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Novel Clusters of Type 2 Diabetes: A Tailored Therapeutic Approach

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A B S T R A C T

Introduction: Type 2 diabetes is a heterogeneous disease for which etiological mechanisms are incompletely understood and subclassification may improve patient care. In this paper, we aimed to stratify a cohort of Portuguese patients with adult-onset diabetes followed at our Diabetic clinic into subgroups and assess the impact of the clusters on outcomes and therapy.
Methods: We performed a cluster analysis on 1280 patients followed at our Diabetic clinic. Clusters were based on three variables: presence of glutamic acid decarboxylase antibodies, age at diagnosis and body mass index. Clinical data was retrieved from patient records. Statistical analysis was performed using SPSS v.25.0.
Results: We identified four replicable clusters of adult-onset diabetes, with significantly different patient characteristics and risk of diabetic complications. Clusters 1 and 2 were characterized by early-onset disease, higher HbA1c and insulin treatment. More than half of patients were included in cluster 3, requiring combined therapy. Cluster 4 was characterized by late-onset disease, low HbA1c and monotherapy. Cluster 1 had the highest risk of retinopathy.
Conclusion: The recently proposed cluster analysis is easily replicable in real-world clinical practice and applicable to different populations, including other Portuguese settings. This new subclassification may contribute patient tailored therapy, therefore representing a first step towards precision medicine in type 2 diabetes.

Palavras-chave:
Análse de Cluster;
Diabetes Mellitus Tipo 2/tratamento farmacológico.

Novos Clusters da Diabetes Tipo 2: Uma Abordagem Terapêutica Personalizada

R E S U M O

Introdução: A diabetes tipo 2 é uma doença heterogénea, sendo o conhecimento da sua etiopatogenia ainda incompleto e a subclassificação pode beneficiar o cuidado aos doentes. Neste artigo, o objetivo foi estratificar uma coorte de doentes com diabetes em subgrupos e avaliar o impacto dos *clusters* nas complicações e terapêutica.
Métodos: Realizámos uma seriação de 1280 doentes seguidos no nosso Departamento de Endocrinologia. Os *clusters* foram baseados em três variáveis: presença de anticorpos GAD, idade ao diagnóstico e IMC. Os dados de cada doente foram obtidos dos processos clínicos. A análise estatística foi realizada no SPSS v.25.0.
Resultados: Identificámos quatro *clusters* replicáveis de diabetes no adulto, com características e risco de complicações significativamente diferentes. Os *clusters* 1 e 2 foram caracterizados por doença de início precoce, maior HbA1c e tratamento com insulina. A maioria dos doentes foi incluído no *cluster* 3, necessitando de terapia combinada. O *cluster* 4 foi caracterizado por doença de início tardio, baixa HbA1c e monoterapia. O *cluster* 1 teve o maior risco de retinopatia.
Conclusão: A análise de *clusters* proposta é facilmente replicável na prática clínica e aplicável a diferentes populações, incluindo outros contextos da população Portuguesa. Esta nova subclassificação irá permitir uma terapêutica mais personalizada para o doente, representando, portanto, um primeiro passo em direção à medicina de precisão na diabetes tipo 2.

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Introduction

Diabetes is traditionally classified into two main forms: type 1 and type 2. Type 1 diabetes, previously called "insulin-dependent diabetes" or "juvenile-onset diabetes," accounts for 5%-10% of diabetes and is due to cellular-mediated autoimmune destruction of the pancreatic beta cells.[1] Type 2 diabetes, previously referred to as "non-insulin-dependent diabetes" or "adult-onset diabetes," accounts for approximately 90%–95% of all diabetes. Beta cell autoimmune destruction does not occur, although its specific etiology remains unclear. Familial predisposition has been observed, but the underlying genetic abnormalities are poorly understood.[2] Type 2 diabetes is a heterogeneous disease with large variation in the relative contributions of insulin resistance and beta cell dysfunction between subgroups and individuals. New data emphasizes that type 2 diabetes is not a single disease entity but that subgroups exist.[3]

