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Original Article

The effect of inflammatory biomarkers on COVID-19 patients with diabetes and comorbidities

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Abstract

The Beta variant Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused a global health emergency, leading to pulmonary lesions and aggravation with preexisting cardiovascular diseases (CVD) and risk factors. A transversal retrospective study has been conducted at the infectious diseases ward of Tlemcen University Hospital Centre (CHU). The survey was conducted on 482 patients divided into 4 groups linked to preexisting cardiovascular diseases and diabetes. The data were collected from the medical records from March 2020–April 2022. The study revealed a high prevalence in patients with cardiovascular comorbidities (68%), including 37% with diabetes and 69% with hypertension. The clinical and biological evaluation of these patients found clearly worse results with a high mortality rate (34%). In the frame of the biological evaluation, we have noticed an unfavorable evolution for patients with cardiovascular disease history (+). The key parameter of the monitoring is oxygen saturation (SpO_2), as the results indicated that persisting hypoxemia in hospitalized patients correlated significantly with C-reactive Protein (CRP) levels (p=0.04) and lymphocytes (p=0.009). Regarding inflammation, CRP levels were higher, exceeding 40 mg/L in subjects with a cardiovascular disease history (CDH+). Furthermore, the results: platelets (<100000/mm^3 in patients with diabetes), fibrinogen (>6 g/L), and D-dimer (>1.5 µg/mL) confirmed high thrombotic risk in patients suffering from CVD regardless of diabetes. This study supports the hypothesis that cardiovascular comorbidities are contaminating and aggravating factors for the COVID-19 condition.

Keywords: Cardiovascular Diseases (CVD), COVID-19, SpO_2, CRP, D-dimer, Fibrinogen.

Introduction

Coronavirus disease 2019 (COVID-19) is a contagious disease that originated and was identified in China in 2019; the disease quickly spread worldwide, resulting in the COVID-19 pandemic and causing the death of 5.4 million people. COVID-19 is a pneumonia caused by SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), which followed the initial animal-to-human spillover through human-to-human transmission. This pathogen involves continual adaptation to humans through specific mutations that modify the virus pathogen to evade the immune system, leading to several variants [1].

SARS-CoV-2 has a devastating effect on human health, causing severe pneumonia and bronchitis. This

is caused by the binding of the spike (S) protein and angiotensin-converting enzyme 2, which is highly expressed in the lungs and heart [2].

The disease may cause cardiovascular lesions due to increased ACE2 receptors (Angiotensin-Converting Enzyme 2) in the cardiovascular system, leading to lung injury and thrombotic events. Cardiovascular diseases, including coronary syndrome and heart failure, hypertension and diabetes, are common pathologies in the elderly, which lead to a higher risk of developing critical or fatal COVID-19 disease. Cardiovascular diseases are the leading cause of death globally, and an estimated 17.7 million people died from CVDs in 2015. Of these deaths, an estimated 7.4 million were due to coronary heart disease, and 6.7 million were due to stroke. Cardiovascular diseases are a group of disorders that include damage to the heart muscle and vascular system that supplies the heart, brain, and other vital organs [3].

In COVID-19 patients, the virus enters the cell via ACE2 receptors, limiting the expression, i.e. ACE1 (Angiotensin-Converting Enzyme 1)/ACE2 imbalance in favor of ACE1 and high angiotensin II. The patients are at a high risk of developing complications. SARS-CoV-2 mechanisms trigger the dysregulation of the host immune response and an elevated release of pro-inflammatory cytokines [4].

As the impact of these preexisting comorbidities on the disease's development is unclear, this study aims to evaluate the effects of cardiovascular diseases on the development of COVID-19. This has been achieved by estimating several biomarkers, and this work is based on evaluating the most relevant.

Material and methods

A transversal retrospective study has been conducted at the infectious diseases ward of Tlemcen University Hospital Centre (CHU). The data were collected from the medical records from March 2020–April 2022. The survey was carried out on 482 patients suffering from COVID-19 or associated with cardiovascular morbidities, 299 men (62%) and 183 women (38%), with a ratio Man/Women of 1.633, the majority of women were at menopause (33%). Our population is divided into 4 groups: G1: patients without cardiovascular history (CDH-/D-) and without diabetes; G2: patients without cardiovascular history (CDH-/D+) and with diabetes; G3: patients with cardiovascular history (CDH+/D-) and without diabetes, G4: patients with cardiovascular history (CDH+/D+) and with diabetes.

Inclusion criteria: Patients involved in this work are those with cardiovascular diseases (hypertension, MI, heart failure etc.), diabetes, diabetes, and supposedly healthy subjects without any comorbidity.

Exclusion criteria: Patients with a history apart from cardiovascular disease and diabetes are excluded.

During the pandemic, COVID-19 was diagnosed using several methods, including radiology (a CT scan) and biological tests (PCR—serological and antigenic tests).

