Association of Autoantibody Levels with the Hazard of Autoimmune Comorbidities in Children with Type 1 Diabetes

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Abstract— **Type 1 diabetes mellitus is an autoimmune disease representing serious burden both on the patients and healthcare systems. Being an autoimmune disease it comes as no surprise that it is often associated with other autoimmune comorbidities, such as coeliac disease or Hashimoto's thyroiditis. As the manifestation of these diseases is often preceded by a longer period of latent (subclinical) autoimmune process, children diagnosed with type 1 diabetes mellitus are often screened for autoantibodies characteristic for these comorbidities to predict their occurrence. Data from $n = 182$ children diagnosed with type 1 diabetes mellitus undergoing such screening program were collected from 2007 to 2012. Cox proportional hazards model with time varying covariate was used to model how autoantibody levels are associated with the hazard of the onset of an autoimmune comorbidity. For coeliac disease, only the IgA subclass of anti-transglutaminase autoantibodies turned out to be relevant ($p < 0.0001$), with a hazard ratio – for being >10 U/ml compared to being smaller – of 44.8 (95% HR: 11.88-168.8). For Hashimoto's thyroiditis, only anti-thyroid peroxidase autoantibodies were significant ($p < 0.0001$), with a hazard ratio raising rapidly to roughly 10 by 100 U/ml, then – after a sharp break in the gradient – raising much more slowly to about 30-40 for the extreme ATPO values greater than 1000 U/ml. This study confirmed the role of autoantibodies in predicting autoimmune comorbidities in T1DM and also demonstrated a way to quantify this effect.**

Keywords— **Type 1 diabetes mellitus, autoimmunity, autoantibody, comorbidities, proportional hazards model with time varying covariates.**

I. Introduction

Type 1 of diabetes mellitus (T1DM) is a prototypical example of autoimmune diseases, in which a T_H1-mediated autoimmune process destroys the insulin-producing β-cells in the pancreatic islets [1]. The resulting absolute or almost absolute deficiency in insulin hinders the entry of glucose from blood to glucose-consuming cells leading to short-term and long-term complications and consequences, even in the presence of adequate treatment [2].

It is very old observation that those patients who have an autoimmune disease (AID) are prone to have a second (or further) AID. In addition to anecdotal evidence, corroborating lessons learned from animal experiments [3] and results of genome-wide association analysis [4, 5], this statement is now supported by epidemiological studies as well, although often of varying quality [6, 7]. Also, it is largely unclear whether this is a general risk among AIDs, or there are certain combinations that emerge more frequently. Nevertheless, it is accepted that in T1DM, the risk of coeliac disease and Hashimoto's thyroiditis is substantially increased [8]; these will be in focus of the present study.

Coeliac disease (CD) is an AID characterized by an abnormal inflammatory response to the dietary exposure to gliadian (a protein found in wheat) and related gluten proteins, which leads to villous atrophy in the small intestine, leading to malabsorption symptoms, among others [9]. Occurrence of CD is strongly associated with T1DM [10] that the screening for the signs of CD, most notably elevated autoantibody-levels is often recommended [11].

Hashimoto's thyroiditis (HT) is an autoimmune thyroiditis, characterized by the lymphocytic invasion of the thyroid tissue, the destruction of which will give rise to hypothyroidism [12]. HT is also strongly associated with T1DM [13, 14].

It is now recognized that the onset of most AIDs is not an abrupt event, rather, a result of a longer process, most of which is subclinical [15]. This gives rise to the possibility to predict the disease before its manifestation. A prime example for such approaches is the screening [16] for autoantibodies: the presence of autoantibodies is not the same as the presence of manifest disease, however they can be used if the presumption is accepted that their level is already elevated in the latent period of the autoimmune process [17].

The present paper will investigate the relationship between autoantibody levels characteristic for CD and HT and the manifestion of these diseases among children suffering in T1DM.

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II. Materials and methods

A. Patient data

$n = 182$ children suffering in T1DM were selected as a convenience sample from patients treated at the 1st Department of Paediatrics of the Semmelweis University. Autoantibodies that are characteristic for CD and HT were measured annually from 2007 (or from the inclusion in the study, i.e. onset of DM) to 2012. Only those subjects were included in the present study who had no clinically manifest CD or HT at the manifestation of the DM, as the "index disease" was DM in the present study. In addition to basic sociodemographic data (sex, date of birth) and the date of DM onset, the onset of clinically manifest CD and HT – if there was – was also recorded. Patient characteristics are summarized in Table 1.

