Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

prevalence POC setting. In this low-prevalence context, due to the lower sensitivity when compared to laboratory RT-PCR, this and similar rapid POC NAT assays may be most useful in enabling the rapid triage of public health and hospital resources while expediting confirmatory PCR testing.

Acknowledgements: We thank the COVID-19 Diagnostics Research Group, which in addition to the listed authors, also comprises Nick Tayler, Jason A. Trubiano, Olivia Smibert, George Drewett, Fiona James, Socheata Chea, Steven Edwards, Nicole Isles, Michelle Sait and Beau Carr, for their laboratory and clinical support for this study. We also thank Kirsten Holden and staff at Clayton and Casey Screening Clinic, as well as Grace Gibney and staff at Austin Health Screening Clinic, for collecting the extra study swabs from patients.

Conflicts of interest and sources of funding: This research was funded by the Medical Research Future Fund (MRFF) 2020 COVID-19 Diagnostics Grant Opportunity as part of the COVID-19 Strategic Planning and Delivery of Testing program and the Victorian Government Department of Health as part of the Doherty Institute Innovative Testing Program. The funders were not involved in data analysis or manuscript preparation. The authors state that there are no conflicts of interest to disclose.

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DOI: https://doi.org/10.1016/j.pathol.2021.07.002

Prolonged PCR positivity in elderly patients infected with SARS-CoV-2

Sir,

The global coronavirus disease 2019 (COVID-19) pandemic has raised many questions around the transmission dynamics of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Among these, defining the period of infectivity and, critically, proving clearance of the virus from the respiratory tract are paramount to public health efforts.

Reverse transcriptase polymerase chain reaction (RT-PCR) assays are now commonplace in diagnostic laboratories, and their limitations are well recognised. Clinicians are aware that common acute respiratory viral infections may be followed by prolonged upper respiratory tract PCR positivity. PCR assays specifically detect viral genetic material but typically cannot distinguish between intact replication-competent virus and residual, non-viable viral fragments. Recent studies have demonstrated that SARS-CoV-2 RNA may be detected well beyond the period in which viable virus can be cultured.[1–3] Shedding of culturable SARS-CoV-2 has been seen up to a median of 9 days from illness onset,[3] with PCR positivity persisting for a median of 17 days, longer in the upper respiratory tract than in the lower airway.[2] In our own centre, viable virus is more likely to be isolated in patients with more severe disease and with lower PCR cycle threshold (Ct) values, with no virus cultured from respiratory samples with a Ct >32.[1]

All respiratory tract samples received for SARS-CoV-2 reverse transcriptase polymerase chain reaction (RT-PCR) testing at the Centre for Infectious Diseases and Microbiology Laboratory Services, NSW Health Pathology – Institute of Clinical Pathology and Medical Research (ICPMR), Westmead, Australia, from 22 January to 30 June 2020 were assessed. Only individuals whose samples were positive for SARS-CoV-2 RNA by RT-PCR testing more than 14 days

from symptom onset were included. Date of symptom onset and dates of each positive sample were recorded. Duration of PCR positivity was defined as number of days from symptom onset until final positive SARS-CoV-2 PCR result.

All samples for SARS-CoV-2 testing were analysed as previously described.[1] In brief, samples were processed within 12 h of receipt in the laboratory. RNA was extracted from 200 µL of sample into an elution volume of 100 µL using the MagNA Pure 96 instrument's Viral NA Small Volume Kit (Roche Diagnostics, Germany) as per the manufacturer's instructions. SARS-CoV-2 nucleic acid amplification and detection by RT-PCR were performed using the LightCycler 480 II System (Roche Diagnostics, Switzerland). SARS-CoV-2 gene targets included between one and four of the following genes: E, N, RdRp, M, ORF1ab and ORF1b. Samples were considered positive with a cycle threshold (Ct) value of ≤40 on any target; Ct values of positive samples were recorded.

Statistical analysis was performed using the R statistical package (https://www.r-project.org/), utilising Welch's two-sided *t* test, with a significance threshold of $p<0.05$.

A non-research determination for this project was granted by the Health Protection NSW as it was a designated communicable disease control activity.

Over the study period, 90,031 samples were received. Of these, 1372 samples tested positive for SARS-CoV-2 (1.5%), with 387 samples (0.43%) from 72 patients displaying prolonged PCR positivity beyond 14 days from the date of symptom onset.

