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Published in:
Biomedicine and Pharmacotherapy

Publication status and date:
Published: 01/12/2022

DOI (link to publisher):
10.1016/j.biopha.2022.113839

Document Version
Publisher's PDF, also known as Version of record

Biomedicine & Pharmacotherapy 156 (2022) 113839

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

The course of C-peptide levels in patients developing diabetes during anti-PD-1 therapy

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ARTICLE INFO

Keywords:
Immunotherapy
Cancer treatment
C-peptide
Islet autoantibodies
Pancreatic enzymes

ABSTRACT

Introduction: Immune checkpoint inhibitor (ICI) associated diabetes is a harmful adverse event (AE) in patients with cancer following anti-programmed (cell) death protein-1 (PD-1) treatment. There are no available biomarkers able to predict this AE. The primary aim of this study was to investigate C-peptide levels as potential predictor for the occurrence of ICI-related diabetes. The secondary aim was to describe the presence of islet autoantibodies and course of pancreatic enzymes in patients with and without ICI-related diabetes.
Methods: From a total of 1318 patients with cancer who started anti-PD-1 treatment 8 cases and 16 controls were studied in this nested case-control study. C-peptide levels, islet autoantibodies, and pancreatic enzymes were measured in prospectively collected blood serum.
Results: In cases versus controls, median C-peptide levels were comparable at baseline and before toxicity or at the corresponding time point in controls. No patient had C-peptide levels below reference range *before* toxicity onset. Two out of eight patients in the ICI-related diabetes group had positive islet autoantibodies, whereas one out of 16 patients in the control group had positive islet autoantibodies. Pancreatic enzymes were elevated before diabetes onset in one patient (13%) and in one control (6%) at the corresponding time point.
Conclusions: In patients developing ICI-related diabetes, changes in C-peptide levels, islet autoantibody positivity, and pancreatic enzymes before ICI-related diabetes onset seem comparable to patients without ICI-related diabetes. (NTR: NL6828)

1. Introduction

PD-1 plays an important role in the inhibition of T-cell activation [1]. ICIs that block the PD-1 pathway are widely used in oncologic practice. This type of treatment, known as ICIs, improve outcomes in various advanced and in early-stage malignancies [2–6]. Currently, ICIs have become standard of care treatment for several types of cancer and the number of indications is expanding.

However, ICIs cause various types of irAEs, such as dermatitis, hepatitis, colitis, and pneumonitis. IrAEs can also present as endocrinopathies, for example thyroid gland disorders, adrenalitis or hypophysitis [7–9]. A rare (<1%), but potentially life-threatening endocrinopathy is ICI-related diabetes [7], which seems to appear more often during anti-PD-(L)1 (or combination) therapy compared to anti-CTLA-4 monotherapy [10]. ICI-related diabetes mimics type 1

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https://doi.org/10.1016/j.biopha.2022.113839
Received 30 July 2022; Received in revised form 1 October 2022; Accepted 6 October 2022
Available online 11 October 2022

Nomenclature

CTCAE	Common Terminology Criteria for Adverse Event.
ECOG	Eastern Cooperative Oncology Group.
IA-2	Islet antigen 2.
ICA	Islet cell antibodies.
ICI	Immune checkpoint inhibitor.
IIF	indirect immunofluorescence.
irAE	Immune-related adverse event.
NSCLC	non-small cell lung cancer.
PD-1	Programmed cell death protein-1.
PD-L1	Programmed cell death-ligand 1.

diabetes and is therefore permanent, in contrast with most other irAEs which resolve after immunosuppressive therapy. Patients need immediate and lifelong insulin treatment and subsequent changes in lifestyle, which results in a considerable longlasting impact on quality of life. However, ICI-related diabetes differs from regular type 1 diabetes as it has a more rapid onset compared to regular type 1 diabetes [11,12], reflected by 39%−71% of the patients developing ICI-related diabetes presenting with a potentially fatal ketoacidosis [13,14]. To improve early detection and clinical management of ICI-related diabetes, it would be therefore of value to predict which patients are at risk of developing this irAE and to develop methods to detect its development at an earlier, perhaps subclinical, stage.

The mechanism via which ICIs lead to diabetes has not completely been elucidated yet, but is thought to be similar to the antitumor mechanism of ICIs, i.e. by suppressing inhibition to a T-cell response against pancreatic islet cells. For other irAEs treatment mainly consists of immunosuppressive agents (e.g. prednisolone), however treating ICI-related diabetes with immunosuppressive agents has not shown to recover beta cell function [15]. Hence, there remains a need to identify predictive biomarkers for ICI-related diabetes in order to be able to mitigate patients' risks for undergoing life threatening situations such as diabetic ketoacidosis or for becoming insulin dependent for the rest of their life.

