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Diagnosing COVID-19; towards a feasible COVID-19 rule-out protocol

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ABSTRACT

Introduction: We present the results of the COVID-19 rule-out protocol at Ghent University Hospital, a step-wise testing approach which included repeat NFS SARS-CoV-2 rRT-PCR, respiratory multiplex RT-PCR, low-dose chest CT and bronchoscopy with BAL to confirm or rule-out SARS-CoV-2 infection in patients admitted with symptoms suggestive of COVID-19.

Results: Between 19 March 2020 and 30 April 2020, 455 non-critically ill patients with symptoms suspect for COVID-19 were admitted. The initial NFS for SARS-CoV-2 rRT-PCR yielded 66.9%, the second NFS 25.4% and bronchoscopy with BAL 5.9% of total COVID-19 diagnoses. In the BAL fluid, other respiratory pathogens were detected in 65% (13/20) of the COVID-19 negative patients and only in 1/7 COVID-19 positive patients. Retrospective antibody testing at the time around BAL sampling showed a positive IgA or IgG in 42.9 % of the COVID-19 positive and 10.5% of the COVID-19 negative group. Follow-up serology showed 100% COVID-19 positivity in the COVID-19 positive group and 100% IgG negativity in the COVID-19 negative group.

Conclusion: In our experience, bronchoscopy with BAL can have an added value to rule-in or rule-out COVID-19 in patients with clinical and radiographical high-likelihood of COVID-19 and repeated negative NFS testing. Furthermore, culture and respiratory multiplex PCR on BAL fluid can aid to identify alternative microbial etiological agents in this group. Retrospective analysis of antibody development in this selected group of patients suggests that the implementation of serological assays in the routine testing protocol will decrease the need for invasive procedures like bronchoscopy.

KEYWORDS
COVID-19; SARS-CoV-2; rRT-PCR; diagnostics; bronchoalveolar lavage

Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, emerged in December 2019 in Wuhan, China and soon spread to a pandemic scale. People with COVID-19 may show a range of symptoms including, but not limited to, cough, shortness of breath, fever, chills, muscle pain and sore throat [1]. During the current SARS-CoV-2 pandemic, rapid and reliable diagnosis of COVID-19 is paramount for appropriate patient care and infection control. Real-time reverse transcription–polymerase chain reaction (rRT-PCR) is considered the gold standard to confirm clinical suspicion [2]. Upper-respiratory tract specimens are commonly used to confirm diagnosis; however, false negativity rates up to 40% have been reported [3]. Both pre-analytical factors such as sampling method, storage and transportation, as well as analytical factors such as kit performance contribute to these numbers [4]. Disease severity also impacts the time frame of viral shedding, with earlier viral clearance in the upper-respiratory tract in mild cases, compared to severe cases [5]. Lower-respiratory tract specimens, such as bronchoalveolar lavage (BAL) fluid, have shown the highest positivity rates of up to 93% [3,6] and prolonged viral shedding in critically ill patients compared to upper-respiratory-tract specimens [7]. Repeat testing of patients with an initially negative upper-respiratory tract rRT-PCR has been put forward as a way to deal with the diagnostic uncertainty imposed by the existing testing methods [8,9]. Also lower-respiratory tract sampling might help the diagnostic process in selected patients, however, due to its aerosol-generating properties, several interventional pneumology societies have dictated caution [10,11]. Bronchoscopy and BAL in the context of

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COVID-19 have therefore been reserved for patients with prior inconclusive COVID-19 testing, especially in the immunocompromised, patients with suspicion of concomitant bacterial or fungal infection, or in case of an urgent need for bronchoscopic intervention [12]. Despite these limitations, several groups have shown the presence of SARS-CoV-2 in BAL samples in patients in which viral RNA was not detected in the upper-respiratory tract [6,13–16]. The structural implementation of invasive lower-respiratory tract sampling in the diagnostic work-up of SARS-CoV-2 has been only reported sporadically [17,18]. This manuscript presents the results of a stepwise diagnostic testing protocol for COVID-19 at Ghent University Hospital during the first wave of the epidemic in Belgium.

