Reduction in white blood cell, neutrophil and red blood cell counts related to gender, HLA and islet autoantibodies in Swedish TEDDY children at increased risk for type 1 diabetes

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Abstract

Islet autoantibodies (IA) precede the clinical onset of type 1 diabetes (T1D), however, the knowledge is limited whether the prodrome affects complete blood counts (CBC) in 4-12 years old children with increased genetic risk for T1D. The aim of this study was to test if CBC was altered in 4-12 years old children without (n=376) or with one or several IA against either insulin, GAD65 or IA-2 (n=72). CBC was analyzed during longitudinal follow up in 448 Swedish children enrolled in The Environmental Determinants of Diabetes in the Young (TEDDY) study. A linear mixed effects model was used to assess potential association between IA and CBC measurements over time. The white blood cell and neutrophil counts were reduced in children with IA, primarily in boys. In contrast, girls had lower levels of hemoglobin and hematocrit. Positivity for multiple IA showed the lowest counts in white blood cells and neutrophils in boys and red blood cells, hemoglobin and hematocrit in girls. These associations were primarily observed in children with the HLA-DR3-DQ2/DR4-DQ8 genotype.

It is concluded that the reduction in neutrophils and red blood cells in children with multiple IA and HLA-DR3-DQ2/DR4-DQ8 genotype, may signal a gender-dependent islet autoimmunity detected in longitudinal CBC.

INTRODUCTION

Autoantibodies against the β-cell autoantigens insulin (IAA), glutamic acid decarboxylase (GADA) or protein tyrosine phosphatase-like (IA-2A) precede the clinical onset of autoimmune type 1 diabetes (T1D). Children at genetic risk for T1D were followed from birth in The Environmental Determinants of Diabetes in the Young (TEDDY) study for a first appearing islet autoantibody (IA) be it either IAA or GADA (1-3). The IAA incidence rate was highest in the first year of life (18 months in Sweden) and declined over the following 5 years, while the incidence rate of GADA increased during the first two years (2.5 years in Sweden) of life and remained seemingly constant until the age of six years (1-3). The appearance of IAA or GADA as the first IA was related to the HLA-DR/DQ genotype (1; 2). Following an initial event that triggers autoimmunity reflected by a first appearing either IAA or GADA, the pathogenesis is progressing towards the clinical onset of disease more rapidly with an increasing number of IA (4; 5), which may be critical as islet β cells are thought to be destroyed by autoreactive T cells, not by autoantibodies (6; 7) . So far, β-cell specific autoantibodies are the best predictors of an ongoing autoimmune process resulting in clinical onset (8). Notwithstanding the critical role of the adaptive immune response in both the etiology and pathogenesis of T1D (reviewed in (9-12)), there is growing evidence that also the innate immune response contributes to the pathogenesis (12-14) . Neutrophils are thought to contribute to both the etiological triggering and the pathogenesis of T1D in mouse models (reviewed in (15)). A reduction in peripheral blood neutrophils has recently been reported in healthy IA positive children all having a first degree relative with the disease (16). Similarly, compared to healthy children, white blood cells, neutrophils and lymphocytes were reduced in IA positive children with a family history of T1D

(17) and there is an unknown impact of the peripheral immune cell counts on the pathogenesis of T1D (18).

We have studied children in Sweden who are enrolled in The Environmental Determinants of Diabetes in the Young (TEDDY) study (19; 20) followed from birth to determine the first appearing IA(1) as well as progression to multiple autoantibodies and clinical onset of diabetes (4; 5). We specifically ask the question whether complete blood count (CBC) was associated with the IA status in 4-12 years old Swedish TEDDY children and whether the association differed by gender and HLA DQ-DR genotype.

RESEARCH DESIGN AND METHODS

TEDDY design

The TEDDY study is a prospective cohort study funded by the National Institutes of Health with the primary goal to identify environmental causes of type 1 diabetes (T1D). TEDDY includes six clinical research centers - three in the US: Colorado, Georgia/Florida, Washington and three in Europe: Finland, Germany, and Sweden. Detailed study design and methods have been previously published (19; 21). Written informed consents were obtained for all study participants from a parent or primary caretaker, separately, for genetic screening and participation in prospective follow-up. The present study was approved by the Regional Ethics Review Board in Lund and is monitored by an External Advisory Board formed by the National Institutes of Health.

TEDDY Children subjected to CBC

The study cohort consists of 448 children, 4-12 years old, from the TEDDY clinic in Malmö, Sweden (Table 1). The CBC measurements were initiated in June 2014 and completed in

February 2017. The samples were analyzed at their scheduled visits and within 8 hours after sample draw. The TEDDY protocol requires every three months visits for those children who were IA positive and every six months for children who remained autoantibody negative.

CBC

CBC was determined in a multi-parameter automated hematology analyzer (CELL-Dyn Ruby, Abbott Laboratories, Diagnostic Division, Abbott Park, IL, USA) (22). The instrument was operated according to the instructions by the manufacturer including a daily calibration. Counts (counts $x10^9$/L) of white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS), basophils (BASO), platelets (PLT), red blood cells (RBC) (counts $x10^{12}$/L) and red blood cell parameters; hemoglobin (HGB) (g/L), hematocrit (HCT)(L/L), mean corpuscular volume (MCV) (fL), mean corpuscular hemoglobin (MCH) (pg), mean corpuscular hemoglobin concentration (MCHC) (g/L) and red cell distribution width (RDW) (%CV) were obtained.

