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Original Article

Role of serum liver markers and elastography in liver fibrosis evaluation depending on body weight

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Abstract

Drug-induced liver injury (DILI) represents a significant yet often underestimated challenge in contemporary medicine, exerting a pivotal influence on the landscape of liver pathology. Acknowledging that official statistics fall short of capturing the genuine prevalence of DILI due to inherent underreporting, this article addresses the issue through a study concentrating on serum liver fibrosis markers among female breast cancer patients subjected to polychemotherapy. The research endeavors to scrutinize the prognostic significance of serum liver fibrosis markers. Additionally, the study investigates correlations among direct fibrosis markers in patients with diverse body weight statuses. The cohort, comprising 123 females, underwent categorization based on the severity of DILI post-polychemotherapy. Employing universal clinically significant indicators, specifically fibrosis indices (FIB-4, Fibrotest), the study comprehensively evaluated liver parenchymal damage using biochemical markers two months post-treatment. Results unveiled a positive correlation between TGF-β1 and Col-4 fibrosis markers and body mass index (BMI). With increasing BMI, levels of TGF-β1 and Col-4 escalated, notably exhibiting a 2.2 and 1.2 times increase, respectively, in obese individuals compared to those with normal weight. Furthermore, heightened TGF-β1 and Col-4 levels correlated with advanced fibrosis stages based on average Fibrotest and FIB-4 index values ($r=0.720$, $p<0.05$ and $r=0.716$, $p<0.05$) and ($r=0.771$, $p<0.05$ and $r=0.799$, $p<0.05$). These findings propose a potential link between stellate cell activation, necroinflammatory fibrosis, and type IV collagen expression initiation. Remarkably, TGF-β1 and Col-4 emerge as prospective markers for monitoring fibrosis progression in DILI post-chemotherapy, providing avenues for refined diagnosis and treatment strategies.

Keywords: drug-induced liver injury, chemotherapy, liver fibrosis, TGF-β1, Col-4.

Introduction

Over the past decades, there has been an increase in the number of adverse effects and complications associated with drug therapy worldwide. Among them, a significant proportion is constituted by liver injuries that arise as a result of treatment with medicinal agents – drug-induced liver injuries (DILI). The clinical experience of physicians suggests that any pharmaceutical product, herbal remedy, or dietary supplement can induce DILI. The diagnosis of DILI poses a complex clinical problem for physicians, as the spectrum of clinicopathological variants of this pathology is extremely diverse. The diagnosis is established through the exclusion of other acute and chronic liver diseases with similar symptoms. However, it is worth diagnosing DILI at earlier stages, as prolonged use of medications can significantly exacerbate the severity of clinical manifestations, which can greatly influence the prognosis of the overall condition [1, 2].

The problem of DILI is important because the use of pharmaceutical agents in therapeutic doses can potentially harm the liver, and the outcomes of their application can be unpredictable [3]. Unfortunately, there are no clear principles of DILI therapy apart from discontinuing the causative drug. Furthermore, the

prevalence of DILI is underestimated, as the diagnosis is rarely established, possibly due to patients' reluctance to report the use of certain medications or physicians' unwillingness to document iatrogenic diseases. Based on population studies, DILI has been identified in 0.139% of participants in France, 0.041% in Italy, 0.03% in Spain, 0.023% in Sweden, and from 0.1% to 1.5% in the United States [4–6].

For practicing physicians and researchers involved in cancer treatment, it is important to understand not only the clinical but also the laboratory characteristics of DILI progression, such as direct biomarkers of liver injury. This knowledge can open up new possibilities in the treatment of comorbid patients [7–10]. In the context of current trends [9–11] in the treatment of oncological diseases, studying DILI is crucial, as it can help improve clinical approaches to DILI treatment and enhance the effectiveness of treating the underlying condition.

The aim of this article is to study and analyze the results of serum markers of liver fibrosis, evaluate their prognostic significance for the development and progression of liver dysfunction and metabolic processes disorders, as well as to search for correlations between direct markers of fibrosis in patients with normal and excessive body weight.

Material and methods

The study was conducted at the Department of Higher Nursing Education, Care for the Sick, Clinical Immunology, and in the conditions of the 1st Surgical Department of the Ternopil Regional Clinical Oncology Dispensary.