The ESMO clinical practice guideline advises to regularly monitor blood glucose levels during ICI treatment to detect the emergence of *de novo* diabetes [7]. However, blood glucose levels fluctuate by food intake and are thus highly dependable on sampling time. Moreover, blood glucose levels are only severely elevated just before clinical presentation [12]. Furthermore, since blood glucose levels are measured every few weeks, an acute onset in between two measurements may be missed. Therefore, blood glucose measurements are not suitable to detect the onset of the disease at an early, subclinical stage.

C-peptide levels are used to determine the residual function of the beta cells [16]. Earlier studies have shown that approximately 85% of patients with ICI-related diabetes have low C-peptide levels at diagnosis of diabetes [10,13]. It has not been studied yet whether C-peptide concentrations in ICI-related diabetes decline acutely or more gradually over a period of weeks or even months. If the latter is true, regular screening for C-peptide concentrations during ICI treatment may prevent a first presentation with ketoacidosis by timely starting insulin treatment.

Islet autoantibodies were detected in 40–53% of the patients at diagnosis of ICI-related diabetes, with a predominance of GAD antibodies [10,13,17]. If the presence of islet antibodies before start of treatment could predict the chance of developing ICI-related DM, this could influence the clinical decision making on starting ICI treatment and monitoring for ICI-related diabetes. Furthermore, the appearance of pancreatic islet antibodies during treatment might help to predict an impending ICI-related diabetes before becoming clinically relevant. This could especially be true for the GAD antibodies as this was associated with earlier onset of ICI-related diabetes [17].

In patients treated with ICIs, it has been shown that 3% had elevated lipase levels [18], while in patients with ICI-related diabetes an elevation is observed in 51% of the patients [17]. If levels of pancreatic enzymes are elevated before onset of ICI-related diabetes as a sign of pancreatitis, this might help to select patients who are prone to develop ICI-related diabetes.

Given abovementioned results and questions, the primary aim of this study is to investigate C-peptide levels as a potential biomarker for 1) early detection of ICI-related diabetes or 2) prediction which patients are at higher risk of developing ICI-related diabetes. The secondary aim is to describe whether there are differences in presence of pancreatic islet autoantibodies and in concentrations of the pancreatic enzymes (lipase and amylase) between patients with ICI-related diabetes and unaffected controls.

2. Materials and methods

2.1. Patient selection

Adult patients with cancer (≥ 18 years) who started with anti-PD-(L)1 treatment after April 2016 at the Erasmus University Medical Center (Rotterdam, The Netherlands) and the Amphia Hospital (Breda, The Netherlands) were eligible for the MULTOMAB-trial (Dutch Trial Register Number NL6828; www.trialregister.nl). In the MULTOMAB-trial, blood serum samples are prospectively collected in order to set up a biobank, e.g. for pharmacokinetic and biomarker analyses related to clinical outcome. All patients starting with anti-PD-(L)1 treatment who provided informed consent were eligible for inclusion. The study was approved by the local ethics committee (MEC 16–011) of the Erasmus University Medical Center.

Out of 1318 patients in the MULTOMAB-trial, 8 patients who developed ICI-related diabetes between April 2016 and December 2020 were included in this nested case-control study. Patients who did not have available blood serum samples before the onset of ICI-related diabetes were excluded from the study. Furthermore, 16 matched control participants without ICI-related diabetes from the MULTOMAB-trial were selected by using a 1:2 ratio (Fig. 1). Patients who had developed treatment-related type I diabetes were matched to their controls by age (+/- 10 years), sex, cancer type, type of anti-PD-1 treatment, anti-PD-1 monotherapy or combination therapy, number of prior treatment lines, and the number of treatment cycles received. Control patients with any types of diabetes were excluded from the matching procedure.

2.2. Data collection

The diagnosis of ICI-related diabetes was determined by the treating physician, based on random glucose levels of > 11.1 mmol/L. Matched control patients were being followed until at least one year after treatment stop or until the cut-off date was reached. In all patients, the following baseline data were collected: age, sex, length, weight, race, tumor type, prior treatments, tumor stage, presence of brain metastasis, performance status, lactate dehydrogenase, glucose, serum albumin, serum CRP, platelet count, and creatinine. The performance status was determined according to the ECOG scale [19]. Glucose, HbA1c, C-peptide, amylase, lipase, the presence of islet autoantibodies, presence of diabetic ketoacidosis at diagnosis, other irAEs, corticosteroid use and dose, and treatment follow-up were obtained in patients who developed ICI-related diabetes at diagnosis. Glucose levels in advance of each treatment cycle were obtained from the patients electronic recording system until onset of diabetes and every three months in control patients. Adverse events were classified according to the National Cancer Institute CTCAE, version 5.0. The presence of diabetic ketoacidosis was determined by the treating physician.

