On the sensitivity and specificity of post-mortem upper respiratory tract testing for SARS-CoV-2.

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Summary: Post-mortem testing of upper respiratory tract samples for SARS-CoV-2 RT-PCR has high sensitivity (96.8%) and specificity (94.2%) if performed within a week after death. It could be a useful diagnostic tool where testing has not taken place ante-mortem.

Footnote Page

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Abstract

Background Post-mortem testing can improve our understanding of the impact of SARS-CoV-2 if it is sufficiently sensitive and specific.

Methods We investigated the post-mortem sensitivity and specificity of reverse transcriptase PCR testing on upper respiratory swabs using a dataset of everyone who had been tested for SARS-CoV-2 before and after death in England between 1st March-29th October 2020. We analysed sensitivity in those who had a positive test before death by time to post-mortem test. We developed a multivariate model and conducted time-to-negativity survival analysis. For specificity we analysed those with a negative test in the week before death.

Results: Post-mortem testing within a week after death had a sensitivity of 96.8% if the person had tested positive within a week before death. There was no effect of age, sex, or specimen type on sensitivity, but individuals with COVID-19-related codes on their death certificate were 5.65 times more likely to test positive after death (95%CI 2.31,13.9). Specificity was 94.2%, increasing to 97.5% in individuals without COVID-19 on the death certificate.

Conclusion Post-mortem testing has high sensitivity (96.8%) and specificity (94.2%) if performed within a week after death and could be a useful diagnostic tool.

Key words: COVID-19; SARS-CoV-2; post-mortem; sensitivity; specificity

Introduction

As of 12th February 2021, there had been 119,627 deaths in England where COVID-19 was documented on the medical certificate of cause of death, of which 14,754 did not have a confirmatory test. In the same period there had been 103,695 deaths within 28 days of a positive COVID-19 test(1). It is recognised that COVID-19 may be an unidentified contributing factor in deaths, possibly leading to an underestimation of its impact(2, 3).

Though it may form part of a coronial[1] post-mortem examination, post-mortem testing for COVID-19 is not routine in England and is not required to confirm cause of death. Anecdotal evidence suggests a small amount of post-mortem testing is taking place, but the limited evidence on its validity has led to significant variation in practice amongst coroners and pathologists, with some testing as routine and others not at all. Introducing post-mortem testing more widely in the community has potential to improve understanding of the prevalence of COVID-19 and its impact on mortality. This is only feasible if non-invasive post-mortem testing, i.e. using nose and/or throat swabs, is sufficiently sensitive and specific in a practicable timeframe following death.

Feasibility of post-mortem testing for detecting the presence of seasonal respiratory viruses in the community is demonstrated by Navascués et al(4). They identified respiratory viruses (including influenza virus A(H3N2), RSV (subgroups A and B), coronavirus (types 229E and OC43), and rhinovirus) in 47% of 57 deceased individuals in Navarre, Spain, despite only 7% being diagnosed prior to death(4). They conclude that reverse transcription PCR (RT-PCR) from nasopharyngeal swabs taken post-mortem provided a sufficiently sensitive method for detecting respiratory viruses in deceased individuals. Similarly, data from Germany, where all patients with RT-PCR–confirmed SARS-CoV-2 infection were required to have an autopsy, showed that that positivity was maintained in post-mortem pharyngeal samples for at least the first week(5) and a study in Zambia detected SARS-

[1] A coroner is an independent judicial officer who has responsibility for investigating deaths which are sudden or suspected to be of violent or unnatural causes. A coronial post-mortem examination is one ordered by the coroner to establish the cause of death. (3)

CoV-2 in 19.2% of post-mortem nasopharyngeal samples taken from individuals enrolled up to 48 hours after death(3).

The aim of this paper is to assess the sensitivity and specificity of post-mortem testing for SARS-CoV-2 using nose and/or throat RT-PCR testing and to make recommendations on the time in which such testing should be conducted.

Methods

Dataset creation

Working in the National COVID-19 Epidemiology Cell of Public Health England (PHE), we created a dataset of everyone PHE had been notified had been tested for SARS-CoV-2 by RT-PCR in England more than once up to 29th October 2020 (n= 19,801,912 tests): the 'multiple samples dataset'. This included hospital and community samples. This was linked on National Health Service (NHS) number to an all-cause mortality dataset provided by the Office for National Statistics (ONS) containing deaths between 1st March 2020 and 23rd November 2020 (n = 385,686). This gave a dataset of everyone who had died and had more than one SARS-CoV-2 test (n = 150,591) and included both positive and negative tests. Of these, there were 1,937 people who had been tested both before and after death.

