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SHORT COMMUNICATION

Parainfluenza viruses: A trigger for type 1 diabetes new onset?

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SUMMARY

Type 1 diabetes (T1DM) ethiopathogenesis is still being studied, since the role of environmental factors , especially viruses, is not yet clear. This study was conducted on 31 paediatric patients with T1DM at onset. We analysed: Coxsackieviruses A (CoxA), Coxsackieviruses B (CoxB), Echoviruses (Echo); Influenzavirus A and B (IV-A and IV-B); Adenovirus (AdV); Parainfluenza viruses 1-2 and 3 (PiV 1-2-3); Cytomegalovirus (CMV) and Respiratory Syncytial Virus (RSV). Enteroviruses, especially CoxB and Echo, are most represented. Unexpectedly, Parainfluenza viruses were detected in seasonal subgroups, with peaks in autumn and spring, and spread homogeneously in different age groups.

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Type 1 diabetes is a chronic autoimmune disease that leads to pancreatic beta-cells destruction with consequent insulin-deficiency in subjects who have predisposing genetics. These genetic factors are acted on by still unclear environmental and viral infectious factors that are capable of inducing an autoimmune response against pancreatic beta cells (Ilonen J *et al.*, 2019, Craig ME *et al.*, 2019). The literature recognizes the role of viral infections, attributing it mainly to some Enterovirus subspecies such as CoxB (Rodriguez-Calvo T *et al*., 2018, Yeung WC *et al*., 2011, Richardson SJ *et al*., 2019, Krogvold L *et al*., 2015, Bergamin CS *et al*., 2015, Yoon JW *et al*., 1979).
Convincing evidence on the role of viruses in the pathogenesis of T1DM has been well studied after cases of fulminant T1DM (Yoon JW *et al*., 1979,Tanaka S *et al*., 2009) reported in Japan during acute infections with viruses such as Mumps, Parainfluenza, Human Herpes Virus 6 and Enterovirus. Usually, the progression of T1DM is much slower (Elshebani A *et al*., 2007, Capua I *et al*., 2018, Oikarinen S *et al*., 2011, Lönnrot M *et al*., 2018). One suggestive hypothesis is that viruses causing T1DM pathogenesis generate a persistent infection of beta cells that precedes the onset of insulitis, with consequent chronic autoimmune attack (Zuniga EI *et al*., 2015, Jean-Baptiste VSE *et al*., 2017, Abdel-Moneim A *et al*., 2018, Filippi C *et al*., 2005, Principi N *et al*., 2017, Op de Beeck A *et al*., 2016). The aim of this study is to describe the viral profile of a cohort of paediatric patients aged between 1 and 18 years, with T1DM at onset.
We conducted a prospective observational study from October 2018 to August 2020 we enrolled 31 patients, 17 males (54.8%) and 14 females (45.2%), aged between 1 and 18 years, mean age 9.47 ± 4.79standard deviation (SD), admitted to the Department of Paediatric Diabetology at "Sapienza" University of Rome for T1DM at onset. Children newly diagnosed T1DM were divided into 4 groups, based on the 4 seasons of the year in which diabetes arose, and into 3 subgroups based on their age at diagnosis: preschool age (1-5 years), school age (6-12 years) and adolescent age (> 12 years).For each patient, after an informed consent had been obtained from their parents, we collected the first serum sample for viral research within 15 days after diagnosis of diabetes (T0) and the second in 15 days (T1). The viruses studied were: Coxsackieviruses A (CoxA), Coxsackieviruses B (CoxB), Echoviruses (Echo); Influenzavirus A and B (IV-A and IV-B); Adenovirus (AdV); Parainfluenza viruses 1, 2 and 3 (PiV 1-2-3); Cytomegalovirus (CMV) and Respiratory Syncytial Virus (RSV).
Samples were frozen at -20°C and later sent to the virology laboratory of the National Institute for In-

