Severe cases of coronavirus disease 2019 (COVID-19) are usually accompanied by an exuberant immune response comparable to cytokine release syndrome (CRS), with markedly elevated serum levels of pro-inflammatory cytokines that are thought to be a major drivers of morbidity and mortality for these patients. Several drugs with anti-inflammatory properties (tocilizumab, situximab, sarilumab, anakinra, among others) have been suggested as adjuncts to supportive care in the management of COVID-19, and several clinical trials are underway (ClinicalTrials.gov Identifier: NCT04315298, NCT04317092, NCT04306705).

The Bruton tyrosine kinase inhibitors (BTKi) ibrutinib, acalabrutinib and zanubrutinib are commonly used to treat chronic lymphocytic leukaemia (CLL), Waldenström macroglobulinaemia (WM), and chronic graft-versus-host disease (GVHD) and have been shown to have potent anti-inflammatory effects resulting in decreased levels of pro-inflammatory cytokines that are commonly elevated in severe COVID-19. Furthermore, these drugs may abate some noxious pulmonary effects of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and other similar viruses, reducing the degree of lung injury and disease-related mortality. Clinical trials examining the potential benefit of BTKi in COVID-19 are underway (ClinicalTrials.gov Identifier: NCT04382586, NCT04346199).

A recent study described the outcomes of six patients with COVID-19 with WM receiving ibrutinib. Five of the six had mild symptoms, did not require hospitalisation, and recovered promptly. One of the six required hospitalisation and mechanical ventilation but eventually recovered fully. Acknowledging the limitations of this small study, the authors hypothesised that ibrutinib may protect against lung injury in patients infected with SARS-CoV-2, and therefore suggest BTKi continuation in patients with WM with COVID-19.

It is unclear if the proposed protective effect of BTKi applies to patients with CLL, who have immune deregulation secondary to the underlying disease process. Also, BTKi use in CLL is associated with increased risk of infection, especially viral. Considering the high prevalence of CLL, studying outcomes of BTKi in patients with CLL with COVID-19 and exploring whether to continue BTKi in this setting becomes highly relevant. We reviewed our institutional experience with this population, examining severity of disease and clinical outcomes. Our present study was approved by the Program for the Protection of Human Subjects at the Icahn School of Medicine at Mount Sinai.

Eight patients with CLL receiving a BTKi were hospitalised for COVID-19 within our healthcare system (seven ibrutinib, one acalabrutinib). The clinical characteristics of the patients are summarised in Table I. The median (range) age was 72 (49–88) years. BTKi was held in six of the eight patients (‘BTKi-held’) and continued in two (‘BTKi-cont’). Two of the eight patients in the ‘BTKi-held’ cohort developed severe respiratory failure and eventually died (Patient 6: ibrutinib for 3+ years, full dose of 420 mg daily, Patient 3: ibrutinib for <4 months, recommended reduced dose of 140 mg due to concomitant use of a strong cytochrome P450 3A4 [CYP3A4] inhibitor). All others had mild-to-moderate disease.

Notably, the two patients who continued on ibrutinib had short hospital stays, minimal oxygen requirements, and have...
Table I. Baseline characteristics of eight patients with B-cell diagnoses on BTKi with COVID-19 infection.

| Demographics | BTKi-held | BTKi-continued |
|--------------|-----------|----------------|
| **Patient**  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| **Age, years** | 72 | 67 | 88 | 49 | 72 | 80 | 56 | 75 |
| **Gender** | M | M | M | M | M | M | F | |
| **Race/ethnicity** | NHB | Unknown | NHW | NHW | NHW | NHW | Unknown | Unknown |
| **Comorbidities** | | |
| **Obesity [BMI > 30 kg/m²]** | N | Y | N | N | Y | N | Y | N |
| **Hypertension** | Y | Y | Y | N | Y | Y | N | N |
| **Diabetes** | N | Y | N | N | N | Y | N | N |
| **Hyperlipidaemia** | N | N | Y | N | N | N | N | N |
| **History of ASCVD** | Y | N | N | N | Y | N | N | N |