Table 1: Characteristics of the patients included in the study. Categorical variables are presented as frequencies and percentages, continuous variables are presented in Mean (Median) ± SD (IQR) [Min-Max] format.

Parameter	Descriptive statistics
Sex	96 male (52.7%), 86 female (47.3%)
Age at the onset of DM	7.7 (8.0) ± 4.3 (7.0) [0-17]
Distribution of manifest autoimmune comorbidities	None: 142 (78.0%), only CD: 22 (12.1%), only HT: 12 (6.6%), both CD and HT: 6 (3.3%)
Time to CD onset (from DM onset) for CD cases	3.0 (1.0) ± 2.3 (2.2) [1-10]
Time to HT onset (from DM onset) for HT cases	5.4 (5.0) ± 3.0 (4.8) [1-11]

The measured autoantibodies were:

- Anti-transglutaminase, both IgA and IgG subclasses (TGIgA, TGIgG). These autoantibodies are characteristic for CD as tissue transglutaminase (tTG) is recognized as the autoantigene in the disease [18]: tTG has a role in the post-translational modification of certain wheat gluten proteins; antibodies directed against them play role in the villous destruction [19, 20].
- Anti-thyroid peroxidase (ATPO) and anti-thyroglobulin (ATG). These thyroid autoantibodies are present in the majority of the HT cases [21].

TGIgA and TGIgG were binarized at 10 U/ml, which was just the upper bound of the reference range, as the vast majority of the measurements were having a value of exactly 5, thus there was no point in handling these as continuous variables. ATPO and ATG were logarithmized to account for their heavily skewed distribution. Scatter plot between the two is show on Figure 1. Note that this scatter plot shows every measurement equally, regardless of the subject and year of measurement, i.e. it neglects the possible intra-individual correlations.

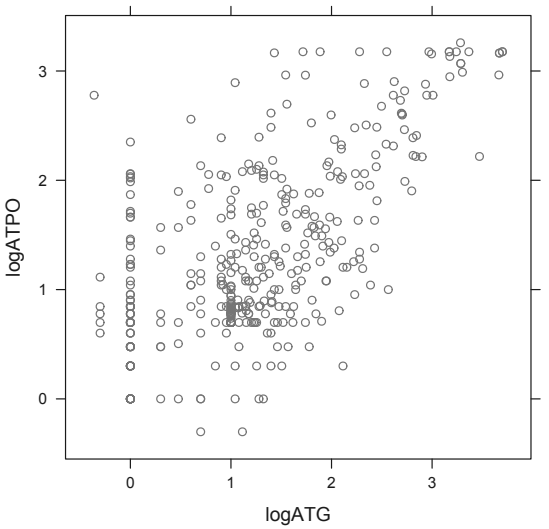

Fig. 1: Scatter plot of logATG and logATPO across the whole sample.

B. Statistical analysis

The collected data can be considered to be time-to-event data, where time is measured from the onset of T1DM, and the – non-recurring – event is the onset of clinically manifest CD or HT. (These two will be considered in two separate analysis.) Thus, the apparatus of survival analysis [22] can be applied, in particular, Cox proportional hazards model [23] will be used, as it casts the problem in regression framework, making it possible to study the effects of certain covariates on survival. These covariates are considered to be multiplicatively affecting the so-called baseline hazard; thus the term "proportional hazards" model. Proportionality will be assessed with the test of Grambsch and Therneua [24]. The achieve our stated aim, the covariates will be the measured autoantibody levels. Sex and age at the onset of T1DM will also be added to the model, to control for their possible confounding effect. For continuous variables, flexibility in functional form specification will be achieved by using spline regression with restricted cubic splines [25]. After model diagnostics and model selection (with LR-test for nested models), the final model will be validated in terms of discrimination using bootstrap [26].

In this setting, autoantibody levels are classical examples of time varying (time dependent) covariates, as their value changes from year to year. Thus, appropriate model has to be applied to incorporate such covariates [23, 27].