The diagnosis of breast cancer was verified at the oncology facility of the Ternopil Regional Clinical Oncology Dispensary of the Ternopil Regional Council based on examination mammography data, and the final diagnosis was made according to the results of the histological (cytological) examination.

The diagnosis of toxic liver injury (drug-induced liver injury, DILI) was verified in accordance with the existing guidelines and the order of the Ministry of Health of Ukraine dated 13.06.2005, No. 271-K 71.7 – Toxic liver injury with liver fibrosis and cirrhosis. This study is based on the results of an examination of patients with breast cancer who are in remission after inpatient treatment in the 1st Surgical Department of the Ternopil Regional Oncology Dispensary.

In accordance with the research objectives, the inclusion criteria for the study were as follows:

- Patients aged 18 or older;
- Presence of a diagnosis of stage I-II breast cancer according to the TNM classification (TNM-8, 2016 edition) without malignancy;
- Belonging to the III clinical group, which includes individuals with confirmed malignant tumors who have completed radical treatment and are in remission.

The exclusion criteria were as follows:

- Presence of a diagnosis of stage III-IV cancer with malignancy;
- Belonging to the I clinical group (patients suspected of having cancer or with precancerous conditions), II clinical group (cancer patients undergoing antitumor treatment), or IV clinical group (patients receiving symptomatic and palliative treatment due to metastatic involvement, tumor decay, cachexia, pain syndrome);
- History of or current viral hepatitis (associated with HBV, HCV, HDV infection), herpes virus infections (EBV, CMV, HHV6), and HIV infection, as well as hepatitis associated with storage diseases and hereditary disorders, autoimmune hepatitis/cross-syndrome (anti-LKM-1, anti-SLA, and anti-LC-1 antibodies), focal liver lesions, chronic pancreatitis, fibrotic processes in other organs and systems;
- Presence of infectious diseases;
- Presence of hereditary diseases;
- Presence of autoimmune diseases;
- Alcoholic liver injury (based on a verified diagnosis of "alcoholic liver disease" and/or daily consumption of alcohol in high doses ≥20 g for women). In addition, the AUDIT test and CAGE questionnaire were used to exclude alcoholic liver disease caused by alcohol consumption.

Overall, none of the patients included in the study had exacerbations of gastrointestinal tract pathology or any other diseases or conditions that could potentially affect the results of the conducted examinations.

Here is the translation of the provided text in a scientific style:

A total of 123 female patients, ranging in age from 35 to 79 years, were examined. The patients were categorized into three main groups: Group I – 33.9% (n=42) of patients who had undergone chemotherapy without signs of liver toxicity, Group II – 41.1% (n=51) of patients with grade I liver toxicity after undergoing chemotherapy, and Group III – 24.2% (n=30) of patients with grade II liver toxicity after undergoing chemotherapy. General clinical laboratory and instrumental methods

were used for patient examination. The examination included collecting and analyzing patients' medical history and complaints, followed by a general examination with subsequent measurement of anthropometric indicators. Fibrosis indices (FIB-4, Fibrotest) were used to comprehensively assess all characteristics (localization, extent, severity) of liver parenchymal damage. The calculation was performed 2 months after the completion of the polychemotherapy cycle to evaluate the hepatological profile objectively. Biochemical parameters were used for their calculation.

Enzyme-linked Immunosorbent Assay (ELISA) was employed to quantitatively determine the levels of transforming growth factor-beta 1 (TGF-β1) and collagen type IV (COL4) in serum samples. The ELISA analysis was conducted using the automatic immunoassay analyzer "Multiskan FC-357" in accordance with the instructions of the test systems "Human TGF-β1 Platinum ELISA (BMS249/4 BMS249/4TEN; eBioscience, Austria)" and "ELISA Kit for Collagen Type IV (COL4), Cloud-Clone Corp., USA". The reference values for TGF-β1 were 5222–13731 pg/mL and for COL-4 were 35.8–134 ng/mL.

Statistical analysis was performed using the SPSS Statistics 26.0 software package and Microsoft Excel. The normality of distribution was assessed using the Kolmogorov-Smirnov test for adherence to the normal distribution law. For continuous variables, differences between independent groups were compared using Student's t-test, and for repeated measurements within the same sample, a paired t-test was employed. Correlation analysis was conducted using Pearson's linear regression for continuous variables and Spearman's rank correlation for non-parametric indicators. The results were considered statistically significant at $p<0.05$.