| CLL disease characteristics | BTKi | BTKi-continued |
|----------------------------|------|----------------|
| **Patient**  | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** |
| **BTKi** | Ibrutinib | Ibrutinib | Ibrutinib | Acalabrutinib | Ibrutinib | Ibrutinib | Ibrutinib | Ibrutinib |
| **Dose of BTKi, mg/day** | 140++ | 420 | 140* | 200 | 420 | 420 | 420 | 420 |
| **Time on BTKi, months** | NA | 18.9 | 3.8 | 16.1 | NA | 44.9 | 29.6 | 18.1 |
| **IVIG in last month** | NA | N | N | N | NA | NA | Y | N |
| **Normal IgG** | NA | NA | Y | NA | NA | NA | Y | N |

| COVID-19 presentation | BTKi-continued |
|-----------------------|----------------|
| **CLL Diagnosis to COVID-19 (months)** | 70-2 | 56-5 | 173-2 | 111-5 | NA | 68-5 | 78-7 | 106-9 |
| **Cough** | Y | Y | Y | Y | N | N | Y | N |
| **Fever** | Y | Y | Y | Y | N | Y | Y | N |
| **Dyspnea** | Y | Y | Y | N | N | N | N | Y |
| **Sore throat** | N | Y | N | N | N | N | N | Y |
| **Fatigue** | N | Y | N | N | N | N | N | Y |
| **Ageusia** | N | N | N | N | N | N | N | Y |
| **Anosmia** | N | N | N | N | N | N | N | Y |

| Data at COVID-19 diagnosis | BTKi-continued |
|----------------------------|----------------|
| **WBC, K/µl** | 4.9 | 10.9 | 28.1 | 8.3 | 7.3 | 9.5 | 34.0 | 7.2 |
| **ALC, K/µl** | 1.2 | 0.9 | 10.3 | 4.4 | 1.7 | 2.1 | 21.7 | 0.8 |
| **Hb, g/l** | 134 | 152 | 88 | 151 | 134 | 152 | 135 | 140 |
| **Platelet count, K/µl** | 145 | 170 | 167 | 150 | 91 | 181 | 207 | 151 |
| **C-reactive protein, mg/l** | 81-3 | 294 | 283-7 | 2-1 | 62-5 | 124-9 | 59-1 | 1-5 |
| **Ferritin, ng/ml** | 13,401 | 1,095 | 295 | NA | NA | 1,113 | 158 | NA |
| **D-dimer, mlFEU** | 3-0 | 5-63 | >20 | NA | NA | 3-08 | 0-4 | 0-28 |
| **IL-1β, pg/ml** | NA | 0-7 | 0-5 | NA | NA | NA | <0-3 | <0-3 |
| **IL-6, pg/ml** | NA | 87-3 | 53-0 | NA | NA | NA | 43-2 | 7-2 |
| **IL-8, pg/ml** | NA | 24-8 | 18-0 | NA | NA | NA | 46-7 | 15-4 |
| **TNF-α, pg/ml** | NA | 34-8 | 100-0 | NA | NA | NA | 22-0 | 16-7 |
| **Multifocal pneumonia** | No | No | No | No | Yes | Yes | No | No |

| COVID-19 outcomes | BTKi-continued |
|-------------------|----------------|
| **Hospitalisation** | Y | Y | Y | Y | Y | Y | Y | Y |
| **Length of stay, days** | 10 | 7 | 9 | 5 | 8 | 19 | 9 | 3 |
| **Max. oxygen requirement** | NC | NC | HFNC | None | None | BIPAP | NC | None |
| **COVID-19 treatment** | HC, AZ | HC | HC | AZ, TOCI | HC, AZ | HC, AZ | HC, AZ | None |
| **Death** | N | N | Y | N | N | Y | N | N |