The blood serum, in which the C-peptide levels, islet autoantibodies, and pancreatic enzymes were measured, was prospectively drawn

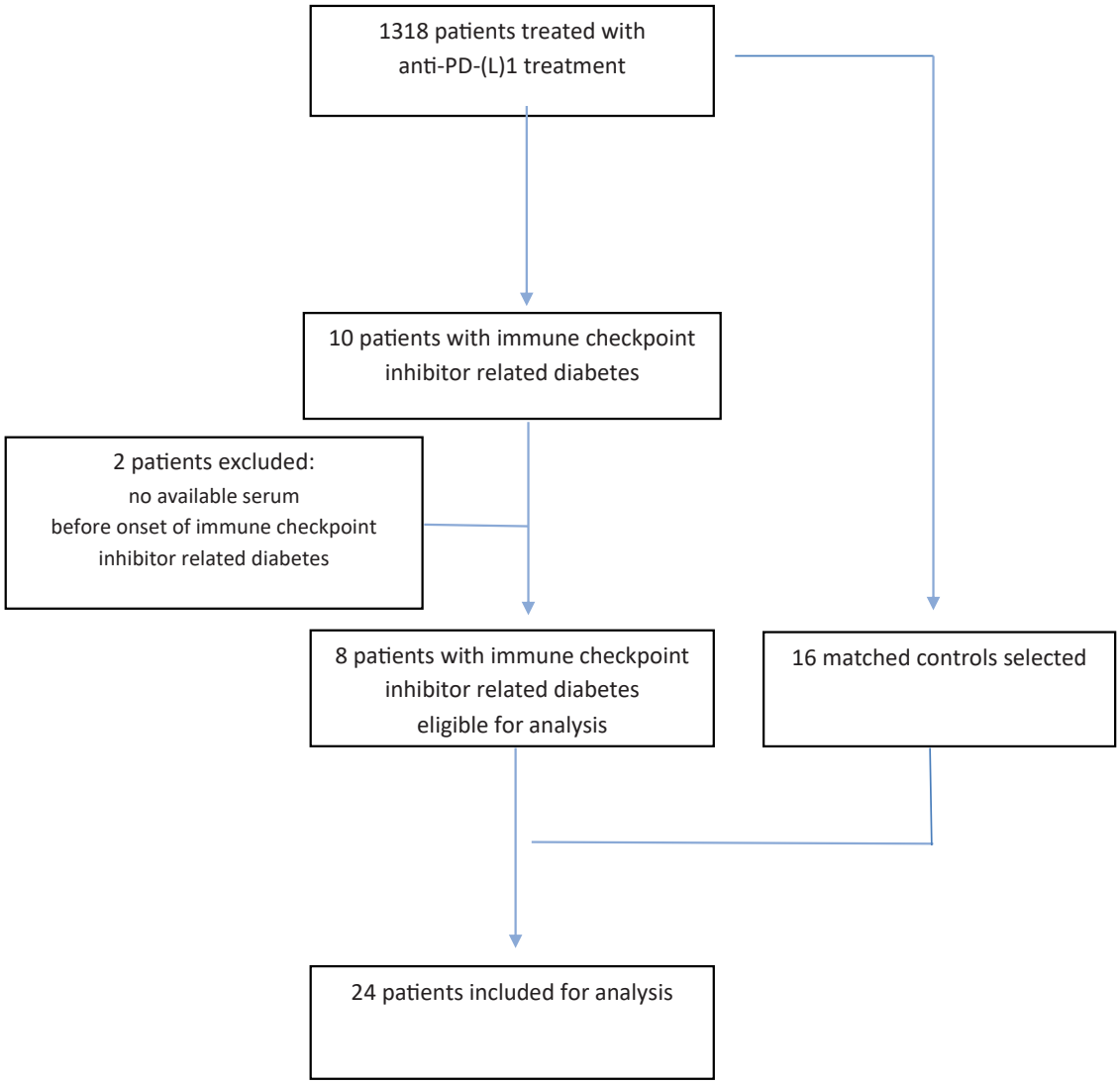

Fig. 1. Flow chart of patient selection.

before the infusion of each consecutive anti-PD-1 treatment cycle. Fasting state was not considered when blood serum was drawn. In patients with ICI-related diabetes, C-peptide levels in blood serum samples until the onset of the toxicity were analysed. Islet autoantibodies and pancreatic enzymes were measured at baseline, in the last available sample just before onset of the toxicity and after the onset of the toxicity. In the control group, C-peptide levels in the blood serum samples were analysed during the first three months after treatment initiation and subsequently every three months until discontinuation of the anti-PD-1 treatment. Islet autoantibodies and pancreatic enzymes were measured at the corresponding time points with the toxicity group. Data were collected until 1st December 2020.