Most patients (56%) left the hospital after one week. However, the patients with diabetes (49%) were hospitalized for 15 days or longer. This research did not contain any studies involving animal or human participants, nor did it take place in any private or protected areas. No specific permissions were required for corresponding locations. This work included a retrospective study of hospitalized or dead patients in the infectious diseases Center at the CHU of Tlemcen (ALGERIA).

Statistical analysis

SPSS version 17.0 has been utilized to conduct statistical analyses of the results acquired in the current research. The results were statistically significant at 5% probability ($p<0.05$).

Results

Oxygen saturation

The oxygen level of a normal saturation is between 95% and 100%. The comparison of SpO_2 results between the four waves shows that there is a desaturation in the study population between 80% and 90% in waves 1, 3 and 4, mainly in diabetics and patients with cardiovascular history (CDH+/D+) (Figure 1).

Lymphocytes

Lymphocytes provide an immune defense barrier for the body against infectious attacks, mainly through B cells and T cells. It should be noted that most patients have a phase of lymphocyte depletion prior to lymphocyte normalization, except for people with diabetes who have severe lymphopenia (<1000/mm^3). The representative graph shows a decrease in lymphocytes during the 4 waves (200 cells/mm^3), and lymphopenia suddenly appears in people with diabetes (D+) during the third wave (Figure 2A).

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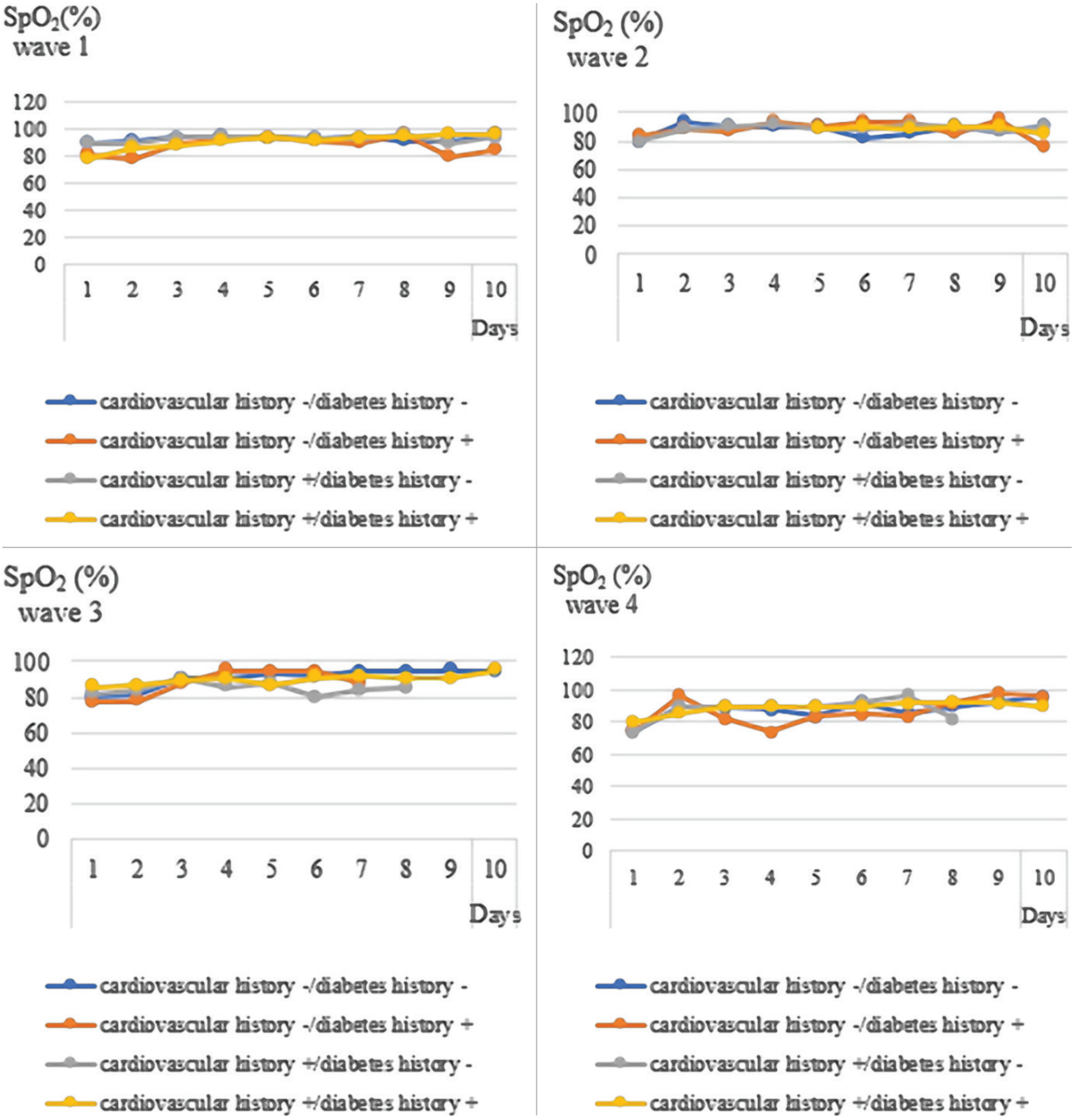

Figure 1: Evolution of lymphocytes in hospitalized patients during the waves. A significant decrease in lymphocytes in all patients is noted, especially in people with diabetes. Patients without cardiovascular history -/and without diabetes history -: CDH -/D -; Patients without cardiovascular history -/and with diabetes history +: CDH -/D +; Patients with cardiovascular history+/and without diabetes history -: CDH +/D -; Patients without cardiovascular history +/and with diabetes history +: CDH +/D +.