HLA-DR-DQ typing

Genotype screening (23) was conducted using either a dried blood spot punch or a small volume whole blood lysate specimen format, as previously published (24). Infants from the general population were eligible for the study if they had any one of the following HLA genotypes (excluding those with DR4 subtype DRB1*04:03):

(i) DR4-DQA1*03:0X-DQB1*03:02/DR3-DQA1*05:01-DQB1*02:01

(ii) DR4-DQA1*03:0X-DQB1*03:02/DR4-DQA1*03:0X-DQB1*03:02[1]

(iii) DR4-DQA1*03:0X-DQB1*03:02/DR8-DQA1*04:01-DQB1*04:02

(iv) DR3-DQA1*05:01-DQB1*02:01/DR3-DQA1*05:01-DQB1*02:01

[1]Acceptable alleles in this haplotype include both DQB1*03:02 and *03:04

Infants who have a first degree relative with type 1 diabetes were eligible for enrollment if they had any of the following HLA genotypes:

(i) DR4-DQA1*03:0X-DQB1*03:02[1]/DR3-DQA1*05:01-DQB1*02:01

(ii) DR4-DQA1*03:0X-DQB1*03:02[1]/DR4-DQA1*03:0X-DQB1*03:02[1]

(iii) DR4-DQA1*030X-DQB1*0302[1]/DR8-DQA1*04:01-DQB1*04:02

(25) DR3-DQA1*05:01-DQB1*02:0[1]/DR3-DQA1*05:01-DQB1*02:01

(v) DR4-DQA1*03:0X-DQB1*03:02[1]/DR4-DQA1*03:0X-DQB1*02:0X

(vi) DR4-DQA1*03:0X-DQB1*03:02[1]/DR12-DQA1*0101-DQB1*05:01[2]

(vii) DR4-DQA1*030X-DQB1*03:02[1]/DR13-DQA1*01:02-DQB1*06:04

(viii) DR4-DQA1*03:0X-DQB1*03:02/DR4-DQA1*03:0X-DQB1*03:04

(24) DR4-DQA1*03:0X-DQB1*03:02[1]/DR9-DQA1*03:0X-DQB1*03:03

(x) DR3-DQA1*05:01-DQB1*02:01/DR9-DQA1*03:0X-DQB1*03:03

Note: [1]Acceptable alleles in this haplotype included both DQB1*03:02 and *03:04. [2]In this DQB1*05:01 haplotype, DR10 was excluded. Only DR1 was eligible.

Autoantibody Measurements

IAA, GADA or IA-2A were measured in two laboratories by radio binding assays (26; 27). In Europe, all sera were assayed at the University of Bristol, Bristol, U.K. All samples positive for IA and 5% of negative samples were re-tested at the Barbara Davis Center for Childhood Diabetes at the University of Colorado, Denver and deemed confirmed if concordant. Both laboratories have previously shown high sensitivity and specificity as well as concordance (28).

IA analyses

Persistent IA positivity was defined as confirmed positive IAA, GADA or IA-2A on at least two consecutive study visits. The first appearance of persistent confirmed IA in the follow-up was considered and counted.

Statistical analysis

Considering correlations between measures from the same subject (within subject correlation), a linear mixed effects model was used to assess the association of islet autoimmunity on each CBC measurement. We first examined whether the association between the status of IA and each CBC measurement was different by sampling age. But no significant difference was noted in all CBC analyses. Hence, the model included random intercept and random slope, as well as sampling age and the status of islet autoantibodies as fixed effects. Unstructured within subject correlation was assumed for the random error. The regression coefficient for the status of IAs was assessed to determine whether the associations of islet autoimmunity were ignorable or not. The association between age and each CBC measurement was assessed among islet autoantibody negative children. Age of initial measurement was compared using Wilcoxon rank sum test and the proportions of girls and HLA-DR3-DQ2/DR4-DQ8 subjects were compared using Fisher's exact test. Age-dependent effects were observed in most CBC parameters and later corrected for in the linear mixed effects model. Due to this procedure, the observed effects of an increasing number of IA on neutrophils and red blood cell parameters were all corrected for age.

Two-sided p-values less than 0.05 were considered for statistical significance. All analyses were performed using the Statistical Analysis System Software (Version 9.4, SAS Institute, Cary, NC).

RESULTS

CBC in relation to age in IA negative children

A total of 448 children participating in the longitudinal TEDDY study were examined for CBC. Children were examined at 1-6 visits if negative (84%) or 1-9 visits (16%) if positive for any islet autoantibody. The number of CBC measurements was therefore higher in the IA subjects (n=72, median=3) than in the IA negative subjects (n=376, median=2), but there was no significant difference in the age when the CBC measurements started (Table 1). We examined whether CBC varies with age in the IA negative children (Table 2). In agreement with earlier studies older age was significantly associated with decreasing cell counts in the different white blood cell types, except for a non-significant association in eosinophils and basophils (Table 2). In contrast to the nucleated cells, red blood cell counts and hemoglobin levels increase with age which is also confirmed in our study(29; 30).

CBC in children with and without IA

In the next step, differences in the peripheral blood cell counts were examined between children with or without IA (Table 3). Children with IA had reduced white blood cell counts ($p=0.046$) as a result from a reduction in neutrophil cell counts ($p=0.017$). The two RBC parameters, hemoglobin ($p=0.026$) and hematocrit ($p=0.031$) were also reduced in children with IA. The neutrophil to lymphocyte ratio (NLR) did not differ between the two groups.

CBC in relation to gender in children with and without IA

Stratified analyses by gender were performed to evaluate gender difference (Table 3). Reduction of white blood cell counts ($p=0.02$) caused by a reduction of neutrophil ($p=0.012$) and basophil ($p=0.029$) counts showed a significant difference between children with IA and without IA in

boys, while a reduction of hemoglobin (p=0.012) and hematocrit (p=0.047) was found in girls with IA.

