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Clinical and biological characteristics of diabetic patients under age 40 in Cameroon: relation to autoantibody status and comparison with Belgian patients

Milca Asanghanwa, MSc¹, Frans K. Gorus, MD, PhD^{1,2}, Ilse Weets, MD, PhD^{1,2}, Bart V. der Auwera, MD, PhD¹, Folefac Aminkeng, PhD³, Eric Mbunwe, MSc¹, Patrick Goubert, MLT², Katrijn Verhaeghen, PhD², Eugene Sobngwi, MD, PhD⁴, Janet M. Wenzlau, PhD⁵, John C. Hutton, PhD^{5†}, Daniel G. Pipeleers, MD, PhD¹, Bart Keymeulen, MD, PhD^{1,2}, Jean-Claude N. Mbanya, MD, PhD, FRCP⁴, and Chris v. Schravendijk, PhD^{1,*§} on behalf of the Belgian Diabetes Registry on behalf of Cameroon Diabetes Epidemiology and Registry Project

¹Diabetes Research Center, Brussels Free University – VUB, Laarbeeklaan 103, B-1090 Brussels, Belgium

²Department of Clinical Chemistry and Radio-immunology – University Hospital Brussels Free University – UZ Brussel, Brussels, Belgium

³The Canadian Pharmacogenomics Network for Drug Safety, University of British Columbia

⁴Faculty of Medicine and Biomedical Sciences, Department of Medicine and Specialties, Laboratory for Molecular and Metabolic Medicine, The Biotechnology Centre, University of Yaoundé 1, Yaoundé, Cameroon

⁵Barbara Davis Center for Childhood Diabetes, University of Colorado at Denver, Aurora, CO

Abstract

Aims—We investigated the prevalence of diabetes autoantibodies (Abs) in Cameroonian patients and controls, assessed their contribution in disease classification and compared results with data from Belgium.

Methods—Abs against GAD (GADA), IA-2 (IA-2A) and zinc transporter 8 (ZnT8A) were assessed in 302 recently diagnosed Cameroonian patients with diabetes and 184 control subjects without diabetes aged below 40 years.

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Corresponding Author: Prof. Chris van Schravendijk Diabetes Research Center – Brussels Free University – VUB, Laarbeeklaan 103, B-1090 Brussels, Belgium Phone 32-2-477 45 49 Fax 32-2-477 45 45 chrisvs@vub.ac.be.

^{5†}We dedicate this paper to the memory of Dr. John Hutton.

*The complete list of members can be found in the supplementary Data

§Names of collaborators are listed in the acknowledgement section

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Conflict of Interest None reported

A preliminary report of this study has been presented by the first author at the African Diabetes Congress in Arusha, Tanzania, held from 25th to 28th July 2012 (Abstract booklet p. 56, #46).

Results—Only 27 (9%) Cameroonian patients were younger than 15 years. Overall, 29% of patients presented at least one diabetes-associated antibody versus 9% in healthy controls (24% versus 7% for GADA ($p < 0.001$), 10% versus 3% for IA-2A ($p < 0.006$); 4% versus 2% for ZnT8A). Ab⁺ patients had lower C-peptide levels ($p < 0.001$), were more often insulin-treated ($p < 0.002$) and were as frequently diagnosed with type 1 diabetes as Ab[−] patients. Only 43% of Ab⁺ patients aged 15–39 years were clinically classified as having type 1 diabetes in Cameroon vs 96% in Belgium ($p < 0.001$). Not one Ab⁺ Cameroonian patient carried *HLA-DQ2/DQ8* genotype versus 23% of Belgian Ab⁺ patients ($p < 0.001$). Younger age at diagnosis and antibody positivity were independent predictors of insulin therapy. Ab⁺ Cameroonian patients were older ($p < 0.001$), had higher BMI ($p < 0.001$) and lower Ab titers than Belgian Ab⁺ patients. In ketonuric patients, prevalence of autoantibodies was similar as in non-ketonuric patients.

Conclusions—In Cameroonian patients with diabetes aged under 40 years, antibody-positivity is not clearly related to disease phenotype, but may help predict the need for insulin treatment.

