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Changes in hematological parameters during first days of diabetic ketoacidosis treatment in children with type 1 diabetes mellitus

ABSTRACT

Background. Diabetic ketoacidosis (DKA) is a life-threatening complication of newly diagnosed type 1 diabetes (T1DM) and is associated with severe dehydration.

The aim of the study was to evaluate the changes in hematological parameters (RBC, Hct, Hb, MCV, PLT, WBC) and their correlations with acidosis level and dehydration during ketoacidosis treatment.

Methods. The study group consisted of 262 children with newly diagnosed type 1 diabetes. Clinical data were collected from hospital discharge charts. Data considering hematological parameters were collected from two timepoints: first at admission and second up to 6 days since admission.

Results. Ketoacidosis was present in 76 patients (29.01%). The DKA group had significantly higher values of baseline RBC ($p = 0.0026$), Hct ($p = 0.0019$), Hb ($p = 0.0235$), PLT ($p = 0.0427$) and WBC count ($p < 0.0001$) *vs.* patients without DKA. Interestingly, baseline MCV level was similar between the groups ($p = 0.9869$). During the first days of diabetes treatment, all hematological parameters such as RBC, Hct, Hb, PLT and WBC significantly decreased in both groups (all p values < 0.0001), while MCV significantly increased after treatment ($p < 0.0001$). However, the latter was evident only in no-DKA group. Changes in all hematological parameters correlated positively with pH (all $R > 0.3$ and all p values < 0.05) in DKA group but not in no-DKA group. However, weak, positive correlations at the margin of statistical significance with pH were observed for changes in PLT ($p = 0.0609$) and WBC ($p = 0.0811$) in no-DKA group.

Conclusion. Monitoring dynamics of hematological parameters at T1DM diagnosis may be useful in estimating patients' hydration status. (Clin Diabetol 2020; 9; 3: 149–160)

Key words: type 1 diabetes mellitus, diabetic ketoacidosis, dehydration, blood cell count, fluid therapy

Introduction

Diabetes mellitus refers to a group of metabolic disorders which are characterized by high glucose concentration resulting from lack of or deficiency in insulin secretion, action or both. In children and adolescents with diabetes, type 1 diabetes (T1DM) is the one most often diagnosed with its underlying cause being autoimmune pancreatic β-cell destruction leading to insulin deficiency [1]. The prevalence of diabetes is dramatically rising, with the projected number of

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patients estimated to increase by 54% from 2017 to 2045 to a staggering 693 million [2]. At the same time the incidence of childhood T1DM increases 3% per year worldwide [2]. Insulinopenia in patients with type 1 diabetes quickly leads to diabetic ketoacidosis (DKA). In this condition insulin deficiency is accompanied by a surge of counterregulatory hormones (glucagon, cortisol, catecholamines and growth hormone), which leads to hyperglycemia, increased lipolysis and ketogenesis. Dehydration sets in soon after, due to glycosuria further aggravated by concomitant vomiting [3]. Eventually, DKA leads to cerebral edema, coma or even death [4–6]. Mild to moderate dehydration in DKA may be managed predominantly by oral rehydration therapy (ORT), which may be bolstered by intravenous (IV) fluid administration in more severe cases, aiming to replenish fluid deficiency over 24 to 48 hours [6]. Cerebral edema is the most severe complication of excessive or too rapid fluid administration. This complication occurs in approximately 0.5% to 0.9% of children with newly onset T1DM and concomitant DKA with mortality rate of cerebral edema reaching 24% [6]. Therefore it is crucial to accurately estimate the degree of dehydration before initiating fluid therapy in DKA. This is not an easy estimate to make though, as dehydration is not directly correlated with the severity of DKA assessed by blood gas values [7].

One of the method used to estimate volume depletion is the Clinical Dehydration Scale (CDS) which takes into consideration: general appearance, sunken eyes, moisture of mucous membranes and tears production. This is a simple and noninvasive indicator but the accuracy of this scale is limited due to the present subjectivity of the performers [8]. Body weight changes during water depletion are also indicative for the degree of dehydration, but given the severity of DKA it is unfeasible in guiding therapy [7]. Moreover, the restitution of initial weight during DKA treatment is also linked to renutrition and insulin administration. Therefore, alternative means are developed to non-invasively, and rapidly evaluate fluid deficiency or overload. One such tool was recently described by Colucci et al. [9]. Their sensor uses nuclear magnetic resonance (NMR) to identify fluid overload in patients with end-stage renal disease (ESRD) at their bedside. Clearly, similar devices may be soon implemented to asses hydration status in children with DKA.

Laboratory measurements are helpful in evaluating hydration in an invasive manner. Plasma osmolality is the most direct test for dehydration, with 300 mOsm/kg being the commonly accepted threshold for dehydration [10]. However, among patients with DKA, equation for plasma osmolality may underestimate patient's osmolality leading to phenomenon known as osmolal gap (difference between calculated and directly measured osmolality) [11].

Serum sodium concentration alone is also used to estimate extracellular fluid (ECF) volume. However, apart from initial measurement, sodium concentration loses its usefulness during treatment. As plasma glucose concentration decreases due to insulin action, serum sodium concentration increases [12]. Therefore, in DKA one has to rely on corrected sodium concentration or bolster it with blood urea nitrogen measurements as described by Ugale et al. [7].

An alternative approach to estimate the extent of dehydration is the use of blood cell count parameters. Hematocrit (Hct) and hemoglobin (Hb) concentration are two such examples [13, 14]. However hemoglobin measurement is believed to be more verifiable indication of hydration status than hematocrit [15]. Dehydration is also reflected by decreased mean corpuscular volume (MCV) of erythrocytes, elevated platelet (PLT) and white blood cell (WBC) counts but all these attributes are influenced by a variety of other clinical factors [16].

Given this data it seems reasonable that hematological parameters such as increased hematocrit, hemoglobin concentration or platelet count might be useful indicators of deficit in extracellular fluid volume in children with DKA, despite their limitations [6, 12]. The benefit of this approach is the serial character of blood cell count parameters, routinely measured several times during DKA treatment. This in turn allows to estimate the baseline values for each patient, i.e. those preceding the onset of DKA, and guide fluid replenishment therapy using this data.