Results and discussion

A significant correlation was found between the levels of transaminases and FIB-4 – ALT ($r=0.738$, $p<0.05$) and AST ($r=0.743$, $p<0.05$). A strong positive correlation was found between FIB-4 and LDL-C ($r=0.818$, $p<0.05$), LDL-NC ($r=0.800$, $p<0.05$), and a negative correlation with HDL-C ($r=-0.571$, $p<0.05$) and platelet count ($r=-0.683$, $p<0.05$). It was established that with an increase in BMI indicators, the levels of TGF-β1 and Col-4 also increased. In the case of obesity, the markers increased 2.2 and 1.2 times for TGF-β1 and Col-4, respectively, compared to the markers' values under normal body weight conditions. It was found

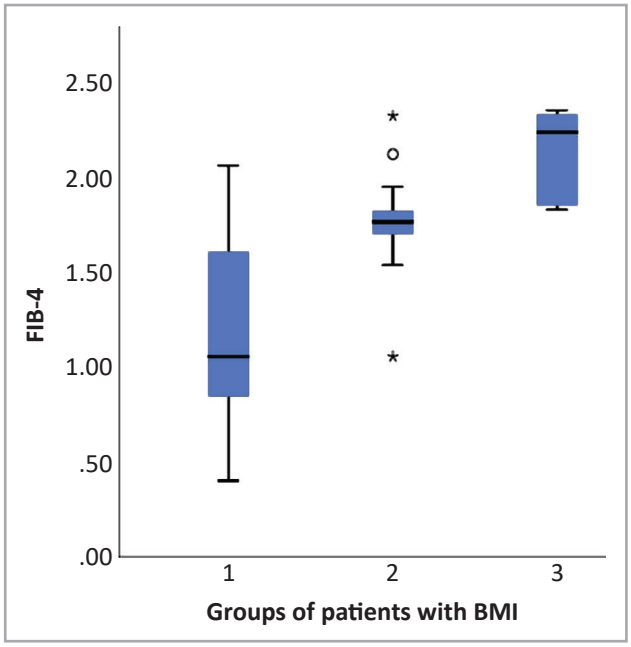

Figure 1: Comparative characteristics of FIB-4 index data in groups.

that the increase in levels of TGF-β1 and COL-4 correlates with higher stages of fibrosis according to the average values of Fibrotest and the FIB-4 index ($r=0.720$, $p<0.05$ and $r=0.716$, $p<0.05$) and ($r=0.771$, $p<0.05$ and $r=0.799$, $p<0.05$).

The FIB-4 index is a method for assessing the condition of liver tissue that is accessible and non-invasive, aiding in the detection of fibrotic processes in the liver. The study showed that the FIB-4 index significantly differs in female patients with normal body weight compared to those with elevated body weight ($p<0.01$), in patients with normal body weight and first-degree obesity ($p<0.01$), as well as in patients with elevated body weight and first-degree obesity ($p<0.01$) (Figure 1). Furthermore, a significant correlation was observed between BMI and the FIB-4 index – as body weight increases, the FIB-4 index also increases linearly ($r=0.695$, $p<0.01$).

A direct linear relationship was found between the levels of transaminases and FIB-4 – ALT ($r=0.738$,

Table 1: Indicators of the FIB-4 index in groups (M±m).

Group	FIB-4	P-value
BMI 18.5–24.9 kg/m²	1.18±0.46	<0.01*
BMI 25–29.9 kg/m²	1.75±0.17	<0.01*
BMI 30–34.9 kg/m²	2.14±0.22	<0.01*

Note: Significance of the difference according to the Kruskal-Wallace test: * – $p<0.01$.

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Table 2: FibroTest index values in subgroups (M±m).

Group	Fibrotest	P-value
BMI 18.5–24.9 kg/m²	1.18±0.46	<0.01*
BMI 25–29.9 kg/m²	1.75±0.17	<0.01*
BMI 30–34.9 kg/m²	2.14±0.22	<0.01*

Note: Significance of the difference according to the Kruskal-Wallace test: * – p<0.01.

Table 3: Levels of TGF-β1 in patients with different degrees of fibrosis.