Keywords

Autoantibodies; classification; diabetes; sub-Saharan Africa

Over the next 20 years, Africa is the continent facing the highest increase in diabetes prevalence with an expected rise from the current 14.7 million to 28 million [1]. Apart from classical type 1 and type 2 diabetes, ketosis-prone atypical diabetes, malnutrition-related diabetes and tropical diabetes have also been described in sub-Saharan Africa (SSA) [2]. These variant disease phenotypes are characterized by mixed features of both type 1 and type 2 diabetes, thus complicating disease classification on clinical grounds [3]. This challenge may lead to the irrational use of insulin therapy in economically disadvantaged SSA [4], and to inadequate disease management with the risk of a future upsurge of diabetes complications. In addition to the increasing burden of diabetes [1,5], the need for the implementation of additional measures for disease classification has never been more relevant.

Autoantibodies against incompletely identified islet cell cytoplasmic antigens or against molecularly defined antigens such as insulin, glutamic acid decarboxylase (GADA), insulinoma-associated protein 2 (IA-2A), and zinc transporter 8 (ZnT8A) constitute the hallmark of immune-mediated type 1 diabetes in Caucasians. They have been instrumental in discriminating between type 1 and type 2 diabetes, especially in early adulthood [6–8]. About 90% of clinically classified type 1 diabetes patients in Caucasian populations are positive for at least one of these autoantibodies at disease onset [9,10]. In sub-Saharan Africa, the impact of the immune markers in disease classification is still uncertain. Two published studies found no association of islet cell cytoplasmic autoantibodies with the disease pattern in SSA [11,12]. Meanwhile, other reports involving molecular Abs have proposed a significant role of these immune markers for disease classification in Africa [13]. To date, no study from SSA has investigated the role of the recently identified ZnT8A. Comparison of study results from SSA is difficult due to the differences in the inclusion criteria of study participants, or the diagnostic methods used for antibody determinations. Moreover, most of the study populations in SSA are handicapped by their low numbers, while including subjects who have longstanding diabetes [14], or are older than the pediatric

population in most Caucasian studies. The present study was undertaken to investigate the occurrence of GADA, IA-2A and ZnT8A in newly diagnosed diabetes patients under age 40 from Cameroon, in relation to clinical disease phenotype and other biological markers of immune-mediated type 1 diabetes (C-peptide, *HLA-DQ*, ketonuria) in order to assess their potential contribution to disease classification and choice of treatment. The data obtained were compared with those of a Belgian cohort using the same methodologies for subject recruitment and immune, genetic and hormonal markers.

Research Design and Methods

Research participants

Between August 2007 and August 2011, 308 patients aged 0–39 years were consecutively recruited in the framework of the “Cameroon Diabetes Epidemiology and Registry (CAMDER)”. This Vlaamse Interuniversitaire Raad (VLIR)-sponsored project is a collaboration between the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1 in Cameroon, and the Diabetes Research Center of the Brussels Free University in Belgium. Patients were recruited from the National Obesity Centre and the National Centre for Hypertension and Diabetes of the Yaoundé Central Hospital in Cameroon. Diagnosis of diabetes was by WHO criteria [15]. Of the 308 patients, 302 were included in the study after verification of the following criteria: i) sub-Saharan African ethnicity ii) registered within 18 months after diagnosis iii) absence of gestational or secondary diabetes iv) residence in Cameroon for at least 6 months before diagnosis and v) availability of demographic data and blood samples for biochemical analysis. A written informed consent was also obtained from all participants and from parents in the case of minors. Patients consisted of 167 males and 135 females (male/female ratio: 1.2) with a median age (interquartile range, IQR) of 31 (23 – 35) years. Median disease duration (IQR) was 23 (5 – 140) days. At diagnosis, patients were clinically classified as having type 1, type 2 or unclassifiable diabetes by their treating physicians according to the clinical criteria recommended in the 1999 WHO report [15]. All subjects fulfilling the entry criteria undertook a clinical examination during which date of diagnosis, type of diabetes (clinical classification), type of symptoms, glycaemia at diagnosis, and family history of diabetes were recorded. Anthropometric parameters including weight and height were also measured and recorded. Venous blood was obtained from an antecubital vein for the determination of autoantibody, genetic and hormonal markers. The control population consisted of 184 healthy volunteers without diabetes aged 0 – 39 (median age [IQR]; 27 [23 – 33]) years and matched for gender and ethnicity. These included hospital workers, university students, blood donors and relatives of hospitalized patients. Controls had no previous medical record of hyperglycaemia, no family history of diabetes, normal glycaemia, fructosamine, HbA1c, and C-peptide levels, and resided in Cameroon for at least 6 months prior to enrolment. Fifty of 184 controls were randomly selected for an OGTT. None of the control subjects showed impaired glucose tolerance, impaired fasting glucose or diabetes with an OGTT. Characteristics of Ab⁺ and Ab[−] patients aged 15 – 39 years were compared with those of a Belgian cohort (median age [IQR]; 29 [22 – 34]) of Ab⁺ (n = 289) and an equally sized Ab[−] (n = 285) patient group, recruited between June 1996 and July 2007 by the Belgian Diabetes Registry (BDR), following a similar recruitment criteria, and submitted to the same tests and