Materials and methods

COVID-19 rule-out protocol

The COVID-19 rule-out protocol at Ghent University Hospital, a 1000+-bed tertiary care facility in Belgium, was developed as a stepwise approach to confirm or rule-out SARS-CoV-2 infection in patients admitted with symptoms suggestive of COVID-19 (Figure 1). The turntable of this testing strategy was the multidisciplinary case discussion (MDCD) held 3 times a day to categorize newly admitted patients into high or low probability, based on clinical presentation and imaging, in order to pro-actively determine which patients should evolve to the next testing step in case of a negative test result. The multidisciplinary team consisted of pulmonologists, infectious disease, internal medicine and intensive care consultants, radiologists and microbiologists. The SARS-CoV-2 rRT-PCR was considered gold-standard for diagnosis and available in-house since the beginning of the epidemic in Belgium, performed 3 times a day. All patients were tested with a nasopharyngeal swab (NFS). The median turn-around time between hospital admission and the result of the first NFS was 10 hours and 20 minutes. In patients with high clinical suspicion for COVID-19, but with a negative initial rRT-PCR on NFS, a repeat NFS for SARS-CoV-2 rRT-PCR, a NFS for respiratory multiplex PCR (run 3 times a week), and low-dose computed tomography (CT), the latter if not yet performed upon admission, were obtained subsequently. The median turn-around time between hospital admission and the second SARS-CoV-2 rRT-PCR result was 1 day, 4 hours and 14 minutes. In case of high clinical suspicion and suggestive imaging, but repeatedly negative rRT-PCR testing results, bronchoscopy with BAL was performed. Patients with pending test results were hospitalized in a COVID-19 transit unit. Depending on the severity, clinically stable patients were hospitalized on a regular ward, patients in need of more intensive monitoring were transferred to a dedicated respiratory intermediate care unit and critically ill patient necessitating more advanced support were transferred to the intensive care unit (ICU). All involved wards were structurally adapted to fulfill requirements regarding SARS-CoV-2 isolation.

Upper-respiratory tract sampling

All NFS samples were collected according to the WHO guidelines [2], by trained personnel.

Bronchoscopy with bronchoalveolar lavage

Bronchoscopy with BAL was performed bedside using a single-use disposable video bronchoscope. Bronchoscopy was only performed in hemodynamically and respiratory stable patients. In patients who were awake and breathing spontaneously, the procedure was only performed in case the oxygen need was less than 3 L/min in rest. Recommended personal protective equipment was used: full face mask, disposable surgical cap, medical protective mask (N95/FFP2/FFP3), work uniform, disposable medical protective gown, disposable gloves. Three to five aliquots of 20 mL sterile normal saline were instilled into the region of the lung with most aberrations on chest CT. Retrieval was done by suctioning of the scope. BAL fluid was sent for bacterial and fungal culture, acid-fast staining, mycobacterial culture, galactomannan antigen testing, respiratory pathogen multiplex RT-PCR and SARS CoV-2 rRT-PCR.

Multiplex RT-PCR for screening of respiratory pathogens

A respiratory multiplex RT-PCR (Seegene Allplex™ Respiratory Panel) was performed as described previously on nasopharyngeal swab and BAL fluid as integral part of the COVID-19 rule-out protocol [19]. The RT-PCR is designed for qualitative detection of the following respiratory pathogens: Adenovirus, Bocavirus, *Bordetella (para)pertussis, Chlamydophila pneumoniae*, Coronavirus (229E, NL63, OC43), Enterovirus, Influenza A virus (H1, H1pdm09, H3), Influenza B virus, Human metapneumovirus, *Mycoplasma pneumoniae*, Parainfluenza 1–4, Respiratory syncytial virus A and B, Rhinovirus, *Legionella pneumophila, Streptococcus pneumoniae* and *Haemophilus influenzae*.

SARS CoV-2 rRT-PCR

Real-time reverse transcription (rRT)-PCR for SARS CoV-2 was performed using an in-house PCR for E-gene (FAM) using primers described by Corman et al [20]. Nucleic acid extraction was performed automatically using NucliSENS Easymag (Biomérieux). Reverse transcription and amplification was performed using

Qiagen One-Step RT-PCR Kit (Qiagen, Hilden, Germany) using Dia ControlRNA (Diagenode, Belgium) (Cy5) as internal control. PCR was performed using a CFX96 real-time cycler and results were analysed with CFX software ((Bio-Rad, Hercules, CA, USA). Positivity was determined based on a cycle threshold (C_t) value below 42.

Chest CT scoring

Low-dose chest CT images at the time of hospital admission were scored retrospectively by a thoracic radiologist, who was blinded to disease outcome, according to the COVID-19 Reporting and Data System (CO-RADS) score [21] and CT severity score [22].

