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Evaluation of SARS-CoV-2 PCR Test Results During Hospital Admission and Positivity Rates in Pre-Operative Screenings

Hastaneye Başvurularda SARS-CoV-2 PCR Test Sonuçlarının ve Preoperatif Dönem Taramalarda Pozitiflik Oranlarının Değerlendirilmesi

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ABSTRACT

Objective: Following the emergence of the first cases in Wuhan, China, in late December 2019, the World Health Organization declared the Coronavirus Disease 2019 (COVID-19) a pandemic which affected the entire world and continues to have an impact. This research aimed to contribute to the epidemiological and present guideline data of the pandemic by evaluating the COVID-19 RT-PCR test results.

Material and Methods: The results of the SARS-CoV-2 RT-qPCR test, which were studied in the Microbiology Laboratory of the Izmir Katip Celebi University Ataturk Training and Research Hospital between 14.09.2020-30.06.2021, were retrospectively analyzed according to the months and reasons for admission(probable case, pre-operative screening).

Results: The total of probable cases and pre-operative tests was 140.249. The ratio of females to males in test requests was 48.4%/51.6%. The test request rates by age groups were 2.1%, 64.2%, and 33.7% for 0-18 years, 19-50 years, and 51-99 years, respectively. November 2020, December 2020, and April 2021 were the months with the highest number of test requests. The rates of positive results from probable cases and pre-operative tests examined in these months were 26.8%, 25.7%, and 16.5%, respectively. The test positivity rate for probable case testing was 19.3% (21.300/110.381), and the positivity rate for female and male cases was 49.5/50.5% (10.546/10.754). The test positivity rate in asymptomatic cases, tested pre-operatively per screening rules in the pre-operative period, was 4% (1.200/29.868), and the test positivity rate of pre-operative positivity for females and males was 51.6/48.4% (619/584) respectively. As a result of analyzing the ten-month period in our region, COVID-19 infection is more common in males and the age range of 19-50 years, and it also spreads faster in November and April.

Conclusion: An important point to be considered to prevent the spread of infection during the pandemic process is the follow-up of asymptomatic cases.

Keywords: *COVID-19, pandemic, pre-operative COVID-19 testing*

ÖZET

Amaç: Aralık 2019 sonlarında Çin'in Wuhan kentinde ilk vakaların ortaya çıkmasının ardından Dünya Sağlık Örgütü tarafından Coronavirüs Hastalığı 2019 (COVID-19) olarak adlandırılan ve tüm dünyayı etkisi altına alan pandemi, etkisini sürdürmeye devam ediyor. İzmir Kâtip Çelebi Üniversitesi Atatürk Eğitim ve Araştırma Hastanesi Tıbbi Mikrobiyoloji Laboratuvarına gönderilen nazofaringeal sürüntülerde çalışılan COVID-19 RT-PCR test sonuçları değerlendirilerek pandemi çalışmasına katkı sağlayacak epidemiyolojik ve güncel kılavuz verilerine katkı sağlanması amaçlanmıştır.

Gereç ve Yöntemler: İzmir Kâtip Çelebi Üniversitesi Atatürk Eğitim ve Araştırma Hastanesi Mikrobiyoloji Laboratuvarında 14.09.2020-30.06.2021 tarihleri arasında çalışılan SARS-CoV-2 RT-qPCR testi sonuçları aylara ve başvuru nedenlerine (olası vaka, ameliyat öncesi tarama) göre retrospektif olarak analiz edildi.

Bulgular: Olası vaka+preoperatif test sayısı 140.249 idi. Test çalışılan kadın/erkek oranı %48,4/%51,6 olarak tespit edildi. Yaş gruplarına göre başvuru oranları 0-18 yaş, 19-50 yaş, 51-99 yaş için sırasıyla %2,1, %64,2 ve %33,7 oldu. En fazla test istemi olan aylar ise Kasım 2020, Aralık 2020 ve Nisan 2021 oldu. Bu aylarda incelenen olası vaka+preoperatif test/pozitif sonuç oranları sırasıyla %26,8, %25,7 ve %16,5 oldu. Olası vaka testlerinde pozitiflik oranı %19,3 (21.300/110.381) ve kadın ve erkek vakalar için pozitiflik oranı %49.5/50.5 (10.546/10.754) idi. Ameliyat öncesi dönemde tarama kuralları gereğince test istenen asemptomatik olgularda pozitiflik oranları ameliyat öncesi dönemde %4 (1.200/29.868), kadın ve erkeklerde ameliyat öncesi pozitiflik oranı %51.6/48.4 (619/584) idi. Bölgemizde 10 aylık dönem incelendiğinde COVID-19 enfeksiyonunun erkeklerde ve 19-50 yaş aralığında daha sık görüldüğü, ayrıca Kasım ve Nisan aylarında daha hızlı yayıldığı görülmektedir.