analyses. Belgian patients were recruited within a week following diagnosis (median disease duration [IQR]; 1 [0 – 4] days). The study was carried out in accordance with the Helsinki Declaration as revised in 2008, and also approved by the Cameroonian National Ethical Committee. All blood samples (except EDTA - anticoagulated blood) were centrifuged within 1 hour of collection, and stored at – 20° to – 80°C after sampling in Cameroon. Aliquots of all samples were transported within 12 to 24 weeks of collection on dry ice to the BDR and preserved at – 80°C until assayed.

Determination of biological markers

IA-2A, GADA, and ZnT8A were determined by liquid phase radiobinding assay [9,16], *HLA-DQA1*, *-DQB1* polymorphisms by PCR allele-specific oligonucleotide method [17] and C-peptide by time-resolved fluorescence immunoassay [18] as previously described. Cut-off values used for this study (except for ZnT8A) were derived from the 99th percentile of antibody levels in 761 Belgian control participants without diabetes after omitting outlying values. This corresponded to 2.6% tracer binding for GADA, and 0.44% for IA-2A. In the 2009 Diabetes Antibody Standardization Program, diagnostic sensitivity and specificity was 66% and 99% for IA-2A, 74% and 97% for GADA respectively. Investigation for ZnT8A was carried out at the Barbara Davis Center for Childhood Diabetes, University of Colorado at Denver as previously described [19]. Samples were centrifuged at 20,000g for 3 minutes to eliminate particulate matter prior to analysis. Using a dimeric zinc transporter-8 carboxy-terminal construct carrying respectively 325Arg and 325Trp, a ZnT8A index of 0.02 was determined as cut-off corresponding to the 99th percentile of ZnT8A levels in 200 Caucasian control subjects from Diabetes Antibody Standardization Program studies. Ketonuria, determined qualitatively using the Bayer ketostix reagent strips by scoring positive at readouts one + (ketonuria levels 20 mg/dl), was recorded whenever available in patient notes.

Exclusion of diabetes in control subjects

A glucose oxidase method (VITROS 5.1 FS/Vitros 4600 Integrated System, Ortho Clinical Diagnostics) was used for the assessment of glycaemia levels. Fructosamine levels were determined by a colorimetric test (Roche/Hitachi Cobas c systems) by reaction with nitroblue tetrazolium [20]. HbA1c levels were determined by immunoassay (Roche, Basel, Switzerland) on a Cobas Integra 400 analyzer (Roche). This method is certified by the National Glycohemoglobin Standardization Program as having documented traceability to the Diabetes Control and Complications Trial reference method. The OGTT was performed in accordance with ADA recommendations for the diagnosis of diabetes or IGT [21].

Statistical analysis

All statistical tests were carried out two-tailed using IBM SPSS statistics version 20.0 (Chicago, IL, USA), EpiInfo version 6 (USD, Stone Mountain, GA, USA) or GraphPad Prism version 5.00 for Windows (San Diego, CA, USA) and considered significant at $p < 0.05$ or for k independent tests, at $p < 0.05/k$ (Bonferroni correction). Differences involving categorical variables were assessed using the Chi-square test or Fisher's exact test if the expected number of subjects in any cell was less than 5. The Mann-Whitney U and

Kruskal-Wallis tests were used for continuous variables involving two groups or more than two groups respectively. Multivariate stepwise logistic regression was used to determine the predictors for the need of insulin therapy (dependent variable). Variables were tested as independent predictors in the model when $p < 0.05$ in univariate analysis. For the descriptive analysis, results were expressed as actual numbers (percentage) or as median (IQR).

