Incidence of cereblon protein in intensive care patients: a cross-sectional study

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Abstract

Background-
Sepsis remains a common cause of death. Involving in important cellular processes, the cereblon (CRBN) protein plays a role in sepsis. This study aimed to show the CRBN protein expression and its effects on the patients in the intensive care unit.

Methods-
Samples were taken by deep tracheal aspiration from patients. The presence of CRBN was pathologically investigated by using immunohistochemical tests and polyclonal antibodies against CRBN. The relationship between gender, sepsis, steroid, survival, and the presence of CRBN was examined.

Results-
Respiratory and neurologic diseases were the most common reasons for admission. Acinetobacter was the most frequent microorganism. In patients with more than normal inflammatory cells, we observed a negative correlation between CRBN expression and PNL rate (p = 0.031). In patients with CRBN, there was no correlation between steroid and mortality, APACHE/Glasgow score, length of stay in hospital and ICU.

Conclusion-
Although the prognosis in sepsis is better in CRBN-deficiency animals, the presence of CRBN in humans does not affect it. The more we saw inflammatory cells in aspiration material, the less there was a CRBN. The response to steroids, an immunomodulator, did not change with the presence of the immunomodulator target molecule, CRBN. This requires us to reconsider using immunomodulators in the treatment of sepsis.

Introduction
Sepsis is still a significant cause of morbidity and mortality[1]. The annual death rate is around 5.3 million [2]. It involves multiple aspects of the interaction between the infecting pathogens and the host. The host response depends on several factors and is vital in mortality [3].

Alveolar macrophages are the main phagocytes of the body's first line of defense against harmful microbes, called the innate immune system. They absorb microorganisms and destroy them. They also produce cytokines that attract other immune cells to the lungs. Macrophages are important for modulating the antibacterial function of neutrophils. A study showed that macrophages are important for modulating the antibacterial function of neutrophils and play an essential role in sepsis [4].
The cereblon (CRBN) protein is involved in critical cellular processes. Multiple disorders (multiple myeloma, acute lung injury, e.g.) are associated with CRBN dysregulation [5–7]. In the heart, liver, and skeletal muscle, disrupting CRBN has been demonstrated to increase AMP-activated protein kinase (AMPK) activity [8–10]. Thus, it slows down the inflammatory process.

CRBN is the primary target of immunomodulatory drugs, especially thalidomide derivatives. Its degradation of transcriptional proteins leads to the progression of multiple myeloma (MM) cells, reducing survival [11]. Thalidomide and a derivative drug called lenalidomide significantly extend patients' survival with MM [12].

CRBN has also been shown to play a role in sepsis. In a septic animal model, CRBN has been found to protect against organ injury by decreasing inflammation [13]. It is also a profibrotic regulator and might be a potential target for treating lung fibrosis [14].

Studies have been conducted on the serological change of CRBN protein, especially in MM cells. However, to date, there is no immunohistochemical study of the localization and presence of CRBN expression in inflammatory cells in non-neoplastic processes in the English language literature. Our primary aim in this study was to demonstrate the presence of CRBN in patients with sepsis and its effect on mortality. Our second goal was to show whether the effect of immunomodulatory drugs used in patients with sepsis changes with the presence of CRBN.

**Material And Methods**

Patient selection: After obtaining the ethics committee permissions (İzmir Democracy University Ethics committee permission no: 2020/21 – 02), (Clinical trials number: NCT05083520), the patients hospitalized in the ICU were examined. Inclusion and exclusion criteria have been determined during the current pandemic period.

Inclusion criteria were being over 18, having a negative COVID-19 PCR test, being intubated, getting permission from the patient's relatives to be included in the study, and having enough secretion to count cells by aspiration through the endotracheal tube.

The exclusion criteria were being under 18, having a positive COVID-19 PCR test, sepsis cases other than pulmonary sepsis, not getting consent from patients' relatives, not having enough secretion to count cells by aspiration through the endotracheal tube, and not having enough cells in the secretion.