Fibrosis stage, METAVIR	TGF-β1	P-value
F0-1	9036.09±2432.11	<0.01*
F1	16024.13±4347.17	<0.01*
F2	21660.11±4371.36	<0.01*

Note: Significance of the difference according to the Kruskal-Wallace test: * – p<0.01.

p<0.05) and AST (r=0.743, p<0.05). A strong positive correlation was observed between FIB-4 and LDL-C (r=0.818, p<0.05), LDL-NC (r=0.800, p<0.05), and an inverse correlation with HDL-C (r=-0.571, p<0.05). Similarly, a reverse relationship was observed between the FIB-4 index and platelet levels (r=-0.683, p<0.05). Furthermore, a significant correlation was found between the FIB-4 index and the presence of hepatomegaly in patients (r=0.610, p<0.05). Of particular interest is the correlation with elastographic liver tissue density (r=0.709, p<0.05), which underscores the diagnostic value of both methods.

Thus, it has been found that as the BMI values increase, the FIB-4 index also undergoes changes with a tendency to increase. Individuals with a normal BMI show no evidence of moderate fibrosis (FIB-4<1.45) (Table 1). Similarly, it can be inferred that significant fibrosis is absent in groups with overweight, where the BMI indicates the presence of overweight and obesity grade I, as the FIB-4 does not exceed 3.25.

In the course of comprehensive computational studies for FibroTest calculation, significant differences were observed in the laboratory parameters of patients with a normal BMI and overweight (p<0.01), normal BMI and obesity grade I (p<0.01), and overweight and obesity grade I (p<0.01). Similarly, an analysis of the FibroTest indicator was conducted based on the BMI levels (Table 2).

It is evident that with the deterioration of the anthropometric profile, the FibroTest data also increased, indicating the presence of F1-F2 fibrosis in the group with the highest BMI value.