Results

Characteristics of Cameroonian patients according to age

Of the 302 Cameroonian patients, only 27 (9%) were aged 0–14 years and 275 (91%) were aged 15 – 39 years (Table 1). There were 167 (55%) male and 135 (45%) female patients with a male/female ratio of 1.2. Of all patients, 115 (38%) were clinically classified as type 1 and 108 (36%) as type 2, while 79 (26%) were unclassifiable. Patients under age 15 were all on insulin therapy and had a significantly lower BMI ($p < 0.001$) than the older patients (Table 1). Although glycaemia tended to be slightly higher in the younger than in the older patient group, C-peptide levels and the presence of ketonuria were similar in both groups. Biological characteristics, especially autoantibody binding characteristics, did not differ between the two age groups (Table 1), allowing comparisons within the whole patient group according to antibody status.

Antibody profile of Cameroonian patients

Abs were determined in all 302 Cameroonian patients. Compared to controls, patients were more often positive for GADA (24% vs 7%, $p < 0.001$) and tended to have a higher prevalence of IA-2A (10% vs 3%, $p = 0.015$), but not of ZnT8A (4% vs 2%, $p = 0.239$, Table 2). Overall, 29% of patients vs 9% of controls ($p < 0.001$) were Ab⁺. Antibody titers did not differ between patients and controls. Ab⁺ patients were mostly positive for only 1 Ab type (76%), mainly GADA. Positivity for 2 Abs (15%) or 3 Abs (8%) was rare (ESM Fig 1A) — unlike in the Belgian cohort (ESM Fig 1B). GADA was present in 71/87 (82%) of Ab⁺ patients, and 12/17 (71%) of Ab⁺ controls (Table 2).

Characteristics of Cameroonian patients according to antibody status

At diagnosis, Ab⁺ patients ($n = 87$) shared some demographic, clinical and biological characteristics with Ab[−] patients ($n = 215$) but differed in others. Ab⁺ patients were more often treated with insulin ($p < 0.002$), had lower C-peptide levels ($p < 0.001$), more often carried *HLA-DQ2/non-DQ8* ($p < 0.001$), tended to have higher glycaemia levels at diagnosis ($p = 0.014$), and were underrepresented in patients with a familial history of diabetes ($p = 0.004$, Table 3). However, both groups showed similar characteristics in terms of age, sex-ratio, number of symptoms prior to diagnosis, clinical classification of diabetes and BMI (all $p > 0.05$, Table 3). Patients positive for 2 or more Abs tended to have a lower BMI, lower C-peptide levels, higher glycaemia and the presence of ketonuria compared to those positive for 1 Ab (ESM Table 1). When we stratified the study cohort into patients aged below ($n = 27$) and above ($n = 275$) age 15, Ab⁺ children (< 15 years) appeared to be more often male ($p = 0.02$), despite the presence of 3-fold more females in this small younger age group. Several other differences according to antibody status were only observed in the larger and older age group — 15 years (ESM Table 2). Patients in this Ab⁺ group tended to be younger

and to have a lower BMI than the Ab⁻ patients. After multivariate logistic regression, age at diagnosis ($p = 0.003$) and antibody status ($p = 0.006$) were predictors of insulin therapy (Table 4).

Characteristics of Cameroonian patients (0 – 39 years) according to the presence or absence of ketonuria

Data on ketonuria were available in a subgroup of patients ($n=167$) with an overall presence of 60%. The presence of ketonuria was associated with lower C-peptide levels ($p < 0.002$) and higher glycaemia levels ($p < 0.0001$) and tended to be associated with younger age ($p < 0.02$) and clinical type 1 diabetes ($p < 0.01$, ESM Table 3); but presence of ketonuria did not differ according to antibody status (Table 3).

Characteristics according to antibody status of Cameroonian and Belgian patients aged 15–39 years

