

# Low 30-day mortality in South African orthopaedic patients undergoing surgery at an academic hospital during the first wave of the COVID-19 pandemic: It was safe to perform orthopaedic procedures at our hospital during the first COVID-19 peak

R Waters,<sup>1,2,3</sup> MSc; R Dey,<sup>1,2,4</sup> PhD; M Laubscher,<sup>1,2</sup> MB ChB, Dip PEC (SA), FC Orth (SA), MMed (Orth); R Dunn,<sup>1,2</sup> MB ChB, FCS (SA) Orth, MMed (Orth); S Maqungo,<sup>1,2</sup> MB ChB, FC Orth (SA), MMed (Orth), PG Dip HPE; G McCollum,<sup>1,2</sup> MB ChB, MMed (Orth), FC Orth (SA); M Nortje,<sup>1,2</sup> MB ChB, FC Orth (SA), MMed (Orth); S Roche,<sup>1,2</sup> MB ChB, FC Orth (SA), LMCC (Canada) (Orth); T Hilton,<sup>2</sup> MB ChB, DA (SA), Dip PEC (SA), FC Orth (SA), MMed (Orth); W Mugla,<sup>1,2</sup> MB ChB, FC Orth (SA); M Held,<sup>1,2</sup> MD, MMed (Orth), FC Orth (SA), PhD, MBA

<sup>1</sup> Orthopaedic Research Unit, Division of Orthopaedic Surgery, Faculty of Health Sciences, University of Cape Town, South Africa

<sup>2</sup> Division of Orthopaedic Surgery, Faculty of Health Sciences, University of Cape Town, South Africa

<sup>3</sup> Wellcome Centre for Infectious Disease Research in Africa (CIDRI-Africa), Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, South Africa

<sup>4</sup> Division of Biomedical Engineering, Department of Human Biology, Faculty of Health Sciences, University of Cape Town, South Africa

**Corresponding author:** R Waters (WTRROB005@myuct.ac.za)

**Background.** Initial local and global evidence suggests that SARS-CoV-2-infected patients who undergo surgery, and those who become infected perioperatively, have an increased mortality risk post surgery.

**Objectives.** To analyse and describe the 30-day mortality, presurgical COVID-19 status and hospital-acquired SARS-CoV-2 infection rates of patients, both SARS-CoV-2-positive and negative, undergoing orthopaedic surgery at a tertiary academic hospital in South Africa (SA) during the first COVID-19 peak.

**Methods.** This single-centre, observational, prospective study included patients who underwent orthopaedic procedures from 1 April 2020 (beginning of the COVID-19 case increase in SA) to 31 July 2020 (first COVID-19 peak in SA). All patients were screened for COVID-19 and were confirmed positive if they had a positive laboratory quantitative polymerase chain reaction test for SARS-CoV-2 RNA on a nasopharyngeal or oral swab. Thirty-day mortality, presurgical COVID-19 status and hospital-acquired SARS-CoV-2 infection were assessed.

**Results.** Overall, a total of 433 operations were performed on 346 patients during the timeframe. Of these patients, 65.9% ( $n=228$ ) were male and 34.1% ( $n=118$ ) were female. The mean (standard deviation) age was 42.5 (16.8) years (range 9 - 89). Of the patients, 5 (1.4%) were identified as COVID-19 patients under investigation (PUI) on admission and tested positive for SARS-CoV-2 before surgery, and 1 (0.3%) contracted SARS-CoV-2 perioperatively; all survived 30 days post surgery. Twenty-nine patients were lost to follow-up, and data were missing for 6 patients. The final analysis was performed excluding these 35 patients. Of the 311 patients included in the final 30-day mortality analysis, 303 (97%) had a follow-up observation  $\geq 30$  days after the operation. The overall 30-day mortality for these patients was 2.5% ( $n=8$  deaths). None of the recorded deaths were of screened COVID-19 PUI.

**Conclusions.** We report a low 30-day mortality rate of 2.5% ( $n=8$ ) for patients undergoing orthopaedic surgery at our hospital during the first COVID-19 peak. None of the deaths were COVID-19 related, and all patients who tested SARS-CoV-2-positive, before or after surgery, survived. Our overall 30-day mortality rate correlates with several other reports of orthopaedic centres analysing over similar timeframes during the first peak of the COVID-19 pandemic. Regarding mortality and SARS-CoV-2 infection risk, we can conclude that with the appropriate measures taken, it was safe to undergo orthopaedic procedures at our hospital during the first peak of the COVID-19 pandemic in SA.

