TrialNet INS ratio analyses

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## Background

The purpose of this analysis is to test whether unmethylated INS ratios and proinsulin ratios differ between 4 groups of TrialNet subjects: T1D, multiple antibody positive, single antibody positive, and negative. The correlation of unmethylated INS ratios and proinsulin ratios with biochemical and ECL antibody levels was also examined.

## Methods

There were 1703 records in the initial longitudinal dataset, and 144 participants in the participant database.

The following data manipulation and cleaning steps were performed:

1. Fasting C-peptide in pM/L was calculated as fasting C-peptide\*331.09.
2. Proinsulin ratios were calculated as proinsulin (pM/L) / C-peptide (pM/L) \*100
3. There were 36 records deleted that were missing both mIAA and ICA512/IA2H results.
4. There were an additional 40 records deleted that were missing just mIAA.
5. Antibody results were classifed as positive/negative using the following cutoff values:

* mIAA, 0.01
* GAD65, 0.032
* GAD65H, 20
* ICA512, 0.049
* IA\_2H, 5
* ZnT8, 0.02

1. The categorical variables (positive/negative) for GAD65 and GAD65H were combined into one variable, as were the categorical variables for ICA512 and IA\_2H.
2. The number of positive antibodies at each visit was calculated as the sum of ICA512/IA2H, GAD65/GAD65H, mIAA, and ZnT8.
3. For analyses using the continuous value of the antibodies, GAD65 and GAD65H were combined by calculating the Z-score of each variable and then combining the Z-scores into one variable. The same procedure was followed for ICA512/IA2H.
4. There were 12 subjects who did not have any proinsulin results who were excluded from analysis.
5. Participants were classified in the T1D group if they ever had a diagnosis of T1D.
6. If a participant was not in the T1D group, they were classfied by antibody status as below. This resulted in 15 participants in the T1D group, 34 participants in the multiple antibody positive group, 68 participants in the single antibody positive group, and 15 participants in the negative group.

* If they were multiple antibody positive at any visit, they were classified as multiple antibody positive.
* If they were never multiple antibody positive, but were single antibody positive at 2 or more visits, they were classified as single antibody positive.
* Otherwise, they were classifed as antibody negative.

1. For cross-sectional analyses, unmethylated INS ratios and proinsulin ratios were selected as follows:

* For the T1D group, the result closest to the date of diagnosis was used.
* For all other groups, the most recent result was used.

## Results

Tables 2 and 3 show the results of the group comparisons for unmethylated INS ratio and proinsulin ratio.

Table 2. Unmethylated insulin ratios.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristic | All (n=103) | 0 (n=13) | 1 (n=42) | 2 (n=33) | 3 (n=15) | P Value |
| **INS ratio** | 0.14±0.04 | 0.14±0.05 | 0.15±0.05 | 0.14±0.04 | 0.13±0.04 | 0.4092 |
|  |  |  |  |  |  |  |

Table 3. Proinsulin ratios.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Characteristic | All (n=98) | 0 (n=9) | 1 (n=44) | 2 (n=32) | 3 (n=13) | P Value |
| **Proinsulin ratio** | 4.27±4.2 | 5.09±5.24 | 3.85±4 | 4.74±4.61 | 3.97±3.18 | 0.7457 |
|  |  |  |  |  |  |  |

Tables 4 and 5 show the correlations of unmethylated INS and proinsulin ratios with continuous antibody levels.

Table 4. Correlation of unmethylated insulin ratios with antibody levels.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | row | column | cor | p |
| 1 | U\_MplusU\_Ratio\_Zen | gadz | -0.0389409 | 0.6961436 |
| 2 | U\_MplusU\_Ratio\_Zen | ia2z | -0.1151146 | 0.2469123 |
| 4 | U\_MplusU\_Ratio\_Zen | mIAA | 0.0438417 | 0.6601357 |
| 7 | U\_MplusU\_Ratio\_Zen | ZnT8 | -0.1786856 | 0.0767954 |
| 11 | U\_MplusU\_Ratio\_Zen | ICA | -0.1318898 | 0.2002195 |
| 16 | U\_MplusU\_Ratio\_Zen | ECL\_GADA | -0.1034135 | 0.3186267 |
| 22 | U\_MplusU\_Ratio\_Zen | ECL\_IA2A | -0.1174538 | 0.2569743 |
| 29 | U\_MplusU\_Ratio\_Zen | ECL\_IAA | -0.1174538 | 0.2569743 |
|  |  |  |  |  |

Table 5. Correlation of proinsulin ratios with antibody levels.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | row | column | cor | p |
| 1 | Pro\_pep | gadz | 0.1317711 | 0.1958802 |
| 2 | Pro\_pep | ia2z | 0.1020925 | 0.3171590 |
| 4 | Pro\_pep | mIAA | 0.1792480 | 0.0773908 |
| 7 | Pro\_pep | ZnT8 | 0.0149201 | 0.8840676 |
| 11 | Pro\_pep | ICA | 0.1227451 | 0.2360095 |
| 16 | Pro\_pep | ECL\_GADA | 0.1883748 | 0.0737462 |
| 22 | Pro\_pep | ECL\_IA2A | 0.2405371 | 0.0216366 |
| 29 | Pro\_pep | ECL\_IAA | 0.2405371 | 0.0216366 |
|  |  |  |  |  |