Progression of Nephropathy in Type 2 Diabetes: The Glycation Gap Is a Significant Predictor after Adjustment for Glycohemoglobin (Hb A₁c)

Santiago Rodrı´guez-Segade, 1,2* Javier Rodrı´guez, 1,2 Jose M. Cabezas-Agricola,3 Felipe F. Casanueva,3,4 and Félix Caminña1

BACKGROUND: The glycation gap has been proposed as an index of nonglycemic determinants of glycated hemoglobin (Hb A₁c). We investigated whether it predicts progression of nephropathy in type 2 diabetic patients.

METHODS: We recorded albumin excretion rate, Hb A₁c, and serum fructosamine in 2314 patients over an average of 6.5 years. Hb A₁c was regressed on fructosamine by using a repeated-measures longitudinal regression model and data for all visits of all patients; the raw glycation gap gg was calculated at each visit, as measured by Hb A₁c minus the value predicted by the regression; and the mean glycation gap (GG) was defined for each patient as the mean of the values for the raw glycation gap (gg) calculated at each visit. The study group was divided into high-, medium- and low-GG groups of equal sizes, which were compared for progression of nephropathy by Cox regression analyses controlling for age, sex, duration of diabetes, initial nephropathy status, therapy, baseline Hb A₁c, mean Hb A₁c, and mean fructosamine. The design of the study was a retrospective cohort study with follow-up for 6.5 (SD 4.2) years.

RESULTS: The gg exhibited considerable stability over time. In the high- and medium-GG groups, the risk of progression of nephropathy was respectively 2.5 and 1.6 times that of the low-GG group (P<0.0001 and P<0.001, respectively) after adjustment as described above.

CONCLUSIONS: GG predicts the progression of nephropathy in type 2 diabetic patients independently of fructosamine and even after adjustment for Hb A₁c. The joint use of the glycation gap and fructosamine as measures of nonglycemic and glycemic determinants of glycation, respectively, may improve evaluation of the risk of nephropathy and of the glycemic control desirable for the individual patient.

* Address correspondence to this author at: Laboratorio de Bioquı ´mica Clı ´nica, Complejo Hospitalario Universitario de Santiago, Travesı ´a de la Choupana s/n, 15706 Santiago de Compostela, Spain. Fax +34-981594912; e-mail ssegade@telefonica.net.

Received February 6, 2010; accepted October 18, 2010.
Previously published online at DOI: 10.1373/clinchem.2010.144949

The association of glycated hemoglobin (Hb A₁c) with the risk of diabetic complications is well established (1, 2) and is the basis of its use to guide the treatment of diabetes. Because the chains of reactions leading to the formation of Hb A₁c and to the formation of glycated proteins involved in diabetic complications all start with glucose, it is a priori reasonable to assume that the association between Hb A₁c and complications is largely due to this common origin and hence to mean blood glucose (MBG). Although a consensus committee acknowledged the flimsiness of the then-available evidence that Hb A₁c reflects MBG (3), a study performed in 1 West African, 3 European, and 6 US centers to provide more robust support (4) revealed close correlation between Hb A₁c and MBG over the previous 3 months (r² = 0.79 among 427 diabetic patients, r² = 0.84 when 80 nondiabetic patients were included). However, even in this study, which applied numerous exclusion criteria, 21% of the variance in Hb A₁c among diabetic patients was not explained by MBG; and in the Diabetes Control and Complications Trial (DCCT), 33% of the variance in Hb A₁c was not accounted for by MBG (5). There is, therefore, little justification for the assumption that the association between Hb A₁c and diabetic complications faithfully and exclusively reflects an underlying association between complications and MBG. In fact, there is growing evidence that there are nonglycemic determinants of Hb...
that should be taken into account in its interpretation (6–14). In keeping with this possibility, blood glucose concentrations account for no more than one-third of the variance in Hb A1c among nondiabetic individuals (15), among whom between-subject variation in Hb A1c is almost 3 times within-subject variation (16, 17).