Antibody testing

Antibody testing was performed retrospectively on samples of the patients who underwent BAL with bronchoscopy using two commercially available ELISA (Enzyme-Linked Immuno Sorbent Assay) kits: Euroimmune Ig A and Euroimmune IgG (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany; # EI 2606–9601 A and EI 2668–9601 G, respectively). Both assays were performed on human plasma or serum, according to the manufacturer's instructions. These two tests, respectively, detect immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies against the SARS-CoV-2 spike protein subunit 1 (S1) [23].

Ethics committee

Ethical approval for this study was deemed unnecessary by the Ethics Committee of the Ghent University Hospital because of its retrospective nature, without deviation from standard of care.

Results

Between 19 March 2020 and 30 April 2020, a total of 455 non-critically ill patients with symptoms suspect for COVID-19 were admitted to the non-ICU COVID-19 transit ward of a tertiary care hospital in Belgium. All patients stayed at this dedicated COVID-19 transit unit until COVID-19 was either confirmed or ruled out. The initial SARS-CoV-2 PCR on NFS was positive in 79 cases (17%) and negative in 376 cases. In 148 cases with a negative initial test, but high clinical suspicion of COVID-19 as determined through MDCD (32% of total), a second NFS for SARS-CoV-2 PCR and respiratory pathogen multiplex PCR was performed, as well as a low-dose CT if not performed yet. Retesting resulted in additional SARS-CoV-2 PCR positivity in 30 cases. Of the 118 cases in which the second NFS was negative, chest CT was normal in 87 cases and suspect for COVID-19 in 31 patients, with characteristic features such as ground-glass opacities and multifocal consolidations. The latter group, accordingly to the stepwise approach, proceeded to bronchoscopy with BAL. In two patients the diagnosis of COVID-19 was eventually established through alternative techniques and in two patients BAL was considered clinically unfeasible. In 27 patients, BAL was performed to formally diagnose or rule out COVID-19 . BAL fluid yielded an additional seven cases of SARS-CoV-2 PCR positivity (Figure 1). In total, COVID-19 was confirmed in 118 cases between 19 March 2020 and 30 April 2020, which corresponds to 25.9% of all patients admitted with symptoms of upper- or lower-respiratory tract infection. The initial NFS yielded 66.9% of COVID-19 diagnoses, the second NFS yielded 25.4% of diagnoses and bronchoscopy with BAL contributed to 5.9% of the confirmed cases.

Demographic characteristics of the patients who underwent bronchoscopy with BAL are summarized in Table 1. The median time between onset of symptoms and the BAL procedure was 9 [IQR 6.5–12] days. In 7/27 (26.9%) patients SARS-CoV-2 rRT-PCR on BAL fluid was positive, confirming the diagnosis of COVID-19 with a median C_T of 32.1 [IQR 25.8–35.2]. In 20/27 (74.1%) cases SARS-CoV-2 rRT PCR was negative.

Besides COVID-19 positivity or negativity, culture and multiplex rRT-PCR of BAL fluid also gave information on the presence of other microorganisms in the respiratory tract (Figure 2). In the rRT-PCR positive group, only in 1 out 7 patients, another microorganism (*E. cloacae*+- in culture) was identified. In the rRT-PCR negative group, other respiratory pathogens were detected in 13 (65%) of 20 patients. Culture of BAL fluid revealed the following pathogens: *Klebsiella aerogenes* (n = 1), *Hemophilus influenzae* (n = 2), *Hafnia alvei* (n = 1) and *Escherichia coli* (n = 2). Respiratory multiplex PCR identified the following bacterial microorganisms: *Mycoplasma pneumoniae* (n = 1), *Streptococcus pneumoniae* (n = 3), *Hemophilus influenzae* (n = 3). In addition, respiratory multiplex PCR detected the following viruses: Human coronavirus OC43 (CoV-OC43) (n = 2), Human metapneumovirus (hMPV) (n = 1), Epstein-Barr virus (EBV) (n = 1) and Rhinovirus A/B/C (n = 1). One patient was diagnosed with *Pneumocystis jirovecii* pneumonia, based on an additional PCR.

