Research Article

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Impact of Antibodies Against the Zinc Transporter 8 (ZnT8) on the Morphotic Elements of Peripheral Blood - Preliminary Report.

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ABSTRACT

The variability in the expression of the gene for the zinc membrane transporter ZnT8 in leukocytes - on the one hand, and the presence of antibodies against this transporter, at a level of 5-6% in the adult population over 30 years of age, on the other - raises the question of the biological and clinical significance of the potential coincidence of these phenomena.

Material and method: It was decided to retrospectively evaluate the relationship between the presence of the serum anti-ZnT8 antibodies (ZnT8Ab) and the parameters of complete blood counts.

Within the period 06/2023 - 04/2024 - 216 ZnT8Ab determinations were performed, mainly in patients in the diabetology department (in patients with type 2 diabetes), of which 15 patients were higher or equal to 10 RU/ml - which represented 7% of the study group of patients.

Results: On the basis of the available data, it can be concluded that the high ZnT8Ab values could be linked to an increased susceptibility to haemolysis, haemolytic anaemia and changes in other morphotic parameters of the peripheral blood - a tendency to lower lymphocyte and platelet levels. That is, the presence of ZnT8Ab could be one of the factors leading to thrombocytopenia and impaired cellular immunity. The retrospective analysis of the data presented here - suggests that high ZnT8Ab values may result in a greater tendency for changes in blood morphotic elements - including erythrocyte, platelet and leukocyte counts (including neutrocytes and lymphocytes) - in the directions observed in the aforementioned analyses. The decrease in platelet count was accompanied by an increase in platelet anisocytosis, while the decrease in leukocytes involved both neutrocytes and lymphocytes. Generally, all quantitative blood count parameters studied showed declining trends.

Conclusions: Generally, in the current study, all quantitative blood morphotic parameters tested tended to decrease with the increasing levels of ZnT8Ab.

Keywords : *antibodies against ZnT8, erythrocytes, leukocytes, platelets, complete blood count.*

INTRODUCTION

The variability in the expression of the gene for the zinc membrane transporter ZnT8 in leukocytes (1), on the one hand, and the presence of antibodies to this transporter, at a level of 5-6% in the adult population over 30 years of age (3), on the other, raises the question of the biological and clinical significance of the potential coincidence of these phenomena. Studies on the importance of inhibition of this receptor have been in progress for some time (2), especially since the extrapancreatic localisation of ZnT8 is a fact and studies are underway trying to determine its significance. (1, 2).

In experimental mice with a genetic deficiency of the ZnT8 gene (Slc30a8 KO Mouse), changes in blood morphotic elements were shown relative to the WT mice (2).

Also in humans, the LabWAS analysis showed that individuals with SNP a of the SLC30A8 rs13266634 'T' allele tended to have lower platelet counts (P<0.006), with no changes in reticulocyte or lymphocyte levels (2).

MATERIAL AND METHOD

It was decided to retrospectively evaluate the relationship of the presence of antibodies against ZnT8 - ZnT8Ab - in serum with the parameters of blood counts.

Since the introduction of the ZnT8Ab determination at the Provincial Hospital in Opole - between 06/2023 and 04/2024 - 216 ZnT8Ab determinations were performed, mainly in patients in the diabetology department (in patients with type 2 diabetes), of which 15 patients were higher or equal to 10 RU/ml - which represented 7% of the study group of patients.

In the ELISA method used, values below 15 RU/ml were considered the within the normal range. The vast majority

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of negative results were reported as <15 RU/ml, with only 2 cases reporting a value of 10 RU/ml. In the remaining cases, no value within the range of 0-15 RU/ml was given, which is an important limitation for interpretation.

According to population data, an average of 16.5 % of subjects over 18 years of age, and about 5-6 % over 30 years of age, without any symptoms of diabetes, were determined to contain ZnT8Ab (3).

In this group, the ZnT8Ab levels were determined by the same ELISA method, assuming a lower limit of antibody detection of 6 RU/ml.ZnT8Ab – limitations of the method - results - <10 can be 0, but also can be 5

RESULTS

The analyses were carried out using the IBM SPSS Statistics 29 software. In the first instance, basic descriptive statistics were calculated together with the Shapiro-Wilk test to assess the normality of the distribution of the test variables. In the next step, analyses of differences were performed using the Welsch t-test, as well as correlation analyses using the Pearson correlation coefficient. The significance level was taken as the threshold $\alpha = 0.05$, the strength of the effect was interpreted following Cohen's (2016).