Platelets

Platelets are the thrombocytes circulating in the blood, they play an essential role in certain stages of coagulation. Normal values are 150,000–45,000,000/mm^3. The number of platelets decreases modestly, except in diabetics (CDH-/D+), who develop thrombocytopenia below 100,000/mm^3 (Figure 2B).

C-reactive protein (CRP)

CRP is an inflammatory marker for assessing the inflammatory state of a human body inflammatory state of a human organism. Normal values are below 0.6 mg/L. CRP values of around 200 mg/dL on the first day of hospitalization were noted during the 4 waves, which decreased in the entire population but remained

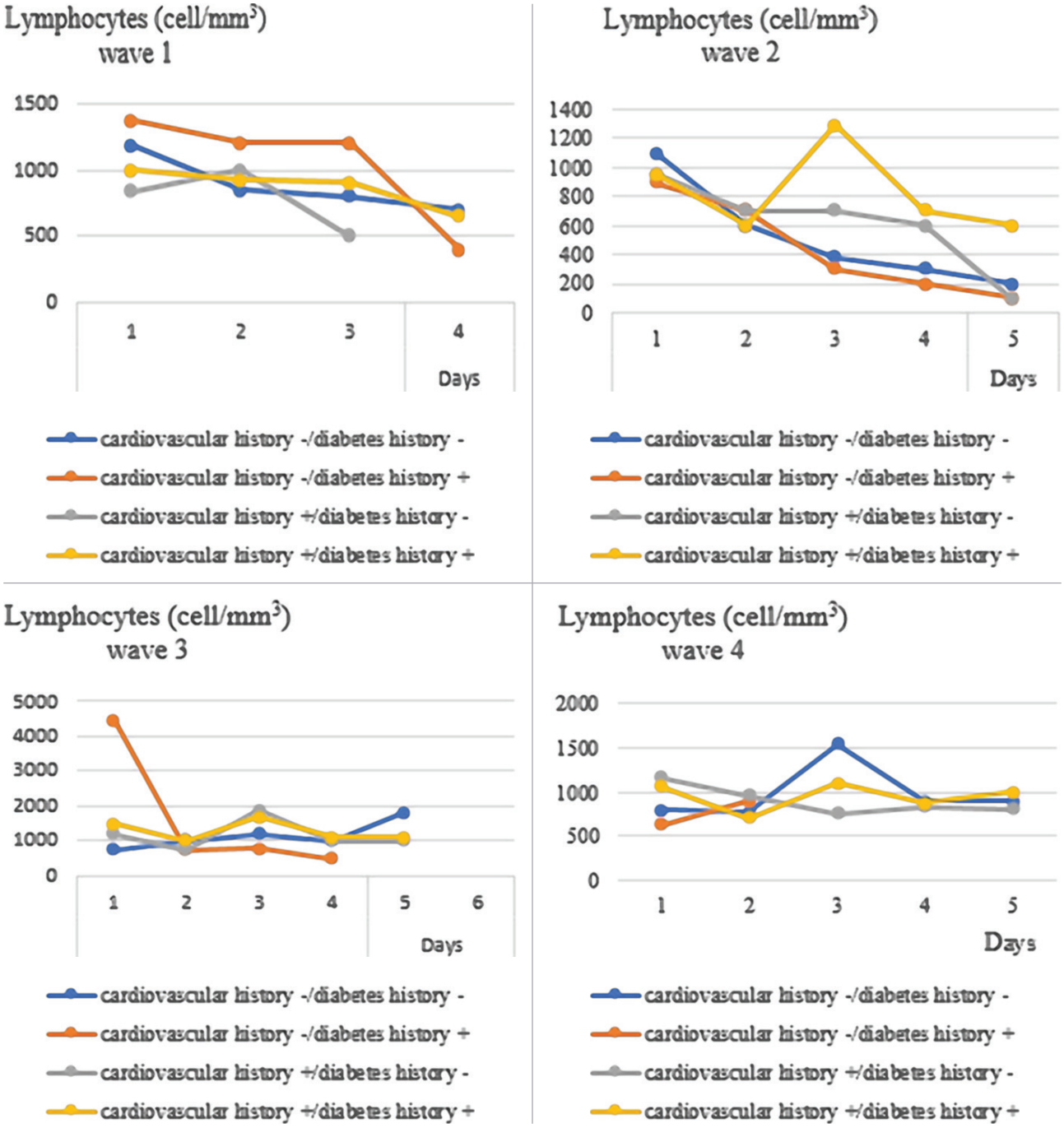

Figure 2A: Evolution of lymphocytes in hospitalized patients during the waves. A significant decrease in lymphocytes in all patients is noted, especially in people with diabetes. Patients without cardiovascular history -/and without diabetes history -: CDH -/D -; Patients without cardiovascular history -/and with diabetes history +: CDH -/D +; Patients with cardiovascular history+/and without diabetes history -: CDH +/D -; Patients without cardiovascular history +/and with diabetes history +: CDH +/D +.

high in patients with cardiovascular comorbidities (CDH+/D-), in the 1st, third and fourth waves (Figure 3).

