Dose the C- Peptide Level Predict Remission Phase in Type 1 Diabetes Mellitus Pediatric Patients

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Abstract

Objectives: To study the C-peptide level for prediction of remission phase and its role in management of newly diagnosed type 1 diabetic children.

Patients and Method: A prospective case-control study was conducted on 64 newly diagnosed type 1 diabetic children. Children were classified into Group 1 (35) patients who experienced remission and Group 2 (29) patients who did not experience remission. Fasting plasma C-peptide, FPG and HbA1c were done for all patients.

Results: Prevalence of remission was 0.546 (35/64). Fasting serum C- peptide of Group 1 at first, last visits and Group 2 were 163.25±120.84, 358.25±184.67 and 86.70±63.51 Pmol/l respectively; P< 0.05. Receiver Operating Characteristic (ROC) showed the prevalence of remission phase was 0.594. Plotting ROC curve for C-Peptide among patients of both groups showed Area under the Curve (AUC= 0.82). The ROC decision plot curve of best C-Peptide true predictive values was 100-200 pmol/l.

Conclusions: Remission phase is prevalent in newly diagnosed type 1 diabetic children. The C-peptide is a sensitive and specific test, so that we can trust its use to predict the remission in newly diagnosed patients. The most considerable readings found 100-200 Pmol/l, as prediction cut-off limit for diagnosis of remission. The C-Peptide may assist physician in adjusting the initial insulin dose early after diagnosis of type 1 diabetes.

Key Words: Type 1diabetes mellitus, remission phase, C-peptide.

Introduction:

Type 1 diabetes mellitus is characterized by selective and progressive autoimmune destruction of beta-cells of the pancreas, before the development of clinical DM, in genetically susceptible individuals. Over the course of the disease, some patients regain their ability to secrete endogenous insulin to some extent for a period of few months to years. [(1)] Moreover, the initial period of type 1 diabetes mellitus is of great importance, since early metabolic adjustment has profound impact on long term control. [(2)] Partial remission was defined as a requirement of insulin < 0.5 U/kg /day. [(3)]

C peptide concentration was found to be within normal levels in the patients under clinical remission phase and in 75% of children during the first 6 months of disease development. [(4)] Moreover, Basal and stimulated C-peptide secretion was higher in patients in clinical remission than in those who were not. [(5)], Endogenous insulin secretion is assessed and determined by measurement of C-peptide, [(6)] which is co-secreted with insulin in one-to-one molar ratio but unlike insulin experiences little first pass clearance by the liver. Measurement of C-peptide under standardized conditions provides a sensitive, well accepted, and clinically validated assessment of β-cell function. [(6)]

A better understanding of the remission phase is very important because of the potential for pharmacological intervention to preserve this function and to evaluate the natural course and characteristics of the remission phase. [(7, 8)]

We tried to study the C-peptide level in newly diagnosed type 1 diabetic children for prediction of the remission phase and its role in management of diabetic children.

Patients and Methods:

Ethical approval

All patients and their families were informed about the aim and the suspected benefit of the study before obtaining their agreements for participation according to the medical research and ethical regulations, thus an oral consent was taken from all enrolled participants and their families.

All the medical research ethics rules and instructions adopted in National Diabetes Center (NDC) regarding patient's privacy, humanity and security; as well as the medical research, laboratory data and investigation results were strictly considered throughout all the steps of study.

Setting

A six months cohort study, from 4th Jan. – 30th Jun. 2009, was conducted for the newly diagnosed type 1 diabetes pediatric patients registered in the National Diabetes Center (NDC)/ Al-Mustansiriya University, Baghdad – Iraq.

Patients

During the study period, the total number of pediatric diabetic patients visits to the National Diabetes Center (NDC) / Al-Mustansiriya University, were 1555 visits. Since the remission lasts from 2-3 months up to a year or more and the study period was six months; for this limitation of the study, our team tried in his design to enroll only the newly diagnosed patients who consulted NDC for treatment of type 1 diabetes for the first time within the first week after diagnosis and followed up until the end of study. Even the registered, newly diagnosed type 1 diabetes mellitus, patients during the same period were 96 patients; but only 64 children and their families agreed to participate in the study. Consents of children and their families

were obtained prior to be enrolled in the study. The newly diagnosed diabetic patients were thoroughly interviewed, examined and followed up during the period of the study by a team formed of consultant pediatrician and clinical nutritionist according to the standard medical and laboratory work up which is adopted in the NDC. Participants were asked, every visit, about any associated disease, side effect, complications, hypo and hyperglycemic events; also they were examined physically and their height, weight and BMI were measured.