As an indication that nonglycemic determinants of Hb A1c may be important for the clinical assessment of diabetic patients, Hempe et al. (18) reported that the hemoglobin glycation index (HGI) (the difference between observed Hb A1c and the value calculated from its regression on MBG) differed among type 1 diabetic patients, was stable over time for a given patient, and was not related to erythrocyte turnover. In fact, when type 1 diabetic patients in the DCCT were ranked by mean HGI during the study, the risks of retinopathy and nephropathy among patients in the top third were at all MBG levels several times those of patients in the bottom third (19). However, because HGI and Hb A1c (as the residual and dependent variable of a regression) are mutually correlated, it has been questioned (20) whether there is any value in attempting to partition observed Hb A1c into a “standard” value for a given MBG level plus a correction determined by greater or lesser propensity for glycation (HGI). For example, reanalysis of the DCCT data shows that HGI is not a significant risk factor for microvascular complications if Hb A1c itself is included in the analysis (20).

Fructosamine is a measure of average glycemia over a shorter period than Hb A1c, but its concentration in plasma is much more stable than that of glucose itself and is much easier to measure than true MBG, which requires continuous blood glucose monitoring or sufficiently frequent 7-point profiles. In a study of 40 type 1 diabetic patients, a 1% increase in a “glycation gap” computed in the same way as the HGI, but using the regression of Hb A1c on fructosamine, was found to increase the risk of more advanced nephropathy 2.9-fold, although there was no significant correlation between more advanced nephropathy and either Hb A1c or fructosamine (21). In the study described here, data on 2314 type 2 diabetic patients followed up for an average of 6.5 years were used to investigate whether the fructosamine-based glycation gap is stable within individuals and is a significant predictor of progression of nephropathy when adjusted for Hb A1c.

Materials and Methods

STUDY POPULATION

Ours is a tertiary center serving a broad cross-section of an almost exclusively white population of about 450,000 individuals including a majority of local diabetic patients requiring insulin or oral antidiabetic medications. In the present study, we included relevant data for all patients who between March 1992 and March 2007 were prescribed insulin or oral antidiabetic drugs for type 2 diabetes and who satisfied the following additional inclusion criteria: (a) the patient had been followed up for at least 1 year; (b) throughout follow-up, nephropathy had been evaluated at least once each year on the basis of albumin excretion rate (AER); (c) each such AER measurement was accompanied by determination of serum fructosamine and Hb A1c; and (d) the patient had no known hemoglobinopathy or erythrocyte disorder.

Like the DCCT (2), we distinguished between a primary cohort of patients with a 1- to 5-year history of diabetes, no ophthalmoscopic retinopathy, and AER <40 mg/24 h at “entry” (i.e., at the date of the earliest determinations included in the study) (1225 patients) and a secondary cohort of patients with a 1- to 15-year history of diabetes, slight or moderate ophthalmoscopic retinopathy, and AER <200 mg/24 h at entry (1089 patients). Other variables recorded at entry included age, sex, duration of diabetes, and type of therapy. Serum fructosamine and Hb A1c levels were included in the study not only at each AER evaluation, but for each occasion on which both had been determined (hereinafter “visit”), except that for patients showing progression of nephropathy (see below), no data were included after the visit on which progression was observed.

As in the DCCT, patients were deemed to have undergone progression of nephropathy if AER was ≥100 mg/24 h and had been <40 mg/24 h at entry, or if AER was ≥300 mg/24 h and had been <200 mg/24 h at entry (19). Following previous authors (19, 20), but with fructosamine as a predictor (21), the (gg) glycation gap was calculated as follows. First, Hb A1c was regressed on fructosamine by using a repeated-measures longitudinal regression model (22), and data for all patients and all visits included in the study, and a “raw” glycation gap (gg), were calculated for each visit as observed Hb A1c minus the Hb A1c value predicted from the regression equation obtained. After verification of the stability of gg (see the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue2), a characteristic mean glycation gap (GG) was then calculated for each patient by averaging his or her raw gg values (19, 20). Finally, the patients were ranked by GG and classified by tertiles as high-, medium-, or low-GG subjects.
study, all Hb A₁c values were converted from Japanese Diabetes Society/Japanese Society for Clinical Chemistry–referenced values to DCCT-aligned units by using the equation Hb A₁c NGSP = 0.985 Hb A₁c JDS/JSCC + 0.46% (where NGSP stands for National Glycohemoglobin Standardization Program) (23). Until January 2001, fructosamine was determined by the nitroblue tetrizolium (NBT) method (Roche Diagnostics), with an interassay CV of 2.9% at 296 μmol/L fructosamine and 1.7% at 521 μmol/L fructosamine and, after that date, by the GlyPro enzymatic method (Genzyme) with an interassay CV of 1.8% at 175 μmol/L fructosamine and 0.91% at 640 μmol/L fructosamine (in both cases, a Cobas Mira analyzer was used). For use in Cox analyses, etc., NBT-derived values were transformed by using the equation Fructosamine₆₉₂₀ = 1.33Fructosamine₆ₙₙ – 127.5, which was obtained by a regression analysis of the results for 188 randomly selected samples for which both methods were used (r = 0.991). Albumin in 24-h urine samples was measured by using a Dade Behring BN nephelometer from Siemens Healthcare Diagnostics with an interassay CV of 2.2% at 79 mg/L albumin and 0.9% at 230 mg/L albumin.