Therefore we aimed in this study to evaluate the changes in hematological parameters (RBC [red blood cells], Hct, Hb, MCV, PLT, WBC) and their correlations with acidosis level and dehydration during ketoacidosis treatment.

Methods

Data collection

All parents gave their informed consent to use their children's medical documentation for clinical studies. Clinical data from hospital discharge charts were obtained from patients hospitalized in the Department of Pediatrics, Oncology, Hematology and Diabetology of the Medical University of Lodz (currently Department of Pediatrics, Diabetology, Endocrinology and Nephrology) between 2009–2015. In our analysis we included patients with age under 18 and newly diagnosed T1DM. DKA and its severity at onset were assessed by using the International Society for Pediatric and Adolescent

Diabetes [6] consensus guidelines (mild: venous pH < 7.3 or serum bicarbonate concentration < 15 mmol/L; moderate: pH < 7.2 or serum bicarbonate concentration < 10 mmol/L; severe: pH < 7.1 or serum bicarbonate concentration < 5 mmol/L). We collected clinical data at T1DM diagnosis about: sex, date of birth, date of diagnosis, diabetes parameters (glucose concentration, HbA_{1c}, C-peptide, daily dose of insulin per kilogram of body weight (DDI), insulin therapy mode, presence of ICA and anti-GAD antibodies), ketosis parameters (pH, HCO_3^-, BE), renal profile (urea, creatinine, urine specific gravity), sodium (Na^+) and potassium (K^+) concentrations, CRP level and hematological parameters (RBC, Hct, Hb, MCV, PLT, WBC). Data considering hematological parameters were collected from two timepoints: first at admission and second up to 6 days since admission. Second measurement was considered as baseline value for each parameter before the DKA onset. Thus, changes of hematological parameters, calculated as a difference between second measurement minus first measurement, may reflect changes in water depletion. We could not provide ketone bodies concentration due to the fact that during diabetes onset they were not routinely examined. Effective osmolality [mOsm/kg] was calculated from following formula [17]: 2 [Na^+] + glucose/18. As acidosis increases the serum potassium concentration independently of intracellular potassium changes, for every 0.1 unit change of extracellular pH, there is an average 0.6 mEq/L inverse change of the serum potassium concentration [18]. Thus, following corrected [K^+] [mEq/L] formula was used for pH < 7.35 : serum potassium + ((pH – 7.35) *0.6 *10). eGFR [mL/min/1.73 m^2] was calculated using the Schwartz formula [19]: 0.413* (height/serum creatinine). Estimated dehydration [%] was calculated from multivariate analysis model of measured dehydration provided by Ugale et al. [7]. Formula was as follow: –22.60 + (0.16 *0.357 *urea at admission) + (0.18 *sodium concentration at admission).

Data analysis

Continuous data were presented as median with interquartile range and categorical data were presented as number with respective percentage. For comparison of continuous variables, we used Mann-Whitney U test and categorical data between two groups were compared with Chi-square, Yates correction and Fisher exact test, respectively. Wilcoxon signed-rank test was used to compare changes in hematological parameters after treatment of diabetic ketoacidosis. Spearman rang correlation coefficients were utilized for analysis of correlation. We used multivariate linear regression models for analysis of factors affecting changes in hematological parameters. In order to select variables entering multivariable linear model building for each hematological parameter, from Table 1. We firstly selected variables with p < 0.15 for correlation in DKA group with each hematological parameter. Afterwards, we checked collinearity of selected variables and excluded one of the variables (with lower R for correlation with outcome) from highly correlated pair (R > 0.4). The final model for each hematological parameter changes was adjusted to age and sex and contained variables with p < 0.15 in multivariate model. A p-value at the level of < 0.05 was considered as statistically significant for remaining analysis. All statistical analysis was performed with STATISTICA 13.1 software (TIBCO Palo Alto, CA, USA).

Results

Study group characteristics

Among 400 young patients (age < 18) diagnosed with new onset of T1DM between October 2009 and October 2015, we excluded 138 (34.5%) patients without two results of blood tests meeting predefined criteria. Characteristics of the remaining study group were shown in Table 1. DKA was present in 76 patients (29.01%).

The DKA group was characterized by significantly higher values of baseline RBC (p = 0.0026), Hct (p = 0.0019), Hb (p = 0.0235), PLT (p = 0.0427) and WBC count (p < 0.0001) *vs.* patients without DKA. Interestingly, baseline MCV level was similar between the groups (p = 0.9869). Children with DKA had also higher glucose concentration, HbA_{1c} level at diagnosis, effective osmolality, and lower eGFR than those without DKA (p < 0.0001, 0.0126, < 0.0001, < 0.0001, < 0.0001 respectively). At the discharged, daily dose of insulin (DDI) was significantly higher in DKA group (Me: 0.79 U/kg (25–75%: 0.62–0.97 U/kg) *vs.* Me: 0.58 U/kg (25–75%: 0.42–0.78 U/kg) p < 0.0001).

During the first days of diabetes treatment, we observed significant decrease of all hematological parameters (all p values < 0.0001) except for MCV which significantly increased after treatment (p < 0.0001). For changes in RBC, Hct, Hb concentration and platelets count there was no significant correlation with time between the blood tests measurements and the magnitude of parameters' change (all absolute R < 0.1 and p values > 0.3). However, the change of MCV correlated positively with time between the blood tests measurements (R = 0.14, p = 0.0303) while WBC change showed a negative correlation (R = –0.21, p = 0.0017). There was no significant difference in time between two hematological parameters measurements between DKA and no-DKA patients (Me: 3.0

