

PANCREATIC AUTOANTIBODIES AND C PEPTIDE IN YOUNG FIRST DEGREE RELATIVES OF TYPE 1 DIABETES PATIENTS FROM TIMISOARA

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REZUMAT

Objective: Obiectivele acestui studiu au fost demonstrarea fezabilității unui screening imunologic și evaluarea prevalenței markerilor imuni și a capacității insulinosecretorii la rudele de gradul I ale pacienților cu diabet zaharat tip 1. **Material și metodă:** Grupul de studiu a fost reprezentat de 66 de rude de gradul I ale unor pacienți cu diabet zaharat tip 1, 30 bărbați (45,5%) și 36 femei (54,5%), având vârsta (medie ± DS) 11,6 ± 5,5 ani. Prin metoda ELISA au fost determinați autoanticorpii antidecarboxilază a acidului glutamic (GADA), autoanticorpii anticelulă insulară (ICA), autoanticorpii anti IA-2 (IA-2A) și peptidul C bazal. Pozitivitatea autoanticorpilor pancreatici (AAP) a fost definită ca valori peste percentila 97,5 a unui grup de control: 3,91 unități pentru GADA, 0,61 unități pentru ICA și 5,55 unități pentru IA-2A. O valoare a p sub 0,05 a fost considerată semnificativă statistic. **Rezultate:** Un număr de 11 rude (16,6%) au fost pozitive pentru AAP: 3 (4,5%) pentru GADA, 6 (9,1%) pentru ICA și 2 (3%) pentru IA-2A. Nici un subiect al lotului de studiu nu a fost pozitiv pentru 2 sau 3 AAP. Nu s-au înregistrat diferențe semnificative statistic între subiecții pozitivi și cei negativi în ceea ce privește valoarea medie a glicemiei a jeun (76 ± 9,8 mg/dl vs. 79,7 ± 9,4 mg/dl, p=0,24) și HbA1c (5,6 ± 0,6% vs. 5,7 ± 0,9%, p=0,71). Rudele pozitive pentru AAP au avut o valoare medie mai mică a peptidului C bazal, comparativ cu cele negative (0,6 ± 0,5 ng/ml vs. 1 ± 0,9 ng/ml, p=0,18). **Concluzii:** Studiul nostru a demonstrat fezabilitatea screeningului imunologic și a arătat o prevalență crescută a pozitivității pentru ICA în rândul rudelor de gradul I ale pacienților diabetici.

Cuvinte cheie: autoanticorpi, peptid C, rude, diabet zaharat tip 1

ABSTRACT

Objective: The purpose of this study was to evaluate the feasibility of an immunologic screening and to assess the prevalence of immune markers and the level of insulin secretion in first degree relatives of type 1 diabetes patients. **Materials and methods:** The study group comprised 66 relatives, 30 males (45.5%) and 36 females (54.5%), aged (mean ± SD) 11.6 ± 5.5 years. Glutamic acid decarboxylase autoantibodies (GADA), islet cell autoantibodies (ICA), IA-2 autoantibodies (IA-2A) and fasting C peptide were measured using ELISA. The positivity for pancreatic autoantibodies (PAA) was defined as values higher than percentile 97.5 from the control group: 3.91 units for GADA, 0.61 units for ICA and 5.55 units for IA-2A. A p value below 0.05 was considered significant.

Results: A subgroup of 11 relatives (16.6%) were PAA positive: 3 (4.5%) for GADA, 6 (9.1%) for ICA and 2 (3%) for IA-2A. None of the relatives tested positive for 2 or 3 PAA. There were no significant differences between the PAA positive and negative subjects regarding mean fasting glycemia (76 ± 9.8 mg/dl vs. 79.7 ± 9.4 mg/dl, p=0.24) and mean HbA1c (5.6 ± 0.6% vs. 5.7 ± 0.9%, p=0.71). PAA positive relatives had lower mean fasting C peptide than those who tested negative (0.6 ± 0.5 ng/ml vs. 1 ± 0.9 ng/ml, p=0.18). **Conclusions:** Our study demonstrated the feasibility of the immunologic screening and showed a high prevalence of ICA positivity among first degree relatives of diabetes patients.