There was a statistically significant trend towards higher Ct values of all tested targets over time with a mean Ct of 30.1±6.6 at >14 days from symptom onset and 27.2±5.5 at 4−14 days, compared to 23.8±3.8 in the first 3 days (both $p<0.001$) (Fig. 1).

The median age of all participants was 51 years (range 14−102 years) (Fig. 2A). The proportion of participants aged 80 years or over with persistently positive PCR results increased over time, from 11% at week 3 to 50% at week 6. Similarly, the mean age of participants with persistent PCR positivity increased over time (50 years in week 3, 74.5 years in week 6) (Fig. 2B). Compared to participants aged under 40 years, the mean duration of PCR positivity in patients aged ≥80 years was significantly prolonged ($p=0.007$) (Table 1); no participants <40 years of age remained PCR positive beyond 33 days from symptom onset.

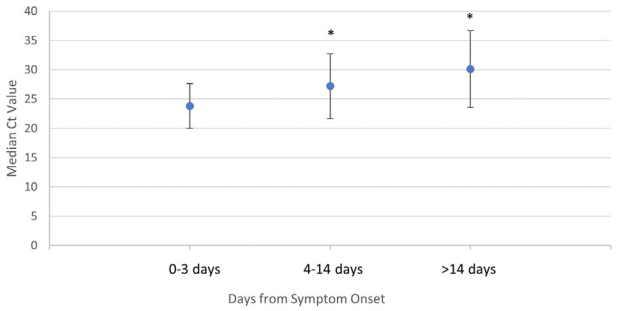

Fig. 1 Mean Ct values (with standard deviations) by day from symptom onset for samples taken 0−3 days, 4−14 days and >14 days from time of symptom onset. * Denotes $p<0.001$ compared to Ct values at 0−3 days from symptom onset.

The number of participants with prolonged SARS-CoV-2 PCR positivity declined with time from symptom onset. Of those positive at 14 days after symptom onset, 51% were still positive at 3 weeks, 25% at 4 weeks, 14% at 5 weeks, 5.6% at 6 weeks and 1.4% at 7−9 weeks (Fig. 2C). SARS-CoV-2 RNA was detected in one patient up to 62 days after symptom onset.

PCR testing for SARS-CoV-2 RNA has a key role in initial diagnosis of COVID-19 disease due to its high analytical sensitivity and specificity, however its role in the latter phases of the infection is unclear. In this case series of adults aged 14−102 years, prolonged PCR positivity for >14 days from symptom onset was seen in <1% of all samples, with persistence for up to 62 days from symptom onset. This is consistent with earlier studies demonstrating prolonged RNA shedding following acute COVID-19 infection.[4,5] In keeping with the current results, one US case series reported persistent PCR positivity rates of 98%, 86%, 56%, 27%, 9% and 5% at 1-week intervals from 1−6 weeks following symptom onset,[6]

