Haematological profile of hospitalised COVID-19 patients from a centre in Singapore

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Abstract

Background: Haematological markers such as absolute lymphopenia has been associated with severe COVID-19 infection. However, the described cohorts were generally unwell with a large proportion of patients requiring intensive care stay. It is uncertain if these markers apply to a population with less severe illness. We sought to describe the haematological profile of patients with mild disease with COVID-19 that were admitted to a single centre in Singapore.

Methods: We examined 554 consecutive PCR positive SARS-COV-2 patients who were admitted to a single tertiary healthcare institution from Feb 2020 to April 2020 2020. We examined patients based on their haematological profile based on full blood count obtained within 24h of presentation.

Results: Patients with pneumonia had higher neutrophil percentages (66.5±11.6 vs 55.2±12.6%, $p<0.001$), lower absolute lymphocyte count (1.5±1.1 vs 1.9±2.1 x109/L, $p<0.011$) and absolute eosinophil count (0.2±0.9 vs 0.7±1.8 x109/L, $p=0.002$). Platelet counts (210±56 vs 230±61, $p=0.020$) were slightly lower in the group with pneumonia. We did not demonstrate significant differences in the neutrophil-lymphocyte ratio, lymphocyte-monocyte ratio and platelet-lymphocyte ratio in patients with or without pneumonia. Sixty-eight patients (12.3%) had peripheral eosinophilia. This was more common in migrant workers living in dormitories.

Conclusion: Neutrophilia and lymphopenia were found to be markers associated with severe COVID-19 illness. We did not find that combined haematological parameters: NLR, MLR and PLR, had any association with disease severity in our cohort of patients with mild-moderate disease. Migrant workers living in dormitories had eosinophilia which may reflect concurrent chronic parasitic infection.

What's already known about this topic?

Haematological markers such as absolute lymphopenia and neutrophilia has been associated with severe COVID-19 infection.

What does this article add?

Other combined haematological parameters such as neutrophil-lymphocyte ratio, monocyte-lymphocyte ratio and platelet-lymphocyte ratios did not reflect any associations with disease severity in our cohort of patients with mild-moderate disease.

In populations with migrant workers such as ours, eosinophilia can reflect concurrent chronic parasitic infections

Introduction

Haematological abnormalities have been described in COVID-19 patients [1]. These patients demonstrate various degrees of leukopenia and lymphopenia. Haematological variations have also been shown to correlate with disease severity and prognosis. The existing literature suggests that neutrophilia and lymphopenia are typically seen in severe cases, and both are early prognosticators of severity. T cell counts (CD4, CD8) were also seen to be decreased in patients with severe disease [2]. The mechanisms behind lymphopenia are not presently fully elucidated, but Tavakolpour et al have postulated that the inflammatory cytokine storm (including elevated interleukin-6 levels) may be closely associated with lymphopenia [3].

Combined haematological parameters including neutrophil-lymphocyte ratio (NLR), monocyte-lymphocyte ratio (MLR) and platelet-lymphocyte ratio (PLR) of COVID-19 patients have been shown to be increased compared to healthy controls in several studies [4, 5]. In a study of 116 Chinese patients of which 23% were considered patients with severe disease (based on the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial version 7) definitions described below), Sun et al. noticed that NLR has greater prognostic value for severe disease than the other two combination parameters MLR and PLR [6].

Despite established haematological abnormalities particularly of the white blood cells, literature on eosinophils and their role in COVID-19 remains sparse. It has been postulated that eosinophils can exert potent proinflammatory effect, and are also implicated in immunoregulation and antiviral activity. In a study of 140 community-acquired COVID-19 infected patients, of which 58 of 140 (41%) were regarded as severe, from Wuhan, China, eosinopenia (defined as < 100 cells/mm^3) was associated with more severe COVID-19 infection, and acute respiratory deterioration [7].

In this study, we sought to profile patients with less severe COVID-19 illnesses and examine associations between their disease severity, sequelae and haematological characteristics.

Methods

In Singapore, all patients with COVID-19 were initially hospitalised as tertiary hospitals had been used as quarantine facilities at the start of the pandemic, prior to the construction of purpose-built community isolation facilities.

We examined the electronic medical records of 554 consecutive patients admitted to our institution from 23rd January 2020 to 30th April 2020 who were confirmed to have COVID-19 based on a positive polymerase chain reaction (PCR) test from a nasopharyngeal swab [8]. There were no patients excluded or lost to follow-up. Data collected for each patient included their demographic backgrounds, past medical history, and their presenting symptoms. The presenting day of illness was derived based on the number of days from symptom onset to the day of hospital admission.