CBC in relation to the number of IA and HLA genotype

We investigated whether the number of IA was associated with CBC by comparing the different cell counts (Table 4). The number of IA was counted as the appearance of any of the following three IA (IAA, GADA or IA-2A) in the follow-up. Of the 72 children with IA, all three IA appeared in 31 children, two IA appeared in 16 children, and one IA appeared in 25 children. The number of IA was inversely correlated with the number of white blood cells, neutrophils and lymphocytes. Gender differences were also identified in this analysis. Children with three IA and therefore at the greatest risk for T1D showed a reduction in white blood cell counts (p=0.007), primarily in boys (p=0.019) and in children with HLA-DR3-DQ2/DR4-DQ8 (p=0.045). The reduction of white blood cells was mainly caused by the reduction in neutrophil counts (p=0.003), primarily in boys (p=0.004) and in children with HLA-DR3-DQ2/DR4-DQ8 (p=0.010), but also the reduction of lymphocyte counts (p=0.038) in all children.

Red cell parameters were also associated with the number of IA, especially when all three IA appeared. The red blood cell count was reduced in all children with two IA (p=0.026), particularly in girls (p=0.002) and in children with the HLA-DR3/4-DQ2/8 genotype and three IA (p=0.006) (Table 4). These reductions in red blood cell counts were reflected in reduced concentrations of both hemoglobin and hematocrit in the presence of two or more IA, in girls and in children with the HLA-DR3-DQ2/DR4-DQ8 genotype (Table 4). Furthermore, in children with the HLA-DR3-DQ2/DR4-DQ8 genotype and one IA the mean corpuscular hemoglobin (MCH) was also decreased (p=0.042). In contrast, boys with one positive IA had increased mean

corpuscular volume (MCV) levels (p=0.044), particular in children not carrying the HLA-DR3-DQ2/DR4-DQ8 genotype (p=0.008).

DISCUSSION

The major findings of CBC in TEDDY children were lower numbers of white blood cells, primarily of neutrophils in boys and in children with HLA-DR3-DQ2/DR4-DQ8 with an increasing number of IA. Also, an increasing number of IA was related to lower numbers of hemoglobin and hematocrit in girls and in children with HLA-DR3-DQ2/DR4-DQ8. These findings may be related to an interaction between HLA risk and development of chronic islet autoimmunity. In TEDDY, chronic islet autoimmunity is defined both as the length of being persistent confirmed IA positive following seroconversion and also in relation to the number of different IA (31; 32). The major finding that levels of neutrophils, primarily in boys, as well as in children with HLA-DR3-DQ2/DR4-DQ8 decreased with an increasing number of IA is a novel finding, which to our knowledge has not been reported before. Our data are otherwise consistent with a reduction in peripheral blood neutrophils as recently reported in healthy IA positive children all with a first degree relative with the disease (16; 17). However, in contrast to one of these reports a reduction in platelet counts in children positive for one or several IA was not detected. The difference may be explained by the fact that our study was longitudinal including a relatively larger number of children. Our results take these previous reports to a next step indicating that the larger the number of IA, the lower the number of neutrophils. Reduction of red blood cells, hemoglobin and hematocrit in girls positive for two or more IA has not been reported previously.

The strength of the present study is that we have been able to determine CBC during almost three years in this TEDDY subset of 448 children who visit the Malmö clinic. They represent about 15% of all children in TEDDY. During this period of investigation children with IA were subjected to CBC more than three times and children with no IA 1-2 times as they only visit every 6 months. Another strength is that the CBC was done at random as our TEDDY laboratory could only perform the CBC in maximally 8 children per day with blood samples collected in the morning. During the current period of investigating these 4-12 year olds, it was not expected that we would come across a child converting to IA because of small numbers of IA positive children followed in this study. Although TEDDY aims to identify the environmental factors behind seroconversion, the strength of the present study was to contribute to the second end-point in TEDDY, which is to identify factors that predispose to T1D, to determine the pathogenic mechanisms that eventually result in clinical onset of T1D.

The weakness was that we did not have any children who have changed IA status during the follow up and therefore we cannot test if the observed changes in CBC may precede seroconversion. Therefore, our data underscore the importance to investigate CBC when children at increased genetic risk for T1D are followed from birth in the future. Another limitation is the number of IA positive children followed for CBC. Therefore, we continue the CBC follow up in children with and without IA in order to understand underlying mechanisms of altered CBC. Validation of these results outside of the TEDDY cohort may be accomplished in studies following newborns, such as in the recently initiated POInT study (33).

Pediatric blood reference intervals are mainly based on retrospective data from hospitalized persons (29; 34) . However, the data in this study are prospective from healthy children with

genetic risk for T1D. This premise makes our data more reliable and useful when IA positive children were compared with those negative for any IA. HLA risk eligibility for TEDDY in Sweden represented 7.5% of all newborns (20). Further CBC analyses are needed as there is an apparent lack of information of CBC neutrophil and red blood cell parameters in relation to HLA-DR-DQ risk for not only T1D but also celiac disease, thyroiditis, multiple sclerosis and other HLA-associated organ specific autoimmune disorders.

In agreement with numerous studies, white blood cell counts varied during the first years of life and decreased slightly thereafter. Moreover, the red blood cell count is known to increase by age in children which was found also in this study (29; 34). Furthermore, we examined the association between age and CBC in the IA negative children considered as the general population with the above limitations. As revealed in Table 2, age-dependent effects were observed in most CBC parameters and later corrected for in the linear mixed effects model. Due to this procedure, the observed associations between the increasing number of IAs and the reduction in neutrophils and red blood cell parameters were all corrected for age.