D-dimers

D-dimers are products of fibrin breakdown during fibrinolysis. They are involved in the coagulation process. The standards are <0.5 µg/mL. Increased D-dimer levels were reported in normal subjects (CDH-/D-) during the third wave (>5 µg/mL) (Figure 4).

Fibrinogen

Fibrinogen is the coagulation factor I. It is a plasma protein that, under the action of thrombin, is transformed into fibrin to ensure hemostasis. The normal

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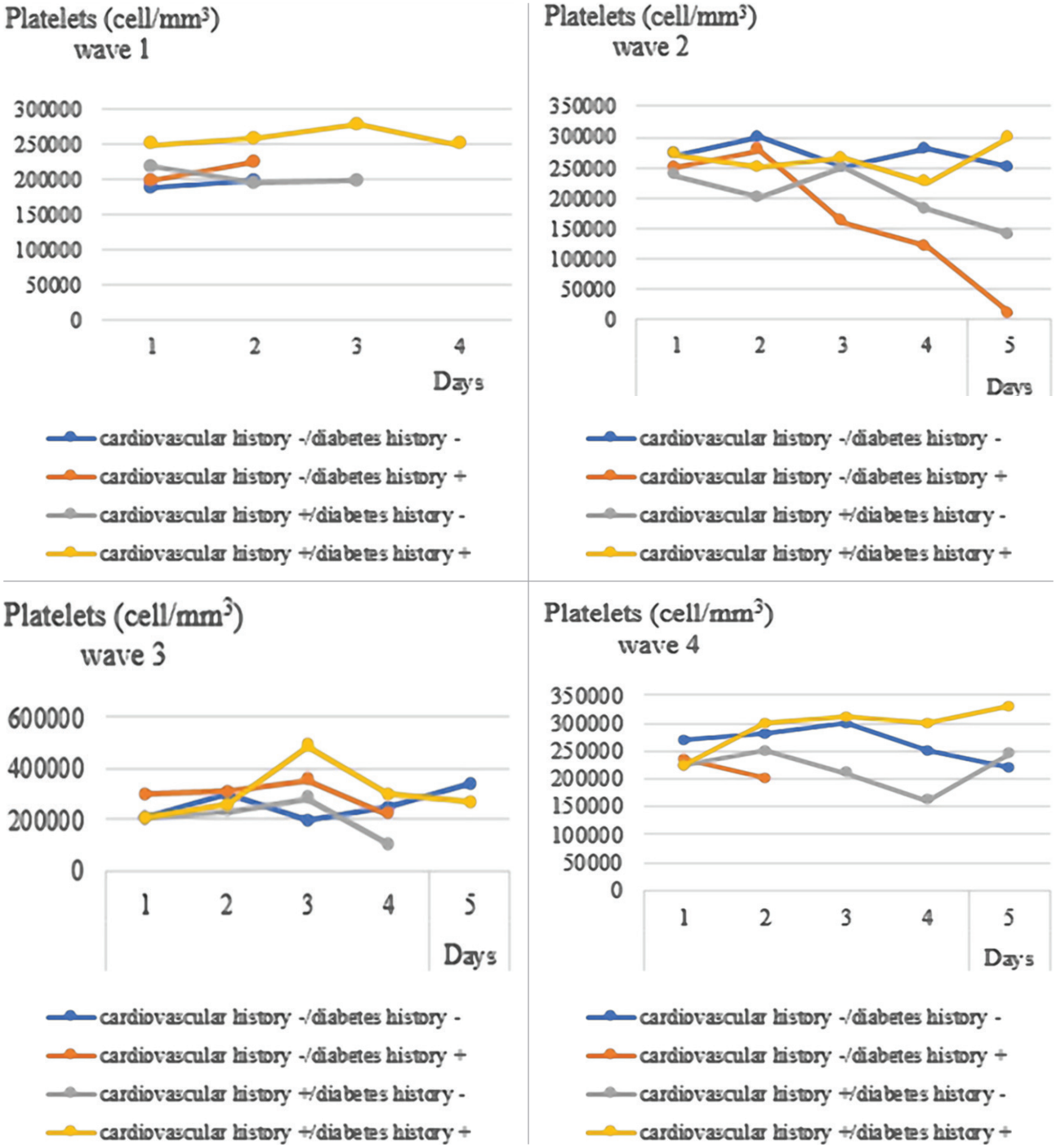

Figure 2B: Evolution of platelets in hospitalized patients during the waves. The platelets decrease slightly, however, a thrombocytopenia was seen in diabetics patients. Patients without cardiovascular history -/and without diabetes history -: CDH -/D -; Patients without cardiovascular history -/and with diabetes history +: CDH -/D +; Patients with cardiovascular history+/and without diabetes history -: CDH +/D -; Patients without cardiovascular history +/and with diabetes history +: CDH +/D +.

values are between (2–4 g/L). The graphs show high fibrinogen values on the first day of hospitalization (up to 9 g/L) and then normalize in the whole population except for patients with cardiovascular disease associated with diabetes (CDH+/D+), where there is a decrease while remaining above the norm (>4 g/L) (Figure 5).