From this dataset we removed the small number of individuals (<0.5%) with discordant age values (>1 year different) in case of mis-matching. Where multiple records existed of the same specimen type on the same date and a discordant positive/negative result, the positive was taken. Tests done on the day of death were considered as post-mortem tests. We included specimens taken from the upper respiratory tract, which included those labelled as "nose", "throat", "combined nose and throat", "upper respiratory tract" or "swab NOS (not otherwise specified)"; all other specimen types were excluded from this analysis. This gave us a dataset of 1,665 deceased persons with a suitable test both before and after death.

Analysis

For the analysis assessing sensitivity, data were restricted to those with a positive test result prior to death. Ante-mortem samples were grouped by the number of days the sample was taken before death: 1-6 days, 7-13 days and >=14 days prior to death. Post-mortem samples were similarly grouped as 0-6 days, 7-13 days and >=14 days after death. Proportions positive post-mortem were then examined according to timing of post-mortem test and last ante-mortem positive test using chi-squared tests. Logistic regression was used to explore the effects of covariates, including age, sex, specimen type, and COVID-19 ICD-10 code (U07x) as the underlying cause on the death certificate. We also considered post-mortem time-to-negativity in a survival analysis framework and examined Kaplan Meier plots according to timing of ante-mortem test.

For the specificity analysis, data were restricted to those who had tested negative in the week before death, and we compared those in which the underlying cause of death included COVID-19-related ICD-10 codes with those that did not. Due to the small numbers of post-mortem positives in this group, we were only able to explore specificity in the first week after death.

Ethics

We received individual records of deaths from ONS which was handled under a data access agreement between ONS and named individuals at PHE for specific agreed analyses, of which this was one. The data were released in accordance with the Statistics and Registration Service Act 2007 s42 (4) as amended by s287 of the Health and Social Care Act 2012 and were processed in accordance with the data protection legislation encompassing the General Data Protection Regulation (GDPR) and the Data Protection Act 2018.

The multiple samples dataset contains identifiable data for all individuals who have had more than one test for SARS-CoV-2 and was handled under the relevant data protection legislation. The linkage of the multiple samples dataset and the ONS all-cause mortality dataset was performed in the PHE

Data Lake, a centralised data repository of SQL databases held securely within PHE networks, by authorised individuals and an anonymised dataset was extracted for analysis using R version 4.0.0. Ethical approval was not required for anonymous secondary data analysis as part of an analysis contributing to pandemic surveillance response.

Results

There were 1,480 post-mortem tests; 56% were male and ages ranged from 0-99 years (median 76, IQR 64-85). The day after death was the most common day that a sample was taken (n=533, 36%), and ranged from the day of death to 90 days after death (median 2, IQR 1-5). Positive post-mortem tests ranged from 0-30 days (median 2, IQR 1-5).

Sensitivity

There were 151 individuals who had a positive test prior to death and at least one post-mortem test; 16 had two tests and two had three tests, giving 171 post-mortem samples. 61% of individuals included were male. Ages ranged from 12 to 99 with a median of 78. More than half (56%) had COVID-19 as the underlying cause of death, as determined by ONS. The majority of swabs were labelled as combined nose and throat (38.6%), site not otherwise specified (23.4%), and throat 19.9%).

Shown in Figure 1 are the number and proportions of positive post-mortem tests with 95% confidence intervals by the timing of their last ante-mortem positive test result (in days) and days since death.

< figure 1 here >

For those testing positive within a week before death, and with a test 0-6 days after death, post-mortem positivity is 96.8%; but this drops to between 71-83% if the last positive ante-mortem test was 1-2 weeks prior to death, or the post-mortem test is at least a week after death. If the last

positive ante-mortem test was more than two weeks before death, positivity post-mortem is low, regardless of how near to death the sample was taken.