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fectious Diseases "Lazzaro Spallanzani" of Rome.
The analysis technique used was the Complement Fixation Assay (CFA), a quantitative test which is able to detect the presence of total immunoglobulin in the serum analyzed, indicating the existence of an acute or sub-acute infection or reinfection.
The choice of performing in parallel CFA was due to the transient expression of the fixing complement antibodies, which make the test more useful in detecting recent infections (Field AM. 1967). Standard reagents for the development of the CFA were from a commercial source (InstitutVirion/Serion GmbH, Germany). Serial twofold dilutions of serum dilutions (1:10 up to 1:40) were tested. Negative and high-positive controls were included in each run.We decided to consider occasional viruses all the viruses found with dilution ratio ≤1:10, and so they are excluded from statistical analysis. Increasing dilutions are indicative of a more recent infection or reinfection. Antibodies movement was analyzed by comparing the results obtained from the T0 and T1 samples.

Table 1 - *Seasonal distribution of T1DM new diagnoses on total population examined.*

Season	*Percentage*
Autumn	29.0%
Spring	22.6%
Winter	22.6%
Summer	25.8%

Table 2 - *Sample characteristic of the children hospitalized for T1DM at onset.*

Characteristics	*Percentage*
Pre school age (1-5)	29.00%
School age (6-12)	38.70%
Adolescence age (>12)	32.30%
DKA on total population	19.35%
IAA/GADA on total population	48.40%

C peptide, diabetes autoantibodies (GADA, IAA, IA2, ICA), HbA1c, presence/absence of diabetes ketoacidosis (DKA) at onset were also analyzed.
Statistical analysis was performed using SPSS version 21 software (SPSS Inc., Chicago, IL, USA). Categorical variables are described with absolute frequencies or percentage frequencies and continuous variables are expressed as mean values ± SD. Differences among groups were assessed with Fisher's exact test for categorical variables, and comparisons of continuous variables were made using Mann-Whitney test. A p value <0.05 was considered statistically significant.
Seasonal distribution of new diagnoses of T1DM shows a peak in autumn (29.0%), followed by summer (25.8%), with the same percentage (22.6%) in spring and winter (*Table 1*).
Among the 31 patients hospitalized for T1DM at onset, at least one positivity for specific antibodies against the analysed viruses was detected in 27 (87.1%). The age distribution of new diabetes diagnoses (preschool, school and adolescent) shows an increase in school age group followed by adolescence and preschool age (*Table 2*). The association between antibody positivity and disease characteristics is not significant. On the contrary, the HbA1c value is statistically significant in relation to sex (p value=0.019) and Echovirus seroprevalence (p=0.047).
Regarding the prevalence of analysed viral infections in the population examined, PiV1, 2, 3 are the most frequent species, followed by Enteroviruses (CoxA and B, Echo), RSV, IV (A and B) and AdV. All viruses have a peak-season in autumn and are homogeneously distributed in the other ones, except for AdV, absent in summer. When analysing the Enterovirus group separately, CoxB is the most represented in autumn and summer. Regarding the Parainfluenza Viruses and Influenza Viruses, PiV-1 and IV-A predominate in every season and PiV-2 and IV-B are absent in winter and summer. The association between season and number of diagnoses is not significant (p=0.073).

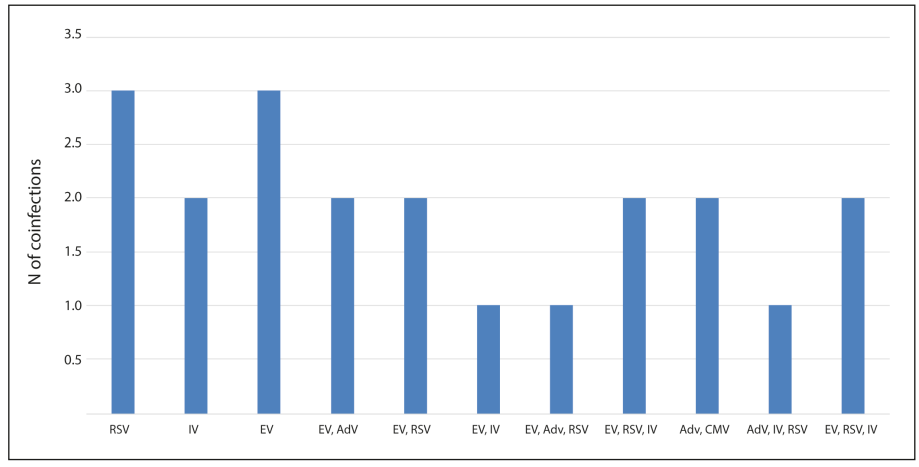

Figure 1 - *PIV 1-2-3 coinfections. Bars show cases of PIV 1-2-3 co-infection, some of which are sustained by one different virus, others by two or three different viruses.*