Serology was not implemented in the original stepwise protocol as it was not available at the beginning of the pandemic but was implemented in the MDCD from the moment this was available. Serum samples taken around the time of BAL sampling were available for 26/27 (96.3%) patients and serology was performed retrospectively. In the rRT-PCR positive group, 2/7 patients had negative IgA and IgG, 2/7 patients had borderline IgA with negative IgG, 1/7 had positive IgA with borderline IgG and 2/7 patients had both positive IgA and IgG. In total 42.9% of the patients had a positive IgA or IgG at this timepoint. In the rRT-PCR negative group, 15/19

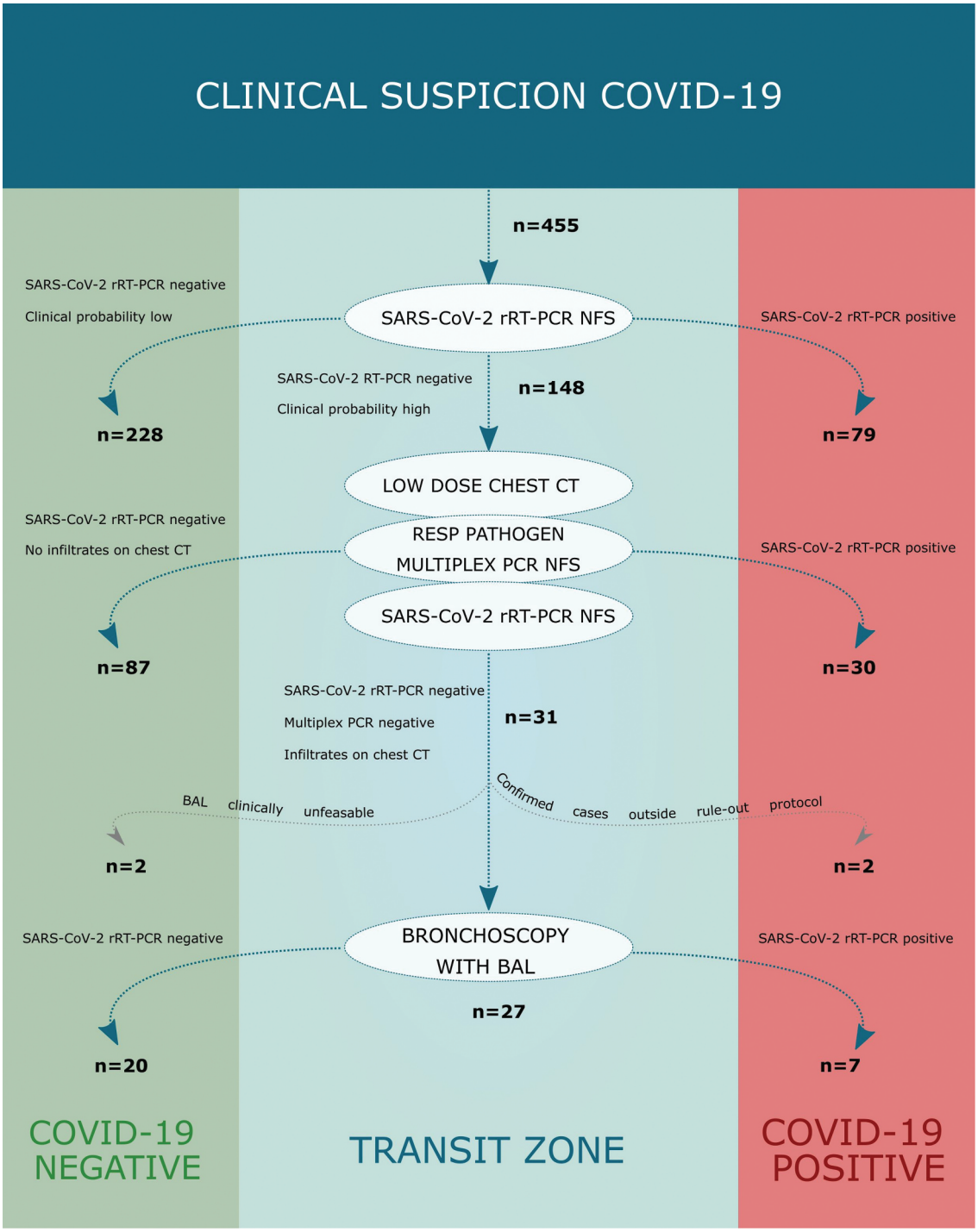

Figure 1. Flowchart of COVID-19 rule-out protocol. BAL clinically unfeasible: one patient already consented to advanced care planning during previous hospitalisations and opted for best supportive care, with a clinical presentation compatible with aspiration pneumonia. The other patient had negative SARS-CoV-2 serology and was previously diagnosed with pulmonary aspergillosis. Confirmed cases outside COVID-19 rule-out protocol: one patient was diagnosed with COVID-19 through a positive rRT-PCR on pleural fluid and another patient, not fit for bronchoscopy, through positive serology. COVID-19 = coronavirus disease 2019; SARS-CoV-2 = severe acute respiratory syndrome coronavirus; rRT-PCR = real-time reverse transcription polymerase chain reaction; NFS = nasopharyngeal swab, CT = computed tomography; BAL = bronchoalveolar lavage