Descriptive Statistics of Variable Ratios

The Table 1 presents the basic descriptive statistics broken down into subsets of patients with the determined positive ZnT8Ab and a control group with a zero ratio of ZnT8Ab. For all variable ratios with a response variance different from zero, a test of normality of the data distribution in the study sample was calculated.

When analysing the results of the normality test presented in the **Table 1**, it was found that for the criterion group there were statistically significant deviations from the normal distribution for the ratios ZnT8Ab, WBC and NEUT. In turn, for the control group, significant deviations were observed for the PLT and LYMPH ratios. Additional assessment of the skewness and kurtosis ratios, confirmed that the vast majority of variables were not characterised by a distribution significantly deviating from the normal distribution, with values falling within the absolute value range |2| (George and Mallery, 2021). Furthermore, researchers showed that the Pearson correlation coefficient does not require the assumption of a normal distribution (Bishara and Hittner, 2012; Havlicek and Peterson, 1977), while the analysis of differences can be carried out using the Welsch's t test, which shows considerable resistance to the assumptions of the classic Student's t test (Derrick et al., 2016).

Table 1: Descriptive statistics of the ratios of the variables tested with the use of the Shapiro-Wilk tests.

Dependent variable	M	Me	SD	Sk.	Kurt.	Min.	Max.	W	p
Positive ZnT8Ab (n = 15)									
ZnT8Ab RU/ml	564.60	50.00	751.34	0.95	-0.82	10.00	1987.00	0.74	<0.001
RBC x10^6/µL	4.68	4.73	0.57	-0.79	0.34	3.44	5.50	0.95	0.459
WBC x10^3/µL	6.05	5.52	2.10	1.39	2.40	3.73	11.65	0.88	0.045
NEUT x10^3/µL	3.42	2.89	1.94	2.21	6.12	1.34	9.36	0.78	0.001
PLT x10^3/µL	233.53	213.00	76.89	1.31	2.83	125.00	441.00	0.91	0.128
MCV fL	86.29	86.70	3.63	0.50	-0.09	81.20	94.10	0.97	0.798
MPV fL	10.46	10.20	0.64	0.48	-0.60	9.50	11.70	0.95	0.554
PCT %	0.24	0.24	0.08	1.28	2.95	0.13	0.45	0.91	0.120
PDW fL	12.07	12.00	1.43	-0.17	0.03	9.10	14.60	0.98	0.992
HCT %	40.71	41.40	4.11	-1.03	2.18	30.30	47.70	0.93	0.284
LYMPH x10^3/µL	1.92	2.03	0.53	0.45	-0.24	1.09	2.94	0.93	0.267
Control group (n = 15)									
RBC x10^6/µL	4.69	4.59	0.68	-0.51	1.47	3.04	5.70	0.90	0.106
WBC x10^3/µL	6.58	6.82	1.67	-0.38	0.12	3.53	9.56	0.94	0.409
NEUT x10^3/µL	3.99	4.10	1.43	-0.55	0.14	1.00	6.15	0.96	0.690
PLT x10^3/µL	233.13	204.00	94.80	1.36	1.35	131.00	463.00	0.85	0.019
MCV fL	85.49	86.20	2.69	-0.25	-1.16	81.40	89.80	0.94	0.329
MPV fL	10.86	10.70	1.18	0.20	0.38	8.70	13.40	0.98	0.964
PCT %	0.24	0.23	0.08	0.31	0.23	0.10	0.40	0.98	0.964
PDW fL	13.14	13.00	2.93	0.87	2.23	8.00	20.60	0.94	0.395
HCT %	40.14	39.30	5.60	-0.88	1.96	25.90	48.40	0.90	0.107
LYMPH x10^3/µL	1.91	1.83	0.52	1.70	3.93	1.37	3.40	0.84	0.013

Annotation. M - mean; Me - median; SD - standard deviation; Sk. - skewness; Kurt. - kurtosis; Min - minimum value; Max. - maximum value; W - Shapiro-Wilk test result; p - p-value for Shapiro-Wilk test.