M, male; F, female; NHB, non-hispanic black; NHW, non-hispanic white; ASCVD, atherosclerotic cardiovascular disease; CLL, chronic lymphocytic leukaemia; WM, Waldenström macroglobulinaemia; IVIG, intravenous immunoglobulin; IgG, immunoglobulin G; WBC, white blood cell count; ALC, absolute lymphocyte count; Hb, haemoglobin; IL, interleukin; TNF, tumor necrosis factor; NC, nasal cannula; HFNC, high-flow nasal cannula; HC, hydroxychloroquine; AZ, azithromycin; TOCI, tocilizumab; ST, steroids, NA, not available. *Dose-reduced due to concomitant use of strong a CYP3A4 inhibitor; ++Reason for dose reduction unclear.

Our observations support continuation of BTKi in patients with CLL throughout COVID-19 infection, as they may provide some protection against noxious viral effects. Our findings concur with Treon et al. in a cohort of patients with WM receiving ibrutinib. We acknowledge the limitations of our present study, namely its retrospective nature and small sample size. Further studies are needed to validate this proposed approach. The results of two clinical trials assessing the effect of zanubrutinib (NCT04382586) and acalabrutinib (NCT04346199) in hospitalised patients with COVID-19 will help clarify the role of BTKi in this setting.
since fully recovered. Neither of them developed significant adverse events (AEs) attributable to BTKi.

Discussion

The BTK pathway is critical to the production of multiple pro-inflammatory cytokines. Inhibition of BTK signalling results in decreased cytokine levels, with a subsequent anti-inflammatory effect. BTK signalling dysregulation in lung macrophages may be a key pathophysiological component of SARS-CoV-2-related lung injury. In mouse influenza models, use of BTKi successfully rescued mice from lethal acute lung injury. Therefore, BTK pathway inhibition is a promising target to lessen the exaggerated immune response of severe COVID-19 and its respiratory complications.

Recent studies show high severity of infection in patients with haematological malignancies who contract COVID-19. Despite the defects in immunity and subsequent infection risk in CLL, six of our eight patients had mild-to-moderate disease severity, minimal oxygen requirements, and short hospital stays. The ‘BTKi-cont’ cohort had particularly prompt recoveries. Apart from hydroxychloroquine, neither received other therapies that could have contributed to their favourable outcomes. Recognising the limitations of a small sample size, our present findings support those of Treon et al., suggesting that BTKi may indeed have protective effects against SARS-CoV-2 virulence.

The ‘BTKi-held’ cohort may still have experienced some benefit from being on BTKi at the time of infection. The half-life of ibrutinib and acalabrutinib with preserved renal function is only 4–7 h, but as BTKi bind covalently to BTK, pathway re-activation requires de novo enzymatic synthesis, which occurs at highly variable rates across patients. Some degree of BTK inhibition may therefore have persisted after drug clearance.

In a document released by the American Society of Hematology (ASH) to provide guidance on CLL management in patients with COVID-19, experts conclude there is insufficient data to determine whether to continue BTKi in this setting. However, they advise caution with abrupt discontinuation of ibrutinib, which has been associated with a form of withdrawal syndrome resulting in significant cytokine release.

BTKi therapy in CLL increases risk of infections, especially pneumonia. However, incidence of infection is highest in the first 6 months and then decreases over time. Furthermore, long-term BTKi therapy may allow for meaningful recovery of humoral immune function, ultimately leading to decreased infection rates.

No significant AEs attributable to BTKi were seen in the ‘BTKi-cont’ cohort (i.e., haemorrhage, atrial arrhythmias). However, neither of them received full-dose anti-coagulation, use of which is becoming increasingly common to target the pro-thrombotic effects of COVID-19. BTKi should be used cautiously in this setting.