had negative IgA and IgG, 1/19 had borderline results for IgA and 2/19 had positive IgA with negative IgG. In total 10.5% of the COVD-19 negative group had positive serology at this timepoint, but this was only based on IgA positivity. Follow-up serum samples, taken 17–40 days after onset of symptoms were available for 25/27 (92.6%) patients. In the rRT-PCR positive group, IgA and IgG became positive for all patients (7/7). In the rRT-PCR negative group, 15/18 patients had negative IgA and IgG at the follow-up timepoint and 3/18 patients kept a positive IgA with a negative, non-evolving IgG (Figure 3).

Table 1. Patient characteristics.

	Negative SARS-CoV-2 on BAL	Positive SARS-CoV-2 on BAL	Total
No. of patients	20	7	27
Sex			
Men	13 (65%)	3 (42.9%)	16 (59.3%)
Women	7 (35%)	4 (57.1%)	11 (40.7%)
Age (y)			
Mean	56.7	57	56.8
SD	13.3	12.9	13.3
Range	35–77	40–73	35–77
Symptoms			
Fever	17 (85%)	5 (71.43%)	22 (81.5%)
Cough	16 (80%)	6 (85%)	22 (81.5%)
Dyspnoea	15 (75%)	4 (57.1%)	19 (70.4%)
Fatigue	12 (60%)	7 (100%)	19 (70.4%)
Chest pain	8 (40%)	3 (42.9%)	11 (40.7%)
Myalgia	3 (15%)	2 (28.6%)	5 (18.5%)
Concomitant disease			
Any concomitant disease	16 (80%)	5 (71.4%)	21 (77.8%)
Hypertension	4 (20%)	3 (42.9%)	7 (25.9%)
Malignancy	7 (35%)	3 (42.9%)	10 (37%)
Chronic kidney disease	6 (30%)	1 (14.3%)	7 (25.9%)
Chronic cardiac disease	6 (30%)	0 (0%)	6 (22.2%)
Chronic pulmonary disease, not asthma	4 (20%)	2 (28.6%)	6 (22.2%)
Asthma	4 (20%)	1 (14.3%)	5 (18.5%)
CT imaging scoring			
CORADS score			
Mean	4	4.43	4.11
SD	0.65	0.53	0.64
CT-severity score			
Mean	10.65	10.43	10.59
SD	5.55	2.82	4.93
Findings on chest CT			
Ground glass opacities	20 (100%)	7 (100%)	27 (100%)
Consolidations	18 (90%)	7 (100%)	25 (92.6%)
Rounded consolidations	13(65%)	3 (42,86%)	16 (59.3%)
(Reversed) halo	14 (70%)	6 (85,71%)	20 (74%)
Vascular thickening	19 (95%)	7 (100%)	26 (96%)
Pleural effusion	6 (30%)	0 (0%)	6 (22%)
Lymphadenopathy	12 (60%)	5 (71,43%)	17 (63.0%)
Days since onset of symptoms			
Median	8	12	9
IQR	5–10,25	9–15	6.5–12

Discussion

Clinical decision-making based on COVID-19 rule-out protocol

The main aim of setting up a testing protocol that included repeated NFS and BAL sampling in case of a first negative PCR on NFS in highly suspect patients, was to identify COVID-19 patients as rapidly and accurately as possible and locate them to designated wards, in order to curtail in-hospital spread of SARS-CoV-2 infection in a high-incidence setting. Repeated testing can overcome the risk of false-negative test results due to variance in specimen collection and the dynamics of viral shedding, but should be balanced against laboratory capacity and scarcity of testing material, which was mainly an issue at the beginning of the pandemic [8]. Other groups have found the diagnostic yield of repeat short-term (within 24 hours) NFS-testing to be low [8]. However, in our experience, repeat NFS testing led to an additional 25.4% of positive diagnoses. In the group classified as negative for COVID-19, retrospective differentiation between true negative and false negative is difficult, since we do not have serological information in this group. However, low-dose chest CT was an integral part of the testing protocol and was performed in all patients with a high clinical likelihood of COVID-19 despite initial negative PCR. Chest CT has a reported sensitivity of 97% for the diagnosis of COVID-19 in hospitalised patients but lacks specificity. Therefore, in patients without CT-graphical anomalies, COVID-19 is much more unlikely [4]. No in-hospital transmissions were detected in the COVID-19 negative group as determined by the MDCD. Furthermore, the pro-active MDCD, organized 3 times a day, proved a very efficient strategy to ensure a fluent patient flow and avoid unnecessary delays or relocation of patients, since the next step in the diagnostic process was anticipated and decisions could be executed as soon after the results became available.