Table 1. Study group characteristics at T1DM diagnosis

Characteristic	DKA (N = 76) N (%)	No-DKA (N = 186) N (%)	P value
Sex			
Males	41 (53.95%)	108 (58.06%)	0.5414
Females	35 (46.05%)	78 (41.94%)	
Type of therapy			
MDI	71 (93.42%)	175 (95.11%)	0.8055
CSII	5 (6.58%)	9 (4.89%)	
The presence of antibodies:			
ICA	36 (69.23%)	84 (70.00%)	0.9196
GAD	40 (75.47%)	90 (74.38%)	0.9705
Severity of DKA			
Mild	30 (39.47%)	NA	**NA**
Moderate	33 (43.42%)	NA	
Severe	13 (17.11%)	NA	
	Me (25–75%)	**Me (25–75%)**	
Age at onset (years)	9.65(4.37–12.54)	9.24 (6.03–13.50)	0.3033
Glucose concentration [mg/dL]	517.50 (390.64–685.00)	402.33 (302.87–550.00)	**< 0.0001**
HbA_{1c} (%)	12.30 (11.10–13.85)	11.70 (10.00–13.30)	**0.0126**
C-peptide [ng/mL]	0.33 (0.21–0.52)	0.37 (0.21–0.64)	0.3787
pH	7.24 (7.17–7.30)	7.38 (7.36–7.41)	**< 0.0001**
HCO_3^- [mmol/L]	9.15 (6.55–12.30)	21.60 (19.00–23.05)	**< 0.0001**
BE [mEq/L]	–16.85 (–21.40 to –12.70)	–3.00 (–5.50 to –1.60)	**< 0.0001**
Effective osmolality [mOsm/kg]	300.71 (291.17–309.67)	292.62 (288.02–300.62)	**< 0.0001**
Na [mEq/L]	135.00 (132.00–138.00)	135.00 (132.90–137.00)	0.6123
K [mEq/L]	4.36 (4.05–4.90)	4.40 (3.98–4.72)	0.2933
Corrected K [mEq/L]*	3.88 (3.02–4.29)	4.37 (3.98–4.70)	**< 0.0001**
Urea [mg/dL]	27.00 (21.40–36.00)	27.91(22.10–34.40)	0.9497
Creatinine [mg/dL]	0.72 (0.55–0.95)	0.60 (0.49–0.78)	**0.0017**
eGFR [mL/min/1.73 m^2]	77.47 (63.83–96.05)	103.25 (88.69–119.55)	**< 0.0001**
Urine specific gravity [kg/L]	1.0225 (1.0150–1.0300)	1.0250 (1.0150–1.0300)	0.6097
Estimated dehydration (%)	3.24 (2.54–4.03)	3.27 (2.77–3.85)	0.8857
CRP [mg/dL]	0.85 (0.20–2.05)	0.30 (0.10–1.47)	0.0769
Before therapy			
RBC [10^{12}/L]	5.15 (4.78–5.42)	4.90 (4.60–5.23)	**0.0026**
Hct (%)	42.00 (39.00–45.50)	40.00 (37.20–42.80)	**0.0019**
Hb [g/dL]	14.30 (13.40–15.50)	13.90 (13.20–14.80)	**0.0235**
MCV [fL]	82.00 (79.00–85.00)	82.00 (78.00–86.00)	0.9869
PLT [$10^3/\mu$L]	315.00 (261.00–354.00)	289.50 (240.00–335.00)	**0.0427**
WBC [$10^3/\mu$L]	13.29 (8.50–18.00)	8.79 (7.30–10.90)	**< 0.0001**
After therapy			
RBC [10^{12}/L]	4.57 (4.32–4.90)	4.65 (4.38–5.00)	0.1401
Hct (%)	37.70 (35.65–39.90)	38.20 (36.40–41.10)	0.0524
Hb [g/dL]	12.80 (12.20–13.70)	13.20 (12.50–14.10)	**0.0143**
MCV [fL]	82.00 (79.00–85.00)	83.00 (79.00–87.00)	0.4655
PLT [$10^3/\mu$L]	237.00 (188.00–289.00)	255.00 (207.00–300.00)	0.0888
WBC [$10^3/\mu$L]	6.70 (5.40–8.20)	6.40 (5.30–8.30)	0.6946

*Corrected by 0.6 [mEq/L] for every 0.1 unit reduction of pH; MDI — multiple daily insulin injections; CSII — continuous subcutaneous insulin infusion; DDI — daily dose of insulin; Hct — hematocrit; Hb — hemoglobin; MCV — means corpuscular volume; PLT — platelets; Me — median; 25–75% — interquartile range; RBC — red blood cells; WBC — white blood cells