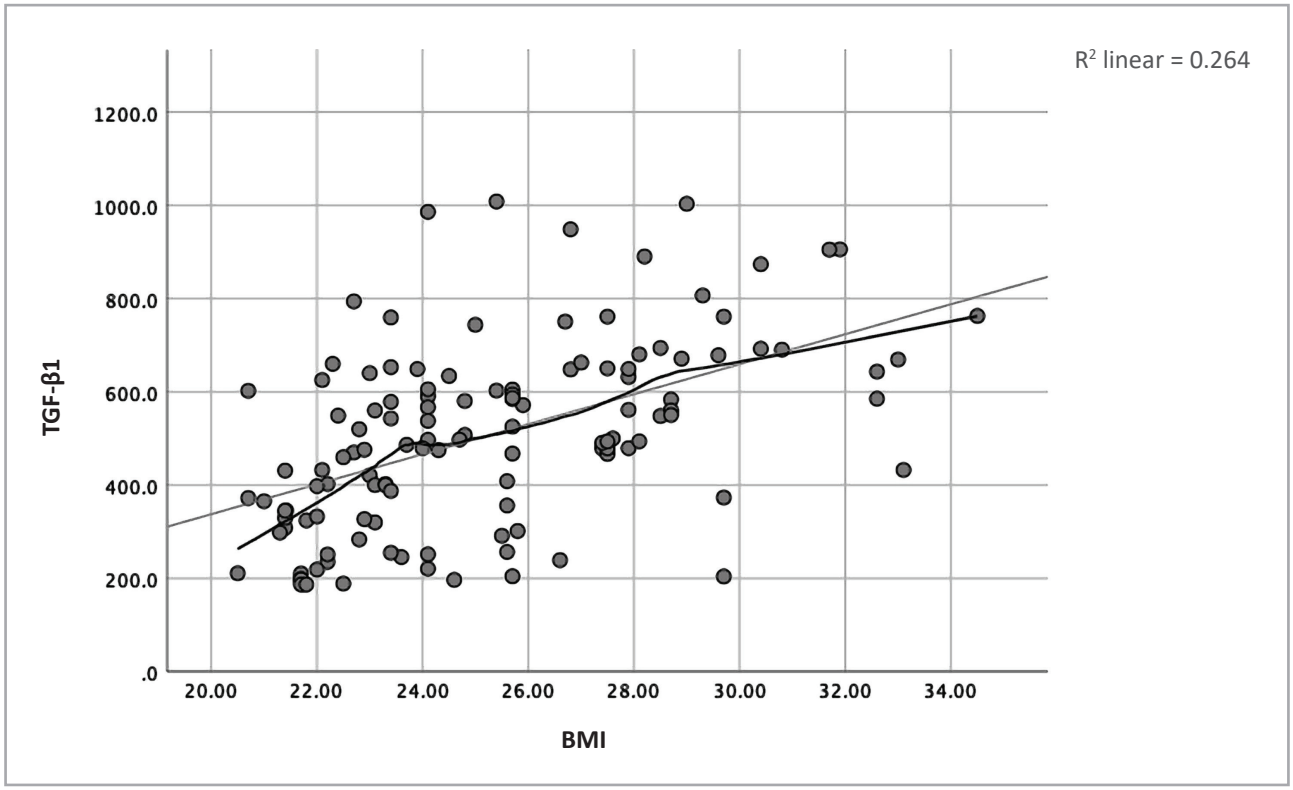

Figure 2: Relationship between the level of TGF-β1 and BMI in patients.

Table 4: Levels of COL-4 in patients with different degrees of fibrosis.

Fibrosis stage, METAVIR	COL-4	P-value
F0-1	124.26±2.01	<0.01*
F1	132.40±4.99	<0.01*
F2	140.26±6.94	<0.01*

Note: Significance of the difference according to the Kruskal-Wallace test: * – p<0.01.

Therefore, for patients under dynamic observation for post-hepatitis liver fibrosis due to received polychemotherapy, it is recommended to perform calculations of such minimally invasive markers as FIB-4 and FibroTest, as they complement each other. In order to confirm fibrotic changes in the liver, an analysis of direct fibrosis markers, TGF-β1 and COL4, was conducted (Table 3).

The obtained results indicate a significant increase in the level of the TGF-β1 marker with the progression of fibrotic changes in the liver, which is likely to be attributed to the activation of hepatic stellate cells (HSCs). Therefore, the relationship between the pro-inflammatory cytokine and anthropometric indicators was investigated (Figure 2).

It is evident that the relationship is linear, indicating a significant increase in the level of TGF-β1 with an increase in BMI (r=0.554, p<0.05).

Similarly, the level of type IV collagen, which is a key structural component of cell basement membranes, was analyzed. The following data were obtained (Table 4).

Thus, with the progression of fibrotic processes in the liver, there is a decrease in the expression of matrix metalloproteinases and, as a result, impaired synthesis of type IV collagen with a tendency to increase. When analyzing Col-4 with anthropometric indicators, a strong significant correlation (r=0.913, p<0.05) was found, which is supported by a linear relationship with an increasing trend (Figure 3).

Furthermore, an analysis of the correlation between the aforementioned direct fibrosis markers and indirect markers (ALT, AST) was conducted, revealing an association between TGF-β1 and the levels of ALT and AST (r=0.401, p<0.05 and r=0.507, p<0.05), as well as between COL-4 and ALT and AST (r=0.531, p<0.05 and r=0.520, p<0.05), respectively.

Since TGF-β1 is a key factor in the development of fibrosis in parenchymal organs and regulates COL-4