2.3. C-peptide, islet autoantibodies, and amylase and lipase level measurements

C-Peptide levels were measured using the chemiluminescence technology-based immunoassay (CLEIA) Lumipulse G C-Peptide on the Lumipulse G1200 analyser (Fujirebio Inc., Belgium). Intra-assay variation coefficients were respectively 1.7%, 1.6%, 1.8% and 2.7% for mean targets 0.54, 2.69, 4.94 and 8.89 nmol/L respectively (N = 80). Inter-assay variation coefficients were CV 4.0% (mean 0.036 nmol/L), CV 2.7% (mean 0.209 nmol/L) and CV 3.4% (mean 4.313 nmol/L; N = 21, as determined over the period of 14 August 2020–28 December 2020). The reference range for fasting C-peptide levels is 0.17 – 0.85 nmol/L.

Antibodies against specific islet antigens GAD(65) and IA-2 were determined by commercial ELISA, according to the manufacturer's instructions (RSR Limited, Cardiff, UK). ELISA was processed and results were analysed using an the automated Evolis system (Bio–Rad Laboratories Inc, Hercules, CA), and reported quantitatively (IU/mL). Antibodies against total islet antigens (ICA) were determined by commercial IIF assay using primate pancreatic tissue sections, according to the manufacturer's instructions (Inova Diagnostics, San Diego, CA). IIF results were visually evaluated by fluorescence microscopy (Zeiss, Breda, The Netherlands), and reported semi-quantitatively (negative – strong positive).

Measurements of lipase (LIPC – Lipase colorimetric assay) and amylase (AMYL2 – α-Amylase EPS ver.2) were performed on a Roche/Hitachi Cobas C502 and C702 system (Co-bas 8000, Roche Diagnostics GmbH, Mannheim, Germany) respectively. The analyses were performed according to the manufacturer's instructions.

2.4. Data analysis

Given the relatively small study population, only descriptive statistics were performed, including frequencies including percentages, and the median including the IQR as normality of the data could not be assumed for most variables or not determined due to the low number of

patients. Scattered dot plots and spaghetti plots were used to visualize data.

2.5. Data and resource availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request. No applicable resources were generated or analyzed during the current study.

3. Results

Between 5th April 2016 and 1st December 2020, a total of 1318 patients treated with anti-PD-(L)1 therapy and included in the MULTOMAB-trial were screened for study participation, of whom ten patients (0.7%) developed ICI-related diabetes. Two of these patients were not evaluable for the primary endpoint due to the absence of available serum before the onset of the adverse event. For every remaining patient (n = 8), two control patients (n = 16) who had not developed ICI-related diabetes were selected. In three cases, matching could not be based on all six matching criteria. As a result, the number of prior treatment lines was not used in the matching procedure in one patient. In the other two patients, one control was selected with differences in sex, primary tumor, and type of anti-PD-1 treatment. For the other patient differences in primary tumor and type of anti-PD-1 treatment existed. Ultimately, 24 patients were included. In two cases, only baseline samples were available for analysis. Therefore, 24 patients were eligible for analysis at baseline and 18 before diabetes onset. A total of 235 serum samples were available for C-peptide measurements, 61 for detection of auto-antibodies, and 58 for pancreatic enzyme measurements.

Baseline characteristics are shown in Table 1. For the eight patients developing ICI-related diabetes, median age at the start of ICI treatment was 69.5 years (IQR: 55.3–75.3). In addition, melanoma and NSCLC were the most prevalent primary tumor types (37.5% each) and most patients were treated with nivolumab monotherapy (62.5%). The median follow-up time was 11.6 months (IQR: 8.3–22.3). The patients' characteristics at time of ICI-related diabetes diagnosis are shown in Table 2. The median time to onset of ICI-related diabetes was 6.9 months (IQR: 0.9–12.4). At clinical presentation, the median glucose concentration was 32.9 mmol/L (IQR: 23.1–44.6), the median C-peptide concentration was 0.34 nmol/L (IQR: 0.15–1.48; n = 5), and the median HbA1c level was 8.5% (69 mmol/mol; IQR: 64.5–76.5). In comparison, the non-fasting median glucose concentration in the control group did not exceed 8.2 mmol/L (IQR: 7.2–9.3). Three patients had diabetic ketoacidosis (37.5%) as presenting symptom of diabetes. In addition, severity of hyperglycaemia was grade 3 (i.e. insulin therapy initiated or hospitalization indicated) in five patients and a grade 4 (i.e. life-threatening consequences or urgent intervention indicated) in three patients. Other grade $\geq$ 3 treatment related adverse events occurred in four patients (50.0%; being pruritus, pancreatitis, colitis, and hypernatremia due to hyperglycaemia). In the control group, two patients experienced severe adverse events (i.e. nephritis and hepatitis). Three patients discontinued ICI treatment after developing ICI-related diabetes due to clinical deterioration, patient request, and disease progression. In two other patients, treatment was temporarily interrupted after diagnosis. Further details on clinical course and laboratory values of the ICI-related diabetes cases are separately described in Supplementary

Table 1
Baseline Characteristics of the Study Cohort.