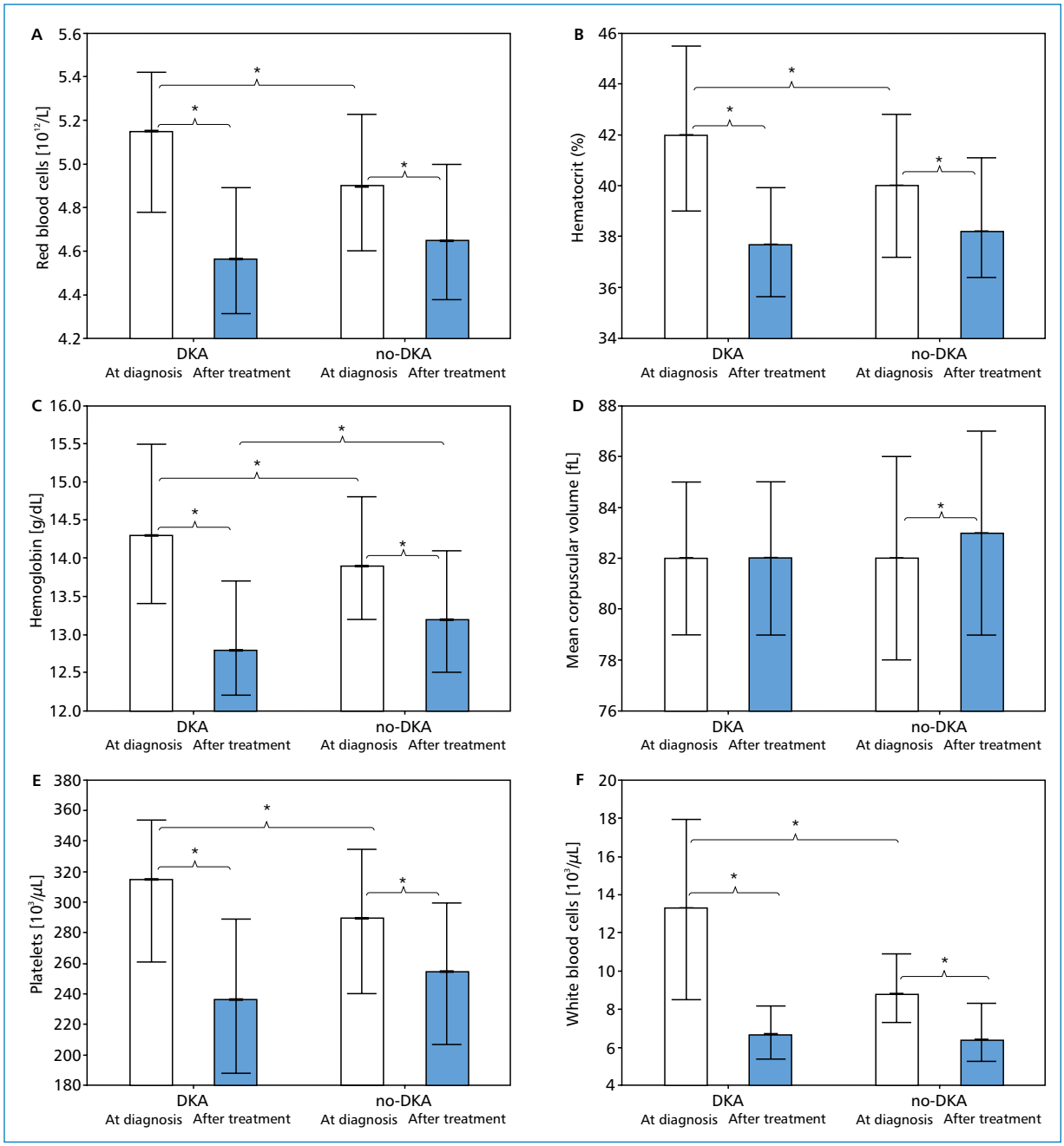

Figure 1. Changes of hematological parameters during first days of newly diagnosed T1DM treatment in children with DKA and without DKA. Red blood cells — **A**; hematocrit — **B**; hemoglobin concentration — **C**; mean corpuscular volume — **D**; platelets — **E**; white blood cells — **F**

days [25–75%: 2.0–4.0 days] *vs.* Me: 3.0 days [25–75%: 2.0–4.0 days] p = 0.2638).

In general linear models analysis with interaction between group allocation and timepoint there was a significant difference in hematological parameters dynamics change between the groups (all p values < 0.01). Parameters such as RBC, Hct, Hb, PLT and WBC were characterized by greater drop from their baseline values in DKA group *vs.* non-DKA group (all p values < 0.0001) (Figure 1 A–C, E–F). MCV level increased significantly but only in the no-DKA group (p < 0.0001) (Figure 1D).

Correlations between changes in hematological parameters and clinical factors

In order to establish the relationship between patients clinical status at admission with hematological parameters in DKA and no-DKA patients we correlated

Table 2. Correlations between blood cell parameters at admission of newly diagnosed type 1 diabetes treatment and clinical parameters

	pH		Effective osmolality [mOsm/kg]		Estimated dehydration (based on Na+ and urea) (%)		eGFR [mL/min/ /1.73 m^2]		C-peptide [ng/mL]	
DKA	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**
RBC [10^{12}/L]	**–0.39**	**0.0006**	**0.31**	**0.0076**	**0.34**	**0.0030**	**–0.36**	**0.0221**	**0.28**	**0.0246**
Hct (%)	**–0.36**	**0.0014**	**0.35**	**0.0022**	**0.32**	**0.0070**	**–0.43**	**0.0046**	**0.36**	**0.0041**
Hb [g/dL]	**–0.42**	**0.0002**	**0.50**	**< 0.0001**	**0.43**	**0.0002**	**–0.51**	**0.0006**	**0.43**	**0.0005**
MCV [fL]	–0.06	0.6327	0.13	0.2734	–0.02	0.8392	–0.07	0.6677	0.25	0.0517
PLT [$10^3/\mu$L]	0.03	0.8247	0.13	0.2866	0.05	0.6780	–0.15	0.3338	0.15	0.2484
WBC [$10^3/\mu$L]	**–0.53**	**< 0.0001**	**0.28**	**0.0155**	0.23	0.0560	**–0.44**	**0.0042**	0.01	0.9119
No-DKA	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**
RBC [10^{12}/L]	0.01	0.9397	0.02	0.8300	0.09	0.2378	**–0.23**	**0.0289**	**0.18**	**0.0255**
Hct (%)	–0.09	0.2309	< 0.01	0.9996	0.01	0.8922	–0.05	0.6385	**0.25**	**0.0023**
Hb [g/dL]	–0.10	0.2089	0.12	0.1256	**0.20**	**0.0115**	–0.12	0.2620	**0.21**	**0.0093**
MCV [fL]	–0.11	0.1343	–0.05	0.5197	**–0.16**	**0.0337**	**0.22**	**0.0301**	0.12	0.1415
PLT [$10^3/\mu$L]	–0.06	0.4582	–0.08	0.3002	0.11	0.1682	–0.11	0.3090	**–0.19**	**0.0204**
WBC [$10^3/\mu$L]	**–0.19**	**0.0122**	0.02	0.8032	0.05	0.4991	**–0.21**	**0.0452**	**–0.25**	**0.0019**