One hundred sixteen patients were followed up during the study period. With the periodic increase of the Covid-19 pandemic, isolated beds in our intensive care unit were reserved for COVID-19 patients in some months. Therefore, these patients were not included in the study. Seventy patients proven non-COVID-19 with negative COVID-19 PCR test were eligible for inclusion in the study. Four of these patients could not be included in the study because their relatives did not consent. Eight patients could not be included because sufficient pulmonary secretion material was not obtained in the aspiration performed during the
intubated follow-up period. The tracheal aspiration procedure was applied to the remaining fifty-eight intubated patients. However, at the stage of microscopic examination, two patients were excluded from the study because there were no cells in the material to be examined. The study continued with the remaining 56 patients (Fig. 1. Flow diagram). Patient samples were taken and numbered by the intensive care physician who was not included in the study to avoid bias.

Tracheal aspiration procedure: The tracheal aspiration procedure was performed in the first 12 hours after the patient was intubated. The patients who were placed in the semi-fowler position were oxygenated with 100% O$_2$ for two minutes before tracheal aspiration. Following aseptic rules, aspiration was performed through the endotracheal tube or tracheostomy cannula. Paul's Tracheal Culture Bottle was used to store secretions. The patients were ventilated with 100% O$_2$ for two minutes.

Storage of samples: An equal volume of pre-prepared fixative (mixture of the same volume of 95% ethyl alcohol and 10% formalin) was added to the patient’s secretion.

Making cell blocks: For preparing cell blocks, each specimen with fixative was centrifuged for 10 minutes at 2000 rpm. The supernatant was thrown away. The cell button was then resuspended in the same fixative, centrifuged at 3000 rpm for 10 minutes. For 4–6 hours, the tube was set aside. After discarding the supernatant, the cell button was gently removed and placed in a labeled tissue cassette. The specimen was processed and implanted. Tissue sections with a thickness of 3–4 µm were cut from the cell blocks. For morphological examination, sections were stained with Hematoxylin and Eosin. Only modest numbers of lymphocytes, neutrophils, and other inflammatory cells are found in bronchial aspiration (BAL) fluid from healthy, nonsmoking adults without lung disease. Alveolar macrophages make up to 90% of the cell population in the lungs. The specimens were considered adequate or unsatisfactory in this study according to certain criteria. These criteria are insufficiency of alveolar macrophages (i.e., less than 10 alveolar macrophages/high-power field), excess of airway-derived epithelial cells (i.e., more than the presence of alveolar macrophages), a mucopurulent exudate, degeneration changes, or laboratory processing artifacts. Specimens found insufficient for evaluation and/ or unsatisfactory were excluded from the study [15].

Immunohistochemical (IHC) tests: The streptavidin-biotin peroxidase method was used (Invitrogen, Camarillo, 85-9043, USA). Serial 4-µm sections, obtained from paraffin blocks, were treated with a microwave with a heat-induced epitope retrieval procedure. Slides were left for 20 minutes in 10mM/L citrate buffer at pH 6.0, cooled at room temperature for 20 minutes, blocked to retrieve endogenous peroxidase and biotin. Polyclonal antibody against CRBN (Invitrogen, PA5-38037, 1/200 dilution) was used. A brown granular or diffuse cytoplasmic and/or nuclear staining for CRBN within inflammatory cells were considered positive. The slides were reviewed by a pathologist who was blinded to the patients' clinical symptoms. Staining patterns were categorized based on the intensity and location of staining.

A single pathologist took part in the study to standardize the evaluation. Our pathology professor had 35 years of professional experience. Cytoplasmic CRBN expression was examined microscopically and
recorded as present/absent.

Statistical analysis: Since there are no clinical studies on the effects of CRBN on sepsis, a power analysis was performed with preclinical studies. Yang H. et al. attempted to explore the effects of CRBN on the progression of acute lung injury (ALI) in mice [7]. In the power analysis based on the number of CRBN knockdown mice they used, the number of patients required for the study to be 80% power was determined as 32. The power analysis program (G*Power 3.1.9.2, Düsseldorf, Germany) was used. Therefore, the research sample will consist of at least 45 patients diagnosed with sepsis hospitalized in the intensive care unit. All statistical analyses were performed using SPSS (version 25.0, IBM). The quantitative data were recorded as mean values ± standard deviation (SD) and analyzed by using the Student’s t-test for analysis. Tukey’s post hoc test was used to validate ANOVA for comparing measurement data among groups. In cases where the necessary conditions for parametric analysis were not met, Mann Whitney U was used to compare the quantitative data of the two groups. The chi-square test or the Fisher’s exact test were used to compare categorical variables, which were recorded as percentages. Differences were considered significant when the p-value was less than 0.05.