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South Africa (SA) remains in a national state of disaster due to COVID-19 and the ongoing detection of active SARS-CoV-2 cases in our population, mainly driven by the local Beta variant 501Y.V2. Our nation's recorded recovery rate remains high at 92%.<sup>[1]</sup> Groote Schuur Hospital (GSH), one of the two state academic hospitals in our area, services patients from vulnerable communities, where infectious diseases such as tuberculosis and HIV continue to dominate the health landscape and multiple comorbidities exist, posing a potential risk for the development of more severe forms of COVID-19-

associated illness. Many of our orthopaedic patients are from a low socioeconomic background and low-income households, associated with potential vulnerabilities.

At GSH, both elective and non-urgent surgeries were cancelled during the initial phases of the hard lockdown, 26 March to the end of May 2020.<sup>[2]</sup> After the initial hard lockdown, we resumed semi-urgent surgeries in lower-risk patients. We used the medically necessary, time-sensitive procedures (MeNTS) scoring system to help select the most appropriate orthopaedic patients to perform surgery

on.<sup>[3]</sup> At the time of writing, elective and non-urgent surgeries were actively resuming and patients were once again returning to hospitals to seek diagnosis and treatment. It remains a top priority to continue ensuring that both patients and healthcare workers are protected from contracting SARS-CoV-2 in the course of patient treatment. A primary focus should be how to safely and fully reinstate elective and non-urgent operating amid the current pandemic and the devastating 'waves'. Here, it is of key importance to provide evidence regarding the safety of surgery in hospitals with high SARS-CoV-2 risk. It is also valuable to assess how COVID-19 may affect surgical outcomes of patients treated during this time, as initial global evidence has suggested that COVID-19-positive patients who undergo surgery have significantly increased mortality and risk of major complications post surgery.<sup>[4-9]</sup> There is limited evidence of the impact of COVID-19 on the mortality of orthopaedic patients undergoing surgery in resource-limited settings.

## Objectives

To assess the pre- and perioperative COVID-19 status and 30-day mortality of all patients who underwent orthopaedic surgery during the first peak of the COVID-19 pandemic in our tertiary academic hospital. To our knowledge, no reports on the 30-day mortality of orthopaedic patients in a level 1 orthopaedic trauma unit in an SA environment are available.

## Methods

### Study design and data source

After obtaining ethical (ref. no. HREC 250/2020) and hospital institutional approval, orthopaedic surgical case data for the timeline 1 April 2020 - 31 July 2020 were extracted from a prospectively collected orthopaedic REDCap database, while maintaining patient confidentiality. All patients presenting to the orthopaedic department were screened for COVID-19 using standardised screening questions and identified as patients under investigation (PUI) if they exhibited COVID-19-related signs and symptoms including a history of fever, shortness of breath, cough, sore throat and/or loss of smell and taste, and/or if they had recently travelled abroad or had contact with an individual with suspected or confirmed SARS-CoV-2. Only the COVID-19 PUI underwent a nasopharyngeal or oral swab for detection of SARS-CoV-2 RNA by quantitative polymerase chain reaction (PCR), as per hospital guidelines.

We attempted to contact by telephone all identified orthopaedic patients ( $n=346$ ) who underwent surgery during the study period. Patients who were successfully contacted provided telephonic consent for the investigators to assess their COVID-19 status and the status of their household members, and if applicable, information on patient mortality. In addition, all identified patients were screened using the Picture Archiving and Communication System (PACS) imaging database to assess the date of latest imaging results for the purpose of understanding patient 30-day mortality. Concurrently, all 346 patients were also screened using the National Health Laboratory Service (NHLS)-LABTRAK (TrakCare) system, both for latest result dates to assess 30-day mortality, for those unsuccessful on the PACS system, and for SARS-CoV-2 PCR results. Patient folders were extracted and Clinicom was accessed for patients who had neither a PACS nor an NHLS follow-up date post surgery, to assess last-seen dates and 30-day mortality, if known to be deceased.

### Statistical analysis

Patient demographic data were summarised using descriptive statistics, including means and ranges, where applicable.

