.

In conclusion, our results confirm the limits of antigenic tests as first line screening tests in settings with high prevalence of disease

Table 1
Ag-RDT negative patients: comparison of patients' characteristics between true negative and false negative patients. Wilcoxon sum rank test (quantitative variables) and chi-square or Fisher's exact test (qualitative variables) are used and multivariable logistic model (including significant variables) to evaluate the association between being a false negative and patients' characteristics.

	True negative ($n = 107$)*Mean (SD), medianor Frequency (%)*	False negative ($n = 42$)*Mean (SD), medianor Frequency (%)*	*P*-values	OR(95% CI)
Age, years	68.4 (18.6), med: 74.4	63.1 (16.3), med: 64.4	0.03	*For 1 year increase* 1.00 (0.96 – 1.03)
Days from symptoms onset	3.9 (6.7), med: 2	6.3 (4.7), med: 6	0.0003	*For 1 day increase* 1.06 (0.97 – 1.16)
NEWS at arrival	2.1 (3.0), med: 1	2.4 (2.5), med: 2	0.14	
Symptoms				
Fever	21 (19.6%)	27 (64.3%)	<0.0001	4.31 (1.30 – 14.28)
Cough	16 (15.0%)	18 (42.9%)	0.0003	5.72 (1.63 – 20.07)
Dyspnoea	33 (30.8%)	16 (38.1%)	0.40	
Respiratory failure	16 (15.1%)	8 (19.1%)	0.56	
Gastrointestinal symptoms	29 (27.1%)	7 (16.7%)	0.18	
Anosmia	0 (0.0%)	2 (4.8%)	0.07*	
Ageusia	6 (5.6%)	3 (7.1%)	0.72	
Asthenia	15 (14.0%)	11 (26.2%)	0.08	
Comorbidities				
Obesity	5 (6.2%)	1 (2.8%)	0.66*	
Hypertension	43 (41.7%)	13 (31.0%)	0.22	
Diabetes	17 (16.5%)	5 (11.9%)	0.48	
Heart disease	26 (25.2%)	7 (16.7%)	0.26	
COPD	17 (16.5%)	2 (4.8%)	0.06*	0.12 (0.01 – 1.29)
Cancer	18 (17.5%)	4 (9.5%)	0.31*	
immunosuppression	8 (7.8%)	1 (2.4%)	0.45*	
neurological disease	14 (13.7%)	4 (9.5%)	0.59*	
Pneumonia				
No	87 (82.1%)	12 (29.3%)	<0.0001	Reference
Monolateral	9 (8.5%)	4 (9.8%)		4.12 (0.59 – 28.60)
Bilateral	10 (9.4%)	25 (61.0%)		14.89(4.14 – 53.52)

* Fisher's exact test.

or in patients with high pre-test odds, where a negative test is not informative (i.e. in ED in a pandemic period). This suggests that in these situations the antigenic test should be integrated in a clinical algorithm.

Declaration of Competing Interest

All authors declare no conflict of interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2021.05.012.

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Clinical efficacy of nitric oxide nasal spray (NONS) for the treatment of mild COVID-19 infection

Dear Editor,

Summary

Baek et al.[1] investigated the duration of COVID-19 virus shedding in infected patients and demonstrated that even in patients demonstrating prolonged viral clearance, the virus was no longer viable after 15 days post onset of symptoms. Our study aimed to measure whether nitric oxide nasal spray (NONS) could accelerate the reduction in SARS-CoV-2 RNA load versus control with a saline spray. Our study recruited 80 participants who were divided into a NONS treatment or a placebo arm to test the efficacy of NONS as a treatment for mild COVID-19 infection.