Results

Considering only patients whose immunohistochemical expression of CRBN could be evaluated, the study population consisted of 56 patients, 32 males (57.1%), 24 (42.9%) females. The mean age of the patients was 70.13 ± 13. The average hospitalization period was 30.04 ± 17.5 days, and the mean follow-up time in the intensive care unit was 27.5 ± 18.3 days.

The diagnostic groups of our patients followed with Apache II scoring were as in Fig. 2. Respiratory (COPD, pneumonia, pulmonary cancer, etc.) and neurologic diseases (cerebrovascular occlusion, hemorrhage, etc.) were the most common reasons for admission (43.1% and 22.4%). The sum of the cumulative percentage of the diagnostic groups is shown with the red line. Accordingly, the total of respiratory and neurologic diseases (65.5%) constitute most of the patients followed.

Forty-two of 56 patients had one or more concomitant chronic diseases. While 36 (64.29%) of our patients had sepsis at the sampling time, 20 (35.71%) did not have. The most frequently grown microorganism was Acinetobacter, encountered in 18 patients.

While complete/partial cure to treatment was obtained in 9 (16.1%) patients, 47 (83.9%) patients died. Different microbiological agents were grown in the cultures of 33 (58.9%) patients in the microbiological examination of bronchoalveolar fluid. According to the antibiograms or with prophylactic aims, 51 (91.1%) patients used different types of antibiotics.

In all patients, examination of immunohistochemically stained cell block materials revealed cytoplasmic or nuclear expression of CRBN in macrophages or polymorphonuclear leucocytes (PMNLs) (Fig. 3).
Cereblon protein was detected in macrophages or neutrophils in 24 patients but not in 32 patients. No statistically significant relationship was detected between the presence of CRBN expression and gender (p = 0.876), presence of sepsis (p = 0.376), use of steroids (p = 0.322), and survival (p = 0.598) (Table 1).
### Table 1
Relationship between presence of CRBN and gender, sepsis, use of steroid, and survival

#### Relationship between presence of CRBN and gender, sepsis, use of steroid, and survival in all patients.

<table>
<thead>
<tr>
<th></th>
<th>Number of patients without CRBN</th>
<th>Number of patients with CRBN</th>
<th>TOTAL</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18</td>
<td>14</td>
<td>32</td>
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<td>Female</td>
<td>14</td>
<td>10</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>32</td>
<td>24</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td><strong>Sepsis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence</td>
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<td>7</td>
<td>20</td>
<td>0.376</td>
</tr>
<tr>
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<td>17</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>32</td>
<td>24</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td><strong>Use of steroid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
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<td>18</td>
<td>38</td>
<td>0.322</td>
</tr>
<tr>
<td>Yes</td>
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<td>6</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>32</td>
<td>24</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td><strong>Survival</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>4</td>
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<td>Ex</td>
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<td>20</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>32</td>
<td>24</td>
<td>56</td>
<td></td>
</tr>
</tbody>
</table>

Relationship between presence of CRBN and gender, sepsis, use of steroid, and survival in patients with high PMNL cells.