STATISTICAL ANALYSIS

Raw Hb A₁c was regressed on raw fructosamine by using a longitudinal linear model following both (a) appropriate data weighting to take between-patient variation in number of visits into account and (b) confirmation of linearity by means of a spline-fitting algorithm that made no prior assumptions regarding slope. A random intercept model was used on the basis of the Akaike information criterion, which showed it to provide a better fit than either the random slope model or the random slope, random intercept model.

In evaluating the relative values of GG and Hb A₁c for assessment of risk of progression of nephropathy by means of Cox regression analyses, Hb A₁c and fructosamine were included as updated mean (u.m.) values, where the u.m. value of a variable (x) at each visit of a patient was obtained by estimation of the mean of all values of each variable in all visits up to and including that visit [the measured value of each variable (x) is termed “raw x” to prevent confusion with u.m. x].

Multivariate Cox regressions adjusted for tied event times were performed with progression of nephropathy as the dependent variable and age at baseline, sex, duration of diabetes at baseline, therapy, and cohort as “basic covariates,” and u.m. fructosamine, u.m. Hb A₁c, and GG as individually optional additional covariates; u.m. Hb A₁c and u.m. fructosamine were treated as time dependent. In addition, Cox analyses in which the study group was trichotomized by GG tertiles, baseline Hb A₁c was also an optional covariate. Continuous covariates were entered as linear terms (nonlinearity was ruled out by examining alternative models by using penalized smoothing splines), and the assumption of proportional hazards was validated before these analyses by examination of Schoenfeld residuals. All statistical calculations were performed by using either SPSS v.15 or Stata v.10.

Results

Table 1 summarizes baseline characteristics of the 2314 type 2 diabetic patients studied. Mean follow-up time was 6.5 years (SD 4.2 years). All patients made at least 1 visit per year during follow-up, and 43.1% of the low-GG group, 47.9% of the medium-GG group, and 47.1% of the high-GG group made an average of 2 visits per year. In all, data collected in a total of 21 960 visits were included in the study (mean 9.5 visits per patient). AER was measured on 17 540 of these occasions (mean 8 measurements per patient).

The stability of the gg was shown by close correlation (r > 0.84) between gg values determined for the same patient on different occasions. Accordingly, the characteristic GG of each patient was calculated by averaging his or her gg values. The distribution function of GG was normal (Gaussian) (P > 0.05), with mean 0.01% and SD 1.01%; for 336 patients (14.5%) GG was >1%, and for 363 (15.7%), <−1%.

Fig. 1 shows the regression of raw Hb A₁c on raw fructosamine in the 3 GG groups (r = 0.800, 0.922, and 0.806 for the low-, medium-, and high-GG group, re-

| Table 1. Baseline characteristics of the study group (n = 2314). |
|-----------------|-------|-------|
| Sex             |       |       |
| Men             | 1118  | 48.3  |
| Women           | 1196  | 51.7  |
| Age (years)     |       |       |
| Primary         | 1225  | 52.9  |
| Secondary       | 1089  | 47.1  |
| White race (%)  | 100   |       |
| Known duration of diabetes (years) | 6.0 (5.6) |
| Treatment       |       |       |
| Insulin         | 687   | 29.7  |
| Oral antidiabetic drugs | 1627 | 70.3  |
| Hb A₁c (%)      | 7.5   | 2.0   |
| Fructosamine (μmol/L) | 322 | 81    |