Discussion

The biological and clinical analysis revealed several parameters, including oxygen saturation, complete blood count, CRP level and hemostasis testing.

In order to improve saturation in COVID-19 patients, the medical staff targeted a range of 92–98% to

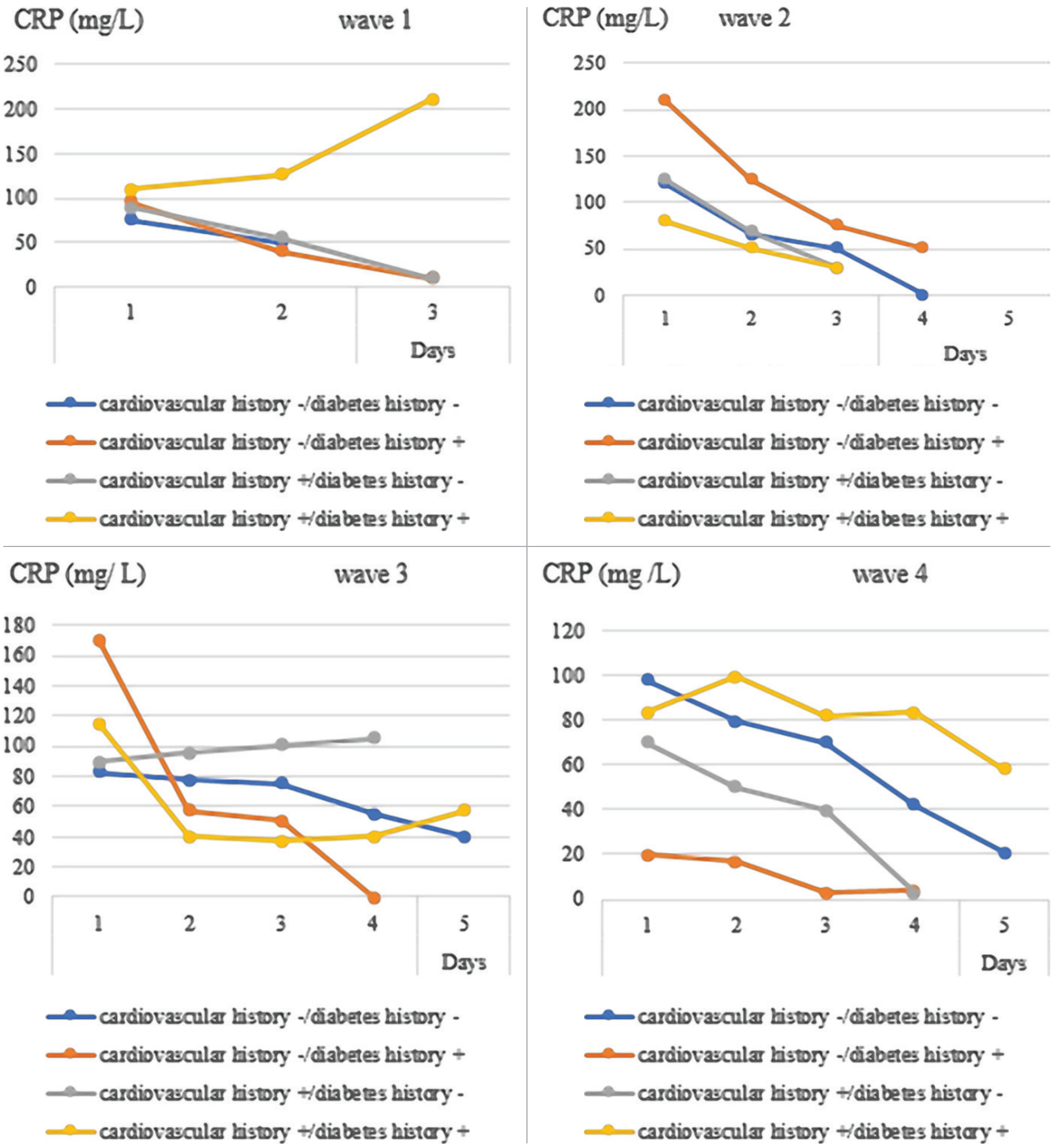

Figure 3: Evolution of CRP during epidemic waves in hospitals. Patients without cardiovascular history -/and without diabetes history -: CDH -/D -; Patients without cardiovascular history -/and with diabetes history +: CDH -/D +; Patients with cardiovascular history+/and without diabetes history -: CDH +/D -; Patients without cardiovascular history +/and with diabetes history +: CDH +/D +.

prevent acute hypoxemia and the risks of hyperoxia by using different respiratory supports of oxygen therapy, especially face masks, high-flow nasal oxygen therapy, Continuous Positive Airway Pressure (CPAP), non-invasive ventilation in case of dyspnea or hypoxemia [5]. The same supports were used at CHU Tlemcen.