Sonuç: Pandemi sürecinde enfeksiyonun yayılmasını önlemek için dikkat edilmesi gereken önemli bir nokta da asemptomatik vakaların takibidir.

Anahtar Kelimeler: *COVID-19, pandemi, preoperatif COVID-19 testi*

Introduction

The coronavirus is an enveloped, single-stranded, positive-sense RNA virus that causes disease by affecting the respiratory, digestive, and nervous systems (1,2). Currently, four coronavirus subgroups are defined as alpha, beta, gamma, and delta (3). Coronaviruses are widely distributed in many animal species, especially camels and bats, HCoV-229E and HCoV-OC43 species can cause respiratory infections in humans (3,4).

Two strains of coronaviruses were associated with fatal diseases until the COVID-19 pandemic emerged in December 2019. One of the zoonotic species is the causative agent of Severe Acute Respiratory Syndrome (SARS-CoV), and the other is Middle East Respiratory Syndrome (MERS-CoV) (5). Cases of severe respiratory syndrome were defined as related to SARS-CoV in China in 2002 and MERS-CoV in the Middle East in 2012 (6-8). Finally, SARS-CoV-2, a new strain of coronavirus, was identified in pneumonia cases in the Wuhan province of China in December 2019 (9). While the disease caused by SARS-CoV-2 was named COVID-19, the World Health Organization (WHO) declared the COVID-19 pandemic on March 11, 2020. By December 2022, over 654 million confirmed cases and over 6.66 million deaths had been reported worldwide, according to data from WHO (10). While the total number of cases in our country was nearly 15 million, more than 98 thousand deaths were recorded (11). For a definitive diagnosis of COVID-19, SARS-CoV-2 RNA assays have been developed using respiratory tract samples (12). Reverse transcription real-time polymerase chain reaction (RT-qPCR) is a primary method for diagnosis. This method can detect viral RNA in nasopharyngeal swabs and all upper respiratory tract specimens, starting three days before the onset of symptoms (13). Study protocols for molecular testing, appropriate kits, and evaluation steps were published by WHO and the Centers for Disease Control and Prevention (CDC) (14,15).

This study aimed to evaluate the epidemiologic features and laboratory findings of cases with probable clinical suspicion of COVID-19 and cases who applied to the COVID-19 pandemic unit of our hospital for pre-operative screening by months.

Material and Methods

The results of the SARS-CoV-2 RT-qPCR test, which were studied in the Microbiology Laboratory of Izmir Katip Celebi University Ataturk Training and Research Hospital between 14.09.2020-30.06.2021, were retrospectively analyzed according to the months and reasons for admission (probable case, pre-operative screening). The SARS-CoV-2 RT-qPCR test was studied using Rotor-Gene Q (Qiagen, USA) with Diagnovital SARS-CoV-2 Multiplex Real-Time PCR (RTA, Turkey) and Bio-speedy® SARS-CoV-2 Double Gene RT-qPCR (Bioeksen, Turkey) kit. This study was approved by the Izmir Katip Celebi University ethics committee of clinical research (August 26, 2021, Approval Number 0357) and conducted in accordance with the guidelines of the 1964 Helsinki Declaration.

Diagnovital SARS-CoV-2 Multiplex RT-PCR study protocol

In diagnosing SARS-CoV-2, a one-step RT-qPCR assay was used to detect the SARS-CoV-2 specific N and Orf1ab gene regions qualitatively. The kit is applied to nucleic acid extracts obtained from the nasopharyngeal swabs, oropharyngeal swabs, bronchoalveolar lavage, nasopharyngeal aspirate, saliva oral/saliva swab, and gargle samples. The total volume of Multiplex RT-qPCR was 20 µl and consisted of 15 µl SARS-CoV-2 multiplex mix, 1 µl enzyme mix, and 4 µl template RNA. Amplification protocol with Rotor-Gene Q (Qiagen, USA) thermal cycler was a cycle at 5 minutes for 50 °C, 5 minutes for 95 °C and 40 cycles at 95 °C for 5 seconds, 60 °C for 30 seconds. Following the manufacturer's recommendations, sigmoidal amplification curves and threshold cycle count (Cq) were reported as ≤38 Cq positive for the target gene region.

