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Predictive factors of hepatotoxicity in immunotherapy with checkpoint inhibitors in patients treated for melanoma and kidney cancer

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ABSTRACT

Introduction. Checkpoint inhibitors immunotherapy (CPI) is widely used in the treatment of malignant tumors and has a positive effect on patient prognosis. CPI treatment is associated with various immunological adverse events (AEs), including a rare one — immunological hepatitis.

Material and methods. This study aims to analyze hepatic AEs in patients undergoing CPI therapy and to attempt to determine hepatotoxicity predictors. A retrospective statistical analysis of medical records of 223 CPI patients treated in the years 2014–2021 in Lower Silesian Oncology, Pulmonology and Hematology Center in Wrocław was performed.

Results. Toxicity grade 1–4 according to the Common Terminology Criteria for Adverse Events (CTCAE) occurred in 26% of patients, of which 6% were grade 3–4. An increased risk of hepatotoxicity was found in the group of patients ≤ 60 years of age compared to the > 60-year-old group (34.1% *vs.* 21.7%, p = 0.0418). It has also been confirmed that the occurrence of hepatic AEs during first-line immunotherapy increases the risk of toxicity recurrence during second-line immunotherapy (58.3% *vs.* 15.4%, p = 0.0199). No significantly increased risk of hepatic AEs has been demonstrated in patients with liver metastases, hepatic steatosis, or other chronic liver disease, or in patients after chemotherapy, with elevated baseline levels of lactate dehydrogenase (LDH), or increased body mass index (BMI).

Conclusions. The hepatotoxicity of CPI immunotherapy poses a significant diagnostic and therapeutic challenge. Its early detection and treatment according to the recommended algorithms increases patient safety for patients and sometimes allows the continuation of treatment.

Key words: hepatotoxicity, immunotherapy, immune checkpoint inhibitors, melanoma, renal cell carcinoma

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Introduction

Immunotherapy with anti-cytotoxic T-cell antigen 4 (anti-CTLA4), anti- programmed cell death protein 1 (anti-PD-1), and anti-programmed cell death ligand 1 (anti-PD-L1) is widely used in the treatment of malignant tumors and has a positive effect on patient prognosis. It has been demonstrated to be effective in improving both progression-free survival (PFS) and overall survival (OS) in the treatment of many cancers, including melanoma and renal cell carcinoma [1, 2].

At the same time, the treatment is associated with the occurrence of immunological toxicities, such as dermatological, endocrinological, pulmonary, or gastroenterological [3, 4]. These include immune-mediated hepatitis (IMH) induced by immune checkpoint inhibitors, which is relatively rare (1–5% depending on the criteria). It most often appears around the 2nd month of therapy and initially is usually asymptomatic, revealing abnormalities only in laboratory tests. However, it can also lead to serious liver damage, including acute failure [5, 6]. Therefore, it is necessary to monitor the patient's condition and laboratory parameters. If abnormalities are detected in tests evaluating liver function, the management recommended by oncological societies depends on the severity of adverse events (AEs) according to the Common Terminology Criteria for Adverse Events (CTCAE). The main treatment is high-dose glucocorticosteroid (CS) therapy, and if steroids fail, non-steroidal immunosuppressants. For grade 1 immune-related liver injury, monitoring of liver enzymes every 1–2 weeks is recommended, with no need to suspend Checkpoint inhibitors immunotherapy (CPI) therapy. For grade 2 immune-related liver injury, temporarily withholding CPI therapy is suggested, with monitoring of transaminases and bilirubin twice weekly. Initiation of CS therapy, preferably (methyl)prednisolone 0.5–1 mg/kg/day should be considered. For patients with grade 3 or 4 immune-related liver injury, hospitalization, and initiation of CS therapy, with (methyl) prednisolone 1–2 mg/kg/day is recommended. If there is no response to CS therapy within 2–3 days, alternative immunosuppressive therapy should be considered, such as mycophenolate mofetil (1000 mg twice daily), tocilizumab (8 mg/kg), tacrolimus, azathioprine, cyclosporine, or anti-thymocyte globulin. Immunosuppressants should be continued until full improvement is achieved, and CS therapy should be maintained for at least several weeks after normalization; dose reduction should be cautious [7–9]. In each case, other causes of liver damage should be excluded, such as viral hepatitis, other hepatotoxic substances/drugs, or disease progression in the liver; however, differential diagnosis is not always conclusive [10]. In the literature on hepatic AEs of CPI, it is difficult to clearly distinguish between IMH-type inflammation and similar liver dysfunction (idiopathic autoimmune hepatitis, drug-induced autoimmune hepatitis), and the differentiation should always take into account malignant liver damage, e.g. hyper progression, especially in patients with liver metastases [11].