Causal mechanisms for type 2 diabetes are incompletely understood and subclassification may improve patient management. In an attempt to deconstruct the heterogeneity of the disease, recent studies have performed cluster analysis of individuals using serum biomarkers and clinical data. Ahlqvist and colleagues[4] proposed five new subgroups for patients with adult-onset diabetes: an autoimmune form, two severe forms (insulin-deficient and insulin-resistant diabetes) and two mild forms (obesity and age-related diabetes). Clusters were based on six clinical variables: presence of glutamic acid decarboxylase (GAD) antibodies, age at diagnosis, body mass index - BMI, HbA1c, homoeostatic model assessment estimates of beta cell function (HOMA-B) and insulin resistance (HOMA-IR). The results revealed a higher prevalence of retinopathy in the insulin-deficient cluster and a higher risk for nephropathy in the insulin-resistant cluster.

Other efforts have tried to identify subtypes of type 2 diabetes. Udler and colleagues stratified individuals by clusters of genetic loci.[5] Out of the five, two clusters presented reduced beta cell function, with marked insulin deficiency, and three clusters displayed features of insulin resistance. The results revealed a higher prevalence of coronary artery disease and stroke in the insulin-deficient cluster. In contrast to serum biomarkers, germline genetic variants associated with type 2 diabetes remain constant regardless of disease stage or treatment. In summary, clustering of genetic variants associated with type 2 diabetes has identified five robust clusters with distinct trait associations, which likely represent different mechanistic pathways.

In this paper, we aimed to stratify a cohort of Portuguese patients with adult-onset diabetes followed at our Diabetic clinic into subgroups and assess the impact of the clusters on outcomes and therapy.

Methods

We conducted a retrospective cross-sectional study and cluster analysis in 1280 patients followed at our Diabetic clinic at the Armed Forces Hospital, in Lisbon, in 2018. We included patients diagnosed with type 2 diabetes and a disease duration of at least 12 months. Diagnosis of diabetes was based on American Diabetes Association criteria. We excluded patients diagnosed with type 1 diabetes under 30 years, early onset of diabetes (<18 years) and diabetes of other causes (monogenic diabetes, diseases of the exocrine pancreas, gestational diabetes, secondary to endocrinopathies and drug-induced diabetes).

Clusters were based on three variables: presence of GAD antibodies, age at diagnosis and BMI. We looked for BMI values at the time of diagnosis. Patients with type 1 features (young, lean, no family history and/or early insulin therapy) had antibody testing years after the initial diagnosis of type 2 diabetes.

Cluster 1 was characterized by presence of GAD antibodies and age at diagnosis over 30 years; Cluster 2 was defined by BMI < 27 kg/m^2 and age at diagnosis before 65 years; Cluster 3 was characterized by BMI > 27 kg/m^2; Cluster 4 was defined by age at diagnosis over 65 years.[6-8]

Data from patient records was collected, particularly focusing on diabetes-related complications, therapy, family history and metabolic control. We selected the most recent HbA1c value for each patient.

Microvascular complications were evaluated on yearly basis with urine albumin and serum creatinine samples to assess the presence of nephropathy. Retinopathy was diagnosed by an ophthalmologist with dilated fundus examination and retinal photography. All patients attended consultation for foot surveillance at least once a year where the presence of neuropathy was assessed with the 10 g monofilament by a foot care nurse.[9,10] Macrovascular complications were screened with an annual electrocardiogram and on individual basis, according with symptoms of angina or claudication, as routine stress tests in asymptomatic patients are not recommended.[11]

Statistical analysis was performed using SPSS v.25.0. A *p*-value of less than *0.05* was *regarded as statistically significant*. Pearson chi-square test for independence was used to study differences in diabetic complications between the clusters. ANOVA test was used to analyse the differences among group means (BMI, HbA1c, age).

The study was approved by the Health Ethics Committee at Armed Forces Hospital. Consent has been obtained from each patient after full explanation of the purpose and nature of the study.