Given the fact that only 9% of patients in the Cameroonian cohort were aged between 0 – 14 years and considering that characteristics and Ab-status of patients diagnosed before or after age 15 are clearly different in Belgium [22], we only compared the data of Cameroonian patients aged 15 – 39 years with those of the Belgian cohort. In both countries, the sex-ratio, number of symptoms, and C-peptide levels of Ab⁺ patients were similar (Table 5). Only 43% of Cameroonian Ab⁺ patients were clinically classified as having type 1 diabetes compared to 96% for the Belgian cohort ($p < 0.001$). In comparison with the Belgian patients, Cameroonian Ab⁺ patients were older ($p < 0.001$) and had a higher BMI ($p < 0.001$), and a lower prevalence of IA-2A ($p = 0.001$) and ZnT8A ($p < 0.001$, Table 4). GADA was the most prevalent autoimmune marker with comparable frequencies in both cohorts (82% in Cameroon vs. 88% in Belgian patients, Table 4). The prevalence of multiple antibody positivity was 18/79 (23%) for Cameroonian patients compared to 198/289 (69%) for Belgian patients (Table 5). In cases of Ab-positivity, titers were higher in Belgian than in Cameroonian patients ($p < 0.003$, Table 5). With respect to genetics, the high-risk genotype *HLA DQ2/DQ8* was completely absent in the Cameroonian cohort but present in 67/289 (23%) of Ab⁺ Belgian patients (Table 5). The frequency of *DQ8/nonDQ2* was also higher in Belgian patients ($p < 0.001$), while Cameroonian Ab⁺ patients showed a higher prevalence of *nonDQ8/nonDQ2* ($p < 0.001$, Table 5). Comparing data of Ab⁻ patients from both cohorts, Belgian patients had higher C-peptide levels ($p < 0.001$), higher frequencies of the *HLA-DQ* high-risk genotypes ($p < 0.001$), and a lower prevalence of *non-DQ8/non-DQ2* ($p < 0.001$). Males were strongly overrepresented ($p = 0.002$) in the group of Ab⁻ Belgian patients (Table 5).

Discussion

A major finding in our study was the low prevalence of children with diabetes (9%) and of Ab⁺ type 1 diabetes (29%) in Cameroon. This low prevalence in patients aged under 40 years at diagnosis is in sharp contrast with the much higher values (> 80%) for Belgian patients in the same age group [23]. Moreover, Cameroonian patients presented Ab positivity at lower titres than Belgian patients. Another striking observation was that ZnT8A could not differentiate Cameroonian diabetes patients from controls. In addition, the high

risk HLA genotype *DQ2/DQ8* appeared to be completely absent in our Cameroonian cohort, while being present in nearly one out of four Belgian autoantibody positive patients. Lower levels of C-peptide and the need for insulin therapy were associated with autoantibody positivity. Antibody positivity and younger age at diagnosis independently predicted the need for insulin therapy.

The strengths of this study are (i) the larger sample size compared to other studies from SSA (ii) the implementation of the BDR methodology for patient recruitment to enhance data comparison (iii) the analysis of Cameroonian samples in the Central BDR laboratory using the same techniques and conditions and (iv) an in-depth coverage and comparison of data. The Cameroonian data were, however, hospital-based and might hence be less representative than the registry-based Belgian data [22]. Although both cohorts comprised of recent-onset patients, the median disease duration for Cameroonian patients was longer, although this slight difference in disease duration probably has had no impact on the autoantibody status of the patients [24]. In the absence of secondary ascertainment, real epidemiologic observations on incidence and prevalence rates could not be made in our study.

The present study supports previous data from SSA [25] of a low incidence of type 1 diabetes in children compared to Caucasian records [23]. In this urban clinic population with well-developed health facilities, it is unlikely that the low incidence is due to early deaths or misdiagnosis [2,3]. Our study also confirms previous data from SSA [12–14] of a lower prevalence of Abs in diabetes patients compared to reports from western populations [6,9,23,26]. In sharp contrast to the observations in Caucasian populations, the ZnT8A marker could not differentiate diabetes patients from controls in Cameroon. This leaves us with GADA as the most significant autoantibody marker for this population, as has been observed in a previous study in Cameroon [14]. Our observation that GADA positivity is associated with the absence of a family history of diabetes, is in agreement with the known higher familial clustering in (Ab⁻) type 2 diabetes patients [27], *versus* a higher prevalence of sporadic type 1 diabetes cases, characterized by lower transmission rates to offspring (5 – 10%) [28]. Taking into account our less clear differentiation between patients and controls observed for IA-2A, we conclude that GADA is indeed the first choice if autoantibodies are to be measured in a population of SSA diabetes patients.

The autoimmune component is clearly much smaller in our Cameroonian study population. Our observations emphasize the heterogeneity of diabetes, and are suggestive of differences in the aetiopathogenesis of the disease across populations. This notion is further corroborated by remarkable differences in the HLA class II-inferred risk between Cameroonian and Belgian diabetes patients, with the high risk *DQ2/DQ8* genotype being completely absent in the whole group of 302 Cameroonian patients. Yongsoo [29] attributed the low incidence of autoimmune type 1 diabetes mellitus in Asia to differences in environmental factors and *DRDQ* linkage disequilibrium; this may also be the case in Cameroon. Even in our small cohort of children with diabetes (aged < 15 years), who were all on insulin therapy, only 30% were Ab⁺. But despite the low Ab prevalence, autoantibody positivity was found to be a predictor of the need for insulin therapy. Although our method for assessing ketonuria was qualitative and not a reliable measure for diabetic ketoacidosis, ketonuric patients had higher glycaemia and lower C-peptide levels and may probably