All the newly diagnosed pediatric diabetic patients enrolled in the study were started on Neutral Protamine Hagedorn insulin, (NPH insulin), therapy based on several factors to match the patients needs and lifestyle, keeping in mind the patients age, weight, blood glucose target and initial C-Peptide levels. Insulin therapy was started, 0.5-1 U/kg/day in three divided doses which was adjusted later according to blood sugar home readings and attacks of hypo and/or hyperglycemia. Patients were seen after one week from the first visit and then monthly by the same team; all patients were capable to consult the NDC out of the usual follow up appointments when needed.

Patients were classified into two groups; Group 1 (35 patients) experienced remission phase criteria (daily required insulin dose < 0.5 U/kg/day) and Group 2 (29 patients) who did not experience remission phase criteria (daily required insulin dose > 0.5 U/kg/day) during the study period.

Laboratory Analysis:

Fasting blood sampels were taken from all patients during their visits for laboratory analysis to measure the fasting plasma C-peptide, fasting plasma glucose (FPG) and glycated hemoglobin (HbA_{1c}).

The C – peptide IRMA kit, Immunotech, A Beckman Coulter Company, immunoradiometric assay kit for the in vitro determination of C – Peptide in human used for the measurement of C-peptide directly in serum and plasma; depending on the principle of immunoradiometric assay of C-peptide, "sandwich" type assay. [9]

Statistical Analysis:

Statistical analysis and reporting of obtained data were carried out by using Microsoft Excel - Windows XP professional program. Statistical tests were performed using a null hypothesis of no difference with F- test; the P values were ≤ 0.05 for the levels of significance.

Receiver Operating Characteristic (ROC) Curves, ROC analysis, was used for assessment of predictive accuracy of test (sensitivity and specificity) for evaluation and comparing data that produce the predictions. *Analyse-it* for Microsoft Excel statistical software was used to conduct the ROC related statistical tests**.**

Results:

The male/female ratio was 1/ 1.2. Mean age of the newly diagnosed patients was 9.45±4.5 years, with age range 2 – 17 years. Patients' BMI was 18.153±4.12 kg/m^2. Frequency of Patients with positive family history of diabetes was 14.06%. The prevalence of patients who experienced partial remission was 0.546 (35/64) and total remission (Insulin treatment was completely stopped) was 0.031 (2/64). Prevalence of diabetic ketoacidosis (DKA) in all patients, Group 1 and Group 2 before starting insulin therapy were 23.43% (15/64), 25.71 (9/35) and 20.68 (6/29) respectively; no more events of DKA was recorded after starting insulin therapy in both groups. In Group 1 frequently experienced attacks of hypoglycemia; about 40.0% (14/35) of them experienced at least one or more attacks of hypoglycemia during the course of study; while there was no such attack noticed among patients of Group 2.

The total daily insulin dose required to achieve glycemic control for all the patients, Group 1 and Group 2 at their first visit was 0.65±0.42, 0.45±0.12and 0.86±0.55U/kg respectively; while at the last visit after three months, were 0.61±0.31, 0.32±0.16 and 0.82±0.20 U/kg respectively. (Table-1)

Fasting plasma glucose (FPG) of all patients enrolled in the study at their first and last visits were 190.44±75.58 and 131.20±56.70 mg/dl respectively; P <0.05, significant statistical difference. The glycated hemoglobin (HbA1c) of Group 1 and Group 2 at the first visit was 9.59±2.46 and 10.62±2.02 %, and at the last visit was 7.81±1.36 and 8.28±1.45 % respectively; there were high statistical significant differences between first and last visit of both Groups, P < 0.05. (Table –2)

Fasting serum C- peptide level of Group 1 at first and last visit and Group 2 at first visit were 163.25±120.84, 358.25±184.67 and 86.70±63.51 Pmol/l respectively; differences between Group 1 and Group 2 were significant at first visit, P <0.05, and highly significant at last visit, P <0.001. (Table –2)