A diminished number of white blood cells were reported in several autoimmune diseases (17; 18; 35). Even though neutrophils are innate immune cells, they are involved in the activation and recruitment of both innate and adaptive immune cells (36; 37). An impaired neutrophil response is thought to cause or initiate several autoimmune diseases including systemic lupus, vasculitis and multiple sclerosis (36; 38). Our results demonstrate a reduction in neutrophils in boys and HLA-DR3-DQ2/DR4-DQ8 children positive for three IA. Recent studies have also reported a reduction in circulating neutrophils in newly diagnosed T1D patients (who did not have diabetes ketoacidosis at onset) and in IA positive healthy persons, suggesting β□cell specific autoimmunity (16; 17; 39). Neutrophils are thought to be recruited to infiltrate pancreatic islets

by the physiological death of β cells and by a signaling crosstalk with other innate immune cells activating the autoreactive T-cells (37). Recently, it has also been suggested that the reduction of neutrophil numbers may be related to an accumulation of neutrophils in the pancreas (16; 17) perhaps associated with insulitis (40). This data would be consistent with the observations that the larger the number of IA, the higher the risk for insulitis (41; 42). However, further studies need to dissect the neutrophil count reduction in relation to pathophysiological changes that occur in the pancreas prior to the clinical onset of T1D.

Mild neutropenia is a common finding in children with viral infections (43). The reduction in the neutrophil counts in healthy Swedish TEDDY boys and children with the HLA-DR3-DQ2/DR4-DQ8 genotype could be due to an impaired hematopoietic cell production in the bone marrow, impaired maturation, apoptosis, peripheral consumption or damage, pooling to other organs like pancreas and perhaps tissue detention (44; 45). The reduction in neutrophil counts associated with a presence of three IA may contribute to an accelerated pathogenesis by potentiating the autoimmune attack on β cells, by increasing the risk for infection or by some other processes. However, it can also be a secondary phenomenon due to detention of neutrophils in the pancreas when the β-cell destruction is more pronounced. Alterations in the CBC among autoantibody positive children may be caused by an impaired hematopoiesis caused by infections or toxins (43; 46).

Under normal circumstances, no gender differences in neutrophil, lymphocyte or basophil counts at the age of 4-12 years have been reported (29). Boys in this study had reduced basophil counts, reduced neutrophil and increased MCHC counts associated with the status of being positive for three IA. The MCHC count is normally decreasing by age (29). Viral and bacterial infections may alter neutrophil, basophil and lymphocyte counts, for instance, whooping cough is known to

decrease basophil numbers. Basophils have been shown to migrate to secondary lymphoid organs where they crosstalk with T- and B- lymphocytes thereby linking the T-helper cell environment as a contributor to the development of autoimmune SLE, and as a consequence the basophils in the circulation decrease (25). Girls with two IA had increased levels of lymphocytes which could be associated with viral infections as many viruses like rotavirus, mumps virus and other have been associated with the pathogenesis of T1D (47; 48).

The reduction of red blood cells, hemoglobin and hematocrit associated with two IA in girls may be explained by a peripheral destruction of red blood cells, impaired hematopoiesis or infections. Previous studies have associated rotavirus and enterovirus with islet β-cell autoimmunity and T1D (49; 50). Hence, it's unclear if reduction in CBC is caused by or contributes to the development of the second or third IA. Our results in TEDDY girls suggest that an alteration of CBC was observed with a second IA.

It is concluded from the present study that deviations in CBC after seroconversion were common primarily in boys and in children with the HLA-DR3-DQ2/DR4-DQ8 genotype. Our statistical models indicate that the reduction of neutrophil counts in boys and HLA-DR3-DQ2/DR4-DQ8 children positive for three autoantibodies may be consequences of islet β-cell autoimmunity, since the reduction in neutrophil levels correlated with the number of IA. Additional factors influencing the CBC in children at genetic risk for T1D and positive for one to three IA were also affected by gender, HLA-genotype and number of IA. The cellular and molecular mechanisms behind the aggravating CBC alterations different in boys or girls with an increasing number of IA need to be further explored.

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Author contribution

F.S. performed CBC analysis, interpreted data and wrote the manuscript. Å.L. conceived the study, contributed to study design, reviewed and edited the manuscript. H.S.L and E.F. carried out statistical analyses, reviewed and edited the manuscript. C.T and H.EL. reviewed and edited the manuscript. Dr. Åke Lernmark is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest

No potential conflicts of interest relevant to this article were reported.

References

1. Krischer JP, Lynch KF, Schatz DA, Ilonen J, Lernmark Å, Hagopian WA, Rewers MJ, She J-X, Simell OG, Toppari J, Ziegler A-G, Akolkar B, Bonifacio E: The 6 year incidence of diabetes-associated autoantibodies in genetically at-risk children: the TEDDY study. Diabetologia 2015;58:980-987
2. Krischer JP, Lynch KF, Lernmark A, Hagopian WA, Rewers MJ, She JX, Toppari J, Ziegler AG, Akolkar B, Group TS: Genetic and Environmental Interactions Modify the Risk of Diabetes-Related Autoimmunity by 6 Years of Age: The TEDDY Study. Diabetes Care 2017;40:1194-1202
3. Krischer JP, Liu X, Lernmark A, Hagopian WA, Rewers MJ, She JX, Toppari J, Ziegler AG, Akolkar B, Group TS: The Influence of Type 1 Diabetes Genetic Susceptibility Regions, Age, Sex, and Family History to the Progression from Multiple Autoantibodies to Type 1 Diabetes: A TEDDY Study Report. Diabetes 2017;
4. Elding Larsson H, Vehik K, Gesualdo P, Akolkar B, Hagopian W, Krischer J, Lernmark A, Rewers M, Simell O, She JX, Ziegler A, Haller MJ: Children followed in the TEDDY study are diagnosed with type 1 diabetes at an early stage of disease. Pediatric Diabetes 2014;15:118-126
5. Vehik K, Lynch KF, Schatz DA, Akolkar B, Hagopian W, Rewers M, She JX, Simell O, Toppari J, Ziegler AG, Lernmark A, Bonifacio E, Krischer JP, Group TS: Reversion of beta-Cell Autoimmunity Changes Risk of Type 1 Diabetes: TEDDY Study. Diabetes Care 2016;39:1535-1542
6. Bulek AM, Cole DK, Skowera A, Dolton G, Gras S, Madura F, Fuller A, Miles JJ, Gostick E, Price DA, Drijfhout JW, Knight RR, Huang GC, Lissin N, Molloy PE, Wooldridge L, Jakobsen BK, Rossjohn J, Peakman M, Rizkallah PJ, Sewell AK: Structural basis for the killing of human beta cells by CD8(+) T cells in type 1 diabetes. Nat Immunol 2012;13:283-289
7. Coppieters KT, Dotta F, Amirian N, Campbell PD, Kay TW, Atkinson MA, Roep BO, von Herrath MG: Demonstration of islet-autoreactive CD8 T cells in insulitic lesions from recent onset and long-term type 1 diabetes patients. The Journal of experimental medicine 2012;209:51-60
8. Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS: Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children. Jama 2013;309:2473-2479
9. Katsarou A, Gudbjornsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, Jacobsen LM, Schatz DA, Lernmark A: Type 1 diabetes mellitus. Nat Rev Dis Primers 2017;3:17016
10. Rewers M, Ludvigsson J: Environmental risk factors for type 1 diabetes. Lancet 2016;387:2340-2348
11. Gomez-Tourino I, Arif S, Eichmann M, Peakman M: T cells in type 1 diabetes: Instructors, regulators and effectors: A comprehensive review. J Autoimmun 2016;66:7-16