Results

In the analysis of our population, 71% of patients were males, with a median age of 69.7 years. The mean duration of disease was 13.7 years. A total of 75% of patients were overweight (BMI 25-30 kg/m^2) or obese (BMI > 30 kg/m^2).

In the analysis of the 1280 patients, we identified four clusters of adult-onset diabetes, with significantly different patient characteristics and risk of diabetic complications. The male to female ratio was similar in all clusters (7:3). Cluster 1 consisted of 2% of all patients, cluster 2 of 22%, cluster 3 of 63% and cluster 4 the remaining 13% (Fig. 1).

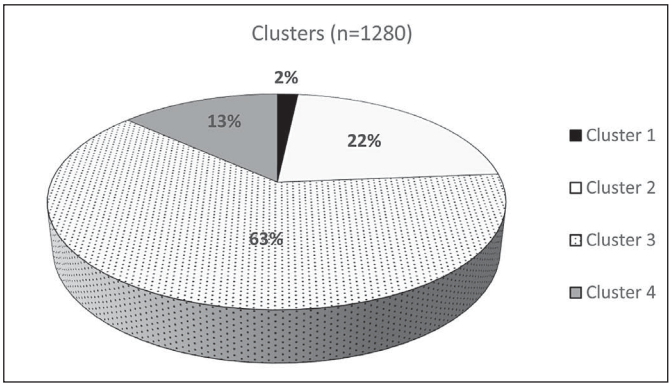

Figure 1. Patient distribution according to cluster classification (n=1280)

Table 1. Cluster characteristics in the Portuguese cohort

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	*p*-value
Number of patients (%)	22 (2%)	282 (22%)	805 (63%)	171 (13%)	---
Mean age at diagnosis (years)	45.8	51.9	54.3	71.9	$p < 0.0001$
Average duration of disease (years)	10.2	18.9	13	8.2	---
Mean BMI (kg/m²)	25.2	23.8	31.7	24.2	$p < 0.0001$
Mean HbA1c % (mmol/mol)	7.3% (56)	7.0% (53)	6.9% (52)	6.6% (49)	$p < 0.033$
Family history of diabetes (%)	38	65	60	34	$p < 0.0001$
Insulin treatment a (%)	73	30	26	14	$p < 0.0001$
Combination therapy b (%)	23	62	58	38	$p < 0.0001$
Male sex (%)	68	73	73	61	---

Cluster 1 (autoimmune); Cluster 2 (adult); Cluster 3 (obesity-related); Cluster 4 (age-related)

a Insulin treatment accounts for single and multiple daily injection regimens.

b Combination therapy is defined as more than one antihyperglycemic agent (nonspecified).

Clusters 1 to 4 patients had a mean age at diagnosis of 46, 52, 54 and 72 years, respectively (Table 1).

Cluster 3 patients displayed the highest mean BMI (31.7 kg/m²). The remaining clusters presented a mean BMI of 24.4 kg/m².

Regarding metabolic control, cluster 1 had substantially higher mean HbA1c throughout follow-up (7.3%), while cluster 4 presented the lowest 49 (6.6%), with a *p*-value of 0.033.

Concerning therapeutics, insulin was prescribed to 73% of patients in cluster 1 *versus* <30% in other clusters ($p < 0.001$). Most patients in clusters 2 (62%) and 3 (58%) required combination therapy, whereas monotherapy was the standard for cluster 4 ($p < 0.001$).

Most patients in clusters 2 and 3 (>60%) had family history of diabetes (nonspecified) *versus* <40% in the other clusters ($p < 0.001$).

Retinopathy was significantly more frequent in clusters 1 (18%) and 2 (16%) than in other clusters (<10%). Nephropathy was the most common diabetic-related complication in this cohort, with a prevalence of 21%, with no significant difference between clusters (Table 2). Moreover, the prevalence of hypertension was 32% in cluster 1, 78% in cluster 2, 86% in cluster 3 and 84% in cluster 4.