There was little evidence of a difference in the positivity by specimen type, except for those labelled 'upper respiratory tract' which were significantly more likely to be positive (p=0.011). This may be because URT samples were more commonly taken 0-3 days post-mortem, (81% compared to 59%, though this was not statistically significant (p=0.157)). Individuals with an underlying ICD10 code of U07x, i.e. COVID-19-related, were significantly more likely to test positive post-mortem (unadjusted odds ratio (OR) 6.98 95%CI 3.47, 14.0). Most people (98%) who tested positive in the week before death and who had an underlying ICD10 code of U07x also tested positive in the three days after death, 83% at four to seven days after death and 80% were positive when tested more than a week after death.

Logistic regression

Age, sex, specimen type and ICD-10 code were considered for modelling. Age was grouped as <60, 60-74, 75-84 and 85+ (roughly equal groups). There was no effect of sex, age or specimen type hence these were removed from this final model, shown in Table 1.

< table 1 here >

The multivariate model showed a significant decline in post-mortem test positivity the longer before death a person had had their last ante-mortem positive test. The reduction in positivity over time after death was not statistically significant but that is likely to be due to the small sample size for those tested more than two weeks after death. The observed differences in post-mortem positivity appear clinically significant. Persons with a COVID-19-related ICD-10 code on their death certificate had five and a half times the odds of testing positive for SARS-CoV-2 after death (aOR 5.65 95%CI 2.31, 13.9).

The timing of post-mortem tests in those previously testing positive, and their *risk of testing negative* was considered in a survival analysis framework. Data were deduplicated to use the latest non-negative post-mortem test, with a negative test being counted as failure (and any subsequent tests discarded). Figure 2 shows the Kaplan Meier plots for the proportion remaining non-negative stratified according to those with a positive test 1-6 days prior to death (N=68) and 7+ days before death (N=83). This confirms that, in someone who has died while infected with SARS-CoV-2 as proxied by a positive test in the week before death, the week after death is a reasonable timeframe in which to expect a post-mortem test to remain positive.

< figure 2 here >

Specificity

313 individuals who had a post-mortem test had tested negative in the week prior to death and are assumed to be true non-cases. After death, 27 had two tests, one had three tests, and one had four tests, giving 345 post-mortem samples. Ages ranged from 0 to 98 with a median age of 73 and 57% of samples were from men. Most swabs were combined nose and throat (36.5%), site not otherwise specified (23.2%), and throat (17.7%).

Of the 313 individuals, 20 (5.8%) had a subsequent positive post-mortem test. These are considered to be false positives, giving an overall specificity of 94.2% (95% CI 91.2-96.2%), as shown in Table 2. The proportion testing positive post-mortem was slightly greater up to a week after death. However, the difference between the specificity at different intervals after death is not significant (p=0.565).

< table 2 here >

28 (8.1%) decedents testing negative ante-mortem had COVID-19 documented on the death certificate. When the analysis is limited to individuals without COVID-19 documented on the death certificate (n=285), i.e. where there was a negative ante-mortem test result and there is presumed

to be no clinical suspicion of COVID-19, post-mortem testing had a specificity of 97.5% (95%CI 95.1-99.9%).

Discussion

These analyses indicate that post-mortem positivity of nose and/or throat swabs for SARS-CoV-2 is likely to remain high up to a week after death, provided the individual had COVID-19 within one, or up to two, weeks prior to death. This may be expected, as most individuals only test positive for SARS-CoV-2 for a week or two after infection(6). Severe cases (who are more likely to die) may test positive for longer(6), and this was seen in the fact that 93% of post-mortem tests were positive in cases who had tested positive the week before death, regardless of timing of the post-mortem test. Persons with a positive test post-mortem were more than five times more likely to have COVID-19-related codes on the death certificate, though this is likely to be due to reverse causation (i.e. that a positive post-mortem test led to COVID-19 being included on the death certificate). SARS-CoV-2 remains present and potentially detectable for up to 30days, but our dataset is not able to shed any light on the conditions under which this occurred.

Analyses of those testing negative in the week prior to death showed few post-mortem positives, with overall specificity exceeding 94%. The assumption that the ante-mortem test is the 'gold standard' leads to the assumption that a post-mortem positive following an ante-mortem negative must be false. This may underestimate the specificity of the post-mortem test by overestimating the sensitivity of the ante-mortem test. Furthermore, those who have died within a week of being tested for SARS-CoV-2 may be more likely to be infected, as the test implies clinical suspicion of illness and the observed positives post-mortem may be true cases who have tested negative previously due to imperfect *sensitivity* of the ante-mortem test, i.e. it may be more likely that an ante-mortem negative is false rather than the post-mortem positive. This could be either because the virus had not yet replicated enough to be detected ante-mortem, or due to imperfect sampling technique or issues with storage or transport. Therefore, true specificity of the post-mortem SARS-CoV-2 testing

may be higher than observed here, as shown in the higher specificity for those without COVID-19-related ICD-10 codes on their death certificate.