12. Price JD, Tarbell KV: The Role of Dendritic Cell Subsets and Innate Immunity in the Pathogenesis of Type 1 Diabetes and Other Autoimmune Diseases. Front Immunol 2015;6:288
13. Dotta F, Fondelli C, Falorni A: Can NK cells be a therapeutic target in human type 1 diabetes? Eur J Immunol 2008;38:2961-2963
14. Wentworth JM, Fourlanos S, Harrison LC: Reappraising the stereotypes of diabetes in the modern diabetogenic environment. Nat Rev Endocrinol 2009;5:483-489
15. Battaglia M: Neutrophils and type 1 autoimmune diabetes. Curr Opin Hematol 2014;21:8-15
16. Valle A, Giamporcaro GM, Scavini M, Stabilini A, Grogan P, Bianconi E, Sebastiani G, Masini M, Maugeri N, Porretti L, Bonfanti R, Meschi F, De Pellegrin M, Lesma A, Rossini S, Piemonti L, Marchetti P, Dotta F, Bosi E, Battaglia M: Reduction of Circulating Neutrophils Precedes and Accompanies Type 1 Diabetes. Diabetes 2013;62:2072-2077
17. Harsunen MH, Puff R, D'Orlando O, Giannopoulou E, Lachmann L, Beyerlein A, von Meyer A, Ziegler AG: Reduced blood leukocyte and neutrophil numbers in the pathogenesis of type 1 diabetes. Horm Metab Res 2013;45:467-470
18. Qin J, Fu S, Speake C, Greenbaum CJ, Odegard JM: NETosis-associated serum biomarkers are reduced in type 1 diabetes in association with neutrophil count. Clin Exp Immunol 2016;184:318-322
19. Group TS: The Environmental Determinants of Diabetes in the Young (TEDDY) study: study design. Pediatr Diabetes 2007;8:286-298
20. Hagopian WA, Erlich H, Lernmark A, Rewers M, Ziegler AG, Simell O, Akolkar B, Vogt R, Jr., Blair A, Ilonen J, Krischer J, She J: The Environmental Determinants of Diabetes in the Young (TEDDY): genetic criteria and international diabetes risk screening of 421 000 infants. Pediatric Diabetes 2011;12:733-743
21. Group TS: The Environmental Determinants of Diabetes in the Young (TEDDY) Study. Ann N Y Acad Sci 2008;1150:1-13
22. Lehto T, Hedberg P: Performance evaluation of Abbott CELL-DYN Ruby for routine use. Int J Lab Hematol 2008;30:400-407
23. Hagopian WA, Erlich H, Lernmark A, Rewers M, Ziegler AG, Simell O, Akolkar B, Vogt R, Jr., Blair A, Ilonen J, Krischer J, She J, Group TS: The Environmental Determinants of Diabetes in the Young (TEDDY): genetic criteria and international diabetes risk screening of 421 000 infants. Pediatr Diabetes 2011;12:733-743
24. Dantonio P, Meredith-Molloy N, Hagopian WA, She JX, Akolkar B, Cordovado SK, Hendrix M, Henderson LO, Hannon WH, Vogt RF: Proficiency testing of human leukocyte antigen-DR and human leukocyte antigen-DQ genetic risk assessment for type 1 diabetes using dried blood spots. Journal of diabetes science and technology 2010;4:929-941
25. Charles N, Rivera J: Basophils and autoreactive IgE in the pathogenesis of systemic lupus erythematosus. Current allergy and asthma reports 2011;11:378-387
26. Bonifacio E, Yu L, Williams AK, Eisenbarth GS, Bingley PJ, Marcovina SM, Adler K, Ziegler AG, Mueller PW, Schatz DA, Krischer JP, Steffes MW, Akolkar B: Harmonization of glutamic acid decarboxylase and islet antigen-2 autoantibody assays for national institute of diabetes and digestive and kidney diseases consortia. The Journal of clinical endocrinology and metabolism 2010;95:3360-3367
27. Babaya N, Yu L, Miao D, Wang J, Rewers M, Nakayama M, Liu E, Barker JM, Eisenbarth GS: Comparison of insulin autoantibody: polyethylene glycol and micro-IAA 1-day and 7-day assays. Diabetes/metabolism research and reviews 2009;25:665-670