As regards to macrovascular complications, the most prevalent was coronary artery disease (9%-15%), with no statistically significant difference among clusters.

Discussion

Clusters 1 and 2 were characterized by early-onset disease, higher HbA1c and low BMI. Furthermore, they presented the highest prevalence of retinopathy. Cluster 4 was characterized by late-onset disease, low HbA1c, low BMI, and monotherapy was the treatment of choice.

In the current analysis, the percentage of males is higher than reported in the Ahlqvist study (70% *versus* 60%). That can probably be explained by our setting – a military hospital.

C-peptide and insulin levels were lacking for most patients, thus we were unable to calculate HOMA index and therefore assess insulin resistance. From a practical point of view, the dosing of C-peptide is of clinical importance in measuring the reserve of beta-pancreatic cells in patients with type 1 diabetes, making differential diagnosis in case of type 1 *versus* type 2 and initiating the suitable treatment or when evaluating insulin resistance in obese patients.[12] In type 2 diabetes, high values of C-peptide are associated with a high risk of macrovascular complications.[13] Also, it is generally accepted that there is progressive ß-cell failure in type 2 diabetes. Therefore, C-peptide measurement can also be a marker for ß-cell function in these patients.

Due to unavailability of C-peptide levels, our study included fewer clusters than those presented by Ahlqvist *et al*.[4] Ahlqvist

Table 2. Prevalence of diabetes-related complications in each cluster

Complications	Cluster 1	Cluster 2	Cluster 3	Cluster 4	*p*-value
Retinopathy (%)	**18**	**16**	10	5	**$p < 0.001$**
Nephropathy (%)	5	21	22	19	NS
Neuropathy (%)	5	3	5	2	NS
Cerebrovascular disease (%)	5	6	7	11	NS
Coronary artery disease (%)	9	14	14	15	NS
Periphery artery disease (%)	9	6	3	4	NS

NS – not significant

Table 3. Management of adult-onset diabetes by clusters

Complications	Clusters 1 & 2	Cluster 3	Cluster 4
Suggested therapeutic approach	Early insulin therapy if **GAD positive or low C-peptide**	Combination therapy: Met+aGLP1 or Met+iSGLT2 Lifestyle interventions	Monotherapy: Met or iDDP4
Active surveillance	Retinopathy screening Follow-up every 3 months	Follow-up every 6 months	Follow-up every 6 to 12 months

Met – metformin; aGLP1 - glucagon-like peptide 1 receptor agonists; iSGLT2 - sodium-glucose cotransporter-2 inhibitors; iDPP4 - inhibitors of dipeptidyl peptidase 4

proposed 5 clusters: autoimmune, insulin-deficient, insulin-resistant, obese-related and age-related.

Nevertheless, most our findings are in line to those published by Ahlqvist.

Patients in the autoimmune cluster (=cluster 1) were also younger with poorer metabolic control, while those in the age-related cluster (=cluster 4) had lower HbA1c. Retinopathy was more frequent in clusters 1 and 2 corresponding to the insulin-deficient cluster proposed by Ahlqvist. Cluster 3 may represent patients in the insulin-resistant and obese-related clusters proposed by Ahlqvist.

Contrarily, we did not find nephropathy to be more prevalent in any cluster. In the Ahlqvist analysis, patients in the insulin-resistant cluster had the highest risk of developing nephropathy. The fact that insulin resistance was not determined in the current study may have contributed to the different outcome.

A significant difference between the studies concerns the timing of HbA1c measurement. Whereas we selected the most recent HbA1c for each patient (with therapy), the previous study used HbA1c at diagnosis. Regardless, the severity of HbA1c was similar in both studies, with poorer metabolic control in the autoimmune and insulin-deficient clusters (=clusters 1 and 2). The mean HbA1c for cluster 1 was 7.3%, whereas in the autoimmune cluster was 9%. For cluster 4, the mean HbA1c was 6.6%, whereas in age-related cluster was 6.7%.