Material and methods

A total of 223 patients were analyzed, including 208 diagnosed with melanoma and 15 with kidney cancer, who were treated in the years 2014–2021 in the Lower Silesian Oncology, Pulmonology and Hematology Center with immunotherapy, i.e. anti-PD-1 antibodies (nivolumab, pembrolizumab) and/or anti-CTLA4 (ipilimumab). In the entire population, 47% of patients received nivolumab, 36% of patients received pembrolizumab, 34% of patients received ipilimumab, and in the subgroup of patients diagnosed with melanoma, 18% received sequentially one of the anti-PD-1 drugs and ipilimumab. In the group of patients with melanoma, patients with advanced disease were analyzed (96%), but also 4% of patients treated with radical intent (adjuvant therapy after optimal surgical treatment).

Clinical data were collected, such as sex (females: 84, males: 139), age (26–92 years, median 65), body mass index (BMI), some comorbidities, baseline lactate dehydrogenase (LDH) (above normal in 26%), presence of liver metastases at the time of therapy initiation (in 27%), previous use of cytostatic chemotherapy for any oncological indication (in 15%), hepatic AEs in previous pharmacotherapy, and for the group treated with anti-PD-1, an increased baseline dose of the drug understood as 480 mg of nivolumab or 400 mg of pembrolizumab from first administration (15%). Before the first analyzed CPI treatment, 44% of patients had previously received first-line systemic treatment for melanoma/kidney cancer, including anti-BRAF +/– MEK (56%), chemotherapy (30%), and tyrosine kinase inhibitors (15%). The study did not include patients treated with combined anti-PD-1 + anti-CTLA-4 immunotherapy due to the limited patient population (the combination was reimbursed in Poland for the treatment of melanoma in 2021), and the difficulty in clearly comparing subgroups. Detailed patient characteristics are presented in Table 1.

The values of selected parameters as predictors of hepatotoxicity were assessed. A retrospective, statistical analysis of the documentation was performed. Correlations between several clinical factors and hepatotoxicity were analyzed by the Chi-square test.

Archival data obtained for the project were anonymized, and ethics approval for the study was granted by the Bioethics Committee in Hirszfeld Institute of Immunology and Experimental Therapy, the Polish Academy of Sciences in Wrocław (No. KB — 4/2023).

Results

In the analyzed cohort, immunotherapy, in general, was associated with hepatotoxicity, defined as an increase in transaminase values above the normal limit and/or hyperbilirubinemia: CTCAE grade 1–4 in 26% of patients, and CTCAE grade 3–4 in 6% of patients. The

Table 1. Patient characteristics

Characteristics	n	[%]
Enrolled	223	100
Sex		
Male	139	62
Female	84	38
Age [years], median (range)	65 (26–92)	
ECOG performance status		
0	28	13
1	191	86
2	4	2
Neoplasm		
Melanoma	208	93
— Stage IV	199	89
— Stage III (adjuvant)	9	4
Renal cell carcinoma (RCC)	15	7
Type of CPI immunotherapy		
Anti-PD-1	34	19
— Nivolumab	105	47
— Pembrolizumab	81	36
Anti-CTLA4 - ipilimumab	75	34
Anti-PD-1 followed by anti-CTLA-4	38	17
Previous systemic treatment due to any oncological disease		
Any	109	49
Chemotherapy	35	15
Previous systemic treatment due to melanoma/RCC		
Any	98	44
BRAF +/− MEK inhibitors	55	25
Chemotherapy	29	13
Other tyrosine kinase inhibitors	15	7
Other immunotherapy (clinical trials)	4	2

Characteristics	n	[%]
Increased starting dose of the drug		
Nivolumab 480 mg	26	12
Pembrolizumab 400 mg	2	< 1
Site of metastasis		
Lymph node	169	76
Lung	136	61
Skin	105	47
Liver	59	26
Brain	42	19
Other	100	45
Pre-existing liver disease		
Hepatic steatosis	42	19
Liver dysfunction on any previous cancer pharmacotherapy	35	16
Viral hepatitis	6	3
Other	6	3
Baseline blood abnormalities		
LDH > ULN	58	26
ALT > ULN	22	10
AST > ULN	15	7
Hypoalbuminemia	12	5
Bilirubin > ULN	6	3
BMI median (range) [kg/m^2]	27 (17–47)	
> 25	141	63
≤ 25	82	37