There is very limited published data on post-mortem testing for SARS-CoV-2. Our results are consistent with the studies in Spain (in pre-COVID-19 times)(4) and Germany (5, 7) (during the COVID-19 pandemic), that show the feasibility of post-mortem sampling, but go further by providing estimates of sensitivity and specificity over a longer period of time since death. This information will be of use to coroners and pathologists when considering whether to conduct a post-mortem test for SARS-CoV-2.

Limitations

Our analyses are limited by the small number of people who have had ante- and post-mortem tests, despite using national level data over the course of the epidemic in England in 2020. Post-mortem testing for SARS-CoV-2 is not systematic, and would have been done at varying levels throughout the epidemic in England; for example, data from March 2020 to August 2020 show a smaller proportion of non-COVID-19-related deaths in England and Wales were subject to a coroner's post-mortem examination than deaths during the same months of the previous five-year average(8). We do not know the reasons why post-mortem tests were done for the people in our sample and so we are not able to explore potential sources of bias arising from the population that have been tested post-mortem. It is not clear why a post-mortem test might be done for someone who has already tested positive before death, unless the result had not been available at the time of death. It is reasonable to think that post-mortem testing may be undertaken where there was a negative ante-mortem test but clinical suspicion of COVID-19 was high, for example in the context of an outbreak in a care home.

We are not able to determine whether samples labelled 'upper respiratory tract' are taken from nose, throat, or combined nose and throat. We have assumed that swab NOS specimens tested for

SARS-CoV-2 PCR are taken from the upper respiratory tract but it is possible that other sites (e.g. post-mortem lung swabs) are included, hence there may be some misclassification. However, there were only 16 of these samples and sensitivity analyses showed that our findings were the same if we excluded these. We are also unable to comment on sensitivity and specificity by PCR assay. The data used in this study were gathered from a national database with reports coming from clinical laboratories across the country using a range of commercial or in-house PCR tests, but all laboratories in England during this time were part of an NHS pathology testing framework, with quality assurance of testing.

There are many other sources of heterogeneity which we are not able to account for or investigate. For example, swabs labelled as "nose" may include those taken from the anterior nose or nasopharynx and our dataset includes swabs performed by health care workers as well as self-taken upper respiratory tract swabs. While a positive PCR test indicates presence of the SARS-CoV-2 virus, we do not have any information on viral load or clinical illness, hence we are not able to comment on the potential infectivity of the deceased persons being tested, nor on whether SARS-CoV-2 contributed to the cause of death.

Conclusion

We have found that post-mortem swabbing of the nose and/or throat has high sensitivity (at least 96%) and specificity (at least 94%) if performed within a week of death. This in line with estimates of the sensitivity (>94%)(9) and specificity (>99%)(10) in live subjects. One week should provide a sufficient time window for post-mortem testing to take place and it is therefore potentially a useful diagnostic tool. Routine surveillance of deaths in the community, i.e. those who have not been to hospital and are less likely to have had an ante-mortem test, would provide more information on the true prevalence of SARS-CoV-2 and the burden of disease in the community, particularly during times of low prevalence or when routine or real-time testing is unavailable, such as in low-resource settings.

In England and Wales it is vital that such testing only takes place on premises licenced by the Human Tissue Authority for this activity to ensure it is conducted in accordance with the law(11). Given the ongoing presence of SARS-CoV-2 after death, and the lack of information on infectivity, suitable infection control measures should continue to be followed when handling the bodies of the deceased.

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Conflicts of interest: All authors declare that they have no conflicts of interest

Data Availability Statement

The data underlying this article cannot be shared publicly due to the legal and policy controls placed on data used as part of the government's response to the COVID-19 pandemic.

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Tables

Table 1 Results of logistic regression model for timing of post-mortem testing

		Odds Ratio	95% Conf. Interval	
	1-6		Baseline	
	7-13	0.13	0.03	0.63
Time before death (days)	**>=14**	0.04	0.01	0.13
	0-6		Baseline	
	7-13	1.12	0.40	3.12
Time since death (days)	**>=14**	0.17	0.01	2.70
	No		Baseline	
U07x on death certificate	**Yes**	5.65	2.31	13.85

Table 2 Post-mortem COVID-19 test results amongst those who tested negative in the week prior to death, by number of days post-mortem the test was taken.