					Patients (n = 24)	
Characteristics	**Cases (n = 8)**		**Controls (n = 16)**		**Total (n = 24)**	
Sex, n (%)						
Male	5 (62.5)		9 (56.3)		14 (58.3)	
Female	3 (37.5)		7 (43.8)		10 (41.7)	
Ethnicity, n (%)						
Caucasian	8 (100)		16 (100)		24 (100)	
ECOG Performance status, n (%)						
0	3 (37.5)		10 (62.5)		13 (54.2)	
1	5 (62.5)		6 (37.5)		11 (45.8)	
Median age at start treatment, years (IQR)	69.5 (55.3–75.3)		68.0 (58.5–72.0)		68.0 (58.3–73.0)	
Median BMI at start treatment, kg/m^2 (IQR)	27.6 (25.2–29.9)		27.3 (24.7–29.0)		27.2 (19.1–33.9)	
Cancer type, n(%)						
Melanoma	3 (37.5)		8 (50.0)		11 (45.8)	
Non-small cell lung cancer	3 (37.5)		4 (25.0)		7 (29.2)	
Renal cell carcinoma	1 (12.5)		2 (12.5)		3 (12.5)	
Urothelial cell carcinoma	1 (12.5)		2 (12.5)		3 (12.5)	
Cancer stage, n (%)						
IV	7 (87.5)		14 (87.5)		21 (87.5)	
IIIB	1 (12.5)*		2 (12.5)		3 (12.5)	
Treatment, n (%)						
Nivolumab	5 (62.5)		8 (50.0)		13 (54.2)	
Pembrolizumab	2 (25.0)		6 (37.5)		8 (33.3)	
Nivolumab and Ipilimumab	1 (12.5)		2 (12.5)		3 (12.5)	
Prior Treatment Lines, n (%)						
None	4 (50.0)		6 (37.5)		10 (41.7)	
One	4 (50.0)		10 (62.5)		14 (58.3)	
Brain Metastasis, n (%)						
Yes	1 (12.5)		1 (6.3)		2 (8.3)	
No	5 (62.5)		9 (56.3)		14 (58.3)	
Unknown	2 (25.0)		6 (37.5)		8 (33.3)	
Laboratory covariates	**Median (IQR)**	**n (%)**	**Median (IQR)**	**n (%)**	**Median (IQR)**	**n (%)**
LD (U/L)	217 (177–329)	8 (100)	217 (200–276)	16 (100)	217 (189–315)	24 (100)
Albumin (g/L)	39 (38–43)	7 (87.5)	44 (41.8–46.3)	14 (87.5)	43 (39.5–45.0)	21 (87.5)
Creatinine (μmol/L)	86 (70–106)	8 (100)	84 (71–96)	16 (100)	86 (70.5–99.8)	24 (100)
Glucose	6.0 (5.7–7.5)	8 (100)	5.7 (5.3–6.7)	14 (87.5)	5.8 (5.4–7.0)	22 (91.7)

Baseline characteristics of all patients included in this study. Abbreviations: n number of patients; ECOG Eastern Cooperative Oncology Group; BMI Body Mass Index, LD lactate dehydrogenase; CRP C-reactive protein.
*This patients received anti-PD-1 therapy in the adjuvant setting

Table 2
Patient characteristics at diagnosis.

n = 8		
Characteristics	**n (%)**	
Median time to diabetes onset, months (IQR)	6.9 (0.9–12.4)	
CTCAE, n (%)		
Grade 3	5 (62.5)	
Grade 4	3 (37.5)	
Median glucose, mmol/L (IQR)	32.9 (23.1–44.6)	
Islet autoantibodies, n (%)		
No*	1 (20.0)	
Yes*	4 (80.0)	
GAD	3 (60.0)	
IA-2	2 (40.0)	
ICA	1 (20.0)	
Not tested	3 (37.5)	
Diabetic ketoacidosis	3 (37.5)	
Use of corticosteroids	0 (0)	
Other AE, n (%)	4 (50.0)	
Treatment interrupted after onset, n (%)	3 (37.5)	
Temporary stop after onset, n(%)	2 (25.0)	
Other laboratory covariates:	**Median (IQR)**	**n (%)**
HbA1c, mmol/mol	69 (64.5–76.5)	6 (75.0)
HbA1c, %	8.5% (8.1%–9.2%)	6 (75.0)
C-peptide, nmol/L	0.34 (0.1475–1.4775)	5 (62.5)

Patients' characteristics at diagnosis of ICI-related Diabetes Mellitus. Abbreviations: n, number of patients; IQR, interquartile range; CTCAE, Common Terminology Criteria for Adverse Events; GAD, glutamic acid decarboxylase; IA-2, islet antigen 2; ICA, AE, adverse event
*Islet autoantibodies were tested in five patients, therefore the denominator is five.