Receiver Operating Characteristic (ROC) Curves showed that the prevalence of remission phase was 0.594 among the population of our study. Plotting the ROC curve for the C-Peptide level among patients with and without remission phase showed the diagonal segment of Area Under the Curve (AUC), true positive rate (sensitivity) versus false positive rate (1- specificity), AUC = 0.82, was quite good; it was close to the ideal value of 1.0 and more than worst value of 0.5. (Table – 3)

Moreover, ROC decision plot curve of C-Peptide true predictive value versus different positive test cutoff values was done and showed prevalence of positive and negative predictive values; the best

predictive values were between C-Peptide levels of 100-200 pmol/l. (Table – 4)

Discussion:

Features of diabetes do not become evident until the majority of β-cells are destroyed (~80%); at this point, residual functioning beta cells still exist but are insufficient in number to maintain glucose tolerance; C-Peptide level reflects the state of residual β-cell function. [10] Lombardo et.al, 2002, found in their study in Italy that Honeymoon frequency and duration are strictly conditioned by both residual beta-cell function and insulin resistance at onset of T1DM; they found that more than 80% of the children experienced partial remission; it lasted more than 12 months in 41.7% and at least 24 months in 16.4%.[11] In Turkey, 2001, Bober found that 56.5% of type 1 diabetics entered partial remission;[8] in Kuwait Majedah Abdul-Rasoul, 2006, found partial remission rate was 68.9% (71/103) and total remission rate was 4.22% (3/71);[12] while in Saudi Arabia, Salman 1991, found that partial remission rate was 30.4% (35/115), total remission rate was 1.7% (2/115). [13] In our study we found that the prevalence of partial remission was 54.6% (35/64) and the total remission rate was 3.1% (2/64). Early diagnosis and rigorous insulin therapy at the time of diagnosis may explain these differences between studies and may have an impact on the beginning and the duration of remission; moreover, Heinze et.al. Found that age and sex were another two important variables for remission which cannot be influenced at all. [14]

The prevalence of DKA in both Group 1 and Group 2 in our study was nearly equal; while, Majedah (2006) found that all children who did not present with DKA entered remission compared with 63.2% of those who presented with DKA. [12] This difference might be explained on the bases of early or delayed start of insulin therapy and seeking medical advice; we noticed in this study in Group 2 even those who experienced DKA their subsequent insulin dosage went down from the initial one but not to the level of partial remission, suggesting some residual β-cell function; Sochett et. al. found that median C-peptide concentration at diagnosis was low reached a maximum at 1-3 months and declined gradually to 1 year. [15] This explains the decline in glucose levels during the first several months after diagnosis as insulin therapy removes the toxic effects of chronic hyperglycemia on b-cells and enhances glucose uptake. [16]

Fasting Plasma C-Peptide levels of Group 1 and Group 2 showed significant statistical difference at the first visit, F-test, $P < 0.05$. Patients of Group 1 who experienced remission showed higher C-peptide level than the Group 2 which improved and raised up after starting insulin therapy; similar to what Heinze and Pelkonen found in their studies, [14, 17] the higher C-peptide level of Group 1 and the raise achieved after insulin therapy showed difference between first and last visit of high statistical significance, F-test, $P<0.001$ due to the improvement in β-cell function. [15, 16]

Receiver Operating Characteristic (ROC) Curves analysis of C-peptide test among Group 1 and Group 2 showed good higher values (tables 3 and 4) and area under the curve (AUC) be 0.82, with 95% confidence interval of 0.72 to 0.92, P value < 0.0001, in conclusion it means that C-peptide test is sensitive and specific test to certain degree that we can trust its use to predict or suspect development of the remission phase in newly diagnosed patients; so in order to decide about the cut-off point of C-peptide level, to differentiate between positive and negative values and to predict the remission phase the readings from 0.01 up to 350 Pmol/l were compared. We found that C-peptide level at a cut-off point of 100 Pmol/l was sensitive (0.763 with 95% C.I 0.598 – 0.889) and specific (0.692 with 95% C.I 0.482 – 0.857) near from what Komulainen et.al.[18] found in their study; the serum C peptide concentration of 0.10 nmol/l (100 pmol/l) or more was associated with a favorable metabolic situation. Agnieszka Zmysłowska et.al. (2007) [19] found that the threshold of C-peptide level for prediction of clinical remission during first year of T1DM was 0.141 pmol/mL (141 pmol/l); however we decide in our study to examine the cut-off point of 200 Pmol/l, although it was less sensitive (0.389 with 95% C.I 0.218 – 0.540) but we got high specificity (0.923 with 95% C.I 0.749 – 0.991); therefore we decided about a cut-off limit of 100 – 200 Pmol/l to predict the remission phase with confidence, because this range of cut-off is well-matched with the results obtained in our study to differentiate between Group 1 and Group 2 and to predict the remission phase depending on the C-peptide level.

