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LETTER TO THE EDITOR

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SARS-CoV-2 pneumonia and atypical lymphocyte morphology in pleural fluid

Dear Editors,

Atypical or reactive lymphocytes circulating in peripheral blood of some patients with COVID-19 infection (hereinafter named COVID-19 RL) have been reported in previous publications.[1-6] In a recent publication, these lymphocytes were correlated with the evolution and prognosis of the disease.[2] In this letter, we present the case of a patient with COVID-19 infection in which a pneumonia complicated with parapneumonic effusion was also demonstrated. Morphological characteristics of the COVID-19 RL found in the pleural fluid are described.

A 76 year-old woman with diabetes mellitus and stage IV breast carcinoma (diagnosed in 2012) was admitted to the Emergency Department of the Hospital for asthenia, vomiting and fever (37.5°C) in the last two weeks. White blood cell (WBC), red blood cell and platelet counts were normal. Differential WBC count showed high values of neutrophils (9.2 × 10^9/L) and low values of lymphocytes (0.5 × 10^9/L). Peripheral blood smear review showed low percentages of normal lymphocytes (5%). Biochemical blood tests showed high values of C-reactive protein (137 mg/L; normal <10), ferritin (2.06 nmol/L, normal<0.44), lactate dehydrogenase (352 U/L, normal <234) and D-dimer (3,200 ng/mL; normal <500) were found (see Table 1). Positive RT-PCR and chest X-ray examination confirmed the diagnosis of SARS-CoV-2 pneumonia, showing the later a right-sided alveolar consolidation and a large right-side pleural fluid collection. Two therapeutic thoracenteses were performed with complete drainage and removal of 500 and 700 mL of pleural fluid, respectively. A pleural fluid sample showed a total nucleated cell count of 830 /μL, as is shown in Table 1. After cytocentrifugation and May-Grünwald-Giemsa staining of pleural fluid sample, morphology of the cells was analysed. Most of the cells in the differential count corresponded to lymphocytes (74%). Among them, COVID-19 RL were the predominant (85%), showing a large-medium size, regular or kidney-shaped nucleus with a spongy chromatin pattern, usually with one nucleolus, with a distinct moderate to deep blue cytoplasm and occasional presence of small vacuoles. Nucleus showed occasionally an eccentric position (see Figure 1). Among all lymphoid cells, small lymphocytes were 10% and large granular lymphocytes were 6%. Malignant cells were absent.

Immunophenotyping of the pleural fluid lymphoid cells by flow cytometry showed high T cell counts (95%), alongside with low B cell counts (3%) and NK cells (2%). Within the T cell population, CD4+T

TABLE 1 Relevant laboratory parameters in pleural fluid and blood

	Result	Normal values
Pleural fluid results		
RBC (/μL)	**270**	-
Nucleated cells (/μL)	**830**	-
Neutrophils (%)	**24**	<1
Lymphocytes (%)	**74**	18-36
Mesothelial cells (%)	0	<2
Macrophages (%)	**2**	64-80
Glucose (mmol/L)	**6.2**	2.2-3.9
Total protein (g/L)	**40**	<30
LDH (U/L)	**1,002**	-
ADA (U/L)	**180**	<33
Blood cell counts		
RBC (x10^{12}/L)	3.90	3.80-4.80
Haemoglobin (g/L)	121	120-150
Haematocrit (L/L)	0.37	0.36-0.46
WBC (x10^9/L)	10.3	4.0-11.0
Neutrophils (x10^9/L)	**9.2**	2.0-7.0
Lymphocytes (x10^9/L)	**0.5**	0.9-4.5
Platelets (x10^9/L)	320	130-400
Blood biochemical tests		
Glucose (mmol/L)	**9.0**	3.6-6.1
BUN (mmol/L)	4.6	2.1-8.9
Creatinine (mmol/L)	0.05	0.03-0.11
GFR (mL/min/1.73 m^2)	>90	>90
LDH (U/L)	**352**	<234
CRP (mg/L)	**137**	<10
Procalcitonin (μg/L)	0.12	<0.50
Ferritin (nmol/L)	**1.17**	0.03-0.44
Cardiac troponin (ng/L)	5.1	<45.2
Coagulation results		
PT (seconds)	14.5	9.9-13.6
D-dimer (μg/L)	**3,200**	<500

Note: In bold, abnormal values.

Abbreviation: ADA, adenosine deaminase; BUN, blood urea nitrogen; CRP, C-reactive protein; GFR, glomerular filtration rate; LDH, lactate dehydrogenase; PT, prothrombin time; RBC, red blood cells; WBC, white blood cells.

