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Original Article

Platelets: A Neglected Cell in Cystic Fibrosis Lung Inflammation

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ABSTRACT

Aim: There is growing recognition of the critical role of platelets in inflammation and immune responses. However, the role of platelets in lung inflammation in patients with cystic fibrosis (CF) is unclear. Therefore, we aimed to investigate platelet count (PC) and mean platelet volume (MPV) in various clinical conditions in CF patients.

Materials and Methods: A total of 53 pediatric patients with CF were enrolled in this study. Data was retrospectively obtained from the patients' medical records for PC and MPV, and then categorized into 6 groups according to their pulmonary exacerbation and non-pulmonary exacerbation status in chronically colonized or non-colonized patients with CF. The groups were then compared statistically.

Results: The mean age of the patients was 8.01±5.34 years with a male to female ratio of 30:23. In the acute pulmonary exacerbation period, all patients with CF had higher PC than those in a non-pulmonary exacerbation period independent of their chronic colonization status ($p<0.05$). However, PC was not different in non-colonized patients whether they were in acute pulmonary exacerbation or non-pulmonary exacerbation periods ($p>0.05$). Importantly, MPV did not show any statistical significance in any compared settings among these CF patients.

Conclusion: Platelets may play an important role along with other inflammatory cells and mediators in CF lung inflammation during pulmonary exacerbations.

Keywords: Cystic fibrosis, thrombocyte, mean platelet volume

Introduction

Cystic fibrosis (CF) is the most common genetic lung disease affecting mainly the Caucasian population worldwide. It is inherited in an autosomal recessive fashion and affects approximately 100,000 people throughout the world. CF is caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), located on the long arm of chromosome 7. To date, more than 2,000 mutations on the CFTR have been reported. Approximately 70% of CF patients in the Caucasian population are homozygous for the F508del genotype. At the lung level, mutations in the CFTR cause reduced chloride in airway secretion, which favors the reabsorption of sodium and results in dried secretions, poor mucociliary clearance and airway obstruction. This results in recurrent airway bacterial infections *[Pseudomonas aeruginosa (P. aeruginosa)* and *Staphylococcus aureus (S. aureus)* being the most common pathogens]. This is associated with inflammatory cell accumulation and the release of proteolytic and other cell contents with resulting damage to the bronchial walls, leading to a loss of bronchial cartilaginous support and muscle tone, and eventually bronchiectasis (1,2).

Airway inflammation in CF is predominantly neutrophilic in nature with increased concentrations of pro-inflammatory mediators including TNF-α, IL-1β, IL-6, IL-8, IL-17, IL-33, GM-CSF and G-CSF. In addition, other cell types including macrophages and T-lymphocytes express CFTR and contribute to the CF inflammatory response due to a lack of CFTR modulation of the inflammation (3).

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The researchers suggested that platelets may also be an important contributor to pulmonary inflammation (4,5). Platelet depletion or antiplatelet therapies attenuate injury and mortality in animal models of acute lung injury (6). More importantly, CFTR expression has been shown on human platelets (7). Recent data suggests that CF patients show an increase in circulating activated platelets and platelet reactivity (8). The soluble form of circulating proinflammatory mediator CD40L, which is mainly derived from platelets, was observed to be increased in those patients with CF and *P. aeruginosa* infection (9).

Taken together, these observations support the hypothesis that platelets play a potentially important role in regulating lung inflammation in CF. However, there are few studies examining platelet and lung inflammation interactions in patients with CF (10,11). Therefore, we aimed to investigate platelet count (PC) and mean platelet volume (MPV) in various conditions in our CF patient population.

Materials and Methods

This was a single-center, retrospective study of patients ≤18 years of age with CF, diagnosed by sweat test (>60 mmol/L) and/or positive genotype. The study period occurred between February 2015 and June 2022 at the Mersin City Training and Research Hospital. The medical records of all of the patients with CF were reviewed mainly for patient demographics, complete blood count analysis via an Auto Hematology Analyzer (Advia2120i, Siemens, IRL) and throat/sputum culture results performed on the same day. Those patients with insufficient data or who were lost to follow-up were not included in this study. The patient demographics included age, sex, current body mass index (BMI), CF mutation analysis, age at diagnosis, chronic colonization for *P. aeruginosa* and *S. aureus* defined by the Leeds criteria (12), inhaled antibiotic treatment, latest year best spirometry (if available), and frequency of hospitalization.

Pulmonary exacerbation was defined by a combination of patient-reported symptomatology (increased cough, change in sputum volume, decreased appetite or decreased weight), a clinician-based evaluation and laboratory data (particularly spirometry) in patients with CF (13).

The CF patients were divided into six groups according to their number of obtained respiratory cultures in various clinical status described below;

Group 1. The number of respiratory culture in CF patients with pulmonary exacerbation; n=175.

Group 2. The number of respiratory culture in CF patients with no pulmonary exacerbation, n=127.

Group 3. The number of respiratory culture in chronically colonized CF patients with no pulmonary exacerbation; n=136.

