Human-Beta-Defensin-1, Ferritin, Interleukin-6 and their Relationship with Clinical and Laboratory Parameters of the Severity of the Tuberculosis Process

Establishing relationships between clinical and laboratory parameters, such as general patient’s state, parameters of complete blood count and blood biochemistry and markers of the course of tuberculosis in the future can be used to predict the severity of dysfunction of various organs and tissues in patients with tuberculosis and in particular in patients who receive anti-tuberculosis treatment.

Objective — to investigate the relationship between biochemical markers, namely Human-beta-defensin-1, ferritin and interleukin-6, and clinical and laboratory indicators of the severity of the tuberculosis process.

Materials and methods. 100 patients diagnosed with pulmonary tuberculosis were included in the study. After receiving 60 doses of anti-tuberculosis treatment, the patients were retrospectively divided into 2 groups. Group 1 (n = 77) consisted of patients in whom sputum conversion was observed after 60 doses of treatment, determined by sputum microscopy. Group 2 (n = 23) comprised patients in whom bacterial secretion was maintained after 60 doses of treatment, as detected by microscopy. In addition to the routine studies provided for the monitoring of patients with tuberculosis by the current orders of the Ministry of Health of Ukraine, the levels of Human-beta-defensin-1, ferritin and interleukin-6 (IL-6) in the fasting blood were additionally measured by ELISA at the beginning of treatment and after 60 days. Statistical data processing was carried out using the Statistica 8.0 software environment.

Results. A comparison of the investigated parameters between groups at the beginning of treatment showed significantly higher values of Human-beta-defensin-1 (Group 1 — (18.97 ± 2.42) pg/ml, Group 2 — (55.02 ± 15.69) pg/ml), ferritin (Group 1 — (94.86 ± 6.02) ng/ml, Group 2 — (141.61 ± 24.66) ng/ml) and IL-6 (Group 1 — (80.33 ± 5.03) pg/ml, Group 2 — (110.13 ± 10.35) pg/ml) in patients with positive sputum microscopy after 60 doses of treatment, p < 0.05. All studied markers demonstrated a reliable positive relationship with the massiveness of bacterial excretion, a conditional indicator of the severity of clinical symptoms and signs, ESR and urea level, as well as reliable negative correlations with creatinine level. In addition, patients with a lower body mass index were found to have higher levels of Human-beta-defensin-1 and ferritin. Higher levels of Human-beta-defensin-1 and ferritin are associated with lower hemoglobin levels (Human-beta-defensin-1 was also negatively correlated with erythrocyte count). An increase in the leukocytes level is accompanied by a significant increase in the level of glucose and ferritin, as well as between the level of bilirubin and ferritin and IL-6.

Conclusions. The determined significantly higher levels of Human-beta-defensin-1, ferritin and interleukin-6 in patients in whom sputum microscopy was positive after 60 doses of treatment allow considering the investigated biochemical parameters as markers of the ineffectiveness of anti-tuberculosis therapy. The identified positive relationships with the severity of clinical symptoms indicate the possibility of using the studied parameters as markers of the severity of the tuberculosis course. The possibility of using Human-beta-defensin-1 and ferritin as markers of anemia was also found. Correlations with parameters of blood biochemistry allow us to talk about increased levels of Human-beta-defensin-1, ferritin and interleukin-6 against the background of kidney damage.

Keywords
Tuberculosis, Human-beta-defensin-1, ferritin, interleukin-6, prognostic markers.
**ORIGINAL RESEARCHES**

**Objective** — to investigate the relationship between biochemical markers, namely Human-beta-defensin-1, ferritin and interleukin-6, and clinical and laboratory parameters of the severity of the tuberculosis process.

**Materials and methods**

100 patients with pulmonary tuberculosis were included in the study. After receiving 60 doses of anti-tuberculosis treatment, the patients were retrospectively divided into 2 groups: Group 1 (n = 77) comprised patients in whom sputum conversion was observed (determined by sputum microscopy) after 60 doses of treatment, while Group 2 (n = 23) comprised patients without sputum conversion (determined by microscopy) after the same duration of treatment.

In addition to the routine studies provided for the monitoring of tuberculosis patients by the current orders of the Ministry of Health of Ukraine, the levels of Human-beta-defensin-1, ferritin and IL-6 in fasting blood by the ELISA method were determined at the beginning of treatment and after 60 days. For the numerical expression of clinical symptoms and signs, a conditional score was used, in which each of the main symptoms of tuberculosis (cough, shortness of breath, chest pain, weight loss, weakness) was evaluated as 1 point.

Statistical data processing was carried out using the Statistica 8.0 software environment using descriptive statistics (mean, standard error, median) and non-parametric statistics (Spearman’s correlation coefficient, Mann–Whitney coefficient); the obtained results were considered statistically significant at p < 0.05.