All patients had a baseline haematological profile performed (full blood count examination, FBC) obtained within 24 hours of admission, and chest X-ray performed. We followed patients for clinical outcomes during the hospital admission, including data on patients who required intensive care, mechanical ventilation and

adverse clinical outcomes such as myocarditis/myocardial injury [9-11] and death. Persistent fever was defined as a fever (37.5 degree Celsius and above) lasting over a 72-hour period. Pneumonia was defined by the presence of radiographic evidence of infiltrates on plain chest radiograph or computed tomography (if performed).

We divided the study population based on the presence of pneumonia, and compared their baseline clinical and haematological profiles. We also compared patients based on the presence of peripheral eosinophilia (>0.5 x 10^9/L) on their initial FBC [12].

The severity of COVID-19 illness was defined based on the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 7) where mild cases were defined as cases where symptoms were mild and there was no evidence of pneumonia on chest radiography. Moderate cases were defined as cases with fever, respiratory symptoms and had radiological evidence of pneumonia. Severe cases were defined as cases with at least one of the three features of: tachypnoea with respiratory rate greater than 30, mean oxygen saturation less than 93%, fractional inspired oxygen less than 300mmHg [13].

Imported cases were defined by those who acquired COVID-19 overseas and returned to Singapore. Locally transmitted cases were defined as those who acquired COVID-19 within the community in Singapore and have not travelled overseas. Dormitory cases were defined as those who acquired COVID-19 as a consequence of living or working in foreign worker dormitories. All treatment with anti-viral drugs were done in consultation with the infectious diseases team and as part of a research trial protocol if the patients met eligibility criteria. All empiric treatment of eosinophilia with albendazole and ivermectin were done in consultation with the infectious disease team.

To compare these groups, Student's t-tests were used for continuous parameters and the data was presented in the form of means (±standard deviation). Categorical parameters were compared by Chi-squared tests, and the data was presented in frequencies and percentages. A p-value of less than 0.05 was considered significant. All data analysis was done on SPSS version 20.0 (SPSS, Inc., Chicago, Illinois). This study was approved by the hospital's institutional review board (National Healthcare Group (NHG) Domain Specific Review Board (DSRB) 2020/00545) prior to the conduct of the study. Data collected was anonymised and a waiver of informed consent had been obtained from the institutional review board.

Results

Demographics and clinical presentation

Of the 554 patients studied, 57 (10.3%) had radiological evidence of pneumonia. These patients tended to be older (49±13 vs 36±11 years, p<0.001) and were more likely to have other medical comorbidities such as hypertension (32.6% vs 9.8%, p< 001), hyperlipidaemia (33.3% vs 5.0%, p< 0.001), and diabetes mellitus (16.3 vs 1.1%, p<0.001). Patients with pneumonia were more likely to require oxygen supplementation (14.0% vs 1.6%, p<0.001), require intensive care (23.2% vs 1.2%, p<0.001) and mechanical ventilation (19.3% vs 1.0%, p<0.001) and receive treatment with lopinavir/ritonavir (26.3 vs 4.4%, p<0.001) and remdesivir (17.5% vs 1.6%, p<0.001). There were 2 deaths (0.5%) out of 554 patients, and both patients had pneumonia (Table 1).

Haematological profiles of patients with pneumonia

There was no significant difference in the total white cell count in patients with or without pneumonia. The absolute lymphocyte count (1.5±1.1 vs 1.9±2.1 $x10^9$/L, p<0.011), absolute eosinophil count (0.2±0.9 vs 0.7±1.8 $x10^9$/L, p=0.002), absolute monocyte count (0.6±0.3 vs 0.9±1.4 $x10^9$/L, p<0.001), absolute platelet counts were lower in COVID-19 patients with pneumonia (Table 1).

We did not find any significant differences in the neutrophil-lymphocyte ratio (NLR) (4.3±4.3 vs 3.0±7.2, p=0.194), lymphocyte-monocyte (LMR) ratio (2.9±1.9 vs 3.1±4.3, p=0.690) and platelet-lymphocyte ratio (PLR) (186.4±95.9 vs 161.2±334.5, p=0.576) between patients with or without pneumonia (Table 1).

Patients with and without eosinophilia

A total of 68 patients (12.3%) had eosinophilia based on their initial FBC

When compared to patients without eosinophilia, fewer patients with eosinophilia had pneumonia (1 (1.5%) vs 56 (11.5%); p=0.005). Patients with eosinophilia were younger (33.2 ±9.4 vs 37.4±11.9 years, p=0.006), and were largely Indian and Bangladeshi migrant workers living in dorms (n=62, 91.2%). Three patients with eosinophilia acquired COVID-19 through local transmission (n=3, 4.4%) and 3 via overseas exposure.

Patients with eosinophilia did not differ significantly based on their medical comorbidities each as hypertension, hyperlipidaemia or diabetes mellitus. None of the patients with eosinophilia reported having atopic conditions such as asthma and atopic dermatitis. The severity of illness did not differ significantly between the group with eosinophilia and the group without (Table 2).