Bio-speedy SARS-CoV-2 Double Gene RT-qPCR study protocol

In diagnosing SARS-CoV-2, a one-step RT-qPCR assay was used to detect the SARS-CoV-2 specific N and Orf1ab gene regions qualitatively. The kit is applied to nucleic acid extracts obtained from the nasopharyngeal swabs, oropharyngeal swabs, bronchoalveolar lavage, nasopharyngeal aspirate, saliva oral/saliva swab, and gargle samples. The total volume of Multiplex RT-qPCR was 20 µl and consisted of 10 µl 2x Prime Script Mix, 5 µl CVD Di Oligo Mix, and 5 µl template RNA. Amplification protocol with Rotor-Gene Q (Qiagen, USA) thermal cycler was 1 cycle at 5 minutes for 52 °C, 10 seconds for 95 °C and 40 cycles at 95 °C for 10 seconds, 55 °C for 12 seconds. Following the manufacturer's recommendations, sigmoidal amplification curves and Cq were reported as ≤ 38 Cq positive for the target gene region.

Statistical analysis

The demographic data of a patient with SARS-CoV-2 PCR test results, admission in the presence of clinical symptoms, and pre-operative test request by month were the analyzed variables. Data were analyzed using SPSS V24.0 (Statistical Package for the Social Sciences, IBM. Corp. Armonk, NY, USA), and results are presented as numbers (n) and percentages (%). A p-value < 0.05 was considered statistically significant.

Results

The total number of SARS-CoV-2 PCR tests for all reasons of admission (probable case, pre-operative, public enterprise, travel, referee, and athlete screening) was 147,907 for the 10-month period. The number of probable cases and pre-operative screening tests was 140,249. The female/male test request ratio was 48.4%/51.6% (71,639/76,268). The difference was not statistically significant (p>0.05) (Figure 1). The months with the highest number of test requests were November 2020, December 2020, and April 2021 (**Figure 1**). The test positivity rates of the total probable cases and pre-operative assessments were 26.8%, 25.7%, and 16.5%, respectively, in these months.

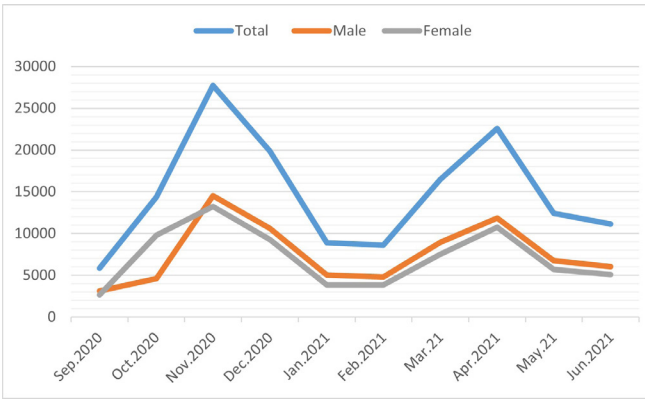

Figure 1. Distribution of SARS-CoV-2 PCR test numbers by months and gender

The distribution of all cases for which the SARS-CoV-2 PCR test was requested according to months and age groups is shown in **Figure 2**. Test request rates according to age groups were 2.1%, 64.2%, and 33.7% for 0-18, 19-50, and 51-99 years old, respectively (p<0.05).