## Results

A total of 433 orthopaedic surgical procedures were performed on 346 patients from 1 April to 31 July 2020. All 346 patients were screened for COVID-19 signs and symptoms on admission. Of these, 36 (10.4%) were identified as PUI and underwent a nasopharyngeal swab for diagnostic testing by the NHLS using a SARS-CoV-2 PCR; 5 (13.9%) were

confirmed SARS-CoV-2-positive, 28 (77.8%) were confirmed SARS-CoV-2-negative, and 3 (8.3%) had inconclusive results or tests rejected due to incorrect sample labelling (Fig. 1 and Table 1). Two hundred and eighty-one orthopaedic patients were not swabbed for SARS-CoV-2 because they screened negative for COVID-19 high risk according to the hospital swabbing guidelines at the time. Assessment of presurgical COVID-19 status for 29 patients was unsuccessful owing to incorrect recording of folder numbers on the REDCap database or lack of 2020 data on the TrakCare system (Fig. 1).

Of the patient cohort, 65.9% ( $n=228$ ) were male. The mean (standard deviation) age of the entire patient cohort was 42.5 (16.8) years (range 9 - 89) (Table 1). The ages of 2 patients were unrecorded or unavailable owing to incorrect folder numbers recorded on the REDCap database.

Of the patients, 56.9% underwent surgery for orthopaedic trauma. There was an equal spread among the rest of the orthopaedic surgical firms, with the exception of spine trauma (2.3%), foot and ankle (1.4%) and paediatric non-trauma (0.3%) (Table 1).

Available data for our orthopaedic surgical cohort for the 30-day mortality assessment are shown in Fig. 2. A total of 35 patients were excluded from the final analysis (dotted line-framed boxes) because the patient hospital folder number was incorrectly recorded on the orthopaedic REDCap database ( $n=6$ ) or the patients were lost to follow-up (LTFU) post surgery ( $n=29$ ). Of the remaining 311 patients, 303 (97.4%) had a  $\geq 30$ -day follow-up date (PACS, NHLS, telephonic follow-up, folder retrieval), of whom 177 (58.4%) were successfully contacted telephonically. The overall 30-day mortality for our patient

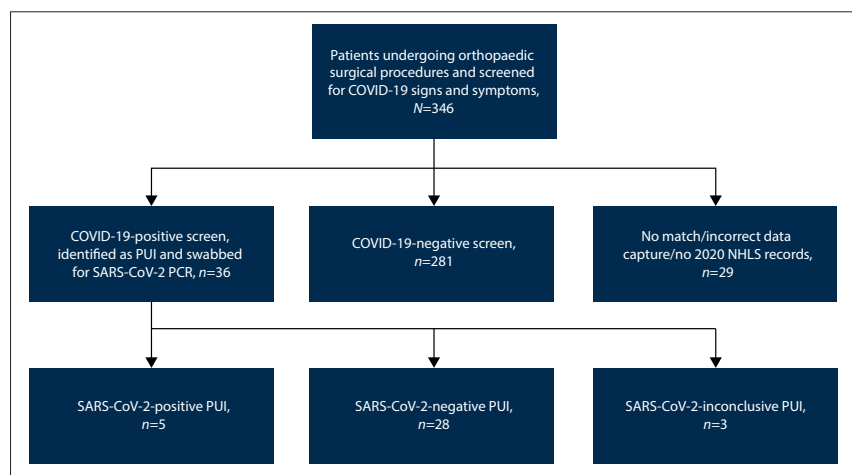


Fig. 1. Flow chart showing screening of our orthopaedic surgical cohort for COVID-19 signs and symptoms on admission. (PUI = patients under investigation; PCR = polymerase chain reaction; NHLS = National Health Laboratory Service.)

**Table 1. Cohort demographics, surgery details, 30-day mortality and pre- and perioperative SARS-CoV-2 infection details for orthopaedic patients operated on during the first wave of the COVID-19 pandemic in South Africa, 1 April 2020 - 31 July 2020**