complement antibody determinations in disease stratification. Such biomarkers of beta cell function and antibody status in this patient group are in agreement with its described heterogeneity. There was no case of diabetic ketoacidosis as none of our patients showed the clinical pattern that characterizes ketosis prone diabetes from patients in the same unit [30,31]. Further steps towards improved diagnosis in this heterogeneous patient group may require both C-peptide and GADA determinations, while additional markers could be developed following the recently described alterations in amino acid metabolism [32], in combination with determination of *PAX4* risk haplotypes [33].

A negative correlation has been reported between C-peptide levels and the number of autoantibody specificities observed in a Swedish study [34]. Despite the high prevalence of solitary Ab⁺ in our Cameroonian patients, their C-peptide levels were similar to those of Belgian patients, which may be suggestive of undiscovered autoimmune or non-autoimmune causes of beta cell failure in SSA. Although we have not always been able, for practical reasons, to determine all biological markers in the same initial patient sample at diagnosis, we observed an association between lower C-peptide levels on the one hand and antibody positivity, need for insulin therapy and ketonuria on the other; this underlines the clinical relevance of measuring C-peptide for diabetes patients in Africa. We therefore agree with the recommendations of Törn C *et al* regarding the use of random C-peptide levels for probing beta cell function in diabetes for low income populations [35].

In conclusion, autoantibodies play a limited role in diabetes classification in Cameroon due to their low prevalence, but support the patients' need for insulin therapy. They could also play a role in more refined classification of ketonuric patients together with C-peptide determinations. Among the biological markers, C-peptide should be the first option for routine laboratory support of diabetes classification and determination of the need for insulin therapy in all patients in Cameroon. This notion is consistent with earlier studies on diabetes in SSA [13,36,37]; it has recently led to the introduction of an assay method for C-peptide determinations for diabetes patients in the region of Yaoundé – Cameroon [38]. Further studies should be undertaken to show that the implementation of better diagnostic tools actually leads to better patient treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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MA coordinated the CAMDER project and recruited subjects, acquired data, analyzed and interpreted data, provided statistical analysis and wrote the manuscript; FKG designed research, obtained funding, supervised the study, analyzed and interpreted data, reviewed/edited the manuscript, IW designed research, supervised activity, reviewed/edited the manuscript; BVD designed research, obtained funding, contributed genetics data, reviewed/edited the manuscript; FA designed research, contributed genetics data, reviewed/edited the manuscript; EM recruited subjects, contributed genetics data, reviewed/edited the manuscript; PG acquired data, reviewed/edited manuscript, KV acquired data, reviewed/edited manuscript; JMW and JCH contributed new reagents/analytical tools, acquired data, contributed to discussion and reviewed/edited the manuscript; ES designed research, contributed to discussion and reviewed/edited the manuscript; DGP designed research, contributed to discussions, reviewed/edited the manuscript; BK contributed to discussions, reviewed/edited the manuscript, JCM designed research, obtained funding, supervised activity, reviewed/edited the manuscript; CvS designed research, obtained funding, supervised activity, reviewed/edited the manuscript.

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Table 1

Demographic, clinical and biological characteristics of Cameroonian patients according to age at diagnosis