**Results**

A comparison of the investigated indicators between the groups at the beginning of treatment (Fig. 1) showed a significantly higher level of Human-beta-defensin-1 (Group 1 — (18.97 ± 2.42) pg/ml (median — 6.81 pg/ml), Group 2 — (55.02 ± 15.69) pg/ml (median — 57.85 pg/ml)), ferritin (Group 1 — (94.86 ± 6.02) ng/ml (median — 78.41 ng/ml), Group 2 — (141.61 ± 24.66) ng/ml (median — 135.81 ng/ml)) and IL-6 (Group 1 — (80.33 ± 5.03) pg/ml (median — 67.93 pg/ml), Group 2 — (110.13 ± 10.35) pg/ml (median — 105.83 pg/ml)) in patients with positive sputum microscopy after 60 doses of treatment (p < 0.05).

A comparison of the studied parameters between the groups after 60 doses (Fig. 2) showed significantly higher levels of Human-beta-defensin-1 (Group 1 — (15.44 ± 2.24) pg/ml (median — 6.76 pg/ml), Group 2 — (71.25 ± 12.53) pg/ml (median — 54.90 pg/ml)), ferritin (Group 1 —...
(60.39 ± 6.60) ng/ml (median — 33.15 ng/ml), Group 2 — (135.63 ± 21.61) ng/ml (median — 115.55 ng/ml)) and IL-6 (Group 1 — (38.04 ± 2.88) pg/ml (median — 24.18 pg/ml), Group 2 — (99.87 ± 15.86) pg/ml (median — 105.80 pg/ml)) in patients with positive sputum microscopy after 60 doses of treatment (p < 0.05).

When studying correlations between Human-beta-defensin-1, ferritin, IL-6 and clinical and laboratory parameters of the severity of the tuberculosis process, all studied markers demonstrated a reliable positive relationship with the massiveness of bacterial excretion, a conditional indicator of the severity of clinical symptoms, ESR and urea level, as well as reliable negative correlations with creatinine level. In addition, patients with a lower body mass index were found to have higher levels of Human-beta-defensin-1 and ferritin. Higher levels of Human-beta-defensin-1 and ferritin are associated with lower hemoglobin levels (Human-beta-defensin-1 was also...
negatively correlated with erythrocytes level). An increase in leukocytes level is accompanied by a significant increase in the levels of Human-beta-defensin-1 and IL-6. In addition, a significant negative correlation was found between the level of glucose and ferritin, as well as between the level of bilirubin and ferritin and IL-6. The obtained data are presented in the Table.

**Discussion**

In previous works, we considered the possibility of using ferritin, Human-beta-defensin-1, and IL-6 as markers of the tuberculosis treatment effectiveness, but their relationship with clinical and radiological parameters of the severity of the tuberculosis process was not sufficiently studied [1, 16].

In the course of the study, significantly higher levels of Human-beta-defensin-1, ferritin, and IL-6 were found in patients who, after 2 months of anti-tuberculosis treatment, maintained bacterial excretion, which allows us to consider the investigated biochemical indicators as markers of the effectiveness of tuberculosis treatment.

The revealed inverse relationship between BMI, Human-beta-defensin-1 and ferritin is probably a consequence of the fact that with a massive tuberculosis lesion, which leads to exhaustion of the patient, the severity of the immune response increases, which is manifested by an increase in the studied inflammatory markers. Interestingly, no significant relationship between BMI and IL-6 was found. This can be explained by the fact that the production of IL-6 is one of the first to increase when immune cells are in contact with *M. tuberculosis*, and IL-6 remains a key inducer of the immune response regardless of the volume of the tuberculosis lesion and the general patient’s state [9].

In all three investigated markers, reliable positive correlations were observed with the severity of clinical symptoms and the massiveness of bacterial excretion, which allows them to be considered as markers of the tuberculosis course severity. At the same time, the highest correlation strength was observed in Human-beta-defensin-1, which makes it the most sensitive of the studied markers.

Ferritin plays an important role in the anti-tuberculosis immune response not only by acting as a pro-inflammatory cytokine [10], but also by reducing the availability of iron to *M. tuberculosis*, as iron is an important cofactor of metabolic enzymes of mycobacteria [1]. In fact, in tuberculosis, there is a transition from available, transferrin-bound iron to accumulated iron, that is, ferritin [14]. However, reducing the availability of iron for the pathogen, ferritin also reduces its availability for the host’s body, which is reflected in the negative relationship between the level of ferritin and hemoglobin that we found. In turn, iron deficiency leads to a violation of the production of antimicrobial peptides, including the family of beta-defensins, and reduces their bactericidal activity [11]. The negative correlations we found between the level of Human-beta-defensin-1 and hemoglobin and erythrocytes confirm this fact.

A positive relationship between the levels of Human-beta-defensin-1, IL-6 and leukocytes was expected, since the latter are the main producers of these cytokines [12, 13].