28. Torn C, Mueller PW, Schlosser M, Bonifacio E, Bingley PJ: Diabetes Antibody Standardization Program: evaluation of assays for autoantibodies to glutamic acid decarboxylase and islet antigen-2. Diabetologia 2008;51:846-852
29. Aldrimer M, Ridefelt P, Rodoo P, Niklasson F, Gustafsson J, Hellberg D: Population-based pediatric reference intervals for hematology, iron and transferrin. Scand J Clin Lab Invest 2013;73:253-261
30. Taylor MR, Holland CV, Spencer R, Jackson JF, O'Connor GI, O'Donnell JR: Haematological reference ranges for schoolchildren. Clinical and laboratory haematology 1997;19:1-15
31. Steck AK, Vehik K, Bonifacio E, Lernmark A, Ziegler AG, Hagopian WA, She J, Simell O, Akolkar B, Krischer J, Schatz D, Rewers MJ: Predictors of Progression From the Appearance of Islet Autoantibodies to Early Childhood Diabetes: The Environmental Determinants of Diabetes in the Young (TEDDY). Diabetes care 2015;38:808-813
32. Kohler M, Beyerlein A, Vehik K, Greven S, Umlauf N, Lernmark A, Hagopian WA, Rewers M, She JX, Toppari J, Akolkar B, Krischer JP, Bonifacio E, Ziegler AG, group Ts: Joint modeling of longitudinal autoantibody patterns and progression to type 1 diabetes: results from the TEDDY study. Acta Diabetol 2017;54:1009-1017
33. Ziegler AG, Danne T, Dunger DB, Berner R, Puff R, Kiess W, Agiostratidou G, Todd JA, Bonifacio E: Primary prevention of beta-cell autoimmunity and type 1 diabetes - The Global Platform for the Prevention of Autoimmune Diabetes (GPPAD) perspectives. Molecular metabolism 2016;5:255-262
34. Mcpherson RA, Pincus MR: *Henry's Clinical Diagnosis and Management by Laboratory Methods Pageburst E-book on Vitalsource Retail Access Card*. Elsevier Science Health Science, 2011
35. Starkebaum G: Chronic neutropenia associated with autoimmune disease. Seminars in hematology 2002;39:121-127
36. Amulic B, Cazalet C, Hayes GL, Metzler KD, Zychlinsky A: Neutrophil function: from mechanisms to disease. Annual review of immunology 2012;30:459-489
37. Diana J, Simoni Y, Furio L, Beaudoin L, Agerberth B, Barrat F, Lehuen A: Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes. Nature medicine 2013;19:65-73
38. Casserly CS, Nantes JC, Whittaker Hawkins RF, Vallieres L: Neutrophil perversion in demyelinating autoimmune diseases: Mechanisms to medicine. Autoimmunity reviews 2017;16:294-307
39. Wang Y, Xiao Y, Zhong L, Ye D, Zhang J, Tu Y, Bornstein SR, Zhou Z, Lam KS, Xu A: Increased neutrophil elastase and proteinase 3 and augmented NETosis are closely associated with beta-cell autoimmunity in patients with type 1 diabetes. Diabetes 2014;63:4239-4248
40. Lundberg M, Seiron P, Ingvast S, Korsgren O, Skog O: Insulitis in human diabetes: a histological evaluation of donor pancreases. Diabetologia 2017;60:346-353
41. In't Veld P, Lievens D, De Grijse J, Ling Z, Van der Auwera B, Pipeleers-Marichal M, Gorus F, Pipeleers D: Screening for insulitis in adult autoantibody-positive organ donors. Diabetes 2007;56:2400-2404
42. Atkinson MA, Gianani R: The pancreas in human type 1 diabetes: providing new answers to age-old questions. Curr Opin Endocrinol Diabetes Obes 2009;16:279-285
43. Dale DC: How I manage children with neutropenia. British journal of haematology 2017;178:351-363

44. Huang J, Xiao Y, Xu A, Zhou Z: Neutrophils in type 1 diabetes. Journal of diabetes investigation 2016;7:652-663
45. Kaplan MJ: Role of neutrophils in systemic autoimmune diseases. Arthritis research & therapy 2013;15:219
46. Kolb-Maurer A, Goebel W: Susceptibility of hematopoietic stem cells to pathogens: role in virus/bacteria tropism and pathogenesis. FEMS Microbiol Lett 2003;226:203-207
47. Op de Beeck A, Eizirik DL: Viral infections in type 1 diabetes mellitus--why the beta cells? Nature reviews Endocrinology 2016;12:263-273
48. Coppieters KT, Boettler T, von Herrath M: Virus infections in type 1 diabetes. Cold Spring Harbor perspectives in medicine 2012;2:a007682
49. Honeyman MC, Coulson BS, Stone NL, Gellert SA, Goldwater PN, Steele CE, Couper JJ, Tait BD, Colman PG, Harrison LC: Association between rotavirus infection and pancreatic islet autoimmunity in children at risk of developing type 1 diabetes. Diabetes 2000;49:1319-1324
50. Honeyman MC, Stone NL, Falk BA, Nepom G, Harrison LC: Evidence for molecular mimicry between human T cell epitopes in rotavirus and pancreatic islet autoantigens. Journal of immunology (Baltimore, Md : 1950) 2010;184:2204-2210

Table 1. Characteristics of TEDDY children (n=448) investigated for Complete Blood Counts (CBC) when negative or positive for one or several islet autoantibodies (IA).