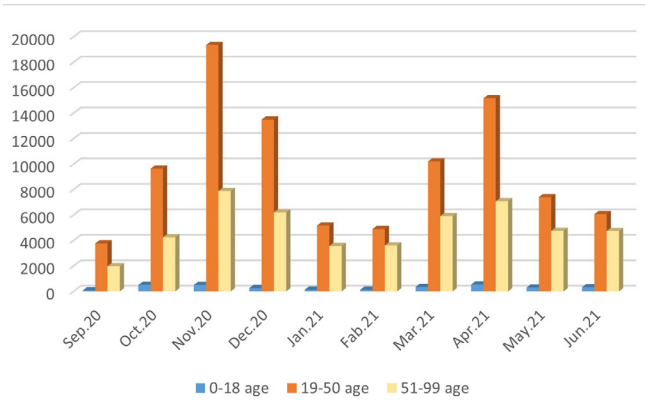

Figure 2. The distribution of all cases for which SARS-CoV-2 PCR test was requested according to months for age groups

The analysis of the SARS-CoV-2 PCR test results according to the month and reason for admissions is shown in **Table 1**. The distribution of SARS-CoV-2 PCR tests studied for probable cases and the positivity rates are shown in **Figure 3**. The positivity rate was 19.3% (21,300/110,381) and the rate of test positivity for females/males was 49.5/50.5% (10,546/10,754). There was no statistically significant difference in terms of gender (p>0.05).

Table 1. The analysis of the SARS-CoV-2 PCR test results by months according to the reason of admissions

	Probable Case		Pre-operative Screening	
	Positive n(%)	Total test n(%)	Positive n(%)	Total test n(%)
Sep.20	332	3527	17	1651
Oct.20	1208	8506	48	2737
Nov.20	6636	22725	286	3053
Dec.20	4808	17202	311	2667
Jan.21	1009	6355	203	2500
Feb.21	573	5619	102	2984
Mar.21	1704	11539	82	4015
Apr.21	3634	18937	100	3698
May.21	1083	8937	36	2727
Jun.21	313	7034	15	3836
Total	**21300 (19.3)**	**110381 (100)**	**1200(%4)**	**29868(100)**

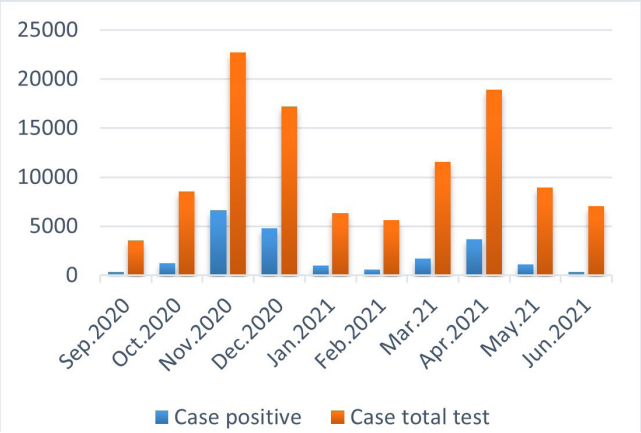

Figure 3. The distribution of SARS-CoV-2 PCR tests studied for probable cases and positivity rate

The positivity rate for pre-operative screening was 4% (1,200/29,868), and the positivity rate for female/male was 51.6%/48.4% (619/584) for pre-operative screening (p>0.05).

The number of tests in the 0-18 age group was 3,117, and the number of positive tests was 434 (13.9%). 49.5% (n= 215) of the positive results were male (**Figure 4**). There was no statistically significant difference in terms of gender under 18 years old (p>0.05).

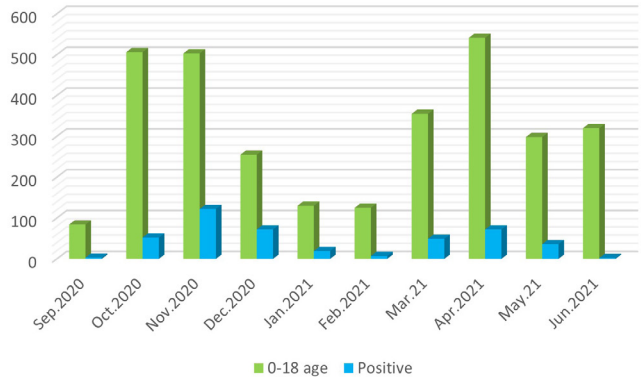

Figure 4. The number of tests in the 0-18 age group and distribution of positivity rates by months

Discussion

In general practice, the gold standard for diagnosing SARS-CoV-2, as in other viral infections, is to detect the causative agent. For this purpose, it may be possible to detect viral particles by electron microscopy and to use tissue cultures for in vitro viral replication. However, these diagnostic methods are not suitable for routine laboratory use because of biosafety levels. In diagnosing SARS-CoV-2 during the pandemic, RT-qPCR is used as a nucleic acid amplification method to identify the virus's genetic material (12). The viral load is highest in bronchoalveolar lavage, sputum, nasopharyngeal swab, and nasal swab, respectively (13).