The revealed negative relationship between the levels of Human-beta-defensin-1, ferritin and IL-6 on the one hand and the levels of bilirubin and creatinine on the other indicates a malfunction of the liver and kidneys against the background of active tuberculosis inflammation and taking anti-tuberculosis drugs.

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<table>
<thead>
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<th>Table. Correlations between clinical and laboratory parameters of the severity of the tuberculosis process and the investigated biochemical markers</th>
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<tr>
<td><strong>Human-beta-defensin-1</strong></td>
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<td>Bilirubin</td>
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Note. All the given parameters have reliable correlations (p < 0.05); ‘–’ the absence of a reliable correlation (p > 0.05).
Conclusions
The determined significantly higher levels of Human-beta-defensin-1, ferritin and interleukin-6 in patients in whom sputum microscopy was positive after 60 doses of treatment allow considering the investigated biochemical parameters as markers of the ineffectiveness of anti-tuberculosis therapy. The identified positive relationships with the severity of clinical symptoms indicate the possibility of using the studied parameters as markers of the severity of the tuberculosis course. The possibility of using Human-beta-defensin-1 and ferritin as markers of anemia was also found. Correlations with parameters of blood biochemistry allow us to talk about increased levels of Human-beta-defensin-1, ferritin and interleukin-6 against the background of kidney damage.

No conflict of interest.

Participation of the authors: concept and design of the study — O.S. Shevchenko, L.D. Todoriko; collection of material — I.A. Ovcharenko; processing of material — O.M. Shvets, R.S. Shevchenko; writing the text — O.O. Pohorielova; text editing — S.L. Matvyeyeva, E. Tudor.

References

ORIGINAL RESEARCHES / ОРИГІНАЛЬНІ ДОСЛІДЖЕННЯ
Матеріали та методи. У дослідження було залучено 100 хворих на туберкульоз легень. Після отримання 60 доз протитуберкульозного лікування пацієнтів ретроспективно розподілили на дві групи: 77 пацієнтів, у яких зареєстрували припинення бактеріовиділення (за даними мікроскопії), і 23 пацієнта, у яких зберігалося бактеріовиділення (за даними мікроскопії). Крім рутинних досліджень, передбачених для моніторингу пацієнтів із туберкульозом наказами МОЗ України щодо ведення хворих на туберкульоз, вимірювали рівень β-дефензину-1, феритину та ЛЛ-6 у крові натис методом імуноферментного аналізу на початку лікування та через 60 днів. Статистичну обробку даних проведено за допомогою програмного середовища Statistica 8.0.

Результати та обговорення. Порівняння досліджуваних показників у групах на початку лікування виявилось статистично значущим (р < 0.05) вищим рівнем у групі 2 β-дефензину-1 ((18,97 ± 2,42) та (55,02 ± 15,69) нг/мл), феритину ((94,86 ± 6,02) і (141,61 ± 24,66) нг/мл) та ЛЛ-6 ((80,33 ± 5,03) і (110,13 ± 10,35) нг/мл). Усі досліджувані маркери мали статистично значущий прямо пропорційний зв'язок з масивністю бактеріовиділення, умовним показником виразності хімічних симптомів, ШОЕ та рівнем сечовини, а також статистично значущий обернено пропорційний зв'язок із рівнем креатиніну. У пацієнтів з меншим індексом маси тіла зареєстровано вище показники β-дефензину-1 і феритину. Більша концентрація β-дефензину-1 та феритину пов’язана з нижчим рівнем гемоглобіну (для β-дефензину-1 також установлена обернено пропорційний зв’язок із кількістю еритроцитів). Збільшення кількості лейкоцитів супроводжувалося статистично значущим підвищенням рівня β-дефензину-1 та ЛЛ-6. Установлено статистично значущий обернено пропорційний зв’язок між вмістом глукози та феритину, а також між рівнем білірубіну і феритину та ЛЛ-6.

Висновки. Статистично значущим було вищим рівнем β-дефензину-1, феритину та ЛЛ-6 у пацієнтів, у яких через 60 доз лікування зберігалося бактеріовиділення, дають підставу розглядати досліджувані біохімічні показники як маркери неефективності протитуберкульозної терапії. Установлено пряме пропорційне зв’язок з тяжкістю клінічної симптоматики свідчить про можливість застосування досліджуваних параметрів як маркерів тяжкого перебігу туберкульозу. Виявлено можливість використання β-дефензину-1 та феритину як маркерів анемії. Наявність кореляції з показниками біохімічного аналізу крові свідчить про підвищення зв’язок β-дефензину-1, феритину та ЛЛ-6 на тлі ураження нирок.

Ключові слова: туберкульоз, β-дефензин-1, феритин, інтерлейкін-6, прогностичні маркери.

Контактна інформація

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