Table 3. Correlations between magnitude of blood cell parameters changes during first days of newly diagnosed type 1 diabetes treatment and clinical parameters

	pH		Effective osmolality [mOsm/kg]		Estimated dehydration (based on Na+ and urea) (%)		eGFR [mL/min/ /1.73 m^2]		C-peptide [ng/mL]	
DKA	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**
Delta RBC [10^{12}/L]	**0.45**	**0.0001**	–0.21	0.0847	–0.19	0.1295	0.20	0.2113	**–0.34**	**0.0084**
Delta Hct (%)	**0.45**	**0.0001**	**–0.26**	**0.0284**	–0.23	0.0648	0.28	0.0737	**–0.35**	**0.0067**
Delta Hb [g/dL]	**0.45**	**0.0001**	–0.21	0.0847	–0.19	0.1295	0.20	0.2113	**–0.34**	**0.0084**
Delta MCV [fL]	**0.30**	**0.0111**	**–0.32**	**0.0075**	**–0.28**	**0.0220**	**0.49**	**0.0012**	–0.11	0.4053
Delta PLT [$10^3/\mu$L]	**0.30**	**0.0136**	**–0.31**	**0.0113**	–0.21	0.0840	**0.37**	**0.0183**	–0.25	0.0535
Delta WBC [$10^3/\mu$L]	**0.59**	**< 0001**	**–0.25**	**0.0354**	–0.13	0.2804	**0.47**	**0.0022**	–0.08	0.5421
No-DKA	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**
Delta RBC [10^{12}/L]	0.02	0.8355	–0.04	0.6014	–0.03	0.7535	0.16	0.1355	0.07	0.4414
Delta Hct (%)	0.05	0.5712	0.04	0.5888	0.04	0.6666	0.08	0.4344	0.10	0.2502
Delta Hb [g/dL]	0.03	0.7131	–0.05	0.5147	–0.03	0.6878	0.14	0.1831	0.08	0.3522
Delta MCV [fL]	–0.06	0.4575	0.16	0.0536	**0.19**	**0.0206**	–0.06	0.5489	0.01	0.8633
Delta PLT [$10^3/\mu$L]	0.15	0.0609	< 0.01	0.9955	–0.02	0.7760	**0.27**	**0.0101**	0.15	0.8633
Delta WBC [$10^3/\mu$L]	0.14	0.0811	–0.13	0.0981	–0.16	0.0555	0.18	0.0841	–0.05	0.5769

them with pH, effective osmolality, estimated dehydration, eGFR and C-peptide (Tables 2 and 3) and with age, HCO_3^-, BE, time between the blood tests results, urine specific gravity and DDI (Tables S1 and S2).

Firstly, we correlated clinical data with hematological parameters at admission (Tables 2 and S1). RBC, Hct and Hb in DKA group correlated significantly, negatively with pH and eGFR and positively with effective osmolality, estimated dehydration and C-peptide. Among those, only correlation between C-peptide with RBC, Hct and Hb were significant also in no-DKA group. WBC correlated negatively and significant with pH and eGFR in both groups. Relationships between BE and hematological parameters at admission (Table S1)

Table S1. Correlations between blood cell parameters at admission during first days of newly diagnosed type 1 diabetes treatment and clinical parameters

	Age at onset (years)		HCO_3^- [mmol/L]		BE [mEq/L]		Time between the blood tests measurements (days)		Urine specific gravity [kg/L]		DDI [units/kg]	
DKA	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**
RBC [10^{12}/L]	**0.51**	**< 0.0001**	**–0.23**	**0.0442**	**–0.30**	**0.0087**	–0.06	0.6161	0.30	0.1000	0.01	0.9219
Hct (%)	**0.67**	**< 0.0001**	–0.10	0.3921	**–0.27**	**0.0189**	–0.05	0.6730	0.17	0.3577	0.03	0.8532
Hb [g/dL]	**0.71**	**< 0.0001**	–0.19	0.1031	**-0.34**	**0.0030**	–0.07	0.5315	0.26	0.1478	0.11	0.4664
MCV [fL]	**0.37**	**0.0011**	0.12	0.2981	–0.06	0.6233	0.07	0.5467	–0.17	0.3525	0.11	0.4429
PLT [$10^3/\mu$L]	–0.11	0.3322	–0.06	0.6354	< 0.01	0.9694	–0.02	0.8444	0.07	0.7220	0.08	0.5990
WBC [$10^3/\mu$L]	0.11	0.3480	**–0.50**	**< 0.0001**	**–0.55**	**< 0.0001**	0.05	0.6673	0.21	0.2409	–0.03	0.8142
No-DKA	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**
RBC [10^{12}/L]	**0.28**	**0.0002**	0.13	0.0975	0.11	0.1612	–0.06	0.4323	0.10	0.3839	0.10	0.3214
Hct (%)	**0.50**	**< 0.0001**	**0.16**	**0.0407**	0.08	0.2736	< 0.01	0.9537	0.14	0.2031	0.12	0.1990
Hb [g/dL]	**0.49**	**< 0.0001**	**0.16**	**0.0343**	0.10	0.1894	–0.04	0.5556	0.15	0.1719	0.08	0.4310
MCV [fL]	**0.28**	**0.0002**	0.02	0.8190	–0.03	0.6974	0.02	0.8150	0.03	0.7765	0.12	0.2055
PLT [$10^3/\mu$L]	**–0.50**	**< 0.0001**	**–0.16**	**0.0305**	**–0.22**	**0.0033**	0.02	0.7451	0.07	0.5156	–0.14	0.1495
WBC [$10^3/\mu$L]	**–0.43**	**< 0.0001**	**–0.38**	**< 0.0001**	**–0.41**	**< 0.0001**	0.02	0.7703	–0.01	0.9314	0.02	0.8612

Table S2. Correlations between magnitude of blood cell parameters changes during first days of newly diagnosed type 1 diabetes treatment and clinical parameters