Considering the fact that the majority of the subjects suffer from hypertension (69%), the study by Xie *et al.* demonstrates, similarly, that hypertension was pre-existing in 43.14% of patients with saturation lower or equal to 90% *versus* 20.22% with saturation higher than 90%. (6). Thus, our findings are alike.

COVID-19 is characterized by endothelial dysfunction and the alteration of the alveolar-capillary diffusion of oxygen, often causing acute respiratory distress syndrome (ARDS), which is a serious complication.

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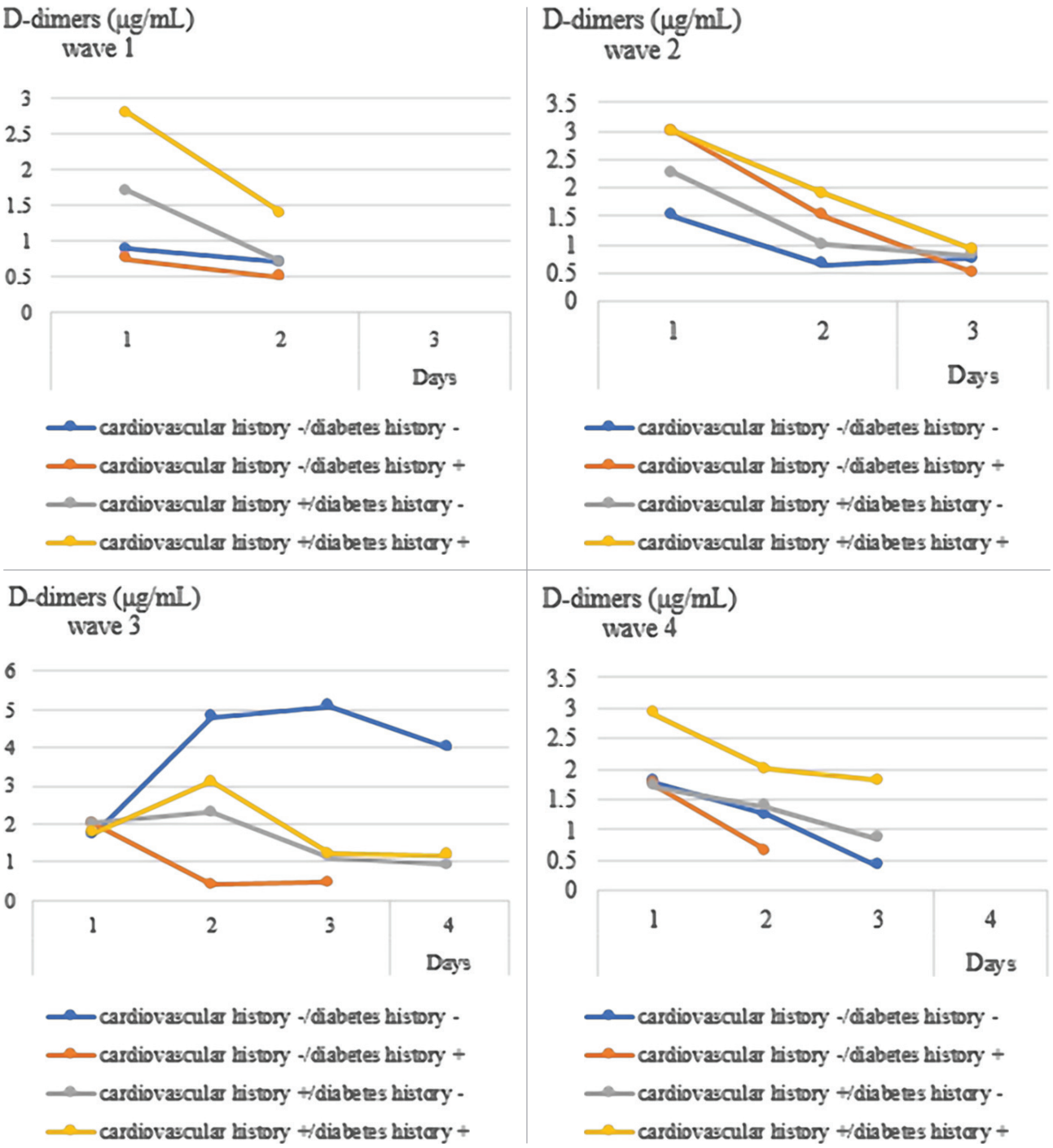

Figure 4: Evolution of D-dimers during epidemic waves in the hospital. Patients without cardiovascular history -/and without diabetes history -: CDH -/D -; Patients without cardiovascular history -/and with diabetes history +: CDH -/D +; Patients with cardiovascular history+/and without diabetes history -: CDH +/D -; Patients without cardiovascular history +/and with diabetes history +: CDH +/D +.