We considered what Oscar Escobar et.al [20] and Alice et.al. [21] recommended as an initial insulin dose 0.5-1.0 U/kg/day with close monitoring; but we noticed that the early insulin doses to achieve full control, of patients who got remission, high C-Peptide level, were ≤ 0.5 U/kg/day; while those who didn't suppose to get remission, low C-Peptide level, were ≥ 0.5 U/kg/day. Good control was achieved by noticing the decline in HbA1c, at last visit, of both groups which correspond to Chase et.al. criteria to redefine the remission phase, as an insulin dose <0.5 U/kg/day and HbA1c below 8 %, [16] like what happened in Group 1. Close monitoring and short interval follow ups for patients with high C-Peptide level, Group 1, to fine tune their insulin dosages and decrease or stop attacks of hypoglycemia helped us to learn that subsequent insulin dosage coincides with C-Peptide level, negative correlation; the higher the C-peptide level the lower the insulin requirement was. So we were able safely to suggest the use of C-Peptide level for judging and fine-tuning the insulin dosage during follow ups in remission phase. These

notes, which learned from our trial, helped us to have a better glycemic control from the start and lessen hypo and/or hyperglycemic attacks and to gain patients and families compliance from the beginning of treatment. Elizabeth et.al., [22] In their study got the same conclusion but in different approach they considered the measurement of urinary C-Peptide excretion as a simple technique that may be useful in assessing endogenous insulin production in T1DM patients; in group of well controlled T1DM patients, those receiving high doses of insulin had low or negligible C-Peptide excretion, while most patients with low exogenous insulin requirement had near normal urinary C-Peptide excretion.

As a limitation, in our study in which we tried to predict remission phase and to have better understanding about the whole course of remission, because of the time limit of the study (six months), we were unable to follow the patients till the end of the remission phase and know for how long was it.

Table – 1: Descriptive criteria of Group 1 and Group 2.

	Group 1 (Honey moon period)	**Group 2 (No Honey moon period)**	**All patients**
Age (years)[a]	8.26±4.19	11.19±4.51	9.45±4.5
BMI (kg/m^2) [a]	17.650±0.221	18.983±4.925	18.153±4.12
Family history of diabetes (%)[b]	10.52	19.23	14.06
DKA (%)[b] (before insulin therapy)	25.71 (9/35)	20.68 (6/29)	23,43 (15/64)
Dose of insulin (U/kg/day) (First visit)	0.45±0.12	0.86±0.55	0.65±0.42
Dose of insulin (U/kg/day) (Last visit)	0.32±0.16	0.82±0.20	0.61±0.31

BMI = Body mass index, DKA = Diabetes ketoacidosis
[a] F-test, P value >0.05, insignificant statistical difference,
[b]Chi- test, P value >0.05, insignificant statistical difference.

Table–2: Fasting plasma glucose glycated hemoglobin and C-Peptide level of patients throughout the study period.

	Group 1 (Honey moon period)	**Group 2 (No Honey moon period)**	**All patients**
FPG (mg/dl) [a] (First visit)	190.93±82.0	189.75±67.25	190.44±75.58
FPG (mg/dl) [a] (Last visit)	133.58±69.18	128.07±37.00	131.2±56.70
HbA1c (%)[a] (First visit)	9.59±2.46	10.62±2.02	10.18±2.26
HbA1c (%)[a] (Last visit)	7.81±1.36	8.28±1.45	8.08±1.41
C- peptide [a] (pmol/l) (First visit)	163.25±120.84	86.70±63.51	129.81±116.46
C- peptide [b] (pmol/l) (Last visit)	358.25±184.67	-------	-------

FPG = Fasting plasma glucose, HbA1c = Glycated hemoglobin
[a] F-test, P value <0.05, [b] F-test, P value ≤ 0.001

Table–3: Area under the curve of Receiver operating characteristic curve and statistical parameters of C-peptide.