	IA	
	Negative (n=376)	**Positive (n=72)**
Number of children	376 (84%)	72 (16%)
Girls/Boys	182/194	30/42
Age at first CBC (months): median(min-max)	91 (52-145)	101.5 (59-139) n.s
Girls/Boys	91 (53-144)/94 (52-145)	103 (59-139)/99 (59-137)
CBC measures per child (min-max)	1-6	1-9
Months of follow up (min-max)	1-30	1-30
Number of IA		
1	0	25
2	0	16
3	0	31
Change in IA status	none	none
HLA DR-DQ (n (%))		
DR3/4 -DQ2/8	151 (40.2%)	39 (54.1%)
DR4/4-DQ 8/8	87 (23.1%)	12 (16.7%)
DR4/8-DQ 8/4	40 (10.6%)	12 (16.7%)
DR3/3-DQ 2/2	91 (24.2%)	9 (12.5%)
DR4/1-DQ 8/5	4 (1.1%)	0
DR4/13-DQ 8/6	2 (0.5%)	0
HLA ineligible	1 (0.3%)	0
Total	376 (100%)	72 (100%)

Table 2. Effects of age (years) on each Complete Blood Counts (CBC) measurement in islet autoantibody (IA) negative subjects (n=376).

CBC	Estimate	standard error	p value
White blood cells (10E9 cells/L)	**-0.123**	**0.044**	**0.005**
Neutrophils (10E9 cells/L)	-0.060	0.031	0.053
Lymphocytes (10E9 cells/L)	**-0.036**	**0.013**	**0.006**
Monocytes (10E9 cells/L)	**-0.010**	**0.004**	**0.015**
Eosinophils (10E9 cells/L)	-0.002	0.008	0.766
Basophils (10E9 cells/L)	-0.001	0.001	0.306
Red blood cells (10E12 cells/L)	**0.040**	**0.015**	**0.010**
Hemoglobin (g/L)	**0.167**	**0.040**	**<.0001**

Table 3. The association between Islet Autoantibodies (IA) and each Complete Blood Counts (CBC) measurement and association to gender.

CBC	Estimate	standard error	p value
White blood cells (10E9 cells/L)			
All subjects	**-0.315**	**0.157**	**0.046**
Girls	-0.025	0.212	0.906
Boys	**-0.540**	**0.229**	**0.019**
Neutrophils (10E9 cells/L)			
All subjects	**-0.239**	**0.099**	**0.017**
Girls	-0.031	0.128	0.807
Boys	**-0.386**	**0.151**	**0.012**
Lymphocytes (10E9 cells/L)			
All subjects	-0.034	0.057	0.553
**NLR (All)*	*-0.115*	*0.067*	*0.090*
Girls	0.048	0.082	0.555
Boys	-0.084	0.080	0.295
Monocytes (10E9 cells/L)			
All subjects	-0.024	0.015	0.108
Girls	-0.024	0.022	0.281
Boys	-0.024	0.021	0.248
Eosinophils (10E9 cells/L)			
All subjects	-0.023	0.030	0.437
Girls	-0.031	0.045	0.490
Boys	-0.028	0.040	0.486
Basophils (10E9 cells/L)			
All subjects	-0.004	0.002	0.064
Girls	-0.001	0.003	0.702
Boys	**-0.007**	**0.003**	**0.029**
Platelets (10E9 cells/L)			
All subjects	-8.190	6.519	0.210
Girls	0.995	8.731	0.909
Boys	-16.260	9.542	0.090
Red blood cells (10E12 cells/L)			
All subjects	-0.089	0.050	0.077
Girls	-0.134	0.077	0.084
Boys	-0.065	0.065	0.322
Hemoglobin (g/L)			
All subjects	**-0.297**	**0.133**	**0.026**

Girls	**-0.491**	**0.192**	**0.012**
Boys	-0.180	0.185	0.337
Hematocrit (L/L)			
All subjects	**-0.800**	**0.366**	**0.031**
Girls	**-1.170**	**0.581**	**0.047**
Boys	-0.518	0.521	0.328
Mean Corpuscular Volume (fL)			
All subjects	0.042	0.374	0.911
Girls	-0.089	0.544	0.870
Boys	0.330	0.500	0.510
Red Cell Distribution Width (%CV)			
All subjects	0.109	0.075	0.146
Girls	0.094	0.121	0.440
Boys	0.130	0.095	0.171

**NLR is Neutrophil to Lymphocyte ratio*

Table 4. Association between the number of Islet Autoantibodies (IA) and each Complete Blood Count (CBC) measurement in 72 subjects (boys n=42).

CBC	n IA	Estimate	Standard error	p value
White blood cells (10E9 cells/L)				
All subjects	1	0.142	0.257	0.582
	2	-0.379	0.293	0.197
	3	**-0.613**	**0.225**	**0.007**
Boys	1	-0.001	0.392	0.998
	2	-0.701	0.404	0.086
	3	**-0.789**	**0.332**	**0.019**
HLA-DR3-DQ2/DR4-DQ8	1	0.512	0.401	0.203
	2	-0.392	0.415	0.347
	3	**-0.729**	**0.360**	**0.045**
Neutrophils (10E9 cells/L)				
All subjects	1	0.081	0.161	0.616
	2	-0.328	0.178	0.068
	3	**-0.427**	**0.141**	**0.003**
Boys	1	0.033	0.251	0.897
	2	-0.479	0.259	0.068
	3	**-0.634**	**0.216**	**0.004**
HLA-DR3-DQ2/DR4-DQ8	1	0.270	0.245	0.275
	2	-0.420	0.246	0.091
	3	**-0.572**	**0.216**	**0.010**
Lymphocytes (10E9 cells/L)				
All subjects	1	0.092	0.091	0.315
	2	0.025	0.109	0.822
	3	**-0.175**	**0.0840**	**0.038**
Girls	1	0.080	0.124	0.519
	2	**0.368**	**0.175**	**0.038**
	3	-0.115	0.118	0.332
Monocytes (10E9 cells/L)				
All subjects	1	0.003	0.0244	0.885
	2	**-0.057**	**0.0281**	**0.044**
	3	-0.026	0.0214	0.231
Red Blood cells (10E12 cells/L)				
All subjects	1	0.019	0.080	0.813
	2	**-0.200**	**0.089**	**0.026**
	3	-0.112	0.073	0.125
Girls	1	0.060	0.119	0.616