Table S1. A total of six patients received corticosteroids during the study period, of whom four were in the control cohort.

At baseline, the median C-peptide concentration in cases was 1.73 nmol/L (IQR: 0.81 – 2.09), and 1.47 nmol/L (IQR: 1.05 – 2.37) in controls. Median C-peptide concentrations were 1.84 nmol/L (IQR: 1.38 – 2.16) before diabetes onset (i.e. median of 8.5 days before onset of diabetes) and 1.46 nmol/L (IQR: 1.26 – 1.78) at the corresponding time points in the control arm. At baseline and before diabetes onset, C-peptide levels were within the reference range for all patients. The different C-peptide courses of all patients with ICI-related diabetes until onset are shown in Fig. 2 and the C-peptide courses of their controls are shown in Supplementary Figure S1. All last study sampling moments in Fig. 2 were measured *before* onset of ICI-related diabetes. Both at baseline and before onset of diabetes, C-peptide levels were comparable between cases and controls (Fig. 3). Two out of five patients in whom C-peptide was measured during routine clinical care had C-peptide concentrations below the reference range at diagnosis of diabetes (Supplementary Table S1), of whom both had diabetic ketoacidosis. The only other patient with diabetic ketoacidosis had a C-peptide level in the reference range at diagnosis. Glucose levels during treatment are depicted in Supplementary Figure S2 (cases) and Supplementary Figure S3 (controls).

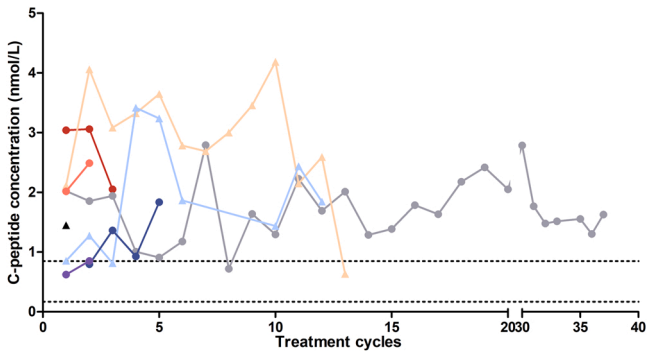

Fig. 2. C-peptide concentration course during anti-PD-1 treatment. C-peptide concentration course in nmol/L during anti-PD-1 treatment before the onset of immune-related type I DM (n = 8 patients). Each dot or triangle represents the C-peptide value just prior to its respective treatment cycle. Each colored line represents a different patient. Dotted lines represent fasting C-peptide reference values. Data points indicated with triangles represents patients with a diabetic ketoacidosis. The last study sampling moment of each patient is *before* ICI-related diabetes onset.

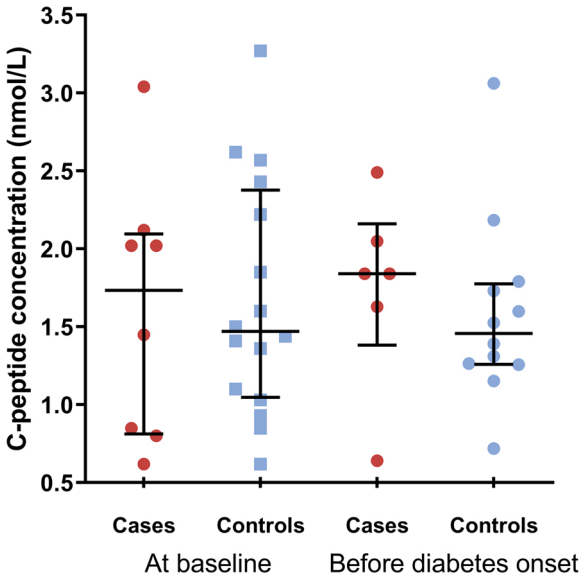

Fig. 3. Scatter dot plot of C-peptide concentrations. Scatter dot plot of the C-peptide concentrations of the ICI-related DM group (n = 8) and the control group (n = 16) at baseline and before onset of diabetes (median of 8.5 days before diabetes onset). The red and blue dots represent the C-peptide concentrations in the ICI-related DM and control groups, respectively. The horizontal lines represent the median values, while the whiskers show the IQR. The lower limit for the fasting C-peptide reference range is 0.17 nmol/L.

Two patients (25%) in the ICI-related diabetes group had positive islet autoantibodies before onset of ICI-related diabetes, one at baseline and one in the last sample before onset of ICI-related diabetes, whereas one patient (6%) in the control group had positive islet autoantibodies at baseline. After onset of ICI-related diabetes, four patients (50%) (of whom two were already seropositive before diabetes onset) had positive islet autoantibodies. During ICI therapy, none of the controls developed elevated islet autoantibodies (Supplementary Table S1).