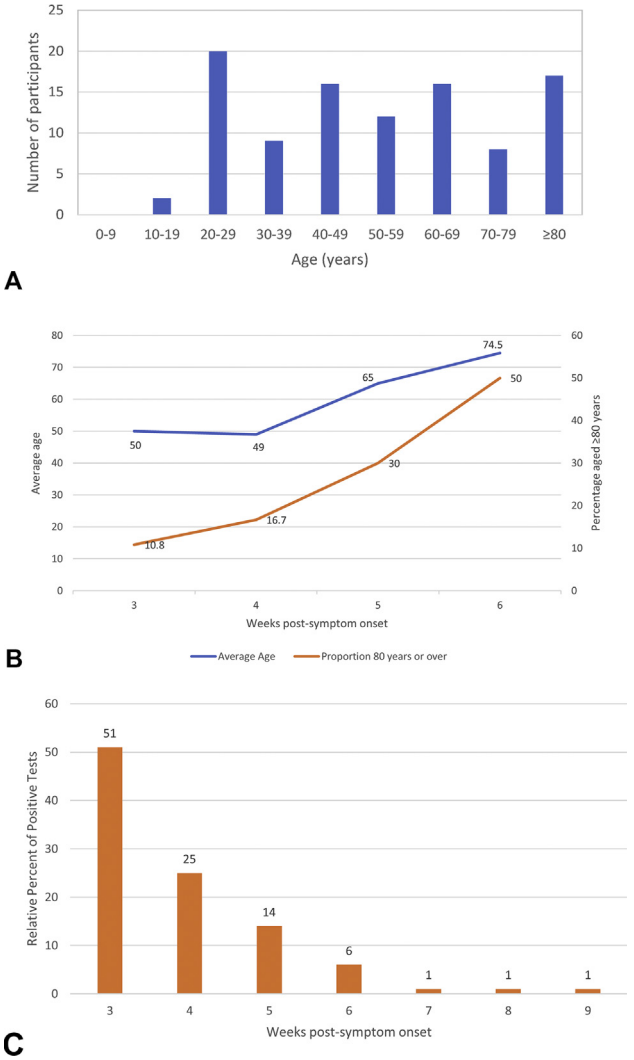

Fig. 2 (A) Age distribution of participants. (B) Average age and proportion of persons aged ≥80 years with prolonged SARS-CoV-2 PCR positivity by number of weeks from symptom onset. (C) Relative proportion of patients with SARS-CoV-2 detected by PCR by week from symptom onset, compared to cohort with positivity at 2 weeks from symptom onset.

Table 1 Duration of SARS-CoV-2 PCR positivity from date of symptom onset, stratified by age

Age group (years)	*n*	Duration of PCR positivity (days)		
Total	100	Mean	Standard deviation	*p* value[a]
0–39	31	19.19	5.26	–
40–59	28	22.96	9.35	0.067
60–79	24	23.21	11.14	0.113
≥80	17	25.88	8.48	0.007

[a] *p* values calculated by comparison to duration of PCR positivity in the 0–39 year age group.

suggesting consistency across geographies despite disparate COVID-19 epidemic curves.

Our data demonstrate that older age (≥80 years) is correlated with prolonged duration of PCR positivity compared to younger persons (<40 years), aligning with earlier meta-analysis data showing a positive correlation of pooled mean duration of PCR positivity with age.[2] This is further corroborated by recent studies showing age,[7–9] residence in long-term care facilities,[10] medical comorbidities (including hypertension, chronic kidney disease, hyperlipidaemia, obesity and coronary artery disease),[7] immunomodulatory therapy[8] and duration of symptom onset[9] are associated with prolonged PCR positivity. By contrast, studies have shown no correlation between duration of PCR positivity and disease severity or clinical outcomes.[9]

In patients with prolonged PCR positivity of at least 2 weeks from symptom onset, a significant trend in Ct toward higher values was seen with increasing time from symptom onset (Fig. 1). These results, from a large cohort comprising hospitalised and non-hospitalised patients, are consistent with data from symptomatic non-hospitalised patients which also showed a trend toward higher Ct values over time.[11]

As PCR assays cannot distinguish between nucleic acid detected from replicating virus and incomplete viral genetic fragments, prolonged PCR positivity may be expected beyond the acute infectious period. However, the pathophysiology underpinning the association with older age remains cryptic. Several possibilities exist, including a contribution from immunosenescence,[12] leading to impaired immune clearance of viral RNA in older people. Whether this association of age with prolonged PCR positivity is generalisable to novel variants of SARS-CoV-2 (including B.1.617.2) or indeed to other common respiratory viruses also warrants further investigation.

Nucleic acid testing should not be used as a proof of cure in COVID-19. Small cohort studies have demonstrated no transmission events to household contacts of patients with prolonged PCR positivity, at least beyond 4 weeks from symptom onset,[13] and clearance of culturable virus occurs many days to weeks prior to cessation of PCR positivity.[3] Awaiting SARS-CoV-2 PCR negativity may unnecessarily delay de-isolation, particularly in older patients, where symptom resolution (and/or viral culture when available) provide more definitive evidence of the end of the infectious period. Larger studies are welcomed linking community-based contact tracing with prolonged PCR positivity to further inform public health policies.

Acknowledgements: We gratefully acknowledge the work of the laboratory scientists of Institute of Clinical Pathology and Medical Research – NSW Health Pathology, Westmead, Australia.

Conflicts of interest and sources of funding: This research did not receive any specific funding from the public, commercial, or not-for-profit sectors. The authors state that there are no conflicts of interest to disclose.

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