	Age at onset (years)		HCO_3^- [mmol/L]		BE [mEq/L]		Time between the blood tests measurements (days)		Urine specific gravity [kg/L]		DDI [units/kg]	
DKA	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**
Delta RBC [10^{12}/L]	**–0.38**	**0.0013**	**0.32**	**0.0073**	**0.42**	**0.0003**	–0.09	0.4690	0.01	0.9470	–0.04	0.7593
Delta Hct (%)	**–0.42**	**0.0003**	**0.30**	**0.0108**	**0.41**	**0.0004**	–0.05	0.6943	< 0.01	0.9979	–0.06	0.6635
Delta Hb [g/dL]	**–0.38**	**0.0013**	**0.32**	**0.0073**	**0.42**	**0.0003**	–0.09	0.4690	0.01	0.9470	–0.04	0.7593
Delta MCV [fL]	–0.12	0.3348	**0.25**	**0.0352**	**0.27**	**0.0224**	0.13	0.2845	–0.17	0.3760	–0.01	0.9359
Delta PLT [$10^3/\mu$L]	–0.22	0.0666	0.23	0.0571	**0.26**	**0.0331**	0.05	0.6758	–0.23	0.2324	–0.01	0.9279
Delta WBC [$10^3/\mu$L]	**–0.24**	**0.0441**	**0.50**	**< 0.0001**	**0.59**	**< 0.0001**	–0.20	0.1002	–0.17	0.3754	0.02	0.9039
No-DKA	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**	**R**	**P value**
Delta RBC [10^{12}/L]	0.10	0.2111	0.12	0.1256	0.13	*0.0994*	–0.07	0.3980	–0.08	0.4853	–0.15	0.1333
Delta Hct (%)	0.06	0.4843	0.10	0.2002	0.14	*0.0798*	–0.01	0.8681	–0.18	0.1304	–0.12	0.2487
Delta Hb [g/dL]	0.11	0.1754	0.12	0.1241	0.14	*0.0800*	–0.04	0.6448	–0.07	0.5784	–0.16	0.1035
Delta MCV [fL]	–0.06	0.4785	–0.08	0.3147	–0.07	0.3583	**0.16**	**0.0443**	–0.04	0.7657	–0.03	0.7290
Delta PLT [$10^3/\mu$L]	0.08	0.3248	0.07	0.3547	0.14	0.0862	–0.10	0.2181	–0.19	0.1038	0.05	0.6280
Delta WBC [$10^3/\mu$L]	0.06	0.4573	**0.16**	**0.0421**	**0.19**	**0.0178**	**–0.18**	**0.0226**	–0.10	0.3919	0.07	0.5052

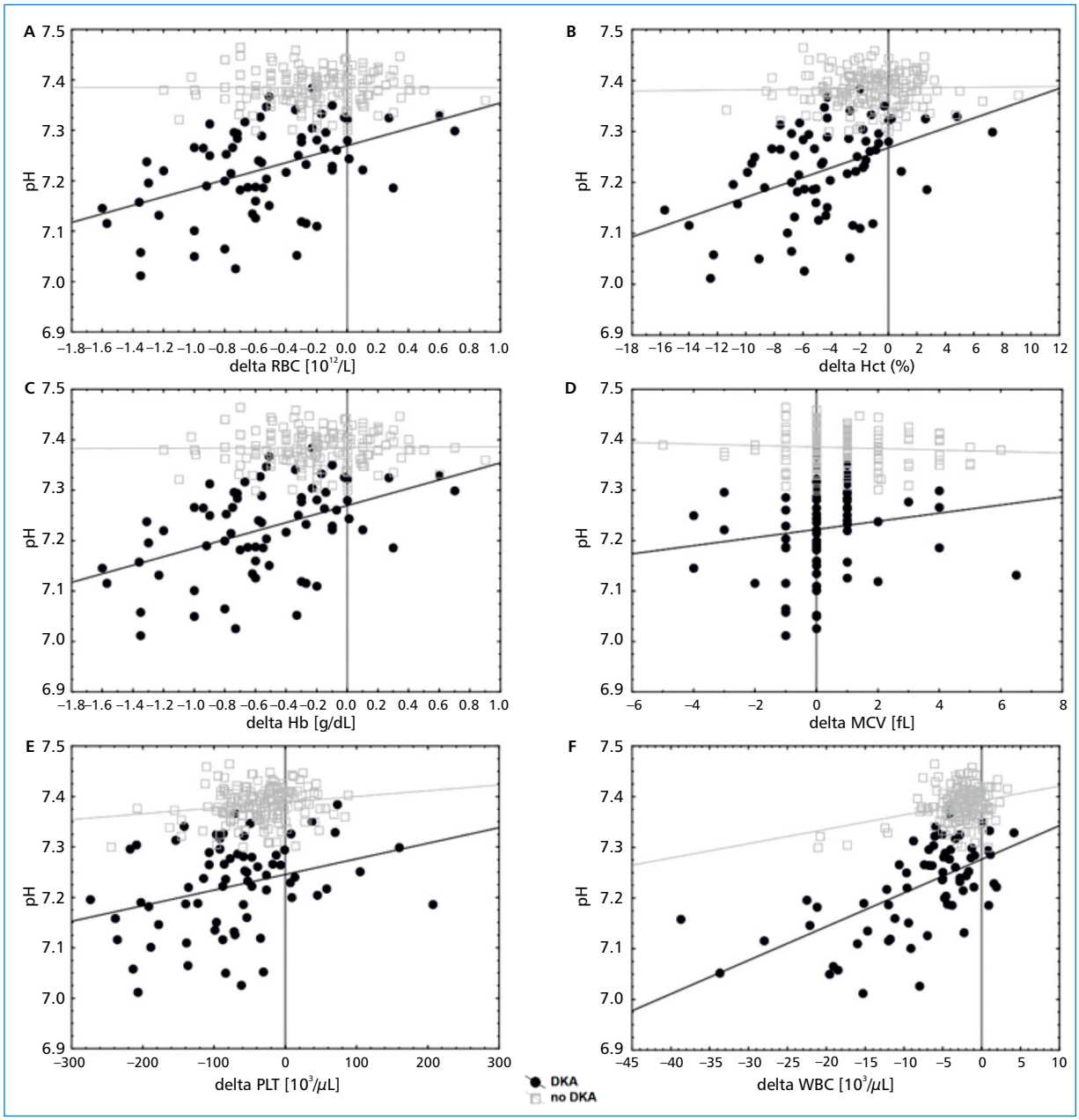

Figure 2. Correlations between pH and changes in morphological parameters during first days of newly diagnosed T1DM treatment in children with DKA and without DKA. RBC (red blood cells) — **A**; Hct (hematocrit) — **B**; Hb (hemoglobin) — **C**; MCV (mean corpuscular volume) — **D**; PLT (platelets) — **E**; WBC (white blood cells) — **F**

showed similar dynamics as correlations with pH in the DKA group. For HCO_3^- only correlations with RBC and WBC were significant. Interestingly, in no-DKA group significant and negative correlations between PLT and both BE and HCO_3^-, were observed. Additionally, also only in no-DKA group significant and positive correlations between HCO_3^- with Hct and Hb were observed.