Variable	Overall	Non-trauma*	Trauma
Gender, <i>n</i> (%)			
Total	346 (100)	149 (43.1)	197 (56.9)
Male	228 (65.9)	89 (39.0)	139 (61.0)
Female	118 (34.1)	60 (50.8)	58 (49.2)
Age (years), mean (SD, range)	42.5 (16.8, 9 - 89)	44.02 (17.61, 9 - 89)	41.26 (16.1, 14 - 88) <sup>†</sup>
Surgical procedures per surgical firm, %			
Arthroplasty	9.0	-	-
Foot/ankle	1.4	-	-
Knee	6.6	-	-
Limb reconstruction unit	7.5	-	-
Oncology	5.2	-	-
Paediatric non-trauma	0.3	-	-
Spine	6.6	-	-
Spine trauma	2.3	-	-
Trauma	56.9	-	-
Upper limb	4.0	-	-
30-day mortality characteristics			
Total, <i>n</i> (% of total patient cohort)	8 (2.5)	1 (12.5)	7 (87.5)
COVID-19 PUI on admission, <i>n</i>	0	0	0
Age (years), mean (SD, range)	57.5 (18.02, 30 - 83)	-	59.14 (18.8, 30 - 83)
Procedures, <i>n</i> (% of total patient cohort)	8 (2.5)	1 (12.5)	7 (87.5)
ORIF (nail), <i>n</i>	2	-	2
ORIF (plate), <i>n</i>	1	-	1
MUA, <i>n</i>	1	-	1
Cervical spine fusion, <i>n</i>	1	1	-
Soft-tissue debridement, <i>n</i>	1	-	1
Removal of hardware, <i>n</i>	1	-	1
Soft-tissue shoulder arthrotomy, <i>n</i>	1	-	1
Pre- and perioperative SARS-CoV-2-positive patient characteristics and surgery detail			
Preoperative SARS-CoV-2 confirmed infection, <i>n</i> (% of total patient cohort)	5 (1.4)	-	5 (100)
Age (years), mean (SD, range)	42.2 (15.97, 21 - 65)	-	42.2 (15.97, 21 - 65)
Procedures, <i>n</i> (% of total patient cohort)	5 (1.4)	-	5 (100)
ORIF (nail), <i>n</i>	2	-	2
ORIF (plate), <i>n</i>	1	-	1
Amputation, <i>n</i>	1	-	1
Cemented bipolar, <i>n</i>	1	-	1
Perioperative SARS-CoV-2 confirmed infection, <i>n</i> (% of total patient cohort)	1 (0.3)	-	1 (100)
Procedure – ORIF (plate), <i>n</i> (% of total patient cohort)	1 (0.3)	-	1 (100)

SD = standard deviation; PUI = patients under investigation; ORIF = open reduction internal fixation; MUA = manipulation under anaesthesia.

\*Non-trauma includes the following surgical firms: arthroplasty, foot and ankle, knee, limb reconstruction unit, oncology, paediatric non-trauma, spine, spine trauma, and upper limb.

<sup>†</sup>2 patients omitted owing to lack of birthdate data.

cohort was 2.5% (*n*=8 deaths). Of the 8 patients who died during the study period, 87.5% (*n*=7) underwent trauma-related surgery and 1 patient underwent a cervical spine fusion. There was an equal ratio between males and females who died postoperatively. None of the recorded deaths were of COVID-19 PUI on admission. Of the patients confirmed positive for SARS-CoV-2 who underwent surgery during the timeframe, 80.0% were male. The most common procedure performed was open reduction and internal fixation of a fracture. One orthopaedic trauma patient (0.3%) contracted SARS-CoV-2 perioperatively (Table 1). After 30 days, 100% survival was recorded for all patients who were admitted with SARS-CoV-2 or contracted it during the postoperative period.

## Discussion

To our knowledge, this is the first SA study to have analysed and described the 30-day mortality and pre- and perioperative SARS-CoV-2 infection status of orthopaedic patients undergoing surgery at a tertiary academic hospital during the first wave of the COVID-19 pandemic.

As we actively resume elective and non-urgent surgical procedures between waves of the COVID-19 pandemic, it is important to acknowledge the continued SARS-CoV-2-exposure risks, both to patients and to surgical staff. Most of the patients in our study cohort underwent surgery for trauma-related injuries, which inherently carries an increased risk to the patient. The risks due to SARS-