Key Words: autoantibodies, C peptide, relatives, type 1 diabetes mellitus

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an organ-specific autoimmune disease with aberrant immune responses to specific pancreatic autoantigens, leading to progressive loss of insulin-secreting β -cells.¹

The disease has a rapidly increasing incidence in many regions and shows a trend towards earlier onset.² It most often interests young children and impairs not only their physical development, but also their psychological and social existence. The life of the whole family is affected. The onset in early childhood also means a threat that severe complications may occur in the most active period of life, that is, in the fourth or fifth decade.³

Diabetology has always been a defensive specialty for the physician. The treatment of T1DM aims to mimic normal physiology, minimize hazards, palliate late complications and offer guidance and support. It is essentially an exercise in damage limitation. Much can be achieved with current therapy and microvascular complications can be prevented – or at least delayed

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– by improved glycemic control. Even so, the means of achieving safe near normoglycemia remain limited at best.⁴

The pathogenesis, prediction and prevention of T1DM constitute one of the great intellectual and human challenges of modern medicine.

Both genetic and environmental factors play roles in pathogenesis. Susceptibility or resistance to the disease are conferred by several genes acting in concert (HLA class II being the most informative genetic locus) and by partly known environmental factors.⁵⁻⁷ The latter stages of the process are mediated by the immune system. Though not directly pathogenic, pancreatic autoantibodies (PAA) provide a practical readout of β -cell autoimmunity. They are easily sampled in venous blood and have become a mainstay of T1DM prediction efforts.⁸ Over time, in individuals with insulinitis there is a progressive decrease of insulin secretion. At the beginning, loss of the first-phase insulin response (FPIR) during intravenous glucose administration is noted; glucose intolerance and overt diabetes finally develop.⁹

It is increasingly practicable to identify first degree relatives at risk of progression to diabetes and to quantify their level of risk, thus producing estimates that can be used to design intervention trials in prediabetes. Models of T1DM prediction in first degree relatives are all based on the presence of PAA and sometimes of the declining insulin secretion (as assessed by loss of FPIR during intravenous glucose tolerance test, low C peptide values or increased seric proinsulin concentration).⁸

Many of us hope and believe that, during the next years, it will be possible to assume the offensive, whether by restoring insulin secretion or halting the incipient disease process before clinical onset of T1DM.¹⁰

The aim of this research is to evaluate the feasibility of an immunologic screening and to assess the prevalence of immune markers and the level of insulin secretion in first degree relatives of T1DM patients from Timișoara. This is only the first part of an intended longitudinal study.

MATERIAL AND METHODS

The study group comprised 66 first degree relatives of T1DM patients, 30 males (45.5%) and 36 females (54.5%). Mean age \pm SD (limits) of the subjects was 11.6 ± 5.5 years (7 months – 23 years 11 months). From the group, 23 individuals (34.9%) had documented T1DM in their mothers, 15 (22.7%)

had diabetic fathers, 26 (39.4%) had one sibling with T1DM and 2 subjects (3%) had one parent and one sibling with T1DM.

Additionally, we examined a control group, represented by 66 healthy non-diabetic subjects, with no family history of T1DM. This group was necessary for establishing the normal range for C peptide and the positivity cut-off for PAA.

The study was approved by the local Ethics Committee. The parents of all participants agreed that their children participate to the research (for subjects aged 18 years or more, informed consent was obtained directly from them).