Group 4. The number of respiratory culture in non-colonized CF patients with no pulmonary exacerbation; n=38.

Group 5. The number of respiratory culture in chronically colonized CF patients with pulmonary exacerbation; n=82.

Group 6. The number of respiratory culture in non-colonized CF patients with pulmonary exacerbation; n=47.

Same day PC and MPV from complete blood analysis and throat/sputum cultures obtained from the patient records were categorized into the 6 groups above. It is important to highlight that the same patient's result could be used in different groups for data analysis as per the study design.

This study was approved by the Mersin University Clinical Research Ethics Committee (approval no.: 138, date: 23.02.2022).

Statistical Analysis

Number and percentage values are given as descriptive statistics for categorical variables. Median and quartile values are given as descriptive statistics since the distribution was not suitable for the normal distribution for the continuous variables. The Mann-Whitney U test was used to identify a statistical differences between the mean PC and MPV values among the groups. Statistical significance was considered when p value was <0.05.

Results

During the study period, 53 of the 56 patients with CF met the inclusion criteria with a male to female ratio of 30:23 and a mean age of 8.01±5.34 years. The latest mean BMI (kg/m^2) of the patients was 15.91±2.79. Genetic analysis demonstrated that 9 (17.0%) patients were homozygous for the F508del/F508del mutation, 14 (26.4%) were heterozygous for delF508del/other, and 30 (56.6%) patients had various other mutations. The additional general characteristics of the CF patients, and the number of complete blood analysis and respiratory culture samples obtained among the groups are given in Table I.

When the data was compared among groups, the PC was significantly higher in all CF patients with pulmonary exacerbation in comparison to all patients without pulmonary exacerbation (Group 1 versus Group 2; p=0.001). When the subset groups were compared, the chronically colonized CF patients with pulmonary exacerbation had significantly higher PC than the chronically colonized CF patients with no pulmonary exacerbation (Group 5 versus

Table I. Demographic characteristics of the patients with CF	
Total patients	53
Age* (years)	8.01±5.34
Male	56.6%
Current BMI*	15.91±2.79
Mutation	
Homozygote F508del	9
Heterozygote F508del	14
Other	30
Age at diagnosis* (months)	15±37.83
Pancreatic insufficiency	39 (73.6%)
Chronic colonization	
P. aeruginosa	14 (26.4%)
S. aureus	1 (1.9%)
None	38 (71.7%)
Inhaled tobramycin/colistin	14 (26.4%)
Spirometry* [if available (n=35), predicted %]	
FVC	73.34±20.80
FEV_1	71.34±22.84
Number of matched results of PC & MPV and respiratory culture samples in groups	
Group 1	175
Group 2	127
Group 3	136
Group 4	38
Group 5	82
Group 6	47
Hospitalization* (times during study period)	2.08±2.74

*Results are given as mean ± SD
CF: Cystic fibrosis, BMI: Body mass index, *P. aeruginosa: Pseudomonas aeruginosa, S. aureus: Staphylococcus aureus*, PC: Platelet count, MPV: Mean platelet volume, SD: Standard deviation

Group 3; p=0.048) and the non-colonized CF patients with no pulmonary exacerbation (Group 5 versus Group 4; p=0.0005). In contrast, the PC was not different in the chronically colonized CF patients with no pulmonary exacerbation and the non-colonized CF patients with no pulmonary exacerbation (Group 3 versus Group 4; p=0.3254), and the non-colonized CF patients with pulmonary exacerbation and the non-colonized CF patients with no pulmonary exacerbation (Group 6 versus Group 4; p=0.382). On the other hand, there were no statistically significant differences in terms of MPV in any comparisons among the groups (Table II).

Table II. Comparison of platelet counts and mean platelet volumes among the groups

	Platelet count ($\times10^3$/μL)	**Mean platelet volume (fL)**
Group 1 vs Group 2 p value	419 (358-485) vs 382 (315-451) **0.001**	8 (7.5-8.6) vs 8.2 (7.6-8.8) 0.267
Group 3 vs Group 4 p value	378 (304-472) vs 390.5 (344.7-466.5) **0.3254**	8.2 (7.6-8.9) vs 8.25 (7.7-8.92) 0.5071
Group 5 vs Group 3 p value	431 (386-482) vs 390.5 (344.7-466.5) **0.048**	7.9 (7.5-8.5) vs 8.25 (7.7-8.92) 0.081
Group 5 vs Group 4 p value	431 (386-482) vs 378 (304-472) **0.0005**	7.9 (7.5-8.5) vs 8.2 (7.6-8.9) 0.1292
Group 6 vs Group 4 p value	393 (302-540) vs 378 (303-472.5) **0.382**	8.1 (7.5-8.9) vs 8.2 (7.6-8.9) 0.966

Results are expressed as median and interquartile range

Discussion

CF is characterized by chronic non-resolving lung inflammation, driven by the continuous recruitment of immune cells into the airways which starts at a very early age. This persistent inflammatory state leads to permanent structural damage of the airways in CF patients. Several defective inflammatory mechanisms have been linked to CFTR deficiency including dysregulation of innate and acquired immunity, cell membrane lipid abnormalities, various transcription factor signaling defects, as well as altered kinase and toll-like receptor responses. The inflammation of the CF lung is mainly dominated by neutrophils which release oxidants and proteases, particularly elastase (14,15).