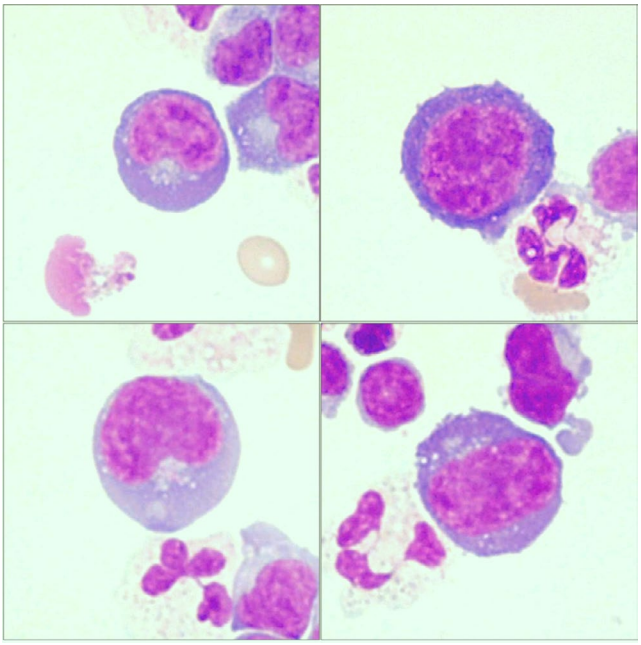

FIGURE 1 Atypical lymphoid cells found in the pleural fluid. Image obtained by cytocentrifugation and May-Grünwald-Giemsa staining (1,000 X)

cells were predominant (81% CD4+ T cells vs. 14.1% CD8+ T cells) and TCR expression showed almost exclusively TCRαβ+ (99.7%). Effector memory T cells represented half of the CD4+ T cells, while 50% of CD8+T cells were effector memory and TEMRA T cells, with similar activation levels within both populations. Comparing with cytological and immunophenotyping findings in the case presented herein, in a previous publication analysing a total of 20 Epstein-Barr virus (EBV) positive pleural effusions,[7] lymphocytosis along with a more polymorphous lymphoid population was found, only four cases showed RL (20%), being T cells 2% - 86%, B cells <0.1% - 15% and NK cells <1% - 16%.

Treatment with hydroxychloroquine, azithromycin, lopinavir/ritonavir and antibiotics (ceftriaxone and teicoplanin) was started. In addition, the patient received a single dose of tocilizumab. No recurrence of pleural effusion was observed and a favourable evolution of the infection was observed over the following days. Nevertheless, two months later she presented a progressive dysphagia and a metastasis of the breast carcinoma in the middle third of the oesophagus was detected by endoscopic biopsy, dying after a short period of time.

As was reported by Weinberg *et al*, atypical lymphocytes in SARS-CoV-2 infection are likely reactive to the virus and, in contrast to the classical Downey type II reactive lymphocytes commonly seen in other viral infections such as Epstein-Barr virus, the morphology of these COVID-19 RL shows larger size, with a nucleolus and abundant deeply basophilic cytoplasm.[1,2,5] To our knowledge, this is the first morphological description of COVID-19 RL in the pleural fluid of a patient with SARS-CoV-2 pneumonia complicated with parapneumonic effusion.

Literature review reveals that prevalence of pleural effusion can be as high as 14% related to SARS-CoV-2 pneumonia.[8-12] In a previous work, the incidence of pleural effusion in patients with acute viral infection increased from 8% incidence to 18% when pleural ultrasonography was used as a screening tool.[13] It would be convenient to discard a parapneumonic effusion in patients with suspicion of SARS-CoV-2 pneumonia through imaging techniques complementary to chest X-ray to improve their management.

Pleural fluid analysis in this patient showed an exudative effusion with high lymphocyte count. In contrast with the low percentages of COVID-19 RL found circulating in peripheral blood,[1,2] these lymphocytes were the predominant cells in the pleural fluid of the case presented herein. Previous immunophenotypic studies revealed that presence of COVID-19 RL suggests an abundant production of *virus-specific T cells*, thus explaining the better outcome of patients showing these cells circulating in blood.[2] Although our patient showed a good evolution of the infection, she died because of the immediate progression of her malignant disease.

KEYWORDS

blood, laboratory practice, morphology, T cells

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CONFLICTS OF INTEREST

Authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Anna Merino: designed the study and wrote the manuscript. Javier Laguna and Angel Molina: collected and analysed the data. Alexandru Vlagea: performed the immunophenotypic study. Oriol Sibila: performed the clinical evaluation and patient management.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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