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Analyses

Analyses of comparisons were performed on two samples of 15 patients each, with 13 males (86.7%) and 2 females (13.3%) in each group, which meant that the groups were comparable in terms of sex (p = 1.000). Furthermore, the criterion group (M = 45.67; SD = 17.86) was not statistically significantly different in terms of age (p = 0.925) compared to the control group (M = 46.27; SD = 16.88). The results of comparisons of individual ratios using the Welsch t test are presented in the **Table 2**.

The analyses performed showed that there had been no statistically significant differences in the vast majority of ratios (Table2), the only statistically significant difference being in the ZnT8Ab ratio. However, attention was drawn to Cohen's d coefficient, which indicated some tendency towards small differences that would be observable as significant with much larger samples (d ≥ 0.20). The magnitude of Cohen's d coefficient suggested that the control group may have had slightly higher white blood cell (WBC) and neutrophil (NEUT) concentrations. Furthermore, the control group may have had slightly higher platelet volume (MPV) and platelet volume variability (PDW) scores. In contrast, the ZnT8Ab-positive group showed a weak trend towards slightly higher mean red cell volume (MCV). However, these effects were characterised by a relatively weak strength of difference effect, meaning that their confirmation would require re-testing on larger patient samples.

Table 2: Differentiation of the ratios tested in the groups under comparison

Dependent variable	Positive ZnT8Ab (n = 15)		Control group (n = 15)		t	df	p	95% CI		Cohen d
	M	SD	M	SD				LL	UL	
ZnT8Ab RU/ml	564.60	751.34	0.00	0.00	2.91	14.00	0.011	148.52	980.68	1.06
RBC x10^6/µL	4.68	0.57	4.69	0.68	-0.07	27.09	0.943	-0.49	0.45	0.03
WBC x10^3/µL	6.05	2.10	6.58	1.67	-0.76	26.67	0.454	-1.95	0.90	0.28
NEUT x10^3/µL	3.42	1.94	3.99	1.43	-0.92	25.67	0.368	-1.85	0.71	0.33
PLT x10^3/µL	233.53	76.89	233.13	94.80	0.01	26.86	0.990	-64.28	65.08	<0.01
MCV fL	86.29	3.63	85.49	2.69	0.69	25.78	0.496	-1.59	3.21	0.25
MPV fL	10.46	0.64	10.86	1.18	-1.15	21.49	0.262	-1.12	0.32	0.42
PCT %	0.24	0.08	0.24	0.08	0.14	28.00	0.888	-0.05	0.06	0.05
PDW fL	12.07	1.43	13.14	2.93	-1.27	20.28	0.217	-2.83	0.68	0.47
HCT %	40.71	4.11	40.14	5.60	0.32	25.69	0.752	-3.12	4.26	0.12
LYMPH x10^3/µL	1.92	0.53	1.91	0.52	0.05	27.99	0.959	-0.39	0.41	0.02

Annotation. n - number of observations; M - mean; SD - standard deviation; t - value of test statistic; df - degrees of freedom; p - statistical significance; CI - confidence interval for the difference between means; LL and UL - lower and upper limits of the confidence interval; Cohen's d - index of the strength of the effect.

In a further stage, analyses of the correlations between the ZnT8Ab ratio and the other morphological parameters were performed, which required the exclusion of the control group from the analyses due to their zero variance for the ZnT8Ab ratio. The analyses were presented in the **Table 3**.

The analyses performed (Table 3) showed only a borderline significance effect for the negative correlation of ZnT8Ab with white blood cell count, meaning that patients with a higher count of the ZnT8Ab antibodies also showed a lower white blood cell count. However, according to Cohen (2016), correlation coefficients above an absolute value of r > |0.10|, already show a kind of trend of correlation in the general population. In line with this assumption, all morphological ratios tended to correlate with the number of the ZnT8Ab antibodies. These correlations suggested that, in addition to a lower white blood cell count, a higher ZnT8Ab level was partly linked to lower red blood cell, neutrophil and platelet counts, as well as to a lower percentage of platelet haematocrit ratio, a lower proportion of red blood cells in blood volume and a lower lymphocyte count. On the other hand, opposite effects were observed for the ratios of mean red blood cell and platelet volume and platelet volume variability, which showed an increasing trend with the increasing ZnT8Ab antibody count. The indicated trends showed a correlation of between 4 per cent and 25 per cent of the variance explained by variation in the ZnT8Ab antibody count, suggesting non-random correlations.