Area under curve (AUC)	**95% CI**	**SE**	**P value**	**conclusion**
0.82	0.72 to 0.92	0.052	<0.0001	Honey moon present have higher values
H0: Area ≤ 0.5. H1: Area > 0.5.				

CI= Confidence interval, SE= standard error, H0= nil hypothesis, H1= acceptance hypothesis.

Table – 4: Coordinate of Receiver operating characteristic curve at different cutoff levels of C-Peptide.

C- Peptide (Positive test >= cutoff) (Pmol/l)	True Positive rate (Sensitivity)	95% CI	True negative rate (Specificity)	95% CI	Predictive value (+)	Predictive value (-)	True positive	True negative	False positive	False negative
0.01	1.000	0.907 to 1.000	0.000	0.000 to 0.132	0.594	-	38	0	26	0
100.00	0.763	0.598 to 0.886	0.692	0.482 to 0.857	0.784	0.667	29	18	8	9
150.00	0.474	0.310 to 0.642	0.923	0.749 to 0.991	0.900	0.545	18	24	2	20
200.00	0.368	0.218 to 0.540	0.923	0.749 to 0.991	0.875	0.500	14	24	2	24
250.00	0.289	0.154 to 0.459	1.000	0.868 to 1.000	1.000	0.491	11	26	0	27
300.00	0.184	0.077 to 0.343	1.000	0.868 to 1.000	1.000	0.456	7	26	0	31
350.00	0.026	0.001 to 0.138	1.000	0.868 to 1.000	1.000	0.413	1	26	0	37

CI= confidence interval.

Conclusions:

Remission phase is prevalent in newly diagnosed type 1 diabetes pediatric patients.

The C-peptide level is sensitive and specific test to certain degree that we can trust its use to predict the remission phase in newly diagnosed patients.

The most considerable readings found to be 100-200 Pmol/l, as prediction cut-off limit for diagnosis of honey moon period.

The C-Peptide level may assist physician in adjusting the initial insulin dosage early after diagnosis of type 1 diabetes mellitus.

Recommendations:

It is important to perform the C-peptide test for all newly diagnosed pediatric patients to predict those who supposed to experience remission and decide about their initial insulin dosage.

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References:

1. Büyükgebiz A, Cemeroglu AP, Böber E, Mohn A, Chiarelli F. Factors influencing remission phase in children with type 1 diabetes mellitus. J Pediatr Endocrinol Metab. 2001 Nov-Dec; 14 (9):1585-96.
2. Dost A, Herbst A, Kintzel K, Haberland H, Roth CL, Gortner L, Holl RW. Shorter remission period in young versus older children with diabetes mellitus type 1. Exp Clin Endocrinol Diabetes. 2007; 115(1):33-7.
3. Muhammad BJ, Swift PG, Raymond NT, Botha JL. Partial remission phase of diabetes in children younger than age 10 years. Arch Dis Child. 1999; 80(4):367-9.
4. Díaz Gómez NM, Doménech Martínez E, Rodríguez Rodríguez I, Díaz Gómez JM. [Metabolic control, C peptide and lipids in insulin-dependent diabetes mellitus]. An Esp Pediatr. 1991; 34(4):276-82.
5. Bonfanti R, Bognetti E, Meschi F, Brunelli A, Riva MC, Pastore MR, Calori G, Chiumello G. Residual beta-cell function and spontaneous clinical remission in type 1 diabetes mellitus: the role of puberty. Acta Diabetol. 1998; 35(2):91-5.
6. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, et. al. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21-22 October 2001. Diabetes 2004; 53(1):250-64.
7. Zmyslowska A, Szadkowska A, Andrzejewski W, Wegner O, Wyka K, Mlynarski W, et. al. Factors affecting C-peptide level during the first year of type 1 diabetes in children. Endokrynol Diabetol Chor Przemiany Materii Wieku Rozw 2004; 10(2)103-11.
8. Bober E, Dundar B, Buyukgebiz A. Partial remission phase and metabolic control in type 1 diabetes mellitus in children and adolescents. J Pediatr Endocrinol Metab 2001 Apr; 14(4):435-41.