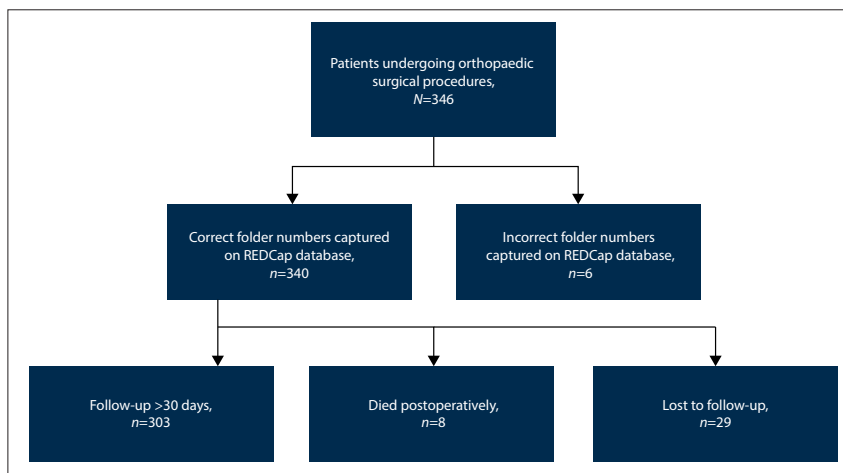


Fig. 2. Flow chart showing available data for our orthopaedic surgical cohort for the 30-day mortality assessment.

CoV-2 infection, in SARS-CoV-2 confirmed positive patients on admission, may compound this general risk, and outcomes could be unfavourable for the patient. Despite these risks, all our SARS-CoV-2 confirmed positive patients undergoing surgery survived 30 days post surgery. Of our cases, 43.1% were non-trauma, as we were actively conducting urgent and semi-urgent orthopaedic procedures during this time.

Globally, several orthopaedic units have assessed the 30-day mortality and SARS-CoV-2 infection rates of patients undergoing procedures during their respective first peaks of the COVID-19 pandemic. Most of the study sites, including in the UK, Europe and the USA, experienced their first COVID-19 peak in March and April 2020, earlier than that experienced in SA in July 2020. We have summarised and described the findings in Table 2. The low 30-day mortality rate of our patient cohort during the first COVID-19 peak is similar to figures published by Sobti *et al.*,<sup>[10]</sup> Karayiannis *et al.*,<sup>[11]</sup> Price *et al.*,<sup>[12]</sup> Hope *et al.*<sup>[13]</sup> and Giorgi *et al.*<sup>[14]</sup> (2.3% v. 4.3%, 1.9%, 3%, 4% and 4.6%, respectively), and is considerably lower than mortality rates reported in other regions in the UK and other developing countries (11.76% - 53%).<sup>[5,7-9,13]</sup> The specific reasons for this lower mortality rate are not clear from our study, but may be attributed to an overall younger population undergoing surgery in our academic hospital and many lower-risk procedures being performed during this timeframe. We show that surgery was, for the most part, safely conducted despite the risk of SARS-CoV-2 infection and postoperative complications. Various factors must be taken into account when allocating theatre resources for elective and non-urgent surgery during the pandemic.

The availability of nursing and surgical staff, limited ward space and decreased oxygen supply, as well as health-seeking behaviour of patients, have influenced surgical processes during the COVID-19 peaks. Yet, owing to the low risk of postoperative complications in our cohort, a fear of higher mortality in well-selected elective surgery was not justified. This specific concern should therefore receive lower priority when considering theatre resource allocation in future waves of the pandemic.

As previously mentioned, our lower 30-day mortality rate was similar to several international studies, including the experience of a UK trauma and orthopaedics unit where 206 trauma and urgent orthopaedic surgical procedures were performed during the peak of the pandemic (1 March - 31 May 2020).<sup>[10]</sup> Of these patients, 4.3% ( $n=9$ ) died postoperatively; 55.5% of the deceased patients were SARS-CoV-2-negative, 1.45% ( $n=3$ ) were SARS-CoV-2-positive postoperatively, and 1 patient presented as asymptomatic and was not tested before surgery. The authors promote the usefulness of comparing and evaluating single-centre practice. A study that collated Northern Ireland regional data showed very low (1.9%) overall 30-day postoperative mortality in trauma and orthopaedic surgery patients. Of the patients, 5.5% ( $n=27$ ) contracted SARS-CoV-2 perioperatively, and in this group the 30-day mortality rate was 14.8% ( $n=4$ ).<sup>[11]</sup> At the Nuffield Orthopaedic Centre in the UK, 9.4% of patients who underwent orthopaedic procedures as SARS-CoV-2-negative ( $n=9$ ) contracted SARS-CoV-2 within 30 days postoperatively.<sup>[12]</sup> Of these patients, 66% ( $n=6$ ) developed significant COVID-19-related complications postoperatively, and there was a 33% ( $n=3$ ) 30-day mortality rate.