a n (%). b Mean (SD).
As was to be expected on statistical grounds, in the whole sample, raw \( gg \) was not correlated with raw fructosamine but was strongly correlated with raw Hb A1c \((r = 0.778; \text{Fig. 2})\). Similarly, the overall mean Hb A1c level in the GG groups increased in the order low-GG \((P = 0.001)\), whereas mean fructosamine did not differ significantly among the GG groups (see the online Supplemental Table). Adjustment for the basic covariates reduced the correlation between raw Hb A1c and raw fructosamine in the whole sample to 0.621 and increased the correlation between raw Hb A1c and \( gg \) to 0.819.

Of the 2314 patients studied, 487 (21%) underwent progression of nephropathy. In the primary cohort, progression affected 170 patients (13.9%), and in the secondary cohort, it affected 317 patients (29.1%). Cox analyses with just 1 covariate in addition to the basic set showed that the risk of progression of ne-

**Fig. 1.** Scatter plots of raw (observed) Hb A1c against raw fructosamine (data for all visits) and the corresponding simple regression lines (solid lines).

(A), whole study group \((\text{Hb A1c} = 0.012 \times \text{fructosamine} + 3.16; R^2 = 0.46)\); (B–D), low-, medium-, and high-GG groups, respectively \((R^2 = 0.64, 0.85, \text{and } 0.65, \text{respectively})\). The dashed lines in (B–D) show the regression for that group, while continuous lines reproduce the regression line for the whole group (A).

**Fig. 2.** Scatter plots of Hb A1c (A) and fructosamine (B) vs \( gg \), showing data from all visits of all patients, with the estimated regression line and correlation.
Phropathy was significantly increased by u.m. fructosamine, u.m. Hb A1c, and GG, with hazard ratios of 1.28, 1.24, and 1.45, respectively, when the fructosamine-Hb A1c and Hb A1c-GG regressions are used to refer all hazard ratios to a 1% increase in Hb A1c (models 1–3, Table 2). When u.m. fructosamine and GG, which derive from uncorrelated raw variables, were both included in the analysis (model 5), both remained significant and their hazard ratios were the same as in models 1 and 3, respectively. By contrast, when redundant covariates derived from closely correlated raw variables were included (models 4 and 6), only the covariate with the greater predictive power emerged as significant: u.m. Hb A1c in model 4 and GG in model 6.

Table 2. Results of various multivariate Cox models of progression of nephropathy.

<table>
<thead>
<tr>
<th>Model number</th>
<th>Variables*</th>
<th>Hazard ratio (95% CI)b</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>u.m. fructosamine</td>
<td>1.283 (1.087–1.394)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>u.m. Hb A1c</td>
<td>1.238 (1.138–1.337)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3</td>
<td>GG</td>
<td>1.447 (1.300–1.615)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4</td>
<td>u.m. fructosamine</td>
<td>1.087 (0.920–1.181)</td>
<td>0.290</td>
</tr>
<tr>
<td>5</td>
<td>u.m. fructosamine</td>
<td>1.201 (1.089–1.320)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6</td>
<td>u.m. HbA1c</td>
<td>1.070 (0.951–1.201)</td>
<td>0.260</td>
</tr>
</tbody>
</table>

* Other than age at baseline, sex, duration of diabetes at baseline, therapy (insulin or oral antidiabetic drugs), and cohort (primary or secondary), which were adjusted for in all models.

b Hazard ratios for GG and fructosamine are given for a 1% rise in Hb A1c.

Discussion

First, the results of this large study confirm that gg values obtained for the same patient at different times are closely correlated (\(r > 0.84\)). Thus, gg is relatively stable, even in this retrospective observational study in which it was subject to marked iatrogenic fluctuation due to the difference between the times taken by serum fructosamine and Hb A1c to reflect changes in antiglycemic medication. Second, we found that GG is a significant predictor of the risk of progression of nephropathy in type 2 diabetic patients and that this predictor is particularly strong when combined with fructosamine, which is a marker of the rate of glycation.