Therefore, we have worked on finding the link between the indicator's parameters of the infection according to the Pearson correlation. SpO_2 is correlated with two parameters: CRP levels and lymphocytes. These results are significant and reveal respiratory failure, the inflammatory phase, which is the first phase of infection and apoptosis accelerated by cell immunity causing lymphopenia. The study by Xie *et al.* [6] revealed that lymphocyte counts, CRP and SpO_2 were found to be closely associated with severe COVID-19, which supports the correlation we have analyzed in our research. Abnormal saturation experienced during the pandemic waves might be explained by the modifications of the viral genome and the mutations of the spike (S) protein, mainly at the level of RBD (Receptor Binding Domain), resulting in a higher variant with a loss of efficiency in

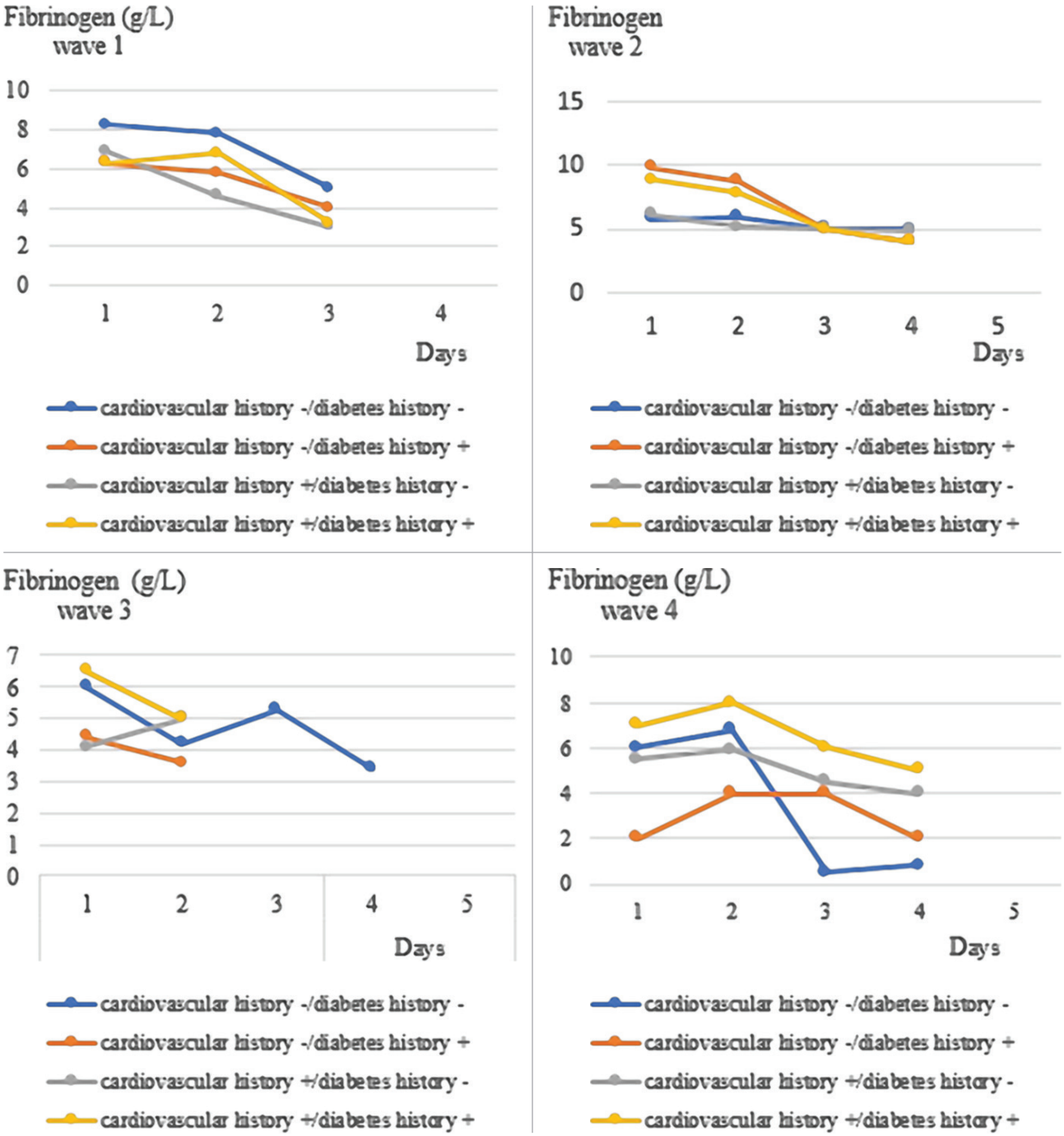

Figure 5: Evolution of fibrinogen during epidemic waves in hospital. Patients without cardiovascular history -/and without diabetes history -: CDH -/D -; Patients without cardiovascular history -/and with diabetes history +: CDH -/D +; Patients with cardiovascular history +/and without diabetes history -: CDH +/D -; Patients without cardiovascular history +/and with diabetes history +: CDH +/D +.

post-infectious and post-vaccine antibodies generated by previous variants, Consoli *et al.*, [7] asserted the same hypothesis (Figure 1).