<table>
<thead>
<tr>
<th></th>
<th>Number of patients without CRBN</th>
<th>Number of patients with CRBN</th>
<th>TOTAL</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
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<tr>
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<td>12</td>
<td>11</td>
<td>23</td>
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<tr>
<td>Female</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>20</td>
<td>19</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td><strong>Sepsis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absence</td>
<td>8</td>
<td>6</td>
<td>14</td>
<td>0.584</td>
</tr>
<tr>
<td>Present</td>
<td>12</td>
<td>13</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>20</td>
<td>19</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td><strong>Use of Steroid</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>13</td>
<td>24</td>
<td>0.297</td>
</tr>
<tr>
<td>Yes</td>
<td>9</td>
<td>6</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05
Although PMNLs were present in some patients, no CRBN expression was determined in PMNLs. PMNL cells were seen in trace amounts in some patients. More than normal PMNL cells (>3% PMNL cells) were detected in the BAL of only 39 patients. When these patients with more than normal PMNL cells were examined, CRBN expression and different clinicopathological parameters were re-evaluated. This group consists of 23 males (59%) and 16 females (41%). The mean age of these patients was 69.1 ± 14 years (range, 25–91 years). In these 39 cases, no statistically significant relationship was detected between the presence of CRBN expression, and gender (p = 0.894), presence of sepsis (p = 0.584), use of steroids (p = 0.297), and survival (p = 0.465). On the contrary, there was a negative correlation between CRBN expression and PMNLs rate (p = 0.031) by chi-square analysis (Table 1). Cereblon protein incidence decreased when the amount of PMNLs in the tracheal aspiration material of the patients increased (Fig. 4).

When these 39 patients were examined, there was no difference between CRBN expressions with age (p = 0.478), Glasgow score (p = 0.647), Apache score (p = 0.478), and hospitalization time (p = 0.627).

It was investigated whether steroid, which is an immunomodulator, makes a difference in patients with CRBN. First, 24 patients with CRBN expression among all patients (56) were examined. The effect of steroid treatment on survival was measured. There was no difference detected between Apache Score, Glasgow Score, length of hospital and the ICU stay (Table 2).
Afterward, 39 patients with PMNLs in tracheal aspiration material were analyzed. Among 19 patients with CRBN expression, the effect of steroid treatment on mortality, intensive care score, and length of stay was evaluated. No significant difference was found.

**Discussion**

This is the first human study on the process of cereblon protein in lung secretions. No difference was observed between the cereblon protein synthesis in the patients with and without sepsis in the ICU. No correlation was observed between the presence of cereblon and gender, survival, and steroid use. However, in patients with more than 3% PMNL cells in BAL, cereblon synthesis decreased significantly as the number of inflammatory cells increased. The more we saw inflammatory cells in BAL, the less cereblon there was. In the presence of CRBN, steroid treatment could not reduce mortality or improve scores in the intensive care unit. While CRBN is the primary target for other immunomodulators, it may not be for steroids. The presence of less cereblon in the patients with more inflammatory cells may explain that steroid therapy, an immunomodulator, did not reduce mortality.

In a sepsis study conducted by Gil et al., the role of CRBN in polymicrobial sepsis induced by cecal ligation and puncture (CLP) was investigated. CRBN-deficient (KO) mice were used for this study.
Resistance to polymicrobial sepsis has been demonstrated in CRBN-deficient mice. Survival 6 days after CLP was found significantly higher in KO mice (50%) compared to wild-type (WT) controls (0%). In KO mice, peripheral blood bacterial load is less; lung damage is more minimal. Activation of AMPK and heme oxygenase-1 (HO-1) in peritoneal macrophages from WT mice was found lower. AMPK has been shown to protect against organ injury by suppressing inflammation. This study demonstrated that CRBN expression plays an attractive role in CLP-induced sepsis and peritoneal macrophage response. This creates a new approach to sepsis [13]. In our study, a relationship could not be established between the presence of CRBN in bronchial secretion and survival. However, as PMNLs increased in the aspiration material, CRBN expression decreased. In cases with a nearly fibrinopurulent exudate and deletion PMNLs, cellular CRBN expression is lost, which means that CRBN cannot have a protective effect against organ damage.

The AMPK, which is negatively controlled by cereblon, has previously been linked to pulmonary fibrosis. Kang et al. interpreted the role of CRBN in bleomycin (BLM)-induced pulmonary fibrosis in mice. In CRBN knockout (KO) mice, BLM-induced fibrosis was significantly reduced. According to this study, CRBN is a profibrotic regulator and might be used as a potential target to treat lung fibrosis [14].