![Fig. 3. Cumulative incidence of progression of nephropathy in the high-, medium-, and low-GG groups (results of fitting a Cox proportional hazards regression model including age, sex, duration of diabetes, therapy [insulin or oral antidiabetic drugs], cohort [primary or secondary], u.m. fructosamine, u.m. Hb A1c, and baseline Hb A1c).](image-url)
A limitation of this study that parallels the cause of criticism (20, 24) of previous similar studies (18, 19) is that gg, as the residual of the regression of Hb A\textsubscript{1c} on fructosamine, contains within-patient as well as between-patient contributions to its variability among all samples. Nevertheless, its observed degree of stability for a given individual in this 15-year study of 2314 type 2 diabetics followed up for an average of 6.5 years supports the thesis that some individuals have an intrinsically greater propensity than others toward glycation of hemoglobin and seems sufficient to allow this propensity to be evaluated by some gg-based measure. The best available measure may well be the value of gg in the untreated patient, averaged over enough measurements to smooth out variations in serum fructosamine. In the present study, following Cohen et al. (21), we made up for the absence of pretreatment measurements the best we could by using GG, the average of the patient’s gg determinations throughout the study.

A potential confounder for this study was between-patient differences in the mean age of circulating erythrocytes; in hematologically normal individuals, a large proportion of variation in Hb A1c is due to this cause (8). However, no significant differences in erythrocyte creatinine level have been observed between glycation groups, and hence there is no indication that the increase in the proportion of individuals with high HGI (7) or high GG (this study) that accompanies increasing mean Hb A1c is due to reduced erythrocyte turnover. In the present study, the low- and high-GG groups included similar proportions of patients with pathological creatinine concentrations (3.5% and 3.9%, respectively), so the prevalence of kidney disease cannot have influenced our results.

The report by McCarter et al. that HGI is a strong predictor of retinopathy and nephropathy among the type 1 diabetic patients of the DCCT (19) has been criticized on the grounds that the correlation between HGI and Hb A1c, which is a mathematical necessity if the statistical model of the regression of Hb A\textsubscript{1c} on MGB is valid, might mean that HGI is merely a surrogate for Hb A\textsubscript{1c}. Reanalysis of the DCCT data shows no significant influence of HGI on the risk of nephropathy if Hb A\textsubscript{1c} is taken into account (20). McCarter et al. acknowledge this, but point out that what is of interest for researchers at this point is not whether Hb A\textsubscript{1c} is associated with risk of diabetic complications, which is well established, but whether this association is due wholly to blood glucose concentrations or is also contributed to by putative nonglucose determinants of Hb A\textsubscript{1c} (25). This is an open question, in part because the DCCT measurements of MBG (7-point profiles recorded on a single day every 3 months), though used successfully to predict the development of retinopathy

![Fig. 4. Mean Hb A\textsubscript{1c} (A) and incidence of progression of nephropathy (% PN) (B) in subgroups defined by the tertiles of both GG and mean fructosamine (where “mean” signifies updated mean at the last visit). For subgroups on the left-to-right diagonals, along which GG increases while mean fructosamine declines, the mean values of the dependent variables are shown on the corresponding column tops, and in (B), the mean values of fructosamine (\mu\text{mol/L}) are shown on the corresponding column side.](image)
(26), may have been insufficiently accurate measures of average blood glucose concentration for the purposes of McCarter et al. In other words, the DCCT data do not unequivocally clarify whether HGI reflects the effects of nonglucose determinants of Hb A1c, or merely inaccuracy in average blood glucose measurement (24). There is little possibility that in the present study the glycation gap merely reflected inaccuracy in the measurement of fructosamine. Unlike Cohen et al. (21) in their study of the glycation gap in type 1 diabetic patients, we found that not only higher GG, but also higher Hb A1c, was associated with more advanced nephropathy; Cohen et al.’s negative result is probably attributable to their small sample size (40 patients). In both studies, however, ordinal logistic regression or Cox analyses showed GG to be closely associated with progression of nephropathy and that significant association persisted when Hb A1c, fructosamine, and other factors were taken into account.