While cluster 1 overlapped with type 1 diabetes, cluster 2 may represent a new form of diabetes, neither related to age nor obesity. These are young, lean individuals who may benefit from early intensified treatment with insulin to prevent diabetic complications. In particular, screening for diabetic retinopathy appears to be of paramount importance.

Most adults with diabetes have overweight or obesity, so those in cluster 3 seem to represent the standard patient in our clinical practice. There is strong evidence that obesity management is beneficial for the treatment of type 2 diabetes.[14] In the latest *ADA-EASD Consensus Report*, efforts targeting weight loss, including lifestyle, medical and surgical interventions, are recommended. When selecting a glucose-lowering medication, we should consider one that promote weight loss, such as GLP-1 agonists or SGLT2 inhibitors, in addition to metformin, as most patients will require combination therapy in order to have an adequate metabolic control.[16] Regarding cluster 4, age-related diabetes is characterized by lower HbA1c and the use of less insulin, suggesting a mild form of diabetes. The aim of the treatment is to protect the quality of life, prevent hypoglycemia and related complications.[8] Metformin is an attractive choice for elderly patients due to low cost, positive effects on cardiovascular disease and low risk of hypoglycemia. However, the most important restricting factor of metformin treatment is glomerular filtration rate and treatment should be stopped if < 30 mL/min. The prevalence of chronic kidney disease (CKD) increases in those over 65 years, so we need to consider other options. DPP-4 inhibitors are an advantageous treatment choice for this population due to the single daily dose, lack of risk for hypoglycemia and neutral effect on weight.[16,17] Monotherapy appears to be sufficient in most of these individuals (Table 3).

Diabetic nephropathy is the most common cause of CKD in those with diabetes.[18] However, it is not the only cause of CKD in diabetic patients. Hypertension is highly prevalent among patients with diabetes, leading to further progression of kidney disease and increased incidence of cardiovascular disease in this population.

Screening for diabetic complications must be initiated at the time of diagnosis in patients with type 2 diabetes. Screening for retinopathy, nephropathy, peripheral neuropathy and foot care should be performed at least once a year.[8]

In asymptomatic patients, routine screening for coronary artery disease is not recommended. However, cardiovascular risk factors should be systematically assessed in all patients with diabetes. There are now several large randomized controlled trials reporting statistically significant reductions in cardiovascular events for SGLT2 inhibitors and GLP-1 receptor agonists. For patients with type 2 diabetes who have cardiovascular disease, it is recommended to incorporate one of these agents, in addition to metformin.[14]

The strengths of this study include adequate sample size, clinical relevance and replication feasibility. Moreover, it supports most findings published by Ahlqvist *et al*.[4] Limitations of this study include its retrospective nature and lack of c-peptide levels to assess insulin resistance. Finally, family history was not studied extensively to exclude a potential MODY.

Conclusion

In summary, this new subclassification is easily replicable in a real world clinical practice setting. We expect to find similar outcomes in other populations if our cluster criteria is employed, regarding patient characteristics, metabolic control and treatment options.

It will be exciting to explore whether individuals respond differently to medications based on the pathway predominantly disrupted or whether they have a variable rate of progression and diabetic complications. Furthermore, classification of patients by clusters of genetic loci may offer individualized treatment choices, therefore representing a first step towards precision medicine in type 2 diabetes.

Responsabilidades Éticas

Conflitos de Interesse: Os autores declaram a inexistência de conflitos de interesse na realização do presente trabalho.
Fontes de Financiamento: Não existiram fontes externas de fi-

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Confidencialidade dos Dados: Os autores declaram ter seguido os protocolos da sua instituição acerca da publicação dos dados de doentes.

Proteção de Pessoas e Animais: Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pelos responsáveis da Comissão de Investigação Clínica e Ética e de acordo com a Declaração de Helsínquia da Associação Médica Mundial.

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Ethical Disclosures

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Protection of Human and Animal Subjects: The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

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