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Evidence of Abnormally Low Lymphocyte-To-Monocyte Ratio In Covid-19-Induced Severe Acute Respiratory Syndrome

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Abstract

It is known that lung injury due to COVID-19 responsible for the respiratory distress would mainly depend on host inflammatory response, depending on an excessive production of inflammatory cytokines, such as IL-6, and TNF-alpha, rather than direct virus-induced tissue damage, as well as for other forms of respiratory distress. Moreover, it is known that the inflammatory cytokines may induce profound changes in the behaviour of the hematic cells, namely neutrophils, monocytes and lymphocytes, with a following enhanced tissue infiltration by their inflammatory cells. According to the data available up to now, IL-6 and TNF-alpha would be the main cytokines involved in determining COVID-19-induced lung injury, as well as in other coronavirus infections, and most in general in all conditions of respiratory distress. Since it is known that the functionless of the whole immune system is namely depending on the interactions between lymphocyte and macrophage system, a preliminary study was planned to analyse the lymphocyte-to-monocyte ratio (LMR) in COVID-19 infective disease. The study included 17 consecutive patients, who underwent ventilator therapy for COVID-19-induced respiratory distress, and 100 healthy subjects, as a control group. Lymphocytopenia and monocytosis occurred in 14/17 (82%) and in 8/17(47%), respectively. Then, abnormally low values of LMR was found in 12/17 (71%) patients, and LMR men values observed in patients were significantly lower than in control ($P<0.001$). Therefore, by reflecting the interactions between lymphocyte and monocyte-macrophage systems, LMR could constitute a simple and less expensive biomarker to monitor the clinical evolution of COVID-19 infection.

Keywords
ARDS; COVID-19; Cytokines; Lymphocyte-to-monocyte ratio; Psychoneuroimmunology.

Introduction
Acute lung injury is characterized by non-cardiogenic edema, pulmonary inflammation and severe systemic hypoxemia. Acute respiratory distress syndrome (ARDS) is the severe form of acute lung injury [1]. Finally, severe acute respiratory syndrome (SARS) is a recently emerged infection disease caused by a novel coronavirus, which has been shown to be characterized by an altered cytokine secretion, mainly consisted of an abnormally high production of inflammatory cytokines, namely IL-6 [2] and TNF-alpha [3,4]. Then, the recent dramatic COVID-19-induced respiratory distress syndrome could be due an altered cytokine secretion comparable to that occurring in SARS due to coronavirus, which has appeared to mainly depend on an exaggerated TNF-alpha secretion [4]. Other studies would suggest a preferential involvement of IL-6 secretion in determining the occurrence of lung injury [2]. Finally, in experimental conditions other authors have observed an increased secretion of both IL-6 and TNF-alpha in SARS-coronavirus-induced SARS [5]. In addition, IL-18 would be also involved in the pathogenesis of ARDS [6]. Finally, the increase in IL-6 blood levels would be more important than the enhanced TNF-alpha secretion in the only case of septic shock, by representing a marker of its severity [7]. In any case, it has been shown that the anomalous secretion of inflammatory cytokines may allow an altered ratio among the different hematologic cells. In fact, an abnormally high neutrophil-to-lymphocyte ratio (NLR) has appeared to predict a worse prognosis in patients with ARDS [8]. However, from a clinical point of view, lymphocyte-to-monocyte ratio (LMR) has been proven to be a more adequate prognostic biomarker than NLR in most chronic inflammatory diseases, including metastatic cancer and autoimmune pathologies [9], even though its profile and prognostic significance in COD-19 induced SARS have still to be investigated and established. On these bases, a preliminary study was performed to evaluate LMR in patients suffering from COVID-19-related SARS.

Materials and Methods

The study included 17 consecutive patients (M/F; median age 63 years, range 33-76), who were hospitalized for COVID-19 infection, and who required ventilator treatment for interstitial pneumonia and important respiratory distress syndrome. In each patient, LMR and C-reactive protein (CRP) were evaluated before undergoing mechanic respiration. Normal values in our laboratory (95% confidence limits) were more than 1,000/m^3 for lymphocytes, less than 440/mm^3 for monocytes, more than 2.1 for LMR, and less than 0.5 mg/dL. The control group consisted of 100 age- and sex-matched healthy controls. NLR and platelet-to-lymphocyte ratio (PLR) were also detected, whose normal values were less than 4.2 and less than 240, respectively. Data were reported as mean +/- SE, and statistically analysed by the chi-square test and the Student's t test, as appropriate.

Results

CRP levels were abnormally high in all patients. Lymphocytopenia occurred in 14/17 (82%) patients. Moreover, an abnormally high monocyte count was found in 8/17 (47%) patients. Therefore, LMR values were abnormally low in 12/17 (71%) patients, and LMR mean values observed in COVID-19 patients were significantly lower than those found in the control group (2.7 +/- 0.6 vs 5.8 +/- 0.4, P<0.001). In any case, no significant negative correlation was observed between CRP blood levels and LMR values (r=-0.1). On the contrary, abnormally high NLR values occurred in 92% patients. Moreover, as far as platelet behaviour is concerned, platelet count was normal in 46%, abnormally high in 23%, and low in the remaining 31% patients, with an association between platelet decline and an increase in D-dimer blood levels. Then, an increase in PLR values occurred in only 38% patients. Finally, the mean values of both NLM and PLR were significantly higher in patients than in the healthy controls (P<0.001 and P<001, respectively). LMR, NLR and PLR mean values observed in patients and in controls are reported in Table 1.