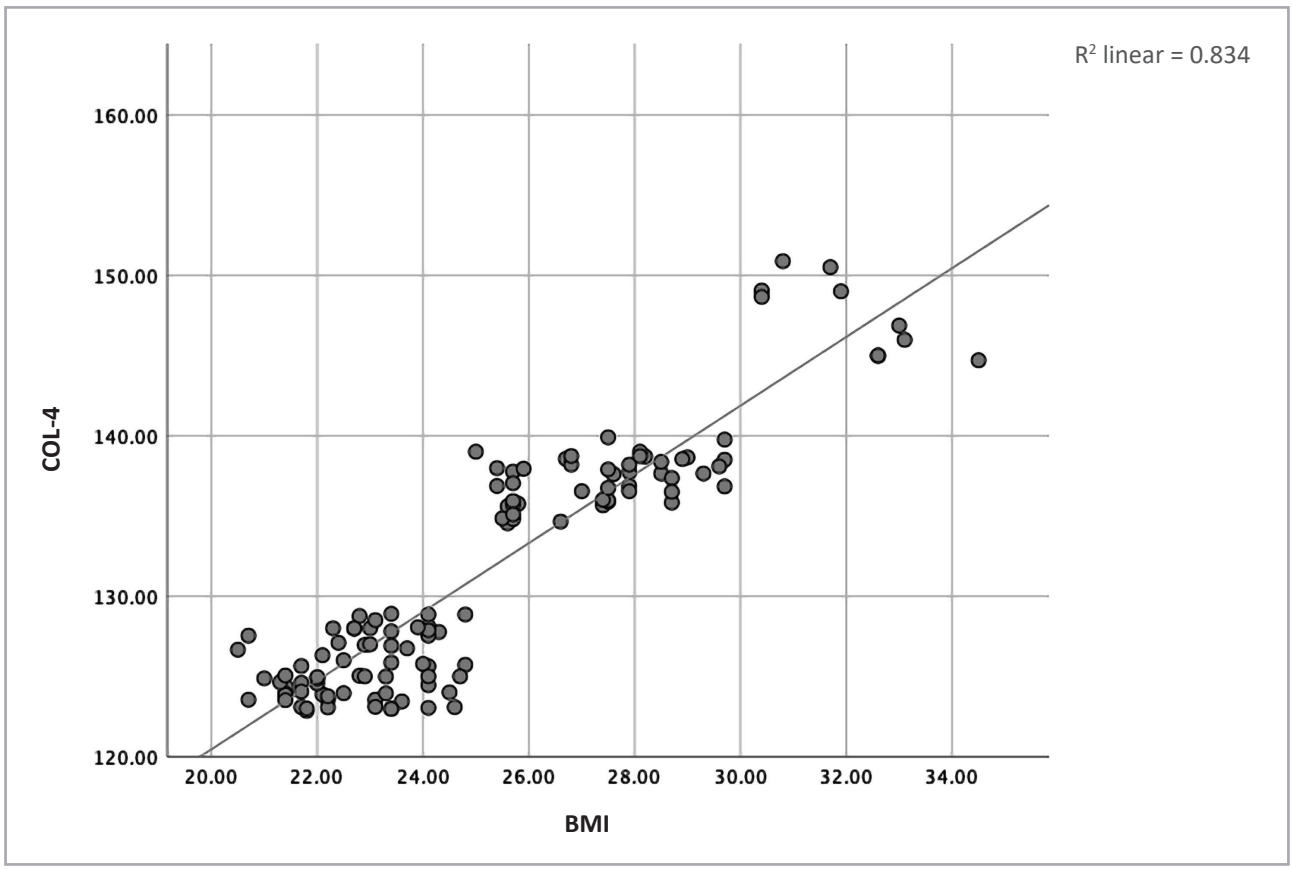

Figure 3: Relationship between COL-4 levels and BMI in patients.