Amylase was not elevated in any of the cases or controls at any time point. Lipase was not elevated at baseline in any of the patients. However, lipase was elevated (163 U/L) in one patient (13%) with ICI-related diabetes in the last sample before diabetes onset, and in one of the patient in the control arm at the corresponding time point (71 U/L; 6%; Supplementary Table S1).

4. Discussion

This is the first longitudinal study in which possible changes in C-peptide levels, islet auto-antibodies, and pancreatic enzymes preceding the onset of ICI-related diabetes were studied. Between patients with and without ICI-related diabetes, no substantial difference in C-peptide levels or course during ICI was seen until toxicity onset. These findings suggest that C-peptide course before onset of ICI-related diabetes is not a predictive biomarker for the onset of this toxicity. Other studies in which the C-peptide concentrations were prospectively determined *after* the onset of ICI-related diabetes have shown that the C-peptide level declines during 2–3 weeks after diagnosis [14,20]; however, those C-peptide levels may have been affected by insulin therapy. This is in line with the results in our study since the median C-peptide concentration at diagnosis was within the normal range as well (Table 2)

indicating that the decline of C-peptide levels occurs after the onset of ICI-related diabetes. This described course of C-peptide concentrations suggest that ICI-related diabetes has a rapid onset, which is in line with current knowledge of this type of diabetes compared with traditional type I diabetes [11,12]. Ideally, we would have obtained fasting C-peptide concentrations, as the wide interindividual variation in C-peptide concentrations we found can partly be explained by the fact that the serum samples were collected just before the infusion of ICI therapy, without regard to the patients' last meal. Therefore, subtle changes prior to the onset of diabetes might have been missed. Moreover, given the retrospective nature of this study, we were not able to correct C-peptide concentrations for simultaneous glucose concentrations, as glucose concentrations at exactly the same time point as blood withdrawals for C-peptide levels were not available. In the randomly sampled glucose levels during routine care, glucose levels prior to onset of diabetes were generally stable (Supplementary Figure S2). Unfortunately, the majority of these samples were not synchronized with the blood draws from which C-peptide concentrations were measured. Hence, we were not able to reliably relate these two parameters. Still, even random sampling for C-peptide measurement is useful in detecting deficient beta cell insulin synthesis [21]. Interestingly, C-peptide courses in the cases with ICI-related diabetes seemed to have more intra-individual variability than those of the control group. A hypothesis that might explain this observation is that the release of insulin fluctuates as a result of beta cell destruction due to anti-PD-1 therapy and subsequent regeneration [22].

In two (25%) cases, islet autoantibodies were present before the diagnosis of diabetes, and in one (6%) patient in the control arm at matching time points. At the time point after the onset of ICI-related diabetes, this proportion rose to 50% of cases (Supplementary Table S1). While being more prevalent than in the general population, it is known that islet autoantibodies are less frequently present in patients with ICI-related diabetes compared to typical type 1 diabetes [17,23,24]. In addition, in type 1 diabetes islet autoantibodies are often present years before the onset of clinical signs and symptoms [25]. These observations may indicate differences in mechanisms of the auto-immune process between both entities and strengthen the hypothesis of a more acute onset in ICI-related diabetes. This theory is further supported by recent observations that ICI-related diabetes routinely presents with diabetic ketoacidosis, but is further accompanied by a distinct clinical presentation than type 1 diabetes[14]. Therefore, it seems that routine measurements of islet autoantibodies before start or during therapy might not be useful for predicting or early detection of ICI-related diabetes, but our study was not powered to draw definitive conclusions on this subject. Interestingly, both patients with positive islet autoantibodies before onset of ICI-related diabetes presented with a diabetic ketoacidosis whereas only one out of the other six patients with ICI-related diabetes experienced diabetic ketoacidosis, suggesting the appearance of islet antibodies to be related with even more fulminant onset of diabetes. The low prevalence of islet autoantibodies in patients with ICI-related diabetes underlines the recommendations of the American Society of Clinical Oncology to start with insulin therapy without delay due to pending results of, amongst others, islet autoantibodies [26].

During therapy with ICIs, it has been shown that 3% of the patients had elevated lipase levels [18], while an elevation was observed in 51% of patients with ICI-related diabetes [17], possibly reflecting pancreatitis as a result of an auto-immune response against the pancreas triggered by anti-PD-1. In our cohort, pancreatic enzymes were measured at onset of ICI-related diabetes in three of the eight patients as part of routine clinical care. In all these three cases, amylase or lipase were elevated. However, at baseline or before onset of ICI-related diabetes, amylase or lipase were elevated in only one out of eight cases and was also elevated in one control. This seems to imply that these parameters do not have a major value in predicting ICI-related diabetes, but this remains to be validated in a sufficiently powered cohort.