	2	**-0.471**	**0.142**	**0.002**
	3	-0.109	0.104	0.295
HLA-DR3-DQ2/DR4-DQ8	1	0.029	0.129	0.826
	2	-0.211	0.136	0.125
	3	**-0.320**	**0.113**	**0.006**
Hemoglobin (g/L)				
All subjects	1	-0.031	0.210	0.882
	2	**-0.635**	**0.235**	**0.008**
	3	-0.282	0.193	0.147
Girls	1	-0.038	0.300	0.900
	2	**-1.248**	**0.335**	**0.0007**
	3	-0.407	0.255	0.116
HLA DR3/4-DQ2/8	1	-0.255	0.372	0.495
	2	-0.614	0.388	0.116
	3	**-0.710**	**0.326**	**0.031**
Hematocrit (L/L)				
All subjects	1	0.147	0.577	0.799
	2	**-1.801**	**0.640**	**0.006**
	3	**-1.034**	**0.525**	**0.050**
Girls	1	0.395	0.907	0.664
	2	**-3.635**	**0.990**	**0.0007**
	3	-1.011	0.776	0.200
HLA-DR3-DQ2/DR4-DQ8	1	-0.375	0.996	0.707
	2	-1.922	1.029	0.065
	3	**-2.514**	**0.853**	**0.004**
Mean Corpuscular Volume (fL)				
All subjects	1	0.854	0.597	0.153
	2	-0.481	0.734	0.513
	3	-0.351	0.544	0.519
Boys	**1**	**1.728**	**0.850**	**0.044**
	2	-0.505	0.900	0.576
	3	-0.118	0.716	0.869
Not HLA-DR3-DQ2/DR4-DQ8				
	1	**2.210**	**0.826**	**0.008**
	2	-0.605	1.246	0.628
	3	-0.192	0.722	0.791
Mean Corpuscular Hemoglobin (pg/cell)				
HLA-DR3-DQ2/DR4-DQ8	**1**	**-0.861**	**0.418**	**0.042**
	2	0.034	0.433	0.938
	3	0.562	0.354	0.116

Supplementary Table 1. Complete blood count (CBC) (median (range)) at first visit in 448 TEDDY children without or with one or several islet autoantibodies (IA).

	IA negative	**IA positive**
n	**376**	**72**
White blood cells (WBC) (10E9 Cells/L)	4.87 (2.09-12.3)	4.54 (2.08-8.02)
Neutrophil (10E9 cells/L)	2.13 (0.59-9.42)	1.95 (0.85-5.05)
Lymphocyte (LYM) (10E9 cells/L)	1.79 (0.50-3.93)	1.81 (0.80-3.22)
Neutrophil Lymphocyte Ratio (NLR)	1.20 (0.28-7.73)	1.18 (0.42-4.45)
Monocyte (MONO) (10E9 cells/L)	0.37 (0.11-1.07)	0.37 (0.18-0.78)
Eosinophils (EOS) (10E9 cells/L)	0.23 (0.01-1.88)	0.21 (0-1.05)
Basophil (BASO) (10E9 cells/L)	0.05 (0.01-0.15)	0.05 (0.01-0.10)
Platelets (PLT) (10E9 cells/L)	255 (99.4-496)	235 (113-350)
Red blood cells (RBC) (10E12 cells/L)	3.99 (2.13-6.66)	3.92 (1.99-5.35)
Red blood cell parameters		
Hemoglobin (HGB) (g/L)	11.4 (6.66-19.7)	11.0 (5.79-14.9)
Hematocrit (HCT) (L/L)	31.1 (17.1-51.7)	30.1 (15-41)
Mean corpuscular volume (MCV) (fL)	77.5 (68.7-88.7)	77.6 (70.6-87.1)
Mean corpuscular hemoglobin (MCH) (pg)	28.7 (23.4-51.3)	28.4 (24.3-31.4)
Mean corpuscular hemoglobin concentration (MCHC) (g/L)	36.9 (33-65.7)	36.7 (34.3-41)
Red cell distribution width (RDW) (%CV)	11.8 (10.5-15.4)	11.8 (10.7-13.5)

Supplementary Table 2. The Association between islet autoantibodies (IA) and each complete blood count (CBC) measurement by HLA-DR3-DQ2/DR4-DQ8.

HLA DR3/4-DQ2/8	CBC	Estimate	Standard error	P-value
Yes	White blood cells (10E9cells/L)	-0.240	0.247	0.333
No		-0.329	0.207	0.116
Yes	Neutrophils (10E9cells/L)	-0.275	0.155	0.078
No		-0.192	0.139	0.171
Yes	Lymphocytes (10E9cells/L)	0.040	0.086	0.640
No		-0.055	0.075	0.461
Yes	Monocytes (10E9cells/L)	-0.006	0.019	0.766
No		**-0.047**	**0.023**	**0.042**
Yes	Eosinophils (10E9cells/L)	-0.020	0.044	0.651
No	Model does not fit			
yes	Basophils (10E9cells/L)	-0.002	0.003	0.557
No		-0.006	0.003	0.071
Yes	Platelets	-4.171	10.201	0.683
No		-13.258	8.721	0.130
Yes	Red blood cells	**-0.177**	**0.080**	**0.030**
No		0.003	0.062	0.966
Yes	Hemoglobin	**-0.548**	**0.224**	**0.016**
No	Model does not fit			
Yes	Hematocrit	**-1.712**	**0.599**	**0.005**
No	Model does not fit			
Yes	Mean corpuscular volume	-0.463	0.539	0.392
No		0.614	0.524	0.242
Yes	Mean corpuscular hemoglobin	-0.010	0.255	0.969
No	Model does not fit			
Yes	Red cell distribution width	0.096	0.107	0.372
No		0.144	0.101	0.156

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