ALT — alanine aminotransferase; AST — aspartate aminotransferase; BMI — body mass index; BRAF — type B rapidly accelerated fibrosarcoma; ECOG — Eastern Cooperative Oncology Group; LDH — lactate dehydrogenase; MEK — mitogen-activated extracellular signal-regulated kinase; ULN — upper limit of normal

median time to the first liver function disorder on anti-PD-1 therapy was 2.3 months, and 1.4 m on anti-CTLA4 therapy. AEs grade 3–4 according to the CTCAE in patients treated with anti-CTLA4 occurred twice as often as in the group treated with anti-PD-1 (12% and 6%, respectively).

In the analysis of predictive factors of hepatotoxicity of any grade during immunotherapy, a statistically significant difference in the frequency of hepatic AEs of the therapy depending on age was demonstrated. The age of 60 was established as a cutoff criterion for old age. An increased risk of hepatotoxicity was found in the group of patients ≤ 60 years of age compared to the group > 60 years of age (34.1% *vs*. 21.7%, respectively, p = 0.0418). Therefore, hepatotoxicity occurred in every third patient up to 60 years of age, and in every fifth patient over 60 years of age.

In the subgroup of 38 patients with melanoma treated with sequential immunotherapy (anti-PD-1 followed by anti-CTLA-4), the occurrence of any grade of hepatotoxicity during first-line immunotherapy significantly increased the risk of its recurrence during second-line immunotherapy (58.3% *vs*. 15.4%, p = 0.0199).

There was no statistically significant effect on the occurrence of hepatotoxicity of any degree for such parameters as liver dysfunction during previous cancer pharmacotherapy (p = 0.4677), presence of liver metastases [not significant (NS)], hepatic steatosis (NS), increased baseline BMI (NS), sex (p = 0.3124), elevated LDH levels (NS), or prior use of any cytostatic chemotherapy (p = 0.3456). In the group treated with anti-PD-1, no association with an increased starting dose of the drug was found (p = 0.5539). Detailed univariate analysis of hepatotoxicity predictors is provided in Table 2.

Table 2. Univariate analysis of hepatotoxicity predictive factors

Covariate	n (%)	Incidence of hepatotoxicity [%]	Chi-square	p value
Liver dysfunction during any previous cancer pharmacotherapy				
Yes	35 (16%)	31.4	0.5273	0.4677
No	188 (84%)	25.5		
Liver metastases				
Present	59 (26%)	27.4	Not tested	NS
Absent	164 (74%)	27.1		
Hepatic steatosis				
Present	60 (27%)	20.0	Not tested	NS
Absent	163 (73%)	17.8		
Baseline BMI				
Increased (> 25)	141 (63%)	27.0	Not tested	NS
Normal (≤ 25)	82 (37%)	25.6		
Sex				
Male	139 (62%)	28.8	1.0204	0.3124
Female	84 (38%)	22.6		
Baseline lactate dehydrogenase				
Increased	58 (26%)	25.9	Not tested	NS
Normal	165 (74%)	26.7		
Age				
≤ 60 years	85 (38%)	36	4.1423	0.0418
> 60 years	138 (62%)	21		
Prior use of any chemotherapy				
Yes	35 (16%)	20,0	0,8897	0.3456
No	188 (84%)	27.7		
Increased starting dose of the drug				
Yes	28 (15%)	21.4	0.3504	0.5539
No	157 (85%)	26.8		
(anti-PD-1 subgroup only n = 185)				
Any hepatotoxicity during the anti-PD-1 therapy				
Yes	12 (32%)	58.3	5.4234*	0.0199
No	26 (68%)	15.4		
(melanoma sequential therapy anti-PD-1 followed by anti-CTLA-4 subgroup only n = 38)				

*Chi-square with Yates correction; BMI — body mass index; NS — non significant

Discussion

There is no consistent definition of hepatotoxicity in the literature, as in some studies, this complication was reported as a single category while in others, it was categorized depending on deviations of various biochemical parameters, such as alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGTP), or bilirubin. Some clinical trials, even those with registration, did not report such AEs in publications at all. For our analysis, we adopted hepatotoxicity defined as an increase of ALT and/or AST and/or bilirubin above the upper limit of normal (ULN) according to the CTCAE, divided by severity: all (grade 1–4) and severe (grade 3–4) or an increase of one or more grades of an initially present disorder. Table 3. presents detailed hepatic adverse event grading according to the CTCAE (version 5.0).