After that, we correlated patients' clinical status with changes in hematological parameters since admission (Tables 3 and S2). In the DKA group changes in all hematological parameters correlated positively with pH (all $R > 0.3$ and all p values < 0.05) (Figure 2). Relationships in the DKA group between changes in hematological parameters and both HCO_3^- (Table S2) and base excess (Table S2), showed similar dynamics to pH. In comparison, in the no-DKA group which was 2.5-times more numerous than DKA group, no correlations with pH were noted for changes in RBC, Hct, Hb and MCV. However, for change in PLT and WBC weak, positive correlations with pH at the margin of

statistical significance were noted. For correlations with HCO_3^- and base excess, no significant associations with hematological parameters changes were found in the no-DKA group, except for WBC and both HCO_3^- and BE (Table S2) and weak positive correlations at the margin of statistical significance of BE and changes in RBC, Hct, Hb and PLT.

Effective plasma osmolality significantly, negatively correlated with all hematological changes but only in DKA group (delta RBC and delta Hb were at the margin of statistical significance). Dehydration estimated by equation provided by Ugale et al. [7] correlated negatively (with borderline significance) with all hematological changes but also only in DKA group. Strangely, the change of MCV correlated significantly but negatively with estimated dehydration in DKA group but significantly and positively in no-DKA group. eGFR correlated strongly positively with the changes of MCV and WBC in DKA group and with the change of PLT both in DKA and no-DKA group. C-peptide correlated significantly and negatively with changes in RBC, Hct and Hb only in DKA group and at the borderline significance with the PLT change.

When correlation between each change in hematological parameters were performed, all but MCV, changes significantly, positively correlated with each other (all $R > 0.4$, all p values < 0.0001). The change of MCV correlated only with the change of Hct ($R = 0.21$, $p = 0.0017$).

Multivariate models explaining changes in hematological parameters during DKA treatment

In order to evaluate which clinical variables can explain changes of hematological parameters in DKA group, we performed multivariate analyses (Tables S3). The change of RBC and Hb were both independently positively associated with pH and negatively with C-peptide. Similar associations were found in the multivariate model for change in Hct which additionally showed significant negative association with creatinine concentration. For the change of RBC, Hct and Hb built models explained more than 30% of parameter variation (adjusted R^2).

The change of MCV was significantly and negatively associated with glucose concentration. Our model explained only 7% of parameter variance suggesting that other variable (not included in our database) are associated with MCV changes during DKA treatment.

PLT changes was associated positively with pH and negatively with glucose concentration. The model explained 17.5% of parameter variation.

For the change of WBC we were able to create model comprising 3 variables: BE (positive association), creatinine level (negative association) and potassium level (negative association). Model explained almost 42% of changes in WBC variation.

Discussion

In this study we found that hematological parameters such as RBC, Hct, Hb, PLT and WBC decreased after fluid therapy among children with newly diagnosed T1DM. At the diagnosis, abovementioned hematological parameters were higher in DKA group compared to no-DKA group. Also, these parameters had greater magnitude of changes from their baseline values in DKA in comparison to no-DKA group. Interestingly, MCV increased after fluid therapy but only in no-DKA group.

MCV changes may be associated with changing plasma osmolality during fluid therapy. Dehydration may influence MCV but the direction of change depends on plasma osmolality. During hyperosmolar dehydration the plasma volume is decreasing (mainly water), which leads to uneven ion concentration between inside of red blood cells and plasma itself. In order to rebalance the concentration, water is moving from the inside of RBC to the extracellular space. As the consequence, RBC shrinks and thus MCV is reduced.

Effective plasma osmolality was higher in DKA group compared to no-DKA group. Hence, odd is the fact that in our study the DKA group had the same MCV values at T1DM diagnosis as no-DKA group. Moreover, in no-DKA group MCV increased significantly after fluid therapy, but this dynamics were not present in DKA group. We believe that the increased MCV may suggests over hydration in patients from no-DKA group. To support this hypothesis, we observed positive correlation of the delta MCV with time between the blood tests measurements suggesting that longer time of fluid therapy is associated with bigger change of MCV. On the other hand, the lack of increase in MCV in DKA group after fluids therapy may suggest improper restoring of plasma osmolality after treatment.

Decreased MCV may also result from iron or copper deficiency and different types of hemoglobinopathies [20]. MCV may increase due to the anemia, vitamin B12 or folate deficiency, alcohol abuse and many other disorders [21]. However, due to short period of time between blood test measurements, we can assume that those factors did not affect MCV in our studies.

Other hematological parameters (RBC, Hct, Hb, PLT and WBC) had the same dynamics of change in both DKA and no-DKA group, but greater magnitude of changes were observed in DKA group. It could suggest that children with DKA were more dehydrated and it was reflected by higher blood condensation at diabetes diagnosis.

Table S3. Multivariate linear models for changes in RBC, Hct, Hb, MCV, PLT and WBC