Role of BAL in COVID-19 diagnostic work-up

Whereas chest CT is very sensitive to diagnose COVID-19, it lacks specificity [4]. The presence of typical radiographic characteristics of COVID-19 such as ground-glass opacities with peripheral distribution [24] in patients with repeatedly negative NFS testing therefore poses a diagnostic dilemma. Especially in

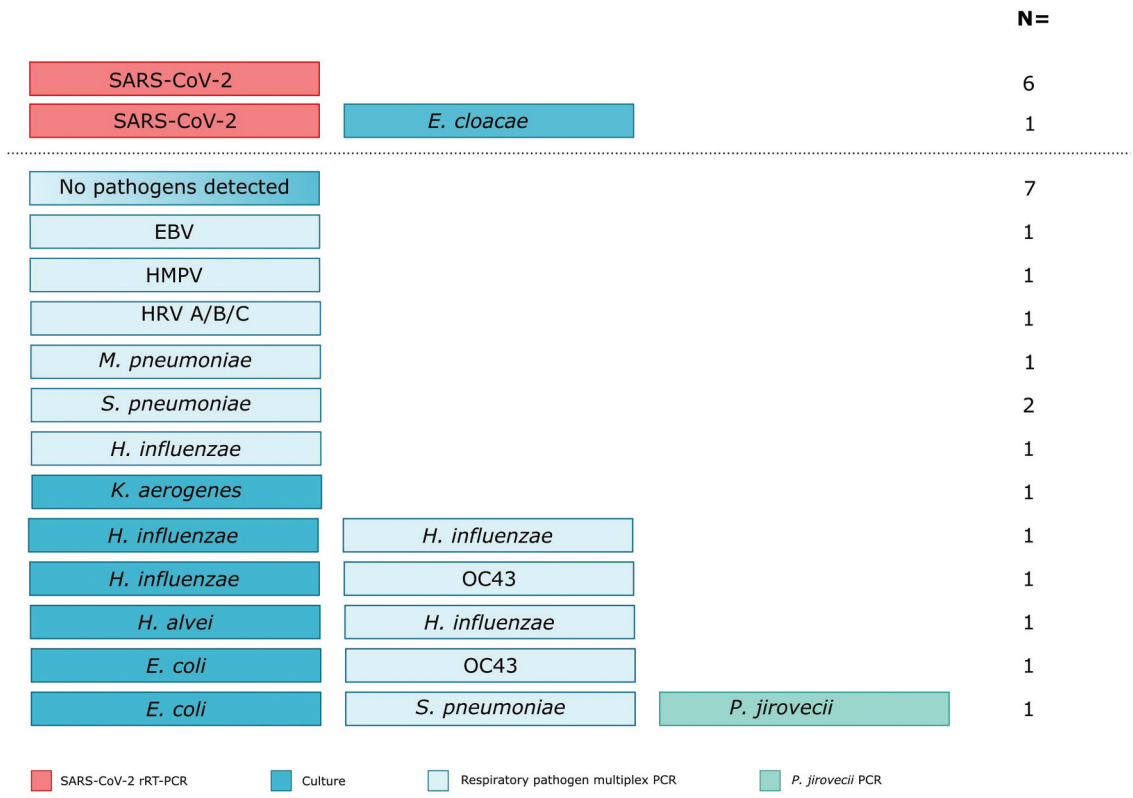

Figure 2. Microorganisms identified in BAL fluid. S
ARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; rRT-PCR = real-time reverse transcription polymerase chain reaction; *E. cloacae* = *Enterobacter cloacae*; EBV = Epstein-Barr virus; HMPV = Human metapneumo virus; HRV A/B/C = Human rhinovirus A/B/C, *M. pneumoniae* = *Mycoplasma pneumoniae*, *S. pneumoniae* = *Streptococcus pneumoniae*, *H. influenzae* = *Hemophilus influenzae*; *K. aerogenes* = *Klebsiella aerogenes*; OC43 = Human coronavirus OC43; *H. alvei* = *Hafnia alvei*; *E. coli* = *Escherichia coli*; *P. jirovecii* = *Pneumocystis jirovecii*