Discussion

The main findings of this study are that patients with pneumonia were more likely to have neutrophilia and lymphopenia. We also found a substantial proportion of patients with a raised eosinophil count (12.3%).

The study finding of sicker patients having neutrophilia and lymphopenia corroborates with the findings of a meta-analysis by Henry B et al (4,969 patients

from 22 studies) studying haematological parameters in COVID-19 patients. In this meta-analysis, lymphopenia conferred more than a 3-fold increase in the odds of both severe and fatal COVID-19, and neutrophilia was associated with a more than 7-fold increase in odds for the same outcomes. [14]. Although the mechanisms for neutrophilia and lymphopenia are not completely elucidated, lymphopenia is thought to arise from direct cytopathic effects and increased apoptosis from deranged cytokine milieu, and results in maladaptive antiviral response, rendering the human host susceptible to severe hyperinflammatory immunopathology [15]. In mouse models of severe acute respiratory syndrome (SARS), CD4+ lymphopenia was shown to cause increased immune mediated pneumonitis [16]. Neutrophilia, as well as neutrophil infiltration in pulmonary capillaries, acute capillaritis with fibrin deposition, neutrophilic mucositis and extravasation of neutrophils into alveolar spaces, have been observed in autopsy studies of COVID-19 patients.

One of the forces of neutrophilic driven lung injury is postulated to be related to neutrophil extracellular traps (NETs) [17]. NETs are extracellular webs of chromatin, proteins, and oxidant enzymes which neutrophils release in order to contain infection. However, when dysregulated, NETs have the potential to propagate inflammation and microvascular thrombosis in the lungs of COVID-19 patients. Recent studies analysing the serum of COVID-19 patients found that COVID-19 sera could trigger otherwise healthy neutrophils to release NETs [18, 19].

Other markers of severity have emerged in literature [20, 21]; notably the neutrophil/lymphocyte ratio had garnered interest where a high NLR was associated with more severe disease. In this study, we were unable to demonstrate significant differences in the NLR between those with pneumonia and those without.

Abnormalities in eosinophil count in our cohort may be better attributed to chronic parasitic infections

Out of 554 patients, 68 (12.3%) patients in this study exhibited peripheral eosinophilia. These patients were younger (33.2 ±9.4 vs 37.4±11.9 years, p=0.006), and were predominantly Indian or Bangladeshi foreign migrant workers residing in dormitories. On account of cost limitations, they were not fully evaluated for the underlying cause of eosinophilia. Most patients came from countries where parasitic infections are endemic, and thus an assumption was made that the eosinophilia seen was likely due to asymptomatic chronic parasitic infection. These patients did not otherwise have or report atopic conditions which might otherwise have accounted for the rise in eosinophils. Given the eosinophilia was largely seen in this group of patients and not in others with mild infection, it is unlikely there was any association between the eosinophilia and their COVID-19 infection. Eleven patients were empirically treated with albendazole or Ivermectin on the assumption of chronic parasitic infection.

Overall, the evidence on the role of eosinopenia reflecting more severe disease is mixed. Henry et al., who had pooled data of four different studies of 347 patients [22], found very modest difference of eosinophil count in COVID-19 patients with severe illness compared to those with milder disease [weighted mean difference (WMD), -0.01×10^9/l; 95% confidence interval (95% CI), -0.02 to -0.01×10^9/l]. There was

notably high heterogeneity (I^2, 74.4%) amongst the pooled studies. Ghahramani et al. in a meta-analysis [23], examined the results of five different studies and found modest and only a marginally significant difference of eosinophil count in COVID-19 patients with severe illness compared to those with milder disease (WMD, -0.03×10^9/l; 95% CI, -0.05 to 0.00×10^9/l), reporting again very high heterogeneity (I^2, 86%). These meta-analyses suggest that the role of eosinophil count in predicting severe COVID-19 illness remains unclear and that further studies may be required.

Strengths and Limitations

Each patient's clinical progress was only evaluated within the hospital admission and we were not able to longitudinally examine patients for longer-term sequelae of the disease. We were not also able to review the effects after treatment with antiparasitic drugs to determine whether migrant workers with eosinophilia had a reduction in their eosinophils. Cohorts analysed in the available literature tend to have cohorts made up of patients with moderate to severe disease. In our study however most patients had mild to moderate disease, with only 11/554 patients (1.8%) with severe disease. This might have impacted the power of our study to validate the use of other haematological biomarkers of severity which were previously found to have clinical utility.

Conclusions

Consistent with other studies, neutrophilia and lymphopenia appear to be reliable markers associated with severe COVID-19 illness. We did not find that combined haematological parameters; NLR, MLR and PLR, had any association with disease severity in our cohort of patients with mild-moderate disease. We found that a substantial proportion of migrant worker living in dormitories had eosinophilia which we assumed to reflect the probability of concurrent chronic parasitic infection.

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