In addition to their well-known role in hemostasis and thrombosis, many studies have identified platelets as playing a key regulatory role in inflammatory reactions (16,17). During neutrophil recruitment to the inflammation site, platelets bind to endothelial cells and interact with leukocytes. The interaction between neutrophil and platelets is mostly mediated by platelet P-selectin binding to P-selectin glycoprotein ligand on leukocytes. Furthermore, firm adhesion of leukocytes to platelets is supported by CD11b/CD18 and CD11a/CD18. In addition to interacting with neutrophils, platelets interact with other leukocyte subpopulations by releasing chemokines thereby activating monocytes. Interestingly, in an experimental mouse model with dysfunctional CFTR, exaggerated acute lung inflammation and platelet activation following

intratracheal lipopolysaccharide or *P. aeruginosa* challenge was seen. This was attributed to the production of aberrant transient receptor potential cation channel 6 (TRPC6)-dependent platelet activation. TRPC6 is thought to be a major driver of lung inflammation and impaired bacterial clearance in CF (18). Autoantibodies to bactericidal/permeability-increasing (BPI) protein, BPI-antineutrophil cytoplasmic autoantibodies (ANCA), are often present in the serum as well as the airways of patients with CF, and have been found to be correlated with airway colonization of *P. aeruginosa*. A study by Hovold et al. (19) found that BPI-ANCA expressed in the airways of CF patients was correlated with *P. aeruginosa* load and PCs. All these studies suggest that the role of platelets in CF lung inflammation is quite complex.

A change in shape also occurs from a disc-shaped cell into an intermediate spherical shaped cell during inflammation (20). It is proposed that platelet volume is correlated with its function (21). Small platelets show lower function than larger sized ones. Previous studies reported conflicting results in MPV values in acute and chronic inflammatory diseases with increased MPV in neonatal sepsis, bronchopulmonary dysplasia, and chronic obstructive lung disease, but decreased MPV in acute appendicitis, Familial Mediterranean Fever, and no change in asthmatic children or chronic inflammatory arthritis (22-26).

A few studies have investigated the values of PC and MPV in limited numbers of children with CF or bronchiectasis (10,27). Nacaroglu et al. (27) examined PC and MPV values in patients with non-CF bronchiectasis. They found that PC in the acute exacerbation and non-exacerbation periods were significantly higher than for those in the control group. Additionally, the average MPV values of the non-exacerbation periods were significantly higher than in the control group. In contrast, the MPV values of the acute exacerbation group were not statistically different when compared to non-exacerbation periods or the control group. A study by Uysal et al. (10) evaluated the relationship between acute exacerbations and the MPV trend in children with CF as a means to predict exacerbations. They reported lower MPV values and higher PC both in the acute exacerbation and the non-exacerbation periods when compared to healthy subjects.

To the best of our knowledge, no studies to date have examined platelet levels and MPV values in different clinical situations in CF patients. The current study suggests a role for platelets in CF lung inflammation by showing that PC was elevated during acute pulmonary exacerbation in patients with CF. This observation is prominent in the chronically bacterial colonized CF population. Interestingly, we did not observe any significant change in PC during acute pulmonary exacerbation in non-colonized CF patients nor in colonized patients in the absence of exacerbation. One speculation in this regard could be that the low grade of lung inflammation present in the absence of exacerbation might not cause prominent changes in PC levels. Notably, our study findings showed no change in platelet size (determined by MPV) in any given scenario. A limited number of studies have reported conflicting results for MPV in CF patients (lower) and in non-CF bronchiectasis patients (higher/non-different) compared to healthy subjects (10,27). The conflicting MPV values in these studies may have been influenced by their different methodological techniques. Based on the current limited data, it can be speculated that MPV values may not be an appropriate marker/predictor for CF lung inflammation.

Study Limitations

The current study has some limitations. First, our clinic is a small center. Therefore, the relatively small sample size of patients with CF might have affected our study results. Second, this study focused only on the correlation of PC and MPV with pulmonary exacerbation, without including other indicators of infection (e.g. white blood cell count, erythrocyte sedimentation rate, C-reactive protein) due to its retrospective nature and incomplete chart data in that regard.

Conclusion

The current study supports the role of platelets in CF lung inflammation during exacerbations with an increase in PC. Further studies with larger CF populations are warranted in order to investigate such a relationship.

Ethics

Ethics Committee Approval: This study was approved by the Mersin University Clinical Research Ethics Committee (approval no.: 138, date: 23.02.2022).

Informed Consent: Not applicable.

Authorship Contributions

Concept: A.Ö., M.E., Design: A.Ö., M.E.,Data Collection and/or Processing: A.Ö., M.E., Analysis and/or Interpretation: A.Ö., M.E., Literature Search: A.Ö., M.E., Writing: A.Ö., M.E.

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