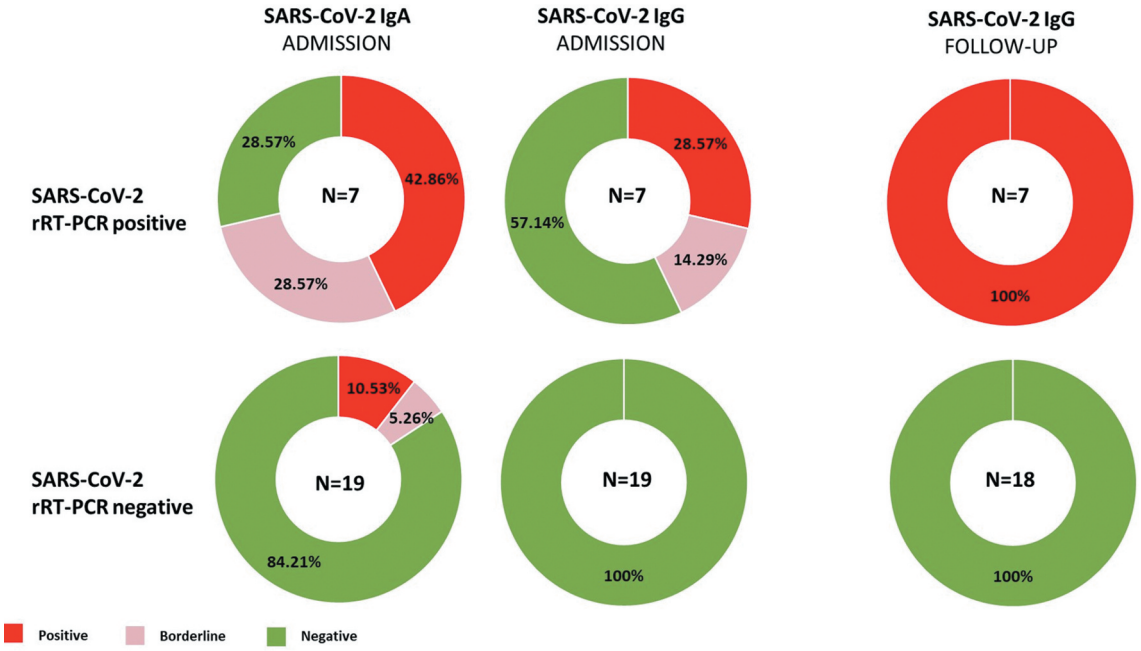

Figure 3. IgA and IgG antibody testing at hospital admission and during follow-up in patients with positive and negative SARS-CoV-2 rRT-PCR of BAL fluid. SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, rRT-PCR = real-time reverse transcription polymerase chain reaction; IgA = immunoglobulin A; IgG = immunoglobulin G

immunocompromised patients, a correct rule-in/rule-out approach is important. The radiographic characteristics of the patients in which bronchoscopy with BAL was performed, were very similar, with comparable CO-RADS and CT Severity Scores in both groups. The majority (77.8%) of the patients who underwent BAL sampling had a concomitant disease and 37% had an underlying malignancy. In this vulnerable population, a false-positive diagnosis solely based on low-dose chest CT, might lead to exposure to the virus by hospitalising the patient on a COVID-19 ward. In our cohort, bronchoscopy with BAL led to the diagnosis of COVID-19 in 7/27 (26.9%) of the patients. The careful selection of patients through multidisciplinary discussion might explain the higher number of positive cases detected with BAL compared to other BAL series published [17,18]. Timing of BAL could also explain the difference. Delay of bronchoscopy and decreased viral shedding with time could explain lower sensitivity. All COVID-19 patients diagnosed by rRT-PCR on BAL had C_T values >24 and had been symptomatic for 7 days or more (median 9) [25]. While rRT-PCR is the prime method for detection of viral RNA to confirm COVID-19 diagnosis, it does not reflect infectiousness of SARS-CoV-2. La Scola *et al*. suggested that C_T values above 33 correspond to non-infectious virus [26]. Another small-sized study by Bullard *et al*. only observed infectious virus in samples with rRT-PCR Ct values <24 and time since onset of symptoms <8 days [25]. This might raise the question whether patients in which rRT-PCR is only positive on BAL fluid and which have C_T values above 30, are still shedding infectious viral particles. Moreover, it is important to note that BAL fluid was always obtained from the lobe showing most radiographical aberrations. It is currently still unclear whether or not indeed more SARS-CoV-2 genetic material can be detected in these zones of ground-glass opacities.