In a study examining the mechanism of acute kidney injury (AKI) in sepsis, the role of CRBN was investigated. Sepsis was induced by applying lipopolysaccharide (LPS) on human kidney 2 (HK2) cells. Circ_0114428 and CRBN levels were higher in septic AKI blood samples and LPS-induced HK2 cells. LPS-induced apoptosis, inflammation, oxidative stress, and ER stress were rescued by CRBN overexpression. The Circ_0114428 knock-down might have deflated this. CRBN expression was significantly raised in serum from septic AKI patients. It was suggested that CRBN played a staminal role in kidney damage due to sepsis, in which circ_0114428 might be related to its function [16]. However, in our study, the presence of cereblon suppressed the increase in PMNLs. This may indicate that inflammation is suppressed. For a reason whose mechanism we do not know, we found that the presence of cereblon and the presence of inflammatory cells showed a negative correlation. In contrast to the protective effect in KO mice without cereblon, we saw nearly fibrinopurulent exudate and abundant PMNL cells in patients without cereblon.

CRBN has a role in chronic inflammation-related conditions and regulates the inflammatory response. CRBN plays a nonenzymatic role in inflammation, leading to suppression of NF-kB activation and increased pro-inflammatory cytokine levels [17].

A study investigating other mechanisms revealed that the transcriptional activity of the activator protein 1 (AP-1) complex is decreased, and CRBN reduced the mRNA expression and the protein levels of several pro-inflammatory cytokines. The researchers introduced a new molecular mechanism by which CRBN adjusts the inflammatory response and apoptosis. CRBN promotes or inhibits the ubiquitination of two critical molecules at different levels of the inflammatory cascade. So, the inflammatory response is suppressed. LPS is an inflammatory stimulus, and it can also provoke the apoptosis of macrophages. Therefore, modulating the AP-1 signaling pathway can be a promising therapeutic strategy for the treatment of inflammation-associated diseases [18].
It is known that CRBN is synthesized in the human retinal cell. In an experimental study, the effect of the absence of CRBN on the condition of retinitis was examined. In that study, retinitis was induced in human retinal cells by LPS. IL-6 and MCP-1 proteins are increased mediators in retinitis. IL-6 and MCP-1 protein synthesis is decreased in CRBN knock-down (KD) retinal cells [19].

In animal studies, CRBN reduced the inflammatory response. However, in our study, as the amount of CRBN in the lung secretion increased, the inflammatory cells decreased. This made us think that the inflammatory response to CRBN might be altered in genetically engineered CRBN KD animals. Other mechanisms may be at play.

CRBN is used as a target port to determine the treatment of inflammatory events and neoplastic diseases. Immunomodulatory drugs (IMiDs) are a class of compounds that can be used to attenuate the inflammatory response. IMiDs such as thalidomide and its structural analogs (lenalidomide, pomalidomide) are also used in cancer therapy, for example, multiple myeloma (MM), myelodysplastic syndrome (MDS) [20, 21].

CRBN has been identified as a mutual direct and major target of IMiDs [22]. A novel CRBN modulator, CC-885, has just been discovered. CC-885, unlike other IMiDs, has a strong anti-solid tumor effect [23]. CC-885 was found to increase the antitumor activity of Volasertib, a drug used in non-small-cell lung cancer (NSCLC). CC-885 works by selectively promoting CRBN, increasing the sensitivity of NSCLC to volasertib. It can be used in combination therapy to treat lung cancer [24].

The relationship between neoplastic diseases and CRBN has been most extensively investigated in MM. It has been suggested that MM patients with high CRBN expression are sensitive to IMiD treatment and show a good clinical course. Decreased CRBN protein levels have also been reported to be specifically associated with the development of lenalidomide resistance during treatment in 77% of lenalidomide-resistant MM patients [25–27].

Western blot, immunoprecipitation, and immunohistochemistry were used to assess CRBN expression in investigations [28, 29]. Xiu-Bao Chang and colleagues used the full-length human CRBN protein as the antigen to create CRBN-specific monoclonal antibodies (mAbs). These mAbs are extremely specific [30]. A commercial antibody developed for research purposes was used in our study, and there was no problem in negative-positive controls.