The overall 30-day mortality rate across the whole cohort of patients ( $n=96$ ) during this period was 3% ( $n=3$ ). Another UK-based study reported an overall survival rate of 96% for patients undergoing emergency orthopaedic trauma surgery during the COVID-19 peak.<sup>[13]</sup> An Italian experience recorded an overall mortality of 4.6% ( $n=3$ ) among fracture trauma patients.<sup>[14]</sup> Of those who died, 5.8% ( $n=1$ ) were SARS-CoV-2-positive and 4.1% ( $n=2$ ) were SARS-CoV-2-negative ( $p>0.05$ ); 17 patients were SARS-CoV-2-positive on admission or became positive within 14 days of admission. It is noteworthy that our perioperative SARS-CoV-2 infection rate for patients we were able to obtain the data for was drastically lower than rates in these studies (Table 2).

Other international studies reported higher 30-day mortality and SARS-CoV-2 infection rates than those reported by this study. These included the COVIDSurg international cohort study, which reported a 30-day postoperative mortality rate of 28.8% ( $n=86/302$ ) in patients undergoing orthopaedic procedures between 1 January and 31 March 2020.<sup>[5]</sup> All these patients had SARS-CoV-2 infection confirmed within 7 days before or 30 days after surgery. In this orthopaedic patient group, 44.3% ( $n=131$ ) exhibited postoperative pulmonary complications, defined as pneumonia, acute respiratory distress syndrome or unexpected postoperative ventilation. A UK-based retrospective cohort study reported an increased postoperative mortality rate of 30.5% ( $n=25/82$ ) in COVID-19-positive patients undergoing hip fracture surgery, compared with a rate of 10.3% ( $n=35/340$ ) in COVID-19-negative patients ( $p<0.001$ ).<sup>[8]</sup> In another fracture study conducted in New York, 12.3% of the patient cohort ( $n=138$ ) were COVID-19-positive and 10.1% suspected COVID-19-positive.<sup>[9]</sup> Increased mortality during hospitalisation and after 30 days (53% of COVID-19-positive and 14% of suspected COVID-19 patients) was reported when compared with COVID-19-negative patients (Table 2).

## Study limitations

This study has limitations. During the study period, the COVID-19 swabbing criteria continued to evolve, and the clinical diagnosis of suspected COVID-19 patients was therefore not standardised. As previously mentioned, only COVID-19 PUI were swabbed for SARS-CoV-2 infection testing by quantitative PCR. We may have missed many negative COVID-19-screened, asymptomatic patients who were in fact positive for SARS-CoV-2 infection, yet this



**Table 2. Summary of the orthopaedic-related studies conducted during the first COVID-19 peak around the world assessing the 30-day mortality, pre- and perioperative SARS-CoV-2 infection rates of patients undergoing surgery, and the percentage of SARS-CoV-2-positive patients who died during the study timeframe**