In the present study, u.m. fructosamine and GG were both significant when both were included in the Cox analysis (as is logical in view of the statistical independence of raw fructosamine and gg), but u.m. Hb A1c was not a significant predictor of progression of nephropathy when GG was included, nor was u.m. fructosamine if u.m. Hb A1c was included. This result suggests that the predictive value of Hb A1c may be due less to its association with fructosamine (a measure of extracellular glycation, and hence of what may be called glycation pressure) than to its relationship with the glycation gap, which may reflect nonglycemic determinants of hemoglobin glycation, or “glycability.”

A substantial proportion of variation in Hb A1c between individuals is of genetic origin, yet is independent of genes influencing fasting glucose (9). Illustrative of this, glycemic control fails to account for consistent differences of up to about 0.4% between the Hb A1c levels of different racial groups (10). These genetic results reinforce the implications of the temporal stability of gg and HGI in the sense that Hb A1c levels must be determined by multiple factors, not all of which are related to glycemia. Candidate factors include erythrocyte pH, and total plasma amino acid levels (8); liperoxides and antagonistic antioxidants (12); glycolytic and deglycating enzymes (6, 7, 21); processes related to glucose transporter 1–mediated transport (11); and erythrocyte transmembrane glucose gradient (27). When factors such as these have been more definitely related to the glycability of hemoglobin and/or of other proteins glycated in diabetes, they will doubtlessly be used in evaluating risk of complications. Meanwhile, it may be asked whether the glycation gap, as a gross measure of glycability, can usefully be employed for this purpose. Our results suggest that it can.

For prediction of diabetic complications in a temporal sense, it is of course necessary to be able to determine an individual’s characteristic glycation gap in a much shorter time than the average 6.5-year follow-up of this study. As noted above, the best measure of the gap may be the value of gg in the untreated patient, averaged over enough measurements to smooth out variations in fructosamine. If pretreatment determinations are not available, a good alternative may be to average 3 or 4 determinations at 1- to 2-week intervals during a period of good, stable glycemic control. We have in fact observed that in the cases of a few patients whose first Hb A1c determinations may have taken place before treatment, the corresponding gg values were indeed very close to those observed during periods of good control.

Finally, it may not be too redundant to point out explicitly the possible implications of the present findings for clinical practice. If neither reliable MBG values nor fructosamine values are obtained, so that the only guiding biochemical parameter is Hb A1c, and the glycation gap is not taken into account in interpreting Hb A1c, then the MBG concentrations of a low-GG patient are probably higher than they appear to be. Although a low-GG patient intrinsically has below-average risk of complications, there might therefore be room to reduce risk further by tighter control of glycemia. On the contrary, the MBG concentrations of a high-GG patient are probably lower than they appear to be, and therapy that does not take this into account may introduce an unnecessarily large risk of hypoglycemia.

In conclusion, the results of the present study of a large sample of type 2 diabetic patients followed up for an average of 6.5 years show that the glycation gap is a stable characteristic of these patients that, when evaluated as GG, predicts the progression of nephropathy, even after adjustment for Hb A1c and fructosamine and independently of the latter. The joint use of the glycation gap and fructosamine as markers of hemoglobin glycability and glycation pressure, respectively, may improve the evaluation of diabetic patients, allowing glycemia control targets to be set in accordance with the extent of the individual patient’s predisposition to protein glycation. Research into the most convenient means of evaluating the glycation gap of new and treated patients is in progress. Future studies should address the possible relationships of the glycation gap with complications of diabetes other than nephropathy and the glycation of proteins other than hemoglobin.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting
or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: None declared.

Consultant or Advisory Role: None declared.

Stock Ownership: None declared.

Expert Testimony: None declared.

Research Funding: S. Rodríguez-Segade and F. Camiña, the Secretariat General for Research and Development of the Xunta de Galicia, Spain (Refs. PGIDIT02BTF20303PR, PGIDIT04BTF203016PR, and PGIDIT06BTF20302PR), the Spanish Ministry of Education and Science (Ref. SAF2004-07602). S. Rodríguez-Segade, Siemens Health-care Diagnostics and Menarini Diagnostics.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: We also acknowledge the statistical guidance of Dr. Juan Manuel Paz and Dr. Francisco Gude of the Clinical Epidemiology Unit of our hospital.

References