9. C-PEPTIDE Kit. Ref.3639, Immunoradiometric assay for the in vitro determination of C-peptide in human. Direction for use. Immunotech, A Beckman Coulter Company. IMMUNOTECH a.s. -Radiová 1 -102 27 Prague 10 -Czech Republic - Phone. +420-267008444 - Fax: +420-267008385.
10. Alvin C. Powers. Diabetes Mellitus, Pathogenesis. In: Dennis L. Kasper, et. al editors of Harrison's Principles of Internal Medicine (2005) 16th Edition; McGraw-Hill, Medical Publishing Division. pp: 2155.
11. Lombardo F, Valenzise M, Wasniewska M, Messina MF, Ruggeri C, Arrigo T, et. al. Two-year prospective evaluation of the factors affecting honeymoon frequency and duration in children with insulin dependent diabetes mellitus: the key-role of age at diagnosis. Diabetes Nutr Metab. 2002 Aug; 15(4):246-51.
12. Majedah Abdul-Rasoul, Hessa Habib, Maha Al-Khouly. The honeymoon phase' in children with type 1 diabetes mellitus: frequency, duration, and influential factors. Pediatric Diabetes 2006: 7: 101–107.
13. Salman H. Remission in diabetic children in Riyadh. Ann Saudi Med. 1991 Sep; 11(5):507-9.
14. Heinze E, Thon A, Honeymoon period in insulin-dependent diabetes mellitus. Pediatrician. 1983-1985; 12(4):208-12.
15. Sochett EB, Daneman D, Clarson C, Ehrlich RM. Factors affecting and patterns of residual insulin secretion during the first year of type 1 (insulin-dependent) diabetes mellitus in children. Diabetologia. 1987 Jul; 30(7):453-9.
16. Chase HP, MacKenzie TA, Burdick J, Fiallo-Scharer R, Walravens P, Klingensmith G, Rewers M. Redefining the clinical remission period in children with type 1 diabetes. Pediatric Diabetes 2004: 5: 16—19.
17. Pelkonen R, Aro A. Factors predicting remission in type I diabetes. Ann Clin Res. 1984; 16(2):94-7.
18. Komulainen J, Lounamaa R, Knip M, Kaprio EA, Akerblom HK. Ketoacidosis at the diagnosis of type 1 (insulin dependent) diabetes mellitus is related to poor residual beta cell function. Childhood Diabetes in Finland Study Group. Arch Dis Child. 1996 Nov;75(5):410-5.
19. Agnieszka Zmysłowska, Wojciech Młynarski, Agnieszka Szadkowska, Jerzy Bodalski. Prediction of clinical remission using the C-peptide level in type 1 diabetes in children. Endokrynologia, diabetologia i choroby przemiany materii wieku rozwojowego: organ Polskiego Towarzystwa Endokrynologów Dziecięcych. 01/02/2007; 13(2):71-4. ISSN: 1234-625X.
20. Oscar Escobar, Allan L. Drash, and Dorothy J. Becker. Management of the Child with Type 1 Diabet. In: Pediatric Endocrinology, Volume 1, edited by: Fima Lifshitz Pediatric Sunshine Academics, Inc. and Sansum Medical Research Institute. 2007 by Informa Healthcare USA, Inc. Informa Healthcare is an Informa business.
21. Alice Y. Y. Cheng, Bernard Zinman. Chapter 39: Principles of Insulin Therapy. In: Joslin's Diabetes Mellitus. 14th Edition. By: Elliott Proctor Joslin, C. Ronald Kahn & Gordon C. Weir. Copyright (c) 2000-2006 Ovid Technologies, Inc.
22. Elizabeth B. Rappaport, Robert A. Ulstrom, Donnell D. Etzwiler, Daniel Fife, Bo E. Hedlund, Michael W. Steffes. Urine C-peptide, β-Cell Function, and Insulin Requirement. Am J Dis Child. 1980; 134(12):1129-1133.

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