Characteristics	< 15 years n = 27	15 years n = 275	p-value
Demographic			
Age, years, P ₅₀ , IQR	12 (11–14)	32 (26–36)	
M/F, n/n (ratio)	7/20 (0.35)	160/115 (1.4)	0.003
Family history of diabetes, n/n (%)	3/15 (20)	91/219 (42)	0.169
Clinical			
BMI, kg/m ² , P ₅₀ (IQR) (n=26 vs 262)	21 (16–24)	26 (22–30)	< 0.001
> 1 symptom ^a , n/n (%)	26 (86)	251 (91)	0.711
Insulin treatment, n (%)	27 (100)	174 (63)	< 0.001
Type 1 diabetes, n (%)	14 (52)	101 (37)	0.181
Type 2 diabetes, n (%)	5 (19)	103 (37)	0.080
Unclassifiable, n (%)	8 (30)	71 (26)	0.841
Biological			
C-peptide, nmol/L, P ₅₀ (IQR)(n=15 vs 223)	0.20 (0.07–0.28)	0.25 (0.11–0.44)	0.159
Glycaemia at diagnosis, mmol/L, P ₅₀ (IQR)	19 (14–22)	17 (12–20)	0.078
Ketonuria ^b , n/n (%)	9/13 (69)	91/154 (59)	0.673
GADA ⁺ , n (%)	6 (22)	65 (24)	0.942
IA-2A ⁺ , n (%)	3 (11)	26 (9)	0.733
ZnT8A ⁺ , n (%)	0 (0)	12 (4)	0.610
1 Abs ⁺ , n (%)	8 (30)	79 (29)	0.901
2 Abs ⁺ , n (%)	1 (4)	18 (7)	1.000
GADA, P ₅₀ (IQR), % tracer bound ^c	34 (6–1836)	9 (4–59)	0.264
IA-2A, P ₅₀ (IQR), % tracer bound ^c	17 (0.45–48)	0.65(0.55–1.0)	0.496
ZnT8A, P ₅₀ (IQR), index ^c	NA	0.08(0.06–0.14)	NT
DQ2/DQ8, n (%)	0 (0)	0 (0)	NT
DQ8/nonDQ2, n (%)	0 (0)	8/273 (3)	1.000
DQ2/nonDQ8, n (%)	9 (33)	35/273 (13)	0.009
nonDQ8/nonDQ2, n (%)	18 (67)	230/273 (84)	0.036

P₅₀, median; Threshold for significance: $p < 0.05/23$ or $p < 0.002$ (Bonferroni correction); NA=not applicable; NT = not tested^aWeight loss, fatigue, polyuria;^bKetonuria only tested in 167 patients;^cAb titer only for Ab⁺;

Table 2

Prevalence of the diabetes-associated autoantibodies in patients with diabetes and a group of control subjects without diabetes from Cameroon (0–39 years)

Antibody marker	Patients n = 302	Controls n = 184	p-value
GADA ⁺ , n (%)	71 (24)	12 (7)	< 0.001
IA-2A ⁺ , n (%)	29 (10)	6 (3)	0.015
ZnT8A ⁺ , n (%)	12 (4)	3 (2)	0.239
GADA, P ₅₀ (IQR), % tracer bound*	9.4 (4.1–59.9)	6.3 (3.3–30.9)	0.166
IA-2A, P ₅₀ (IQR), % tracer bound*	0.7 (0.5–1.2)	1.1 (0.8–4.6)	0.092
ZnT8A, P ₅₀ (IQR), index*	0.08 (0.06–0.17)	0.05 (0.03–0.13)	0.279
1 Ab ⁺ , n (%)	87 (29)	17 (9)	< 0.001
2 Abs ⁺ , n (%)	19 (6)	3 (2)	0.030

P₅₀, median; Threshold for significance: $p < 0.05/8$ or $p < 0.006$ (Bonferroni correction),

* Ab titer only for Ab⁺;

Table 3

Demographic, clinical and biological characteristics of patients with diabetes from Cameroon (0 – 39 years) according to antibody status.

Characteristics	Antibody-positive n = 87	Antibody-negative n = 215	p-value
Demographic			
Age, years, P ₅₀ (IQR)	30 (19–34)	32 (25–35)	0.053
M/F, n/n (ratio)	54/33 (1.6)	113/102 (1.1)	0.168
Family history of diabetes, n (%)	17/68 (26)	77/166 (46)	0.004
Clinical			
BMI, kg/m ² , P ₅₀ (IQR) (n=84 vs. 207)	24 (20–28)	26 (21–29)	0.083
> 1 symptom [*] , n (%)	80 (92)	197 (92)	1.000
Insulin treatment, n (%) (n=86 vs. 211)	70 (81)	131 (61)	0.002
Ketonuria, n (%)	35/51 (69)	65/116 (56)	0.175
Type 1 diabetes, n (%)	37 (43)	78 (36)	0.378
Type 2 diabetes, n (%)	29 (33)	79 (37)	0.326
Unclassified, n (%)	21 (24)	58 (27)	0.424
Biological			
C-peptide, nmol/L, P ₅₀ (IQR) (n=70 vs. 168)	0.16 (0.06–0.36)	0.30 (0.16–0.47)	< 0.001
Glycaemia at diagnosis, mmol/L, P ₅₀ (IQR)	18 (13–23)	16 (12–20)	0.014
DQ2/DQ8, n (%)	0/87 (0)	0/213 (0)	NT
DQ8/nonDQ2, n (%)	3/87 (3)	5/213 (2)	0.695
DQ2/nonDQ8, n (%)	24/87 (28)	20/213 (9)	< 0.001
nonDQ8/nonDQ2, n (%)	60/87 (69)	188/213 (88)	0.043