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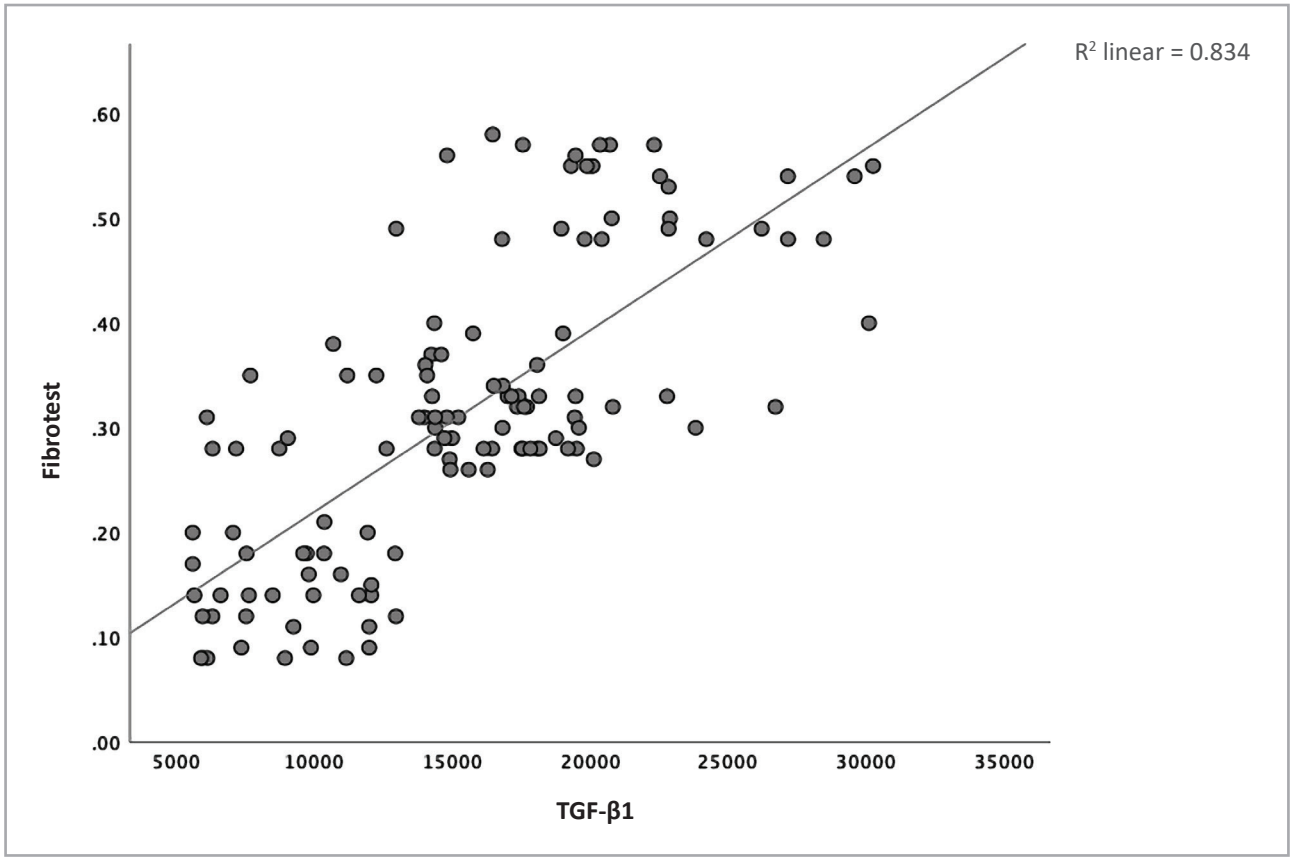

Figure 4: Dependence of TGF-β1 level on fibrotest indicators.