The diagnosis criteria for DM were those proposed by American Diabetes Association in 1997 and adopted by World Health Organization in 1999.^{11,12}

The clinical criteria for establishing the diagnosis of T1DM were: diagnosis before 30 years, normal or low body mass index (BMI), acute onset of disease, marked classical symptoms and tendency towards ketosis.¹³

Some baseline clinical parameters of the subjects from the study group were noted: gender, age (years), height – H (m), weight – W (kg) and BMI (calculated by the formula $BMI = W/H^2$) (kg/m²). The study and control groups were matched for gender, age, H, W and BMI. (Table 1)

Table 1. Baseline clinical characteristics of the study and control groups.

| Parameter | Study group (n = 66) | Control group (n = 66) | p |
|----------------------------|-------------------------|---------------------------|----|
| Gender:* | | | |
| - male | 30 (45.5) | 30 (45.5) | NS |
| - female | 36 (54.5) | 36 (54.5) | NS |
| Age (years)** | 11.6 ± 5.5 | 11.9 ± 2.8 | NS |
| H (cm)** | 142.9 ± 27.4 | 149.4 ± 15 | NS |
| W (kg)** | 41.8 ± 22 | 41.7 ± 15.6 | NS |
| BMI (kg/m ²)** | 19 ± 5.1 | 17.9 ± 3.8 | NS |

*: values expressed as number (percent)

**: values expressed as mean \pm SD

The following laboratory investigations were performed: fasting plasma glucose – FPG (mg/dl) (measured by glucosoxidase); glycated hemoglobin – HbA1c (%) (using immune turbidimetric method); PAA (units of optic density), using ELISA: glutamic acid decarboxylase autoantibodies – GADA, islet cell autoantibodies – ICA and IA-2 autoantibodies – IA-2A; fasting basal C peptide (ng/ml) (by ELISA). The positivity for PAA was defined as values higher than percentile 97.5 in the control group: 3.91 units of optic density for GADA, 0.61 units of optic density for ICA

and 5.55 units of optic density for IA-2A. In order to establish the importance of the cut-off limit, a second analysis was performed by defining positivity for PAA as values higher than percentile 99: 4 units of optic density for GADA, 0.64 units of optic density for ICA and 7.79 units of optic density for IA-2A.

Normal C peptide values were considered between percentile 5 and 95 of the control group, i.e., between 0.67 ng/ml and 4.57 ng/ml.

The statistical analysis was performed using Microsoft Excel 2002 and GraphPad InStat version 3.05. Unpaired t test and Fisher's exact test were used. A p value below 0.05 was considered significant.

RESULTS

From the study group, 11 participants (16.6%) were positive for 1 PAA: 3 subjects (4.5%) were GADA positive, 6 (9.1%) were ICA positive and 2 (3%) were IA-2A positive. No participant had 2 or 3 PAA above the cut-off limit. According to the presence or absence of PAA, the study group was divided into 2 subgroups: PAA positive and PAA negative, respectively. These data are depicted in Figure 1.

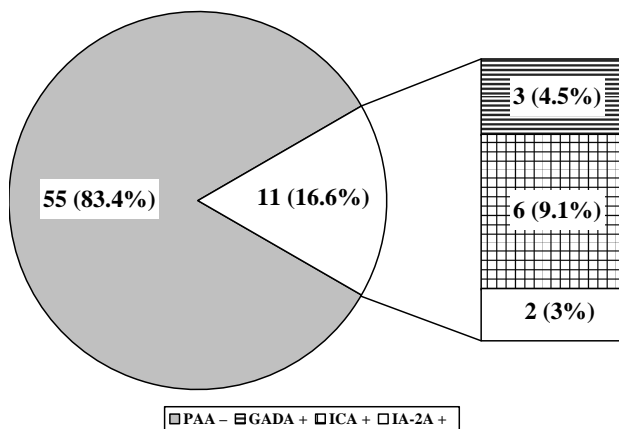


Figure 1. Presence of pancreatic autoantibodies into the study group.

From the 11 PAA positive subjects, 4 (36.4%) had the mother, 4 (36.4%) had the father, 2 (18.2%) had a sibling and 1 (9%) had the mother and one sibling with T1DM.