Delta RBC	**R^2**	**R^2 adj.**	**P value for the model**	**Delta MCV**	**R^2**	**R^2 adj.**	**P value for the model**
	38.91%	34.47%	**< 0.0001**		11.18%	7.15%	**0.0484**
Variables	**Beta**	**Beta***	**P value**	**Variables**	**Beta**	**Beta***	**P value**
Intercept	**–16.7650**		**0.0004**	Intercept	**1.5844**		**0.0053**
Sex — male	–0.0093	–0.02	0.8607	Sex — male	–0.0886	–0.05	0.6466
Age (years)	**–0.0248**	**–0.25**	**0.0345**	Age (years)	–0.0190	–0.06	0.6428
pH	**2.2952**	**0.42**	**0.0004**	Glucose concentration [mg/dL]	**–0.0020**	**–0.30**	**0.0145**
C-peptide [ng/mL]	–0.3138	–0.21	0.0720				
Delta Hct	**R^2**	**R^2 adj.**	**P value for the model**	**Delta PLT**	**R^2**	**R^2 adj.**	**P value for the model**
	44.44%	39.20%	**< 0.0001**		22.35%	17.50%	**0.0025**
Variables	**Beta**	**Beta***	**P value**	**Variables**	**Beta**	**Beta***	**P value**
Intercept	**–127.4183**		**0.0027**	Intercept	–1549.9744		0.0883
Sex — male	0.0433	0.01	0.9252	Sex — male	8.5233	0.09	0.4130
Age (years)	–0.1601	–0.18	0.1607	Age (years)	–3.0931	–0.16	0.1659
pH	**17.6857**	**0.36**	**0.0023**	pH	216.9799	0.21	0.0817
Creatinine [mg/dL]	–2.9730	–0.23	0.0897	Glucose concentration [mg/dL]	**–0.1094**	**–0.29**	**0.0162**
C-peptide [ng/mL]	–2.7970	–0.20	0.0661				
Delta Hb	**R^2**	**R^2 adj.**	**P value for the model**	**Delta WBC**	**R^2**	**R^2 adj.**	**P value for the model**
	38.91%	34.47%	**< 0.0001**		46.26%	41.92%	**< 0.0001**
Variables	**Beta**	**Beta***	**P value**	**Variables**	**Beta**	**Beta***	**P value**
Intercept	**–16.7650**		**0.0004**	Intercept	**17.1585**		**0.0021**
Sex — male	–0.0093	–0.02	0.8607	Sex — male	–0.7409	–0.10	0.3040
Age (years)	**–0.0248**	**–0.25**	**0.0345**	Age (years)	–0.0899	–0.06	0.6173
pH	**2.2952**	**0.42**	**0.0004**	BE [mEq/L]	**0.7697**	**0.51**	**< 0.0001**
C-peptide [ng/mL]	–0.3138	–0.21	0.0720	Creatinine [mg/dL]	–4.7149	–0.21	0.0931
				K [mEq/L]	–1.6080	0.15	0.1326

Based on correlations between changes in each hematological parameters (RBC, Hct, Hb, MCV, PLT and WBC) with pH, we assumed that severity of DKA is associated with dehydration expressed as greater change of each parameter from the baseline level. Our findings are not consistent with those provided by Ugale et al. [7]. This difference might arose from fact that they measured dehydration as a change of body mass. Measuring change of body mass as a dehydration status may be affected by e.g. body fat percentage. Thus, when patient has higher percentage of body fat and loses e.g. 10% of total body water, the change in total body weight will be smaller in comparison to the lean patient with the same percentage of lost total body water. Additionally, renutrition and insulin administration may affect body mass. These might be the reasons of the different results between our study and Ugale's one. Also, estimated dehydration provided in Ugale's study did not differ significantly between DKA and no-DKA group in our study. This result is rather strange and we believe that it may indicate that this equation is not accurate in estimating dehydration in newly diagnosed T1DM patients as the difference in hydration status between those two group was shown by lower eGFR and higher effective plasma osmolality in DKA group.

Furthermore, our study showed that only RBC, Hct and Hb at admission correlated significantly and negatively with eGFR and pH and positively with estimated dehydration, effective osmolality and C-peptide. This could further support hypothesis that higher RBC, Hct and Hb at diagnosis and their further drop after fluid administration is strongly associated with patients

hydration status and could help clinicians to estimate patients hydration status at T1DM diagnosis. However, those correlations were not evident in no-DKA group what could emerge from lesser dehydration in this group of patients (eGFR and plasma osmolality were mostly within normal limits in this group).

Interestingly, even though most of above-mentioned correlations were not present in no-DKA group, positive correlation between C-peptide and RBC, Hct, Hb was significant in both groups. We presume that higher C-peptide concentration at admission (preserved residual beta-cell function) was associated with longer development of full symptomatic type 1 diabetes that forced patients and their parents to seek medical counselling. Thus, with longer time, those children may develop greater dehydration as shown by lower RBC, Hct and Hb without much changes in plasma osmolality and eGFR due to compensatory mechanisms.

Also we observed increased level of PLT count at T1DM diagnosis and higher levels of this parameter in DKA group. Venous thrombosis compilations are rare but well-known consequences of DKA [22]. Increased PLT count may partially explain this phenomenon in addition to already known increased platelet aggregation, elevated levels of procoagulants and decreased activity of anticoagulants in patients with DKA [23]. All of this, could suggest consideration of antithrombotic treatment among patients with DKA and high PLT count.

Despite above-mentioned parameter, also WBC shared the same dynamics of change (significant drop after treatment and higher levels in DKA group at admission). It is known that WBC reflects systemic inflammation level but taking into consideration that most of our patient had CRP levels with normal limits, it suggest that here higher WBC could be also a marker of blood condensation. Interestingly, WBC values most strongly correlated with pH level in DKA group and this association was also present in no-DKA group and this phenomenon was also observed by others [24].

The main limitation of our study was lack of directly measured dehydration. Thereby, we were not able to associate blood morphology parameters and their changes with true patients' hydration status. Additionally, the amount of administered fluids was not registered and we draw our conclusion on effect of fluid therapy using time between the blood samples collection as a surrogate of administered fluid volume. Also, absence of ketone bodies concentration can be consider as a study limitation. Due to retrospective character of our study we were not able to obtain measurements of this parameter. However taking into consideration that all patients were newly diagnosed with T1DM and improved with insulin therapy we may assume that only diabetic ketoacidosis was an underlying cause of acidosis among those patients. Finally, we used hematological parameter after treatment as baseline values as we were not able to obtain their values shortly before development T1DM. Another solution would be to verify hematological parameters 6 months after hospitalization, but we believe that other factors (e.g. diet) might influenced them in such a long period of time and disturbed the results.

Conclusions

Hematological parameters measured at T1DM diagnosis may be useful in estimating patients' hydration status. Monitoring of their dynamics during fluid therapy may inform about the treatment effectiveness in restoring total body water.

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Statement of competing interests

The authors have no conflicts of interest to disclose.

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