Our research reveals that aggravated respiratory illness may lead to ARDS, a syndrome, during the three pandemic waves, mainly the third wave, through which the complications are more significant due to the shortage of oxygen and Delta's higher transmissibility [1, 7].

The subjects revealed other severe complications, especially cardiac injury, such as myocardial infarction (MI), arrhythmia, and cardiogenic shock. These could be related to the indirect interaction between COVID-19 and the cardiovascular system since the pathogen agent SARS-COV-2 causes an elevated level of cytokines with hypoxemia, provoking several tissue damages [8].

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Regarding the complete blood account, most patients went through a phase of lower lymphocyte levels before their normalization. SARS-COV-2 causes apoptosis in the respiratory system cells and circulating immune cells (lymphocytes, monocytes, and macrophages) [9].

Furthermore, it is hypothesized that the human organism cannot reconstitute the lymphocytes killed by the virus after aging, and the immune response is weaker in elderly patients in the frame of the conducted analysis. Our results converge with the study conducted by Wu *et al.*, which reveals higher levels of lymphocytes in surviving patients (Figure 2A) [10].

Many studies confirm our results. The research conducted by Marzouki *et al.* [11] revealed lymphopenia in 78% of patients. However, the study by Touahri *et al.* [12] comparing the three waves of the pandemic describes lymphopenia (56% during the 3rd wave *versus* 43% during the 1st and 2nd waves).

In addition to this, we have noticed a significant decrease in platelets in all patients, besides the fact that platelets were low in patients with "CDH+/Diabetes" over the second and third waves. The interpretation of our findings can be made through hemostasis parameters. We have discovered, with thrombocytopenia, high levels of D-dimers and thrombocytopenia. This confirms that variations are due to coagulation and fibrinolysis mechanism dysfunctions caused by SARS-COV-2.

Furthermore, we have noticed highly elevated D-dimers in healthy subjects during the 3rd wave, *i.e.* Delta is a severe variant at the origin of the severe form of the disease (Figure 4) [12, 13].

Subjects with cardiovascular diseases are predisposed to thrombotic risk, as SARS-COV-2 induces pro-thrombotic risks through the interaction of the viral agent and the vascular endothelium, which triggers the tissue factor (TF) associated with coagulation activation markers.

Moreover, NET's immune response (NETosis) releases mediators that cause inflammation, thrombotic risks, antifibrinolysis, and platelet activation. These cells (NETs) secrete cytokines leading to over secretion of TF and reinforcing NETosis aspect; this converges with Levi *et al.* [14] results indicating that 5% of patients with platelets level <100*10^9 cell/L (≈100000 cell/mm^3) (Figures 2B).

Medical literature indicates that hypertensive patients have an elevated CRP concentration, likely due to the prevalence of inflammation in this category [15]. These findings are similar to our results, as 69% of the subject patients suffer from hypertension. Moreover, the pandemic's first and third waves witnessed a very high level of CRP as the variant is less severe, according to medical literature (Figure 3) [16].

COVID-19 was associated with several inflammatory and thrombotic (hypercoagulation) disorders. In this context, hospitals proceeded to D-dimer monitoring to evaluate thrombotic risks.

Meanwhile, considering the strong relationship between COVID-19 and Disseminated Intravascular Coagulation (DIC) [17], fibrinogen was the most required parameter at CHU Tlemcen during the pandemic.

A similar study has assessed the aggravation of hypercoagulability in the presence of cardiovascular diseases and related risk factors. The study revealed that elevated fibrinogen levels are at the origin of this complication, which implies the key role of this parameter in the pathogenesis of endothelial vascular damage, hypercoagulation and thrombosis (Figure 5) [18].

Conclusions

Underlying cardiovascular diseases and risk factors (male sex, age, diabetes etc.) are at the origin of severe progression of COVID-19. Our results have revealed that most affected patients by COVID-19 are those with cardiovascular diseases associated or not with diabetes, mainly hypertension and myocardial infarction, which confirms that patients with cardiovascular disorders history are more vulnerable. Moreover, they experienced complications, especially respiratory distress, renal failure, as well as cardiac and thrombotic complications in comparison with healthy patients. Our findings regarding the explored parameters reveal low oxygen saturation, CRP and high fibrinogen levels in subjects with preexisting comorbidities, which reflect the severe form of the disease in comparison with patients having no medical history. Meanwhile, highly elevated D-dimers associated with thrombopenia in these patients imply that preexisting disorders increase the risk of thrombotic complications, including disseminated intravascular coagulation and venous thromboembolic diseases in COVID-19 patients, which require awareness and appropriate care of cardiovascular risk factors likely to reduce underlying complications and SARS-COV-2 contamination.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the Faculty of Medicine of Tlemcen University (approval ID: ED.Covid. 09.21).

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