Compared to the PAA negative individuals, the PAA positive relatives were younger (8.9 ± 5.5 years vs. 12.1 ± 5.4 years, $p = 0.08$). The differences did not reach the significance threshold. (Fig. 2)

BMI was higher in the subgroup of patients having signs of humoral autoimmunity (19.7 ± 7.1 kg/m² vs. 18.9 ± 4.6 kg/m²), but this was not statistical significant ($p = 0.65$).

FPG and HbA_{1c} had almost the same value in

the 2 subgroups. Table 2 presents the comparative parameters of the PAA positive and negative subjects.

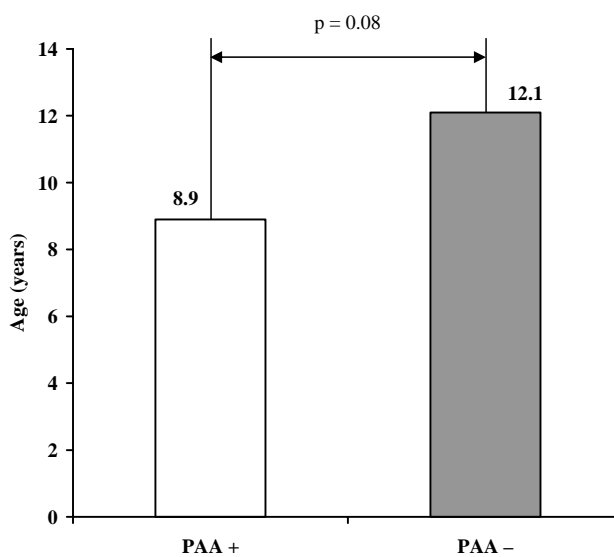


Figure 2. Mean age of the subjects from the 2 subgroups.

Table 2. Comparative parameters of the PAA positive and negative subjects (percentile 97.5).

| Parameter | PAA + subjects (n = 11) | PAA - subjects (n = 55) | p |
|--------------------------|----------------------------|----------------------------|------|
| Age (years) | 8.9 ± 5.5 | 12.1 ± 5.4 | 0.08 |
| BMI (kg/m ²) | 19.7 ± 7.1 | 18.9 ± 4.6 | 0.65 |
| FPG (mg/dl) | 76 ± 9.8 | 79.7 ± 9.4 | 0.24 |
| HbA _{1c} (%) | 5.6 ± 0.6 | 5.7 ± 0.9 | 0.71 |

Values are expressed as mean \pm SD

Taking into account the value of fasting C peptide, the subjects from the study group were divided into the low (<0.67 ng/ml), normal (0.67-4.57 ng/ml) and high (>4.57 ng/ml) categories.

From the whole study group, 28 subjects (42.4%) had low C peptide (<0.67 ng/ml) and 38 (57.6%) had normal C peptide (0.67-4.57 ng/ml). No individual had high C peptide.

It was found that in the PAA positive subgroup, 5 subjects (45.5%) had low C peptide and 6 (54.5%) had normal C peptide. In the PAA negative subgroup, 23 relatives (41.8%) had low C peptide and 32 (58.2%) had normal C peptide ($p = 1$, relative risk = 1.13). (Table 3) The relatives who were PAA-positive had a lower mean fasting C peptide (0.6 ± 0.5 ng/ml vs. 1 ± 0.9 ng/ml, $p = 0.18$). (Fig. 3).

The statistical analysis was repeated considering abnormal PAA values higher than percentile 99 from the control group.

In this case, only 7 relatives (10.6%) were PAA positive: 2 subjects (3%) were GADA positive, 4 (6.1%) were ICA positive and 1 (1.5%) was IA-2A positive.

From these subjects, 2 (28.6%) had the mother, 3 (42.8%) had the father, 1 (14.3%) had a sibling and 1 (14.3%) had the mother and one sibling with T1DM.