	Post-mortem test result			
Days post-mortem test	**Positive**		**Negative**	
taken	**N**	**%**	**N**	**%**
0-6	17	6.16	259	93.8
≥7	3	4.35	66	95.7
Total	20	5.80	325	94.20

Figure 1

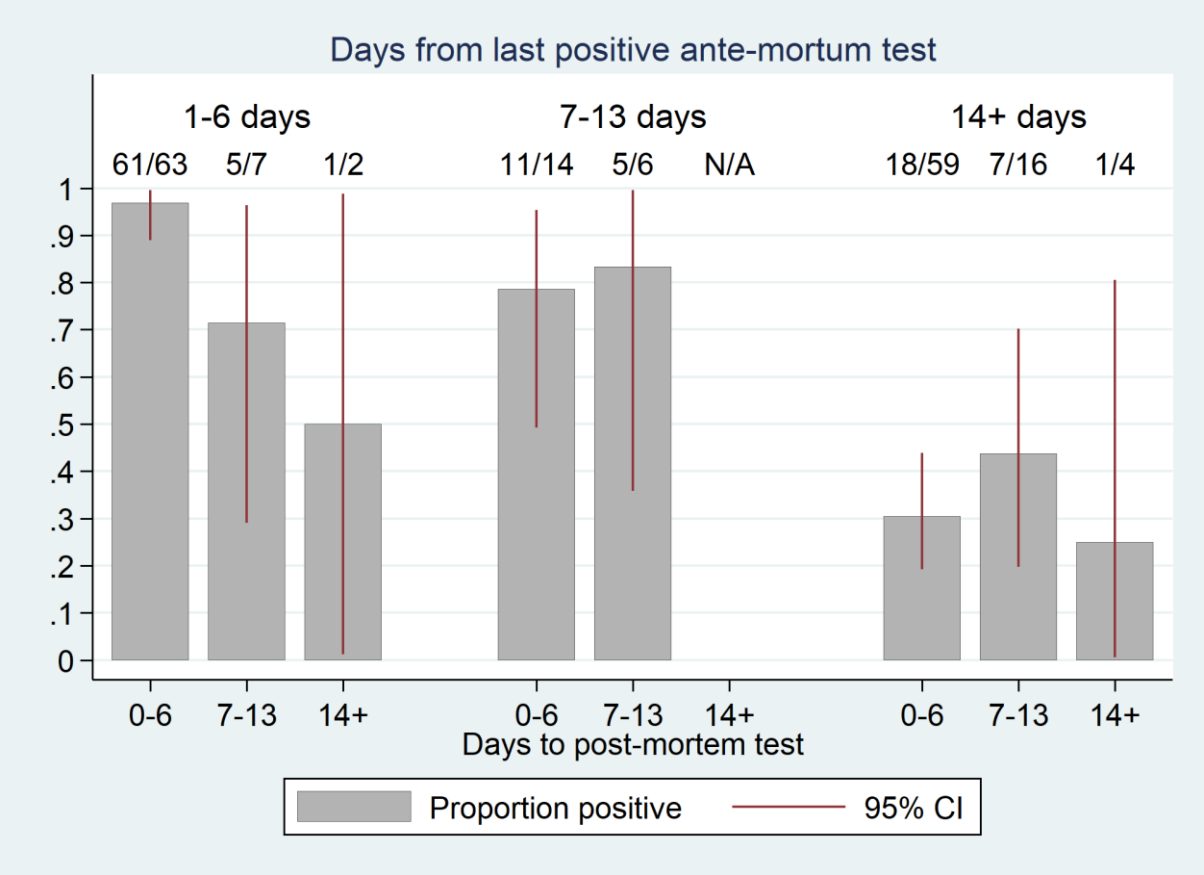
Days from last positive ante-mortum test
1-6 days
7-13 days
14+ days
61/63
5/7
1/2
11/14
5/6
N/A
18/59
7/16
1/4
1
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
0-6
7-13
14+
0-6
7-13
14+
0-6
7-13
14+
Days to post-mortem test
Proportion positive
95% CI

Figure 2