To perform the calculations, version 3.1.1 of the `R` program package [28] together with the `survival` [29] and `rms` [30] libraries was applied, using a custom script developed for this purpose which is available at the corresponding author on request.

III. Results

A. Coeliac disease

The interaction between the subclasses of anti-transglutaminase autoantibodies, and sex and age at DM onset are irrelevant ($p = 0.9758$). Interestingly, IgG is also irrelevant in the obtained model ($p = 0.1186$), and also with the aforementioned covariates ($p = 0.4979$), leaving IgA subclass of anti-transglutaminase autoantibody the only covariate, which is however relevant ($p < 0.0001$).

The obtained model passes the proportionality test ($p = 0.699$), and has an R^2 of 43.7%, which is still 41.8% with bootsrap validation.

In this final model the coefficient of anti-transglutaminase IgA is 3.8, giving rise to a hazard ratio of 44.8 (if the autoantibody level is above 10 U/ml), with a 95% confidence interval of 11.88-168.8.

B. Hashimoto's thyroiditis

Sex and age at DM onset, and nonlinearities in ATG were irrelevant even together ($p = 0.4726$). Even after leaving out these covariates, ATG itself is still irrelevant ($p = 0.1173$, and $p = 0.2639$ together), so the final model only includes ATPO (but with splines).

This model passes proportionality test ($p = 0.731$) and has an R^2 of 29.5%, which is 26.3% under bootstrap validation.

Overall, ATPO is relevant at $p < 0.001$; with hazard ratio given on Figure 2.

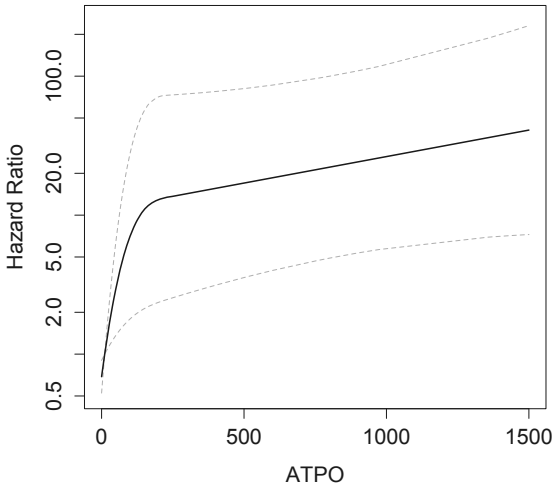

Fig. 2: Hazard ratio for different levels of ATPO, estimated with restricted cubic spline.

IV. Discussion and conclusion

Interestingly IgG subclass of anti-transglutaminase autoantibodies did not turn out to be a relevant factor in explaining the hazard of CD in children with T1DM. While it might be due to the relatively low sample size, it also worth mentioning that several other studies also found that IgG is less reliable for CD [31]. In contrast, IgA had a hazard ratio in excess to 40 (if it is above 10 U/ml, compared to being smaller than this threshold).

As far as HT is concerned, only ATPO turned out to be relevant. This had a nonlinear impact on hazard ratio: until about 100 U/ml (note that the upper bound of the reference range is 35 with the exception of the first year, when it was 63) it raises rapidly to roughly 10, then there is a sharp break in the gradient, and raises much more slowly to about 30-40 for the extreme ATPO values greater than 1000 U/ml. It is important to mention that at this range, the confidence interval is very large (even for 1500 U/ml, it spans from 5 to 200) due to the very small number of observations with such extreme ATPO values.

Naturally, our study also has certain limitations. The first and foremost – in addition to the convenience sampling – is perhaps the fact that subjects were followed only from 2007, irrespectively of the onset of their DM. It would have given a more accurate picture, if subjects were all followed right since the onset of their DM.

Another limitation is the fact that the values of antibody levels were considered themselves, without regard to the previous/further measurements within the same subject. In other words, the possible effect of certain time-patterns – if there is any – were neglected.

Nevertheless, this study confirmed the role of autoantibodies in predicting autoimmune comorbidities in T1DM and also demonstrated a way to quantify this effect.

Conflict of Interest

The authors declare that they have no conflict of interest.

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