Keywords: BTK inhibitors, CLL, COVID-19

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Since the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019, the coronavirus disease 2019 (COVID-19) has spread to most of the world. The understanding of the pathomechanism of SARS-CoV-2 is extremely limited.

SARS-CoV-2 is a member of β-coronavirus family, which includes SARS-CoV and Middle East respiratory syndrome (MERS)-CoV. Studies have found lymphocytes play a significant role in the anti-viral reaction against coronavirus. Lymphocytes, especially T cells, are also destroyed by the virus. The interaction between lymphocytes and SARS-CoV-2 in vivo is largely unknown. Epidemiological investigation has found blood lymphocytes are decreased in patients with COVID-19. As to lymphocyte subgroups, CD8+ T cells are decreased during the course of COVID-19, while the percentage of exhausted CD8+ T cells (NKG2A+ T cells) is increased. The mechanism underlying the lymphopenia in patients with COVID-19 is that lymphocytes, especially T cells, are attracted into the infection sites or are killed by the coronavirus; described in detail in a recent review. B cells are the source of antibodies to SARS-CoV-2 and are a subtype of lymphocyte, but the levels of circulating B cells, especially activated B cells or plasma cells, are unclear. One study found that B cells were decreased substantially in patients with severe COVID-19, while another study suggested the level was not changed.

High-fluorescent lymphocytes (HFLs) in the blood are cells related with activated B cells or plasma cells. HFLs can be easily counted by an automated haematology analyser as one of the parameters of a full blood count. In the present study, we conducted retrospective analyses of the HFLs counts of 111 patients with COVID-19. The HFLs levels of patients with COVID-19 were compared with those of healthy individuals. To our knowledge, this is the first study to count and compare the numbers of HFLs in patients with differing severity of COVID-19. The present study may provide insight into understanding the interaction of B cells with SARS-CoV-2 and a clue to monitoring disease severity in patients with COVID-19.

We retrospectively analysed the full blood count results of patients with COVID-19 admitted to Wuhan Union Hospital, China, from 29 January to 8 March 2020. The diagnosis of COVID-19 was based on the Guideline provided by the National Health Commission of China. Patients with COVID-19 were classified into ‘mild’ or ‘severe’ subgroups according to the Guideline. Severe cases were defined as having any of the following features: (i) respiratory rate ≥30 breaths/min, (ii) oxygen saturation at rest of ≤93%; (iii) ratio of arterial oxygen partial pressure to fractional inspired oxygen (PaO2/FiO2) ≤300; (iv) pulmonary imaging showing that patients’ lesions had increased >50% within 24–48 h. Cases with other than a severe condition were classified as ‘mild’. Full blood count results including HFLs count were performed using Sysmex XE-5000 automated cytometry (Sysmex, Kobe, Japan). The healthy controls were enrolled before the SARS-CoV-2 outbreak in late November 2019. Healthy controls were from regular physical examination groups. Subjects with a fever, cough, or diarrhoea symptoms were excluded. Subjects with any lung, heart, liver, kidney and other infectious diseases were also excluded. All the results for the full blood count, including HFLs counts, were retrospectively retrieved from the information-processing unit (IPU) of Sysmex XE-5000 automated haematology analyser.

The study was approved by the Ethics Committee of Wuhan Union Hospital. Non-normal data were described as medians with interquartile ranges. The Kruskal–Wallis test and Dunn’s multiple comparisons test were used to compare variables among groups. All statistical analyses were performed by GRAPHPAD PRISM version 8.0 (Graphpad Software Inc., La Jolla, CA, USA). A P < 0.05 was considered as statistically significant.

There were 111 patients with COVID-19 enrolled in the present study (Table I). All were from the epicentre of Wuhan city at the beginning of the COVID-19 pandemic and 46 (41.4%) were male. The median (range) overall age of the patients with COVID-19 was 48.6 (24–89) years. In all, 19 patients (17.1%) were classified into the ‘severe’ group and five (4.5%) of them died. Patients in the severe group were older than patients in