In a meta-analysis investigating the rates of COVID-19 cases by gender, using data from 57 studies comprising 221,195 cases, the prevalence of males/females whose positivity was confirmed by PCR test was reported to be 55%/45% (16). In our study, the positivity rate for male/female cases was found to be 50.5%/49.5%.

Person-to-person transmission of SARS-CoV-2 occurs through direct contact with an infected person or inhalation of airborne droplets. The fact that the virus can be easily transmitted from person to person in this way during the pandemic makes it challenging to control the epidemic (17). The study conducted by Lavezzo et al. in the city of Vo, where 3,275 people live, showed that the spread of the virus could be effectively and quickly prevented by isolating the sick people early and implementing the quarantine procedure. The study, which included at least 71% of the population during the quarantine period, assessed the presence of SARS-CoV-2 using PCR testing and collected data on demographic characteristics and contact environment.

In this analysis, it was found that 43.2% of the cases diagnosed with COVID-19 and undergoing SARS-CoV-2 PCR

test at the initial and end of the 14-day quarantine period were asymptomatic. Contact tracing and chain of transmission screening studies have shown that newly detected cases are caused by contact with asymptomatic cases living in the same household. In a meta-analysis by Sah et al., more than 350 studies were evaluated and they found that 35.1% (95% CI: 30.7% to 39.9%) had SARS-CoV-2 PCR positivity but did not develop any clinical symptoms (18). While the average rate of asymptomatic cases among the elderly was 19.7%, this rate was 46.7% among children. In addition, cases with comorbidities were found much less likely to be asymptomatic.

The possibility of SARS-CoV-2 transmission to the healthcare workers on duty and the patients receiving treatment in clinics due to different health problems is of particular importance in the hospital environment. In order to maintain the reliability of the working environment of all healthcare workers and to protect other patients from COVID-19, individuals who are asymptomatic despite carrying SARS-CoV-2 should be identified by screening. For this purpose, pre-operative SARS-CoV-2 PCR testing is requested from all patients admitted to the hospital with an indication for surgery. According to the Ministry of Health data, as of 12 December 2020, 130,000 healthcare workers were infected with COVID-19 and 225 died in Turkey (19). In addition to the screening test requirement, guidelines to be used in emergency surgery cases were prepared and the patient decolonization process with chlorhexidine bath, chlorhexidine mouthwash, and nasal sprays before the operation was also recommended. The aim is to protect other patients and healthcare workers from the transmission of COVID-19 (20). According to Patkar et al., 200 (9.5%) of 2,108 asymptomatic patients were positive according to the results of the SARS-CoV-2 PCR test performed before the planned surgical procedure in cancer patients in India (21). This rate was found to be higher when compared to our data.

Colosimo et al. reported that 1451 (26%) pre-operative SARS-CoV-2 PCR tests were performed in surgical interventions (n=5,547) and 39 (2.7%) of them were positive in 8 hospitals in the state of Kansas in the first months of the pandemic in 2020 (22). This study showed that the need for intensive care, length of stay, and need for ventilators after surgery in positive cases were higher compared to the negative group. Pre-operative SARS-CoV-2 PCR test positivity for asymptomatic cases scheduled for surgery in Iowa was 0.35% (26/1,997) (23). In Germany, SARS-CoV-2 positivity was detected in 39 (0.5%) of 5,985 patients screened before surgery. Of these operations, 2,833 (37%) were emergency surgical procedures and 4,912 (63%) were elective surgical procedures. The SARS-CoV-2 PCR test was positive in 25 (0.9%) emergency operations and 14 (0.3%) elective surgeries (24). Our study's positivity rate for pre-operative screening was 4% (1,200/29,868), which is higher than other studies. According to Hoyos et al. study, the risks of contracting COVID-19 within 14 days following the surgery in cases undergoing thoracic surgery, they emphasized that if strict contact precautions and protective infection control protocols are not applied during the pre-operative stay in the hospital, the postoperative recovery process may be disrupted by various complications due to COVID-19 (25).