Although CRBN increased the response in cancer treatment in these studies, our study found no statistically significant relationship between CRBN expression and clinicopathological and prognostic parameters. This may be because we found lower CRBN synthesis in patients with increased inflammatory response. We could not observe a difference in mortality since this decrease did not contribute to the effect of IMiDs and other drugs used. This situation caused us to question the use of IMiDs like steroids in patients with severe inflammation in BAL. If CRBN, the primary target of IMiDs, is low in these patients, should we use IMiDs such as steroids and risk side effects? In addition, there is a tendency to initiate IMiDs according to the presence of CRBN in cancer patients. However, in non-
neoplastic inflammatory events, there is no practice to start treatment based on the presence of CRBN. More comprehensive studies are needed to enlighten these topics.

Because our study coincided with the pandemic period, our intensive care unit served as a corona intensive care unit during the peak periods of the epidemic. This caused us difficulty in finding non-COVID-19 cases. In addition, in this challenging period when the workload of anesthesiologists has increased, patient follow-up for scientific research became a luxury. Temporary assignments, difficulties in supplying materials, loss of motivation of staff, approaching every patient as a possible corona case, and cautious approach to procedures such as tracheal aspiration that cause an increase in the number of droplets were some of these difficulties. Despite all of these, my team continued to work with great motivation.

This study was conducted in non-COVID-19 patients. Due to the pandemic, our intensive care unit served as COVID-19 intensive care in some periods, which caused the prolongation of the case collection process for the research. In this study, some of the patients were in the "sepsis" stage, some in the "severe sepsis" stage, and some in the "septic shock" stage. In future studies, it is recommended to examine patients with sepsis of the same severity. Although there are many publications on Cereblon, there are no similar studies in humans. Therefore, the hypothesis was based on only animal or cell studies in mind. It would be nice if Cereblon could also be investigated in the blood. However, our hospital did not have the necessary materials. In our study, immunohistochemical cytoplasmic Cereblon expression was categorized as present/absent by an experienced pathologist. It is recommended to perform further studies in which digital images are obtained by virtual microscopy and quantitatively graded according to the intensity of staining.

**Conclusion**

CRBN expression is not a biomarker to be used as an indication for the treatment of IMiDs. In particular, cells expressing high amounts of CRBN are resistant to proteasome inhibitor-induced death in MM investigations, indicating that CRBN is important in chemotherapeutic treatment-induced cell death. However, the situation may not be similar in sepsis. The non-specificity of the most widely used polyclonal CRBN antibodies on the market requires a reproducibly accurate method of detecting cereblon protein in inflammatory cells. In addition to developing new monoclonal CRBN antibodies and optimizing the immunohistochemical staining method, further studies are needed to determine the most appropriate treatment strategy for CRBN expression.

**References**


Figures
During the study period, 116 intensive care patients were followed up in the anesthesia intensive care unit.

46 patients could not be included in the study because they tested positive to COVID-19.

70 patients were eligible for inclusion in the study

Relatives of 4 patients could not be included in the study because they did not give consent.

As a result of tracheal aspiration performed in 8 patients, adequate tracheal aspiration was not obtained, so they could not be included in the study.

Tracheal aspiration was obtained from 58 patients for research.

Tracheal aspiration material taken from 2 patients was excluded from the study because no cells could be seen in the microscopic examination after cell blocking.

The results of 56 patients were examined and statistics were made. (First stage)

Since PMNLs could not be seen in the material taken from 17 patients, it was not evaluated in the second stage.

Statistics were performed again with the results of 39 patients. (Second stage)

**Figure 1**

Flow diagram of the study
Figure 2

The diagnostic groups of patients
Figure 3

Histopathological evaluation reveals the differences of the CRBN expressions according to the cell types: (A) note the presence of CRBN expressions in both macrophages and neutrophils (DAB x 400), (B) note the presence of CRBN expressions in only macrophages (DAB x 400)

![Bar Chart](image)

CRBN expression

Figure 4

Negative correlation between the presence of CRBN expression and PMNLs rate (p=0.031) by chi-square analysis.