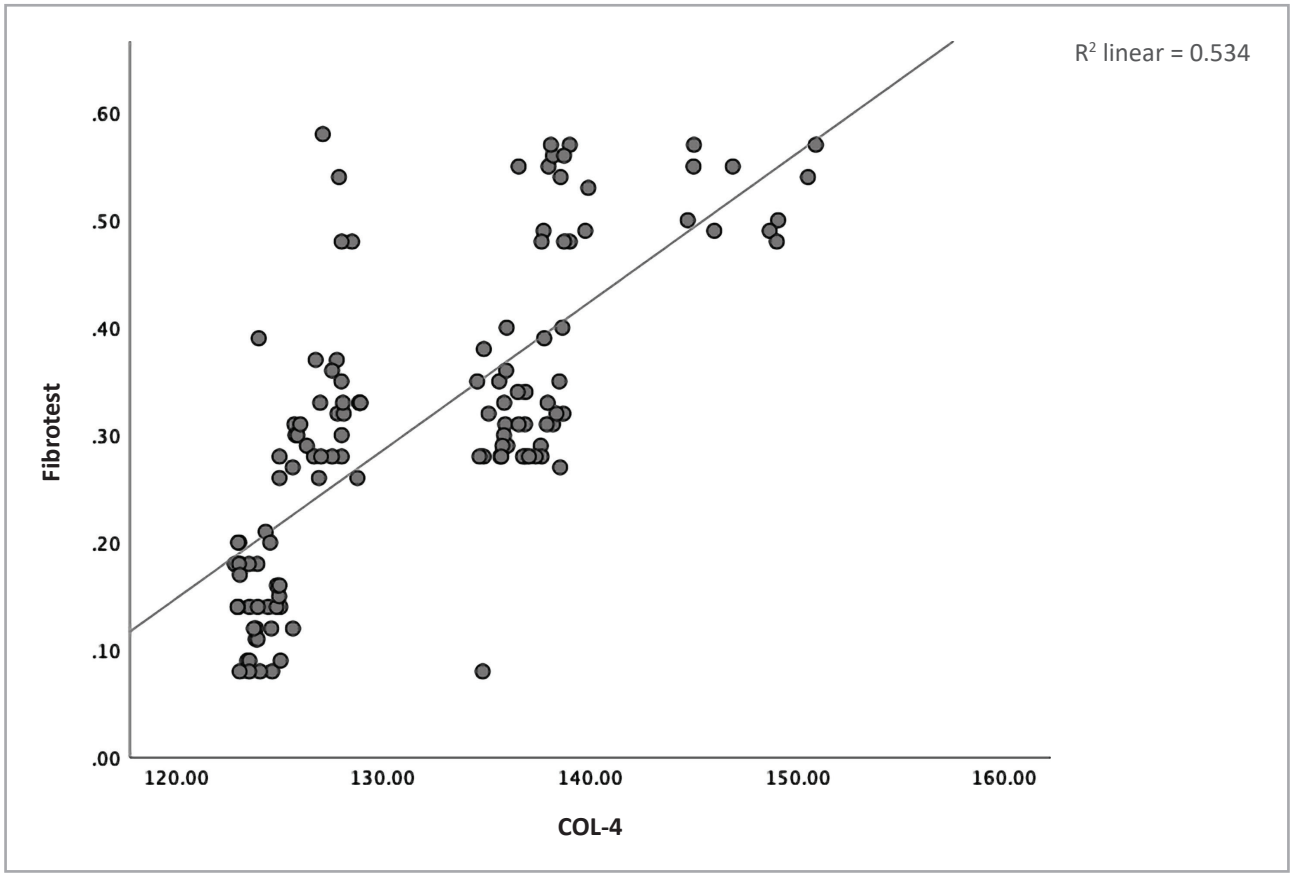

Figure 5: Dependence of TGF-β1 level on fibrotest indicators.

synthesis, a relationship was found between their levels and ultrasound data. It was found that patients with enlarged liver size and changes in liver tissue echogenicity also had elevated levels of TGF-β1 ($r=0.629$, $p<0.05$ and $r=0.738$, $p<0.05$). Similar dynamics were demonstrated for the level of type IV collagen in relation to ultrasound parameters ($r=0.682$, $p<0.05$ and $r=0.693$, $p<0.05$), respectively. As the direct markers increase, indicating the progression of fibrogenesis, the obtained data should be considered as evidence that direct markers can serve as indicators of liver fibrosis before the presence of ultrasound signs. By using the pairwise correlation analysis, a close relationship was found between the direct fibrosis markers TGF-β1 and COL-4 and the fibrosis stage according to Metavir, as well as the FIB-4 index, with significant correlations ($r=0.720$, $p<0.05$ and $r=0.716$, $p<0.05$) and ($r=0.771$, $p<0.05$ and $r=0.799$, $p<0.05$), respectively (Figures 4 and 5).

An analysis was conducted of the strong correlation between direct and indirect markers of liver fibrosis in patients following chemotherapy with normal and excessive body weight, allowing for the conclusion of a mutually reinforcing effect of these conditions. This supports the likelihood of activation and progression of fibrosis processes in liver tissue [12].

Conclusions

Fib-4 and Fibrotest are sensitive and reliable laboratory screening methods for detecting severe liver fibrosis in oncology patients following chemotherapy.

The presence of excess body weight in oncology patients in a state of stable remission correlates with the severity of liver fibrosis ($r=0.75$) and may be an important factor in predicting the development of severe fibrosis in patients in the long term following treatment for breast cancer. Therefore, patients with BMI>25.0 should be prioritized for screening for liver fibrosis.

The levels of direct fibrosis markers, TGF-β1 and COL-4, correlate with the severity of liver fibrosis based on Fibrotest data ($r=0.720$, $r=0.771$, $p<0.05$) and can serve as sensitive predictors of fibrosis progression, contributing to the improvement of diagnostics and treatment of patients with metastatic invasive urothelial carcinoma (MIUC), particularly in cases where Fib-4 and Fibrotest have limited informativeness.

In further research, an elastography examination is planned for patients with MIUC to analyze and explore the correlations between its results and the data of direct fibrosis markers.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The approval for this study was obtained from the Ethics Committee of the I. Horbachevsky Ternopil National Medical University (approval ID: 0121U100066, May 03, 2019).

Consent to participate

Written informed consent was obtained from all participants in this study.

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