The data from different regions, the proportion of children among COVID-19 patients is quite low (2.4% in China, 1.3% in Italy, 2.8% in Australia, and 7.0% in Korea) (26). In SARS-CoV-2 PCR tests performed in children, 180/14,419 (1%) tests were found positive in probable cases who applied to a pediatric hospital in Australia in the early stages of the pandemic. In China, SARS-CoV-2 PCR positivity was 34.1% in 2,135 clinically suspected cases and 56.6% of these pediatric COVID-19 cases were male (27). In the first two weeks of the pandemic in Madrid, 11.2% of 365 pediatric patients screened for clinical suspicion were positive for SARS-CoV-2 PCR (28). Kaba et al. found 85/1,076 (7.9%) cases with PCR positivity in pediatric patients with suspected COVID-19 in a tertiary hospital in Istanbul (29). 49.4% of the cases were reported as boys. In our study, the PCR positivity rate was 13.9% and the male rate was 49.5% among the probable COVID-19 cases (n=3,117) included in the study over 10 months period in the pediatric population. It has been reported that 67.5% of pediatric patients who were COVID-19 confirmed by PCR in Egypt were male (30). In the study by Ennab et al., 53.2% of the children were males in COVID-19 cases (31).

According to an analysis performed on pediatric cases from 32 hospitals in Turkey, 50.3% of 1,156 pediatric COVID-19 patients confirmed by PCR test were male, with a mean age of 10.75 years (32). Out of the 237 symptomatic patients (median age 8.5) who applied to the pediatric emergency service in the first period of the pandemic in İzmir and underwent a SARS-CoV-2 PCR test, 45 (18.9%) were positive and 25 (55.0%) of them were girls (33). Studies have shown that the spread of the virus can be prevented effectively and quickly with the early isolation and quarantine process of individuals who do not show any signs of disease but carry the SARS-CoV-2. Community surveillance with improved screenings, early detection of the spread of SARS-CoV-2, and timely implementation of interventions are the keys to control (34). These methods are essential in reducing the economic and social burden of the COVID-19 pandemic on public health worldwide.

In the analyzes where approximately 396,000 cases of COVID-19 and 12,800 deaths were reported in the United States, regional differences were prominent in the same pe-

riod (35). Monitoring changes in reported case numbers and disease incidence over time is critical to understanding and responding to the evolving local epidemiology of this outbreak. COVID-19 modeling estimates suggest that monitoring and enrollment of infection could lead to significant reductions in hospitalization, critical care, and death rates. The effectiveness of these strategies in reducing infection rates and adverse outcomes depends on their timely implementation before high levels of community contamination are observed.

A clear picture of changing incidence will determine decisions regarding the correct implementation of collective living strategies, including social distancing, and the more appropriate use of resources such as human and capital that support healthcare infrastructure.

Study Limitations

The limitations of our study are its retrospective nature. Multi-center studies on a much larger number of cases will significantly contribute to this issue.

The positivity rate is important in individuals with symptoms who apply to the hospital. However, the presence of the virus in the asymptomatic population is not known unless PCR testing is performed. In this regard, pre-operative screening may guide the frequency of positivity in asymptomatic individuals.

Conclusion

The COVID-19 pandemic, which we have been experiencing in our country since March 2020, with all its negative effects on the health system and social life, continues. According to our monthly analyses, an increase in cases was detected with the arrival of the winter period, like other seasonal respiratory tract infections. However, it is predicted that we will be able to prevent the increase in the numbers in the winter period with careful compliance with the mask and hygiene rules. Vaccination is the most critical factor in ensuring herd immunity. In the near future, we must take the necessary precautions to overcome this difficult period and return to normal life before the pandemic.

Ethics Committee Approval: This study was approved by the Izmir Katip Celebi University ethics committee of clinical research (August 26, 2021, Approval Number 0357) and conducted in accordance with the guidelines of the 1964 Helsinki Declaration.

Data-sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Concept FBB and BOP; Design FBB and BOP; Supervision FBB and BOP; Materials FBB and BOP; Data Collection and/or Processing FBB, SK, BOP, TM, SGY, EU, EO, OUB, IA, AGKS, HE, NG, BP, RO; Analysis and/or Interpretation FBB; Literature Review FBB; Writing FBB and BOP; Critical Review FBB and BOP.

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