Twenty-four patients (88.8%) were positive to Parainfluenza viruses and 21 (87.5%) showed coinfections with other viruses analysed: Enteroviruses (61.9%); RSV (52.4%); IV A and B (38.1%); AdV (33.3%) (*Figure 1*). From the comparison of the samples collected at T0 and T1, no significant antibodies movement was detected.

Focusing only on the 24 patients with PiV infection (dilution ≥1:40), 22 (91.6%) were positive to PiV-1 (7 of these featured coinfections with PiV-2 or -3, probably as a cross-reaction) and only PiV-3 was detected in the last two (8.3%). Comparing PiV and EV in the 27 patients with at least one virus positivity, the coinfection between PiV-2 and CoxA is statistically significant (p=0.017). When analyzing C peptide, a significant correlation with PiV infection was found, specifically with PiV-1 (p=0.012). The prevalence of Parainfluenza viruses is 24/27 (0.8889) with a confidence interval at 95% equal to [0.7084; 0.9765].

Among the 27 patients who tested positive for at least one virus, 6 (22.2%)were hospitalized for diabetes ketoacidosis (DKA); all cases of DKA were associated to PiV-1 infection. In two patients with mild ketoacidosis (33.3%) only PiV-1 was found, while 3 patients with moderate ketoacidosis (50.0%) had a PiV-3 co-infection. The only patient with severe ketoacidosis (16.7%) had only PiV-1 infection (coinfected with IVA). Regarding the diabetes autoimmunity pattern, 15 out of 27 patients were anti GAD/IAA antibodies positive (55.6%). Among 6 patients with DKA, 4 were antiGAD/IAA antibodies negative (66.7%). C peptide is lower in patients tested for Parainfluenza virus (p=0.029) than for other viruses tested and, in particular, in the Parainfluenza virus family, C peptide is lower in P1.

Our study confirmed that there are more new diabetes diagnoses in autumn, contemporary to high circulation of respiratory viruses and Enteroviruses, as already known (Field AM *et al*., 1967, Ruiz PLD *et al*., 2018). To our knowledge, Enteroviruses, which appear to have a trigger role in the pathogenesis of T1DM, were the most represented, especially CoxB, followed by Echo (Laitinen OH *et al*., 2014, Ashton MP *et al*., 2016, Vehik K *et al*., 2019). But unexpectedly, our study revealed that Parainfluenza viruses, with a dilution ratio ≥1:40, were the most represented viruses in all seasons and age groups, with peaks in autumn and spring. Regarding viral coinfections in T1DM at onset, we found a strong association between Parainfluenza viruses and Enteroviruses, especially PiV-1 and CoxA, followed by RSV, IVA-B, and AdV. This study adds a new consideration about the association between virus and T1DM: in the literature, the most represented enterovirus envolved in T1DM pathogenesis is CoxB. Parainfluenza viruses are less represented in the literature, but CoxA and PiV1 coinfection is to be taken into consideration as a possible trigger. Moreover, considering this historical period, patients with new onset T1DM from March to August 2020 were tested with molecular swab for Covid-19, but no one was positive.

Finally, our study has several limitations. First, the study lacked healthy controls; second, the sample size was small.

The relationship between Parainfluenza viruses and new onset diabetes has been described in the literature but without a significant correlation between Parainfluenza viruses seroprevalence and new onset type 1 diabetes (Harms RZ *et al*., 2020).

In conclusion, our study demonstrates that Parainfluenza viruses can be detected in children with T1DM at onset, both as single agent and as co-infection. Interestingly, Parainfluenza viruses were founded in all cases of DKA. In light of the results obtained, our future project will be to increase the sample size and provide a control group.

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