Introduction

The coronavirus (COVID-19) pandemic has had a profound impact on the world, resulting in a worldwide death toll of over 2.6 million and global cases in excess of 119 million as at March 2021.[2] These figures demonstrate the necessity of rapidly developing new and effective ways in which to control and treat the virus in support of the emergency use of already-available COVID-19 vaccines.[3]

There are currently no evidence-based treatments for mild COVID-19 infection. This double-blind phase IIb clinical trial used a placebo control to evaluate the efficacy of nitric oxide in the treatment of mild, symptomatic COVID-19 infection in the form of a self-administered nasal spray. Nitric oxide (NO) is a free radical gas molecule involved in innate immunity, as well as wound healing, vasodilation, neurotransmission, and angiogenesis.[4] Although produced physiologically, NO has been shown to exhibit a number of antimicrobial actions at therapeutic dosage regimens both *in vitro* and *in vivo*.[5–7]

Materials and methods

This trial was carried out at Ashford and St. Peter's Hospitals NHS Foundation Trust (ASPHFT). 80 adults (18–70 years) who were isolated with mild COVID-19 infection confirmed by laboratory SARS-CoV-2 RT-PCR nasal and throat swab within the 48 h of randomisation were eligible for recruitment. Participants were randomised 1:1 to receive NONS ($n=40$) placebo ($n=40$). The nasal sprays were self-administered 5–6 times daily (two sprays per nostril/dose, 120–140 μL of solution/spray) for 9 days.

Treatment with NONS or placebo commenced on day 1. Participants took self-sampled nasal and throat swabs on days 1 (at baseline, before initiating treatments), 2, 4, and 6 in the mornings, prior to treatment. Quantitative RT-PCR was carried out at Berkshire Surrey Pathology Services Virology laboratory to determine SARS-CoV-2 RNA levels. SARS-CoV-2 sequencing for variants was performed at Public Health England Colindale. Daily self-reporting questionnaires on symptoms, compliance, and treatment tolerance were completed by patients and follow-up continues for a total of 18 days.

Results

Patients in both trial groups started on NONS or placebo at least 4 days after the onset of symptoms and were well balanced in terms of risk factors (Table 1). 34 (85%) of the NONS group and the placebo group were determined to be lineage B.1.1.7 (VOC202012/01) and the remainder were not determined to be a variant of concern. There were no serious adverse events in patients within either trial group. NONS versus placebo started on at least day 4 of symptom onset was independently associated with an accelerated decrease in log(10) SARS-CoV-2 RNA concentration of −1.21 (95% CI, −2.07 to −0.35; $P=0.01$) and −1.21 (95% CI, −2.19 to −0.24; $P=0.02$) on days 2 and 4 respectively (Fig. 1). Mean SARS-CoV-2 RNA concentration was lower on NONS by a factor of 16.2 at days 2 and 4. A rapid reduction (95%) in the SARS-CoV-2 viral load was observed within 24 hours, with a 99% reduction observed within 72 hours with NONS treatments.

The mean SARS-CoV-2 RNA concentration at day 6 was lowered to −3.32 on NONS, with a treatment difference of −0.98 (95% CI, −2.04 to 0.08; $P=0.069$). The mean treatment difference using an area under curve estimate from baseline through day 6 was −5.22 with a 95% CI, −9.14 to −1.31; $P=0.001$), where the mean change was −10.17 for the NONS group and −4.95 for the placebo group.

40 subjects (15 NONS and 25 placebo subjects) completed and returned the trial assessment questionnaire. A total of 46.7% (7 of 15) of NONS respondents reported feeling better versus 8% (2 of 25) of placebo respondents on treatment. NONS subjects typically reported being better by day 2-4 on treatment, whereas the placebo subjects typically did not report feeling better until after day 5.

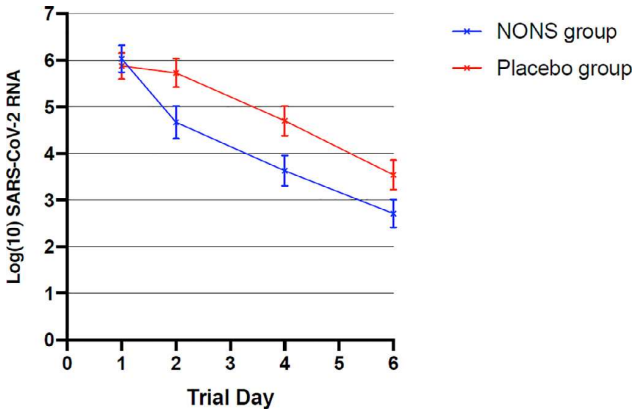

Fig. 1. Mean Log(10) SARS-CoV-2 RNA at days 1 to 6
Shown is the difference in the change from baseline in SARS-CoV-2 RNA between the active (NONS) group and the placebo (saline) group from day 1 to day 6. The I bars represent standard error.