Table 1: Mean values of lymphocyte-to-monocyte ratio (LMR), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLT) in healthy controls (n=100) and in patients with COVID-19-induced respiratory distress (n=17).

	LMR	NLR	PLR
CONTROL SUBJECTS	5.8 ± 0.4	3.3 ± 0.7	128 ± 23
PATIENTS	2.7 ± 0.6*	12.5 ± 2.8**	306 ± 56***
***P<0.001 vs controls; P<0.01 vs controls; P<0.05 vs controls.**			

Discussion

According to previous clinical investigations (1-6), the results of this preliminary study show that lymphocytopenia and neutrophilia represent the most evident hematic cell alterations occurring in patients with coronavirus-induced respiratory distress, as well in other forms of ARDS. Therefore, according to previous clinic investigations in other forms of ARDS (1-6), this preliminary study would confirm that COVID-19- induced acute respiratory syndrome may primarily depend on host excessive immune response rather than to be a direct effect of viral replication itself. In addition, this study shows that lymphocyte decline occurring in patients with COVID-19 induced respiratory distress is associated with a concomitant increase in monocyte count, with a following decrease in LMR values. Moreover, it is possible to suggest that COVID-19-related lymphocytopenia would be due to lymphocyte exit from blood circulation to infiltrate pulmonary tissue and to determine pulmonary damage-related respiratory distress rather than a diminished lymphocyte production. Therefore, LMR decline would constitute a more adequate clinical biomarker to monitor the evolution of COVID-19 infection and its prognosis with respect to other less specific parameters, such as NLR and PLR value. In any case, the important COVID-19-induced

changes in hematic cell population would depend on an altered secretion involving several cytokines, namely TNF-alpha and IL-6, whose secretion is promoted by IL-1 beta [9]. Then, further clinical studies will be required to establish whether COVID-19-induced respiratory distress may mainly depend on IL-6 or TNF-alpha enhanced endogenous secretion. Because of the reciprocal stimulation occurring between IL-6 and TNF-alpha [4,9], it is probable that COVID-19- induced SARS may depend on both IL-6 and TNF-alpha. In more detail, because of CRP hepatic production is under an IL-6-induced stimulation [9], the evidence of abnormally high CRP blood concentrations is obviously associated with an enhanced IL-6 secretion, whereas the increased TNF-alpha endogenous production would be mainly connected to the direct pulmonary tissue damage responsible for the severe respiratory distress, as observed in other experimental forms of ARDS [1-6]. On the other side, the measurement not only of the inflammatory cytokines, but also of the anti-inflammatory ones, namely IL-10 and TGF-beta, whose increase could protect against virus-induced inflammatory response, would have to be concomitantly clinically investigated. In any case, COVID-19 infection could obtain therapeutic benefits from both strategies carried out to counteract to control IL-6 activity by anti-IL-6 receptor monoclonal antibodies, such as tocilizumab, as well as pharmacological approaches to inhibit TNF-alpha secretion and activity, including infliximab, adalimumab and etanercept [4]. Moreover, it has to be considered that the recent advances in the area of the Psycho-neuro-endocrine-immunology (PNEI) have demonstrated that the endogenous cytokine secretion is physiologically under a neuroendocrine regulation, and in particular it has been shown that both pineal hormone melatonin [10] and cannabinoid agents [11] may inhibit the secretion of the inflammatory cytokines, including TNF-alpha and IL-6. In addition, the pineal hormone indole MLT has also appeared to reduce tissue damage induced by several virus species, including Ebola virus, and several virus infection responsible for brain inflammatory reactions occurring during the encephalitis induced by several viruses, such as West Nile virus (WN-25), Semiki Forest virus (SFV), Venezuelan equine encephalitis virus (VEEV), Eastern equine encephalitis virus (EEEV), Aleutian mink virus disease (AMVD), and probably coronavirus-induced infections themselves [12]. The apparently greater resistance of children, whose endogenous MLT production is very high [13], to COVID-19-induced tissue damage would also confirm the protective role of MLT against the viral infection. Therefore, the evaluation of the physiological light/dark rhythm of MLT [12,13] would also be included in the clinical evaluation of COVID-19 infected patients. Unfortunately, despite the well demonstrated potential efficacy of MLT as a new anti-viral agent, these evidences did not generate much scientific attraction, and at present no clinical study has been performed in humans. In any case, the neuroimmune approach of PNEI may constitute a new interesting strategy in the treatment of viral infection, including COVID-19 infection. Not only, but several plants may contribute to counteract virus-induced tissue damage itself. Lotus extracts, commonly used in the traditional Chinese Medicine, could be particularly effective in the control virus-induced tissue damage by down-regulating TNF-alpha secretion [14]. Then, the too low interest during the last past years in the clinical investigation of cytokine secretion and its

neuroendocrine regulation, which has been shown to be namely under a pineal central control [14,15], may be considered as one of the main causes responsible for the lack of the elaboration of the new science of the cytokines, and consequently of the evolution of Medicine itself.

Conclusion

The evaluation of LMR values, by monitoring their changes during the clinical course of the infective disease, could constitute a simple and less expensive biomarker to monitor the evolution of COVID-19 infection and its respiratory complications. Therefore at present the LMR value may be considered a clinical marker to show the severity of disease. Successive studies by monitoring the changes of LMR during the clinical cause of COVID-19 infection will be needed to confirm its importance in evidencing the severity of disease. In successive studies we will demonstrate the prognostic significance of LMR as a clinical index of the disease severity. Biological strategies able to modify this index value could become a possible therapy.

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