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Table 3: Dependence of the ratios tested on the severity of ZnT8Ab (n = 15).

Variable	ZnT8Ab RU/ml		
	PCC (r)	r^2	p
RBC x10^6/µL	-0.37	0.14	0.173
WBC x10^3/µL	-0.50	0.25	0.056
NEUT x10^3/µL	-0.43	0.19	0.107
PLT x10^3/µL	-0.44	0.20	0.098
MCV fL	0.19	0.04	0.494
MPV fL	0.32	0.10	0.243
PCT %	-0.42	0.18	0.120
PDW fL	0.37	0.13	0.178
HCT %	-0.34	0.12	0.215
LYMPH x10^3/µL	-0.40	0.16	0.137

Annotation. r^2 – coefficient of determination.

DISCUSSION

In humans an analysis of LabWAS showed that individuals with the SNP of the SLC30A8 rs13266634 'T' (gain-of-function) allele tended to have lower platelet counts (P<0.006), and higher MCV and MCH, with no change in reticulocyte or lymphocyte levels (2). They showed a lower risk of type 2 diabetes and congenital haemolytic anaemias (including spherocytosis, elliptocytosis, and haemolytic anaemias resulting from enzymatic deficits in glutathione metabolism). In contrast, subjects suffering from the SNP of the SLC30A8 rs13266634 'C' allele (impaired function), showed not only a tendency to develop type 2 diabetes, but also haemolytic anaemia and a decrease in mean haemoglobin per cell (MCH). In mice congenitally lacking ZnT8 - SLC30a8 KO - MCH and MCV were unchanged, while levels of reticulocytes, platelets and lymphocytes were elevated. Counts of neutrophils, monocytes, eosinophils and basophils were not changed (2)

These data suggest differences in the effects of the ZnT8 expression on the blood morphotic parameters between laboratory mice and humans provided that in humans, comparative studies addressed the activity of the ZnT8 gene and its rs13266634 'C' allele as the less active variant - and in mice, with the complete absence of ZnT8. Overall, the PheWAS and LabWAS analyses and the analyses in the SLC30a8 KO mice (without the ZnT8 gene) are consistent (concordant) in showing extrapancreatic effects of the ZnT8 function (2).

The group of people described in the analysis were patients with type two diabetes; the lowest determined ZnT8Ab result was 10 RU/ml, representing 7% of the study population. In the study by Grace et al. (3) - in an analysis of people without diabetes - in a similar age group, the lowest ZnT8Ab result of 6 RU/ml was about 5-6% of the study population.

It is therefore reasonable to believe that with a lower threshold of determination, the detection rate of the ZnT8Ab antibodies in people with type 2 diabetes would be higher.

The occurrence of autoantibodies is an element of autoimmunity, and is indicative of immune dysfunction, but does not equate to a clinical manifestation of the problem.

The influx/efflux of zinc ions is regulated by zinc transporters (Zip1-14 and ZnT1-8, 10) (4,5).

It is probable that in the deficiency or absence of ZnT8 - its function may be partly replaced by the ZnT7 (2).

On the basis of the available data - experimental data, the analyses of PheWAS and LabWAS (2) - it can be concluded that high values of ZnT8Ab could be associated with an increased tendency to haemolysis, haemolytic anaemia (in mice an increase in reticulocytes - as a secondary sign to haemolysis?) and changes in other morphotic parameters of the peripheral blood - a tendency to lower lymphocyte and platelet levels. That is, the presence of ZnT8Ab could be one of the factors leading to thrombocytopenia and impaired cellular immunity.

The current retrospective analysis of the data - presented here - suggests that the high ZnT8Ab values may result in a greater tendency for changes in blood morphotic elements - including the number of erythrocytes, platelets and leukocytes (including neutrocytes and lymphocytes) - in the directions observed in the aforementioned PheWAS and LabWAS analyses and experimental studies. The decrease in platelet counts was accompanied by an increase in platelet anisocytosis, and the decrease in leukocytes involved both neurocytes and lymphocytes. Overall, all quantitative parameters of complete blood counts studied showed declining trends.

Possible prospective studies in this respect would need to take into account, in addition to blood morphotic parameters, biochemical parameters of haemolysis - such as, inter alia, LDH

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and haptoglobin, the selected platelet function parameters and the selected parameters of immune functions.

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