Table 3. Distribution of subjects in the two subgroups (defined by percentile 97.5) according to C peptide.

| C peptide | PAA + subjects (n = 11) | PAA - subjects (n = 55) |
|--------------------------|----------------------------|----------------------------|
| Low (<0,67 ng/ml) | 5 (45.5%) | 23 (41.8%) |
| Normal (0,67-4,57 ng/ml) | 6 (54.5%) | 32 (58.2%) |
| High (>4.57 ng/ml) | 0 (0%) | 0 (0%) |

Values are expressed as number (percent)

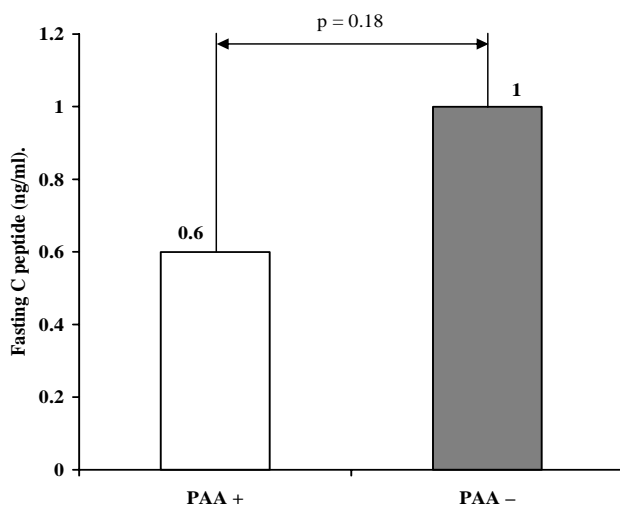


Figure 3. Mean fasting C peptide in the 2 subgroups.

The analysis of PAA positive and negative subgroups did not show important differences compared to the case when the cut-off limit for positivity of PAA was given by percentile 97.5 in the control group. (Table 4) However, the difference of age between the 2 subgroups reached the threshold for significance and there was a tendency towards grouping in the low C peptide category of the PAA positive subjects.

Table 4. Comparison between PAA positive and PAA negative relatives when percentile 99 was chosen.

| Parameter | PAA + subjects (n = 7) | PAA - subjects (n = 59) | p |
|---------------------------|---------------------------|----------------------------|--------------|
| Age (years) | 6.1 ± 3.7 | 12.2 ± 5.4 | 10.01 |
| BMI (kg/m ²) | 19.3 ± 8.1 | 19 ± 4.7 | 0.88 |
| FPG (mg/dl) | 74.2 ± 8.7 | 79.7 ± 9.5 | 0.14 |
| HbA _{1c} (%) | 5.5 ± 0.5 | 5.7 ± 0.9 | 0.56 |
| Fasting C peptide (ng/ml) | 0.4 ± 0.4 | 1 ± 0.9 | 0.09 |
| Categories of C peptide: | | | |
| - low | 4 | 24 | 0.44 |
| - normal | 3 | 35 | (relative |
| - high | 0 | 0 | risk = 1.81) |

DISCUSSIONS

Single PAA positivity may represent an incipient phase of the autoimmune attack directed against the pancreas or may result from persistent memory B cells in lymph nodes or bone marrow after brief transient insulinitis not resulting in clinical diabetes.¹⁴ The different PAA appear sequentially over time during prediabetes; the presence of multiple PAA in one subject marks a more persistent insulinitis and a greater diabetes risk.¹⁵⁻¹⁸ The subjects from the study group were positive only for 1 PAA, regardless the cut-off level (percentile 97.5 or 99). Thus, it is not possible to say that they are at risk of progressing to diabetes from a single determination.

The prevalence of positivity for GADA and IA-2A among first degree relatives was similar to that provided by literature. Regarding ICA, we obtained a higher prevalence than other authors, independent of the cut-off limit.^{9,18-20} This could be explained by the different laboratory techniques used: in our study ELISA for determining ICA was performed, in contrast to immune fluorescence method usually used in other clinical trials.