Besides control of disease transmission, the accurate diagnosis of patients with COVID-19 is also important to tailor patient-specific care. As the scientific community is rapidly pushing forward the search for new therapeutic approaches to COVID-19, rapid identification of patients who might benefit from these therapeutics is paramount, especially since certain drugs will probably only be beneficial when administered within a specific timeframe. Besides the higher sensitivity of molecular SARS-CoV-2 detection on BAL fluid as compared to upper respiratory tract samples, other respiratory pathogens can be detected with greater sensitivity in BAL fluid as well. This approach proved in particular useful in patients with significant comorbidity, such as the immunocompromised. The use of multiplex nucleic acid amplification testing (NAAT) enhances the detection of both respiratory viruses, as well as bacteria [27]. In our cohort, BAL sampling provided an alternative microbial diagnosis in 63.2% of the rRT-PCR negative group. We found only 1 patient in whom both COVID-19 and a bacterial pathogen were identified at diagnosis. A necessary side-note to these results is the fact that neither culture, nor NAAT can distinguish between colonizing and invasive pathogens and critical clinical interpretation of the results is needed [27]. A recent meta-analysis found that bacterial co-infections occur in 7% of hospitalised COVID-19 patients, a number which increases to 14% if only patients admitted into the ICU are considered [28]. Kim *et al*. found co-infection between SARS-CoV-2 and other respiratory pathogens (including viral pathogens) in 20.7% of the study population[29]. Both seasonality and timing of bronchoscopy, are significant factors which might influence the pathogens identified. In general, it is important to note that the added value of performing BAL does not only lie within the diagnostic yield of a positive SARS-CoV -2 rRT-PCR, but also within the diagnostic value of a confirmed negative SARS-CoV-2 rRT PCR, as well as the many other respiratory pathogens that can be identified in BAL samples. Especially in high-risk patients, these are important factors to guide further clinical decisions.

Could implementation of antibody testing decrease diagnostic uncertainty?

Whereas the sensitivity of nucleic acid testing declines after the first week of COVID-19 symptoms, possibly resulting in false-negative test results, SARS-CoV-2 antibody testing becomes more sensitive in the course of the infection [30]. Serological testing was not part of the routine testing protocol, since it was not validated at the time of data collection. Retrospective Ig A and IgG antibody testing was performed on samples from the subgroup of patients who underwent bronchoscopy with BAL, to evaluate the added diagnostic value of serology in these specific cases. Our results show that IgA antibodies were present in almost half of the patients who were later confirmed to be positive by rRT-PCR on BAL. Follow-up serology showed development of IgG antibodies in all the rRT-PCR positive patients, despite a high inter-patient variability in timing of antibody development. In the rRT-PCR negative group, positivity for IgA was seen in 2 patients, but none of them developed IgG antibodies in the follow-up sample (Figure 2). Interestingly, in one of the patients with a positive IgA testing result, an infection with human coronavirus OC43 was identified, a cross-reactivity pattern which has been described [22]. The Euroimmun IgA and IgG ELISA tests have sensitivities of 90% and 65%, with specificity of 93% and 96%, respectively [31]. IgM antibodies have not been evaluated in this cohort, but recent findings have shown that combined rRT-PCR and IgM testing provides better sensitivity than rRT-PCR alone [30,32]. The use of serology as the sole diagnostic tool for COVID-19 is impractical because of its time- and host factor-dependent nature, however, our

results suggest that the combination of NAAT with serological testing, might reduce the need for invasive procedures like bronchoscopy with BAL.

Conclusion

Accurate and rapid diagnosis of COVID-19 is paramount in preventing in-hospital spread and tailoring patient care in the context of new therapeutic developments. The COVID-19 rule-out protocol and multidisciplinary approach that was used in Ghent University Hospital, was able to diagnose COVID-19 patients in a fast and efficient way. No nosocomial SARS-CoV-2 infections were noted during the studied timeframe. In our experience, bronchoscopy with BAL can have an added value to rule-in or rule-out COVID-19, as well as to identify alternative microbial etiological agents, in patients with clinical and radiographical high-likelihood of COVID-19 and repeated negative NFS testing. With the necessary safety measures in place and in well-identified patients, BAL can be performed safely, with no bronchoscopy-related adverse events or transmission towards involved staff reported in this cohort. Retrospective analysis of antibody development in this selected group of patients, suggests that the implementation of serological assays in the routine testing protocol will decrease the need for invasive procedures like bronchoscopy.

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