**Author’s response to reviews**

**Title:** The course of diabetes in children, adolescents and young adults: does the autoimmunity status matter?

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**Author’s response to reviews:**

Dear Editor-in Chief,

Many thanks for the evaluation of our manuscript.

Please find below the answers to the reviewers.

Reviewer #1: The article presents important information regarding type 1 Diabetes.

My main considerations are about the results and the limitations of a crosssectional analysis.
Concerning results presentation:

1. I would suggest not to repeat in the text information that has already been presented in tables and figures.

Answer: The information that is repeated in the text has been deleted (page 10 and 12). Otherwise, a short interpretation of results that seems important to highlight to the authors have been kept in the text.

2. Tables should not have horizontal lines nor external lines

Answer: corrected.

3. All the abbreviations in tables must be described in notes under these tables.

Answer: Done

4. In page 9, line 42, is the median value = 24.73 correct? "The mean duration of diabetes was 5.05±4.97 yrs (0.01-3.77, median 24.73 yrs".

Answer: The typos mistake in values was corrected as follows: The mean duration of diabetes was 5.05±4.97 yrs (0.01-24.73, median 3.77 yrs).

5. Means followed by standard deviation should not be presented with the sign +/- . It would be better to indicate that they are means and SD and present the SD in between brackets, or to present the 95% CI (usually mean-2dp; mean+2dp).

Answer: corrected as suggested.

6. In table 2, the p values should be presented, not only <0.05, since the reader must be able to evaluate the value's magnitude (0.06 is is more close to 0.05 than to 0.60) an alternative would be to present the frequencies and means 95% CI.

Answer: corrected as suggested by the Reviewer.
7. In table 2, the frequencies of patients with ketosis is confusing.

The text says: "Ketosis was significantly more frequent in patients with no family history of diabetes (91.2% vs 78.3%, p<0.05) and in the antibodies-negative group (88.1 vs 73.5%)"

Those %s are not referring to the total amount of patients in these groups, 25 to 87 and 517 to 1079 respectively. Since the text says only 621 patients had this information, it gets confusing. One would expect that only patients with a suspicious clinical presentation would be investigated. Therefore, it would be more informative to present the % of patients from each group that were investigated, and how many were positive.

The information would be: Out of all the patients with >1 Ab, 54.4% were tested for ketosis, and out of those, 88.1% of them were positive, and 39.1% of the patients from the group without Ab were investigated, and out of those, 73.5% were positive.

There must be an * on the table next to ketosis, explaining that this information was only available to 621 patients.

Answer: Corrections in the Table 2 were made according to reviewer's comments.

8. On table 3 there should be a column with the patients without any microvascular alteration.

Answer: The column with data of patients without complications was added in Table 3, as suggested by the Reviewer.

9. The frequencies presented in the graphics should have 95% CI (vertical lines).

Answers: Since the data are presented as frequencies (percents), not the means, confidence intervals are not available.

Regarding the study's sample and limitations to be considered in the discussion:

The study population is a national register (part of?) with 100% of the pediatric patients and 70% of the patients between 18-25 years.

1. What would be the reasons for those 30% of patients between 18-25 years with type 1 diabetes to not be registered? Are the non-included diabetics different from the ones included? This discussion should be included on the study's limitations;
2. The analysed patients were registered in a 25 years period, from 1990 to 2015. It is probable that during this time, other patients were registered and were not alive at the time of the study.

- If this information is available, how many they would be?

- What are the clinical and demographical characteristics of the patients who died?

- Can survival bias explain the study results?

Consider that in a cross sectional study the participants are usually "survivors", since individuals that stay diseased for a short time (due to death in chronic disease) have a smaller chance of participating in a cross sectional study. Characteristics that are frequent in sick individuals in a cross sectional study may be associated to the disease's longer duration, and not with the disease's incidence, and therefore, it can be a prognostic factor, instead of a risk factor.

Answer: the authors completely agree with the reviewer's point of missing data from the adult 18-25 years old population. This reflection is added to the study limitations part (page 16). Unfortunately, we do not have mortality data in this population, that would no doubt be of great value to analyze. However, and it would be another subject of research, not in the scope of the present manuscript.

Reviewer #2: This is an in-depth study of autoimmune and clinical parameters and their correlation in young T1D patients. The cohort represents almost all T1D cases in 0-24 year old patients (n=1209) in Lithuania diagnosed between 1990 and 2015.

The stated purpose of the study is the identification of patients that were misdiagnosed as T1D and could benefit from a re-classification and different treatment.

Indeed, ~10% of children diagnosed with T1D are later reclassified as having monogenetic forms of diabetes.

Their investigation finds 7.5% of the cohort to test negative for islet autoantibodies.

The correlation analysis confirms several previous findings: association of GAD65Ab with older age at onset, presence of GAD65Ab in patients after long duration of disease, inverse correlation of islet autoantibodies and disease duration, higher incidence of ketosis in islet autoantibody-positive patients, association of T1D with thyroid autoimmunity.
There are a few comments, which may help improve the study in its present form:

* The finding of higher frequency of autoantibody-negative individuals with retinopathy (after age adjustment) is of interest. The authors may want to discuss related findings (inverse relationship between GAD65Ab levels and severe retinopathy (Agardh D et al Diabetes Res Clin Pract. 1998, Miruma T et al Ophthalmology 2005).

Answer: we analyzed levels of GAD65 Ab, IA-2 Ab, IAAs and compared the levels between patients with/without microvascular complications. We also compared our findings with the findings of proposed articles in the discussion (page 15, highlighted in yellow).

* The phenomenon of autoantibody negative T1D (Type 1B or idiopathic) patients without monogenic diabetes has been described in the literature and accounts for ~5% of T1D patients. Inclusion of ZnT8 autoantibodies have further reduced the frequency of autoantibody-negative T1D patients. The possibility that some of the autoantibody-negative patients in the current cohort have in fact Type 1B diabetes needs to be addressed.

Answer: the authors completely agree with the Reviewers comment and this was included in the discussion (page 16, highlighted in yellow)

* Monogenic forms of diabetes often have normal C-peptide levels. It would be useful to know whether the autoantibody-negative patients in the current study have normal C-peptide levels.

Answer: unfortunately, C-peptide levels were not available in this cohort.

* The authors mention in the discussion that insulin therapy in the majority of the patients may affect the measurement of insulin autoantibodies in these patients. Indeed, the detection of insulin autoantibodies (IAA) after initiation of insulin therapy is impossible as most patients develop insulin antibodies (IA) to endogenous insulin. To avoid confusion, the authors may use IA when referring to measurements made in patients that are on insulin treatment for at least 2 weeks and IAA when referring to those patients that are newly diagnosed and have either not yet received any insulin therapy, or have been on insulin treatment for less than 2 weeks.

Answer: there were only 3 patients with positive only insulin antibodies in newly diagnosed diabetes patients, therefore we didn’t performed a subcohort analysis.
The authors point out that ZnT8 autoantibodies were not determined in the study, therefore it is possible that some of the autoantibody-negative patients have undetected islet autoimmunity.

Answer: the authors completely agree with the Reviewers comment and this was included in the discussion (page 16, highlighted in yellow)

As the authors discuss, the lack of autoantibodies in patients with long duration of disease may be due to disappearance of autoantibodies over time. It would therefore be important to describe the findings of islet autoimmunity and correlations with age etc in newly diagnosed T1D patients (n=205 in the study) as a subcohort.

Many thanks for your kind consideration of our revised manuscript.

With best regards,

Rasa Verkauskiene

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