Study reference	Study design	Timeframe	Sample size, N	SARS-CoV-2 patients on admission/at the time of surgery (if reported), n (%)	30-day mortality of total cohort, n (%)	Postoperative SARS-CoV-2 positivity, n (%)	Deceased with confirmed SARS-CoV-2 infection perioperatively, n (%)
The present study, Cape Town, South Africa	Single-centre, observational prospective study of patients	1 April - 31 July 2020	346	5 (1.4)	8 (2.5)	1 (0.3)	0
Karayannis <i>et al.</i> <sup>[11]</sup>	Retrospective multicentre review	18 March - 27 April 2020	484	5 (1)	9 (1.9)	21 (4.3)	4 (44.4)
Price <i>et al.</i> <sup>[12]</sup>	Retrospective, single-centre observational cohort study	7 March - 3 May 2020	96	0	3 (3)	9 (9.4)	3 (100)
Hope <i>et al.</i> <sup>[13]</sup>	Retrospective review	23 March - 4 April 2020	76	10 (13)	3 (4)	3 (4)	2 (66.7)
Sobti <i>et al.</i> <sup>[10]</sup>	Prospective database	1 March - 31 May 2020	206	Not reported	9 (4.3)	3 (4.3)	3 (33.3)
Giorgi <i>et al.</i> <sup>[14]</sup>	Retrospective case-control study	8 March - 4 May 2020	65	17 (26)	3 (4.6)	3 (4.6)	1 (33.3)
Wright <i>et al.</i> <sup>[18]</sup>	Retrospective audit analysis	11 March - 30 April 2020	68	17 (25)	8 (11.8)	Not reported	5 (62.5)
Macey <i>et al.</i> <sup>[19]</sup>	Retrospective departmental audit	20 March - 25 April 2020; 20 March - 25 April 2019	76	1 (1.3)	11 (14)	9 (11.8)	2 (20)
Thakrar <i>et al.</i> <sup>[7]</sup>	Single-centre, observational prospective study of patients	15 March - 15 April 2020	43	11 (26)	7 (16.3)	Not reported	4 (57)
Nepogodiev <i>et al.</i> <sup>[5]</sup>	International observational cohort study	1 January - 31 March 2020	302	Not reported for orthopaedic patients specifically	86 (28.8)	Not reported for orthopaedic patients specifically	Not reported for orthopaedic patients specifically
Kayani <i>et al.</i> <sup>[8]</sup>	Multicentre cohort study	1 February - 20 April 2020	424	9 (11)	30.5%	73 (17.2)	25 (30.5)
Egol <i>et al.</i> <sup>[9]</sup>	Prospective cohort study	1 February - 15 April 2020; 1 February - 15 April 2019	138	17 (12.3)	17 (12.3)	Not reported	9 (53)

would not have changed our primary outcome of overall mortality. It is important to note that asymptomatic carriers can transmit the virus during the incubation phase, posing a risk to patients in the ward, and to nursing and surgical staff.<sup>[4]</sup> Patient follow-up remains complex in the population serviced by our academic hospital. We were not able to successfully contact every patient who underwent surgery to assess their and their household contacts' COVID-19 status postoperatively, which would have given our data more strength and improved accuracy of reporting. In addition, several patients were LTFU because they did not attend their follow-up appointments. In our case, LTFU does not mean 'no death'. Although reporting of LTFU is quite rare in all the COVID survival-related studies to date, a study that excluded 21 patients and calculated the mortality percentage for the remaining patients reported a mortality rate of 5.4%,<sup>[15]</sup> while ours, for patients we were able to obtain 30-day mortality data for, was 2.5%. If we were to assume that all 35 patients for whom we are missing data died, our mortality rate would equate to 10%. This is still lower than the pooled prevalence mortality rate of 20% that took into account reports from UK, China, Turkey, India, Italy, Spain, the USA and France,<sup>[16]</sup> and an overall mortality rate of 15% reported by a Canadian study.<sup>[17]</sup> Our mortality rate, inclusive or exclusive of the missing data, falls within the globally reported rates. Further prospective studies with larger cohorts, comparing 30-day mortality data with previous years, and throughout the different COVID-19 peaks and in the presence of different SARS-CoV-2 variants, are needed for a deeper understanding of the risk associated with pre- and perioperative SARS-CoV-2 infection. In addition, we did not assess any postoperative complications in our SARS-CoV-2-positive patients.

### Conclusions

We report a low 30-day mortality rate of 2.5% for patients undergoing orthopaedic surgery at our hospital during the first peak of the COVID-19 pandemic in 2020 and a 30-day mortality rate of 0% for preoperatively confirmed SARS-CoV-2-positive patients undergoing orthopaedic procedures. Of the 8 patients who died, 7 (87.5%) underwent surgery for orthopaedic trauma. We can conclude that it was safe to operate on orthopaedic patients at our academic hospital during the first COVID-19 peak in SA. COVID-19 status and 30-day mortality data are important, as they will guide our surgeons on the most appropriate way to rapidly and safely resume elective and non-urgent surgery, with careful consideration of both patient and surgical staff risk.

**Declaration.** The research for this study was done in partial fulfilment of the requirements for RW's PhD degree at the University of Cape Town.

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**Author contributions.** MH conceptualised the manuscript. RW extracted and prepared the data, accessed all databases, reviewed folders, prepared figures and wrote the manuscript. MH, ML, RDe, RDu, TH, WM, SR, GMcC, MN and SM performed critical revision of the manuscript. All authors read and approved the final manuscript.

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