The lower mean age in the positive PAA subgroup, in both analyses, could be due to the fact that PAA titer is inversely correlated to the age of the investigated subjects.⁹

It is known that the onset of T1DM is frequently associated to physiological states of insulin resistance, such as puberty and pregnancy. Furthermore, it was shown that insulin resistance is a risk factor for progression to T1DM.²¹ BMI represents an anthropometric parameter that suggests the presence of insulin resistance. In our research, relatives positive for one PAA had a higher BMI compared to those negative for PAA, meaning an increased risk of diabetes. The role of BMI as a risk factor for progression to disease has to be clarified by the longitudinal follow-up.

Mean values of FPG and HbA_{1c} were similar in the 2 subgroups. The explanation is that, even in the positive PAA subjects, the disease did not progress to the stage characterized by the occurrence of glucose abnormalities.⁹

In the course of the evolution of the autoimmune process, as β -cell destruction progresses, the insulin secretion begins to decrease. This is usually assessed by decrease of FPIR, a test that is difficult to perform.⁹ Another possibility is the measurement of C peptide. The normal range for basal C peptide is not standardized, but depends on the laboratory method used. There is no consensus regarding the cut-off value

that would identify patients with low insulin secretion, i.e., who are in danger of progressing in short time to insulin deficiency.

In March 2001, Marian Parrot, a world known diabetologist, stated that "...if we were to chose a cut-off value that would include all type 1 diabetes patients, it would be somewhere between 0.8 and 1 ng/ml".²² In our study, it was noted a lower mean fasting C peptide and a higher percentage of subjects from the low C peptide category in the PAA positive, compared to the PAA negative subgroup. This could mean that the positivity of PAA is caused by persistent insulinitis. Sequential follow-up of the participants is needed in order to settle this issue.

It is well known that the cut-off limit of a test defines the sensitivity and specificity of the method, these parameters being inversely correlated.²³ The high cut-off limit for PAA positivity, given by percentile 99, confers a higher specificity and a lower sensitivity to the method, i.e., the existence of prediabetes in the positive subjects is more probable, but a smaller proportion of the relatives at risk will be identified. In this cross-sectional study, there were no significant differences between the subgroups when different cut-off limits were chosen. The evolution of the patients will answer to the question regarding the best choice for PAA positivity.

CONCLUSIONS

Our study demonstrated the feasibility of an immunologic screening of first degree relatives of T1DM patients.

The results obtained showed a similar prevalence of GADA and IA-2A as described in literature, but a higher percentage of ICA positive subjects.

Insulin secretion, assessed by fasting C peptide, was lower in relatives positive for PAA.

The two different cut-off limits for PAA, given by the two chosen percentiles (97.5 and 99, respectively) did not determine important changes in the results.

A clear conclusion cannot be drawn from these data. The follow-up will continue with annual visits and measurements of PAA titers and C peptide concentrations. The baseline sample will be expanded by including more relatives, especially children.

REFERENCES

1. Paronen J, Eisenbarth GS. Immunopathogenesis of type 1 diabetes in western society. In: DeFronzo RA, Ferrannini E, Keen H, Zimmet P (eds). International Textbook of Diabetes Mellitus, Third Edition. John Wiley & Sons, Ltd, Chichester, West Sussex, 2004, p. 495-513.