The incidence of ICI-related diabetes in this study (0.7%) corresponds with the incidence known in the current literature. However, a different median time to onset (30 versus 8.5 weeks) was found in our study [12]. This might be due to the fact that previous reviews are based on case reports and as a consequence only the more severe and cases with an early onset of this toxicity may have been described. Baden et al., who selected patients with ICI-related diabetes using a literature search and a national survey, described a median onset of 22 weeks, which is more in line with the findings in this study [14]. The proportion of patients with diabetic ketoacidosis was relatively low in our cohort compared to other studies (38% versus 39%−75%) [10,12,14]. However, this proportion of patients might reflect a true incidence, since we selected cases from over 1300 prospectively included patients.

Due to the retrospective nature of this analysis, several pitfalls in the selection of our cohort might be relevant. For example, this study was not powered to perform statistical analyses on the studied parameters. Additionally, anti-PD-1 with anti-CTLA4 combination therapy generally causes more severe adverse events than anti-PD-1 therapy alone. Therefore, we aimed to match cases with controls who received the same type of treatment. As such, it is not expected that the type of ICI therapy influences the outcomes of the study. However, only three patients received the combination therapy, therefore our results might mostly be a reflection of anti-PD-1 monotherapy. Moreover, the use of corticosteroids and other severe adverse events, such as endocrinopathies other than diabetes, might affect glucose levels. While cases and controls were not matched on those parameters, patients with elevated glucose levels were excluded as control patients. Therefore, no patients with prior or current type 2 diabetes were included in this study.

In this study of 1318 patients who were treated with ICIs, we demonstrated that patients with ICI-related diabetes do not have declined C-peptide levels before onset of ICI-related diabetes, nor is the course of C-peptide levels altered compared to patients who did not develop ICI-related diabetes. Therefore, we do not expect any additional value of serial C-peptide measurements in patients receiving ICI therapy. Moreover, islet autoantibodies and pancreatic enzymes were not clearly related to the onset of ICI-related diabetes, but our study was not sufficiently powered to exclude a predictive value for these parameters. Despite the low incidence of ICI-related diabetes, its clinical course can be severe. Our results confirm the more acute and rapid onset of ICI-related diabetes compared to type 1 diabetes and underline recommendations of clinical guidelines emphasizing the need for regular glucose measurements and immediate initiation of insulin treatment even in case of negative autoantibodies, normal pancreatic enzymes or normal C-peptide.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

CRediT authorship contribution statement

EAB, KdJ, TU, LC, RAF, AJ, RHJM, SB contributed to the conception and design of the content of this manuscript. EAB, KdJ, TU contributed to the data acquisition of the content of this manuscript. EAB, kdJ, TU, SB contributed to drafting of the content of this manuscript. kdJ, RvdW, MWJS, SAAvdB, EOH, CHvdL, LC, RAF, AAMvdV, AJ, SLWK, JGJVA, RHJM, SB contributed to the critical revision of the content of this manuscript. RvDW, MWJS, SAAvdB provided technical contributions to the content of this manuscript. RHJM and SB contributed to the supervision of the content of this manuscript. All authors contributed to the data analysis and interpretation and final approval of the content of this manuscript.

Conflict of interest statement

EAB, KdJ, TU, RvdW, MWJS, SAAvdB, EOH, SLWK, SB declares having no relevant conflict of interest. CHvdL declares having received consulting fees from Amgen, Pfizer, MSD, payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from MSD. LC declares having received support for attending meetings and/or travel from Ipsen. RAF declares having received consultancy fees from Recordati, support for attending meetings and/or travel from Ipsen. AAMvdV declares having received consulatency fees (paid to the institute) from BMS, MSD, Merck, Eisai, Ipsen, Pfizer, Pierre Fabre, Novartis, Roche, Sanofi. AJ declares having received support for attending meetings and/or travel from Ipsen. JGJVA declares having received consulting fees from MSD, BMS, Boehringer Ingelheim, Amphera, Eli-Lilly, Takeda, Bayer, Roche, Astra Zeneca, BIOCAD, payment or honoraria for lectures, presentrations, speakers bureaus, manuscript writing or educational events from MSD, BMS, Boehringer Ingelheim, Amphera, Eli-Lilly, Takeda, Bayer, Roche, Astra Zeneca, BIOCAD, having patents planned, issued or pending regarding biomarkers for immunotherapy, allogenic tumor cell lysates. RHJM declares having received grants or contracts (paid to the institute) from Astellas, Bayer, Crital Therapeutics, Novartis, Pamgene, Pfizer, Roche, Sanofi, Servier.

Acknowledgements

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2022.113839.

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