Due to a significant clinical problem such as liver dysfunction during immunotherapy, risk factors for its occurrence are researched. It has been shown that the risk of hepatotoxicity increases when a similar AE occurs during previous immunotherapy treatment and is higher when using CTLA-4 inhibitors compared to treatment

Table 3. Hepatic adverse events grading according to the Common Terminology Criteria for Adverse Events (CTCAE) (version 5.0)

CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Alanine aminotransferase increased	> ULN — 3.0 × ULN if baseline was normal; 1.5–3.0 × baseline if baseline was abnormal	> 3.0–5.0 × ULN if baseline was normal; > 3.0–5.0 × baseline if baseline was abnormal	> 5.0–20.0 × ULN if baseline was normal; > 5.0–20.0 × baseline if baseline was abnormal	> 20.0 × ULN if baseline was normal; > 20.0 × baseline if baseline was abnormal	–

Definition: A finding based on laboratory test results that indicate an increase in the level of alanine aminotransferase (ALT or SGPT) in the blood specimen

CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Aspartate aminotransferase increased	> ULN — 3.0 × ULN if baseline was normal; 1.5–3.0 × baseline if baseline was abnormal	> 3.0–5.0 × ULN if baseline was normal; > 3.0–5.0 × baseline if baseline was abnormal	> 5.0–20.0 × ULN if baseline was normal; > 5.0–20.0 × baseline if baseline was abnormal	> 20.0 × ULN if baseline was normal; > 20.0 × baseline if baseline was abnormal	–

Definition: A finding based on laboratory test results that indicate an increase in the level of aspartate aminotransferase (AST or SGOT) in the blood specimen

CTCAE Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Blood bilirubin increased	> ULN — 1.5 × ULN if baseline was normal; > 1.0–1.5 × baseline if baseline was abnormal	> 1.5–3.0 × ULN if baseline was normal; > 1.5–3.0 × baseline if baseline was abnormal	> 3.0–10.0 × ULN if baseline was normal; > 3.0–10.0 × baseline if baseline was abnormal	> 10.0 × ULN if baseline was normal; > 10.0 × baseline if baseline was abnormal	–

Definition: A finding based on laboratory test results that indicate an abnormally high level of bilirubin in the blood. Excess of bilirubin is associated with jaundice

ALT/SGPT — alanine transaminase; AST/SGOT — aspartate transaminase; ULN — upper limit of normal

based on PD-1 inhibitors. At the same time, there are reports of an increased risk of hepatic AEs when using anti-PD-1 immunotherapy at an increased initial dose [12], which is inconsistent with our results. There is no definite link between chronic liver disease or the presence of liver metastases and an increased risk of toxicity [13]. Interestingly, CPI therapy in melanoma is associated with higher risk of hepatotoxicity than in other cancers — odds ratio 5.66 *vs*. 2.71 [14], which may be caused by the relatively frequent presence of liver metastases, as well as the originally registered "high" dose of ipilimumab (3 mg/kg). The positive correlation between the risk of hepatotoxicity and the younger age of patients, as demonstrated, has not been mentioned in the literature and needs to be confirmed in further studies.

The main limitation of this study is a relatively small population, and consequently a small percentage of patients with higher-grade hepatotoxicity according to the CTCAE. All non-baseline serum ALT, AST, or total bilirubin elevations during immunotherapy were included in the analysis. Of 59 patients, 32 (54%) had only grade 1 toxicity.

Conclusions

Immune hepatitis is a potentially serious complication of immunotherapy. This toxicity is more likely to occur with CTLA-4 inhibitors alone than with PD-L1 inhibitors. Earlier occurrence of hepatic AEs, during first-line immunotherapy, predisposes to the occurrence of this complication also during subsequent immunotherapy. Patients younger than 60 years of age may be at higher risk of immunotherapy-induced hepatotoxicity. There was no evidence of an increased risk of hepatic AEs in patients with chronic liver disease, hepatic steatosis, liver metastases, prior chemotherapy, elevated LDH, or BMI.

Article Information and Declarations

Data availability statement
The data that support the findings of this study are available from the corresponding author, M.M., upon reasonable request.

Ethics statement
The publication of the results was approved by the Bioethics Committee in Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wrocław.

Author contributions
M.M.: conceptualization and design, investigation, data curation and original draft preparation; Z.C.: investigation, data curation and original draft preparation;

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