2. Karvonen M, Tuomilehto J, Podar T. In: Pickup JC, Williams G (eds). Textbook of diabetes, Third Edition. Blackwell Science Ltd., Massachusetts, Oxford, Victoria, Berlin, 2003, p. 5.1-14.
3. Dahlquist GG. Primary and secondary prevention strategies of pre-type 1 diabetes. Potentials and pitfalls. Diabetes Care 1999;22(Suppl 2): B4-6.
4. Gale EAM, Bingley PJ. Can we prevent IDDM? Diabetes Care 1994;17:339-44.
5. Atkinson MA, Eisenbarth GS. Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001;358:221-9.
6. Kelly MA, Barnett AH, Bain SC. Molecular genetics of type 1 diabetes. In: DeFronzo RA, Ferrannini E, Keen H, Zimmet P (eds). International Textbook of Diabetes Mellitus, Third Edition. John Wiley & Sons, Ltd, Chichester, West Sussex, 2004, p. 515-32.
7. MacFarlane AJ, Scott FW. Environmental agents and type 1 diabetes. In: Pickup JC, Williams G (eds). Textbook of Diabetes, Third Edition. Blackwell Science Ltd., Massachusetts, Oxford, Victoria, Berlin, 2003, p. 17.1-16.
8. Șerban V. Predicția diabetului zaharat. In: Șerban V (ed). Actualități în diabetul zaharat. Editura Brumar, Timișoara, 2002, p. 81-110.
9. Petrovsky N, Schatz DA. The immunology of human type 1 diabetes. In: Pickup JC, Williams G (eds). Textbook of Diabetes, Third Edition. Blackwell Science Ltd., Massachusetts, Oxford, Victoria, Berlin, 2003, p. 18.1-14.
10. Șerban V. Prevenția diabetului zaharat. In: Șerban V (ed). Actualități în diabetul zaharat. Editura Brumar, Timișoara, 2002, p. 111-38.
11. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care 1997;20:1183-97.
12. World Health Organization Consultation. Definition, diagnosis and classification of diabetes mellitus and its complications, Part 1. Diagnosis and classification of diabetes mellitus. Report of a WHO Consultation. World Health Organization, Geneva, 1999.
13. Slama G. Type 1 diabetes: an overview. In: Pickup JC, Williams G (eds). Textbook of Diabetes, Third Edition. Blackwell Science Ltd., Massachusetts, Oxford, Victoria, Berlin, 2003, p. 3.1-17.
14. Yu L, Rewers M, Gianani R, et al. Anti-islet autoantibodies usually develop sequentially rather than simultaneously. J Clin Endocrinol Metab 1996;81:4264-7.
15. Eisenbarth GS. Prediction of type I diabetes: the natural history of the prediabetic period. In: Type 1 Diabetes: Molecular, Cellular, and Clinical Immunology, Online Edition 2.5, Chapter 11. <http://www.uchsc.edu/misc/diabetes/oxch11.html>, accessed on July 21st, 2005.
16. Kimpimäki T, Kupila A, Hamalainen AM, et al. The first signs of beta-cell autoimmunity appear in infancy in genetically susceptible children from the general population: the Finnish type 1 diabetes prediction and prevention study. J Clin Endocrinol Metab 2001; 86:4782-8.
17. Kupila A, Keskinen P, Simell T, et al. Genetic risk determines the emergence of diabetes-associated autoantibodies in young children. Diabetes 2002;51:646-51.
18. Winter WE, Harris N, Schatz D. Immunological markers in the diagnosis and prediction of autoimmune type 1a diabetes. Clinical Diabetes 2002;20:183-91.
19. Bonifacio E, Genovese S, Braghi S, et al. Islet autoantibody markers in IDDM: risk assessment strategies yielding high sensitivity. Diabetologia 1995;38:816-22.
20. Leslie RDG, Atkinson MA, Notkins AL. Autoantigens IA-2 and GAD in type I (insulin-dependent) diabetes. Diabetologia 1999;42:3-14.
21. Fourlanos S, Narendran P, Byrnes GB, et al. Insulin resistance is a risk factor for progression to type 1 diabetes. Diabetologia 2004;47:1661-7.
22. Medicare Coverage Policy Decisions. C peptide level as a criterion for use of the insulin pump. www.hcfa.gov, 2001, accessed on May 9th, 2002.
23. Goldman L. Quantitative aspects of clinical reasoning. In: Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo DL (eds). Harrison's Principles of Internal Medicine, 14th Edition. McGraw-Hill, New York, 1998, p. 9-14.