P₅₀, median; threshold for significance: $p < 0.05/16$ or $p < 0.003$ (Bonferroni correction); NT = not tested

* weight loss, fatigue, polyuria;

Table 4

Binary stepwise logistic regression analysis for prediction of insulin therapy in 222 diabetic patients from Cameroon (Insulin treated=74/222)

Insulin therapy = dependent variable	Univariate	Multivariate forward stepwise	
	<i>p</i> -value	<i>p</i> -value	OR (95% CI)
Sex	0.505	NT	-
Age at diagnosis < 31 vs ≥ 31 ^a	0.001	0.003	0.41 (0.22 – 0.74)
BMI, kg/m ²	0.344	NT	-
Family history of DM	0.923	NT	-
Type of diabetes	0.133	NT	-
Antibody status ^b	0.002	0.006	2.80 (1.34 – 5.86)
C-peptide, nmol/L	0.026	NS	-

NS = not significant. DM, diabetes mellitus

^aMedian age = 31 years,

^bAntibody status: Ab⁻ = 0 and Ab⁺ = 1, NT= not tested;

Table 5

Demographic, clinical and biological characteristics of antibody-positive (Ab^{+}) and negative (Ab^{-}) patients aged 15–39 years at diagnosis

Characteristics	Ab ⁺		Ab ⁻		p-value	p-value
	Cameroon n = 79	Belgium n = 289	Cameroon n=196	Belgium n = 285		
Demographic						
Age, years, P ₅₀ (IQR)	30 (21–36)	25 (20–31)	33 (28–36)	33 (27–36)	<0.001	0.412
M/F, n/n (ratio)	49/30 (1.6)	168/121 (1.4)	111/85 (1.3)	201/84 (2.4)	0.621	0.002
Clinical						
BMI, kg/m ² , P ₅₀ (IQR) (n=77 vs 269 and 190 vs 269)	25 (21–29)	21 (19–24)	26 (22–30)	26 (23–32)	<0.001	0.370
> 1 symptom ^a , n/n (%)	66/71 (93)	271/281 (96)	178 (91)	261 (92)	0.196	0.899
Type 1 diabetes, n (%)	34 (43)	276 (96)	67 (34)	127 (45)	<0.001	0.029
Biological						
C-peptide, nmol/L, P ₅₀ (IQR) (n= 64 vs 223 and 159 vs 124)	0.17 (0.06–0.36)	0.17 (0.09–0.25)	0.29 (0.16–0.47)	0.42 (0.24–0.82)	0.794	<0.001
GADA ⁺ , n (%)	65 (82)	254 (88)	-	-	0.265	-
IA-2A ⁺ , n (%)	26 (33)	156 (54)	-	-	0.001	-
ZnT8A ⁺ , n (%)	12 (14)	180 (62)	-	-	<0.001	-
2 Abs ⁺ , n (%)	18 (23)	198 (69)	-	-	<0.001	-
GADA, P ₅₀ (IQR), % tracer bound ^b	8.8 (3.9–59)	40 (10–207)	-	-	<0.001	-
IA-2A, P ₅₀ (IQR), % tracer bound ^b	0.6 (0.6–0.9)	40 (4.0–274)	-	-	<0.001	-
ZnT8A, P ₅₀ (IQR), index ^b	0.08 (0.06–0.14)	0.47 (0.20–0.89)	-	-	0.002	-
DQ2/DQ8, n (%)	0 (0)	67 (23)	0/194 (0)	20 (7)	<0.001	<0.001
DQ8/nonDQ2, n (%)	3 (4)	85 (29)	5/194 (3)	41 (14)	<0.001	<0.001
DQ2/nonDQ8, n (%)	19 (24)	92 (32)	16/194 (8)	72 (25)	0.231	<0.001
nonDQ8/nonDQ2, n (%)	57 (72)	45 (16)	173/194 (89)	152 (53)	<0.001	<0.001

P_{50} , median; threshold for significance: $p < 0.05/17$ or $p < 0.003$ (Bonferroni correction)

^a weight loss, fatigue, polyuria;

^b Ab titer only for Ab^{+} ;