Antibodies against Synthetic Deamidated Gliadin Peptides for Celiac Disease Diagnosis and Follow-Up in Children

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Celiac disease is a common medical problem, its estimated prevalence being 0.5% and 1.26% (1:200 and 1:79) in North America and Western Europe populations, respectively (1, 2). Although in Northern European countries the prevalence of celiac disease is high, in Italy it is reported to range from 0.2% to 0.94% (3). Although fully developed gluten-induced villous atrophy associated with clinical symptoms of malabsorption (classic celiac disease) is frequently found in patients with celiac disease, atypical or silent disease is not uncommon in children or adults (4, 5). In the absence of overt clinical signs, celiac disease may escape diagnosis and therefore may not be treated appropriately, with a consequent increase in morbidity and mortality (6, 7).

The gold standard for diagnosing celiac disease is histology. Patients with this condition have an increased intraepithelial lymphocyte (IEL)5 count, crypt hyperplasia, and villous atrophy in the duodenal mucosa. The development of mucosal lesions, a dynamic process, covers a broad spectrum of histologic forms, including normal mucosa with increased intraepithelial lymphocytes on the one hand and classic flat mucosa on the other (8, 9). Histology is the first tool in the diagnosis of this condition, and serological tests are the second (1, 2, 10–14). Serological tests comprise a series of assays, including antigliadin antibodies, antienthymic antibodies (EMA), antitissue transglutaminase antibodies (tTG) of the IgA class, and the recently introduced antibodies antisynthetic deamidated gliadin peptides (AGA) (10–29). Antigliadin antibodies have a sensitivity and specificity of about 80%, which is lower than that of EMA or tTG IgA, thus allowing the clinical laboratory counselor to avoid antigliadin antibody measurement for celiac disease diagnosis (10, 12, 21). Several researchers have found that tTG

BACKGROUND: AGA IgA II and AGA IgG II have recently been suggested as reliable tools for celiac disease (CD) diagnosis. We compared their utility for diagnosis and monitoring CD in children with that of tTG IgA, an established CD marker.

METHODS: We studied a cohort of 161 CD and 129 control children in whom CD was histologically confirmed or ruled out. We followed 37 children with CD on a gluten-free diet for 12–84 months. In fasting sera, we measured AGA IgA II, AGA IgG II, and tTG IgA using ELISAs.

RESULTS: The best sensitivity (92.5%), specificity (97.6%), positive predictive value (98%), and negative predictive value (91.2%) were obtained using tTG IgA. AGA IgG II correctly identified 3 of 3 children with CD with total IgA deficiency who had negative AGA IgA II and tTG IgA results. In children <2 years old without total IgA deficiency, AGA IgG II and tTG IgA performed equally well (sensitivity 96.4% and specificity 100%). AGA IgA II, AGA IgG II, and tTG IgA concentrations diminished significantly (P < 0.0001) after 1 year of a gluten-free diet, reaching values below the cutoff in 87%, 70%, and 51% of cases, respectively.

CONCLUSIONS: The best available index for diagnosing CD in children was tTG IgA. In infants <2 years old, AGA IgG II performed as well as tTG IgA in cases without total IgA deficiency and allowed detection of CD when total IgA was <0.06 g/L. Gluten-free diet monitoring can be achieved using any of the studied serum markers.

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IgA overlap with, or even surpass, EMA sensitivity and specificity, which are close to 95%–100% (12, 15, 17–21, 24, 25); such findings are to be expected, given that the dominant antigen of endomysial antibodies is transglutaminase, and both methods measure anti-transglutaminase antibodies (30). Some authors, including ourselves, suggest that tTG IgA alone might be used in the flow chart of celiac disease diagnosis, because this type of measurement is accurate and not operator-dependent (20, 21, 25, 31–33). A diagnostic ELISA for celiac disease, which uses a synthetic deamidated peptide derived from gliadin as its antigen, has recently been suggested as a reliable tool for celiac disease diagnosis (22, 26–29). However, it has yet to be established whether this test has a role in the diagnosis and monitoring of celiac disease independent of tTG IgA.

The aim of the present study was to ascertain the diagnostic utility of tTG IgA and of antibodies of the IgA and IgG classes directed against synthetic deamidated gliadin peptides (AGA IgA II and AGA IgG II) in the diagnosis and follow-up of celiac disease in children.

Materials and Methods

We conducted this retrospective cohort study of 290 children [110 boys, 180 girls; mean age (SD) 7.65 (4.4) years, median 8, range 1–17] on a gluten diet who consecutively underwent upper gastrointestinal endoscopy in 2002–2007, during which multiple gastric and duodenal biopsies were obtained for histology. A diagnosis of celiac disease (CD) based on histology was made in 161 patients [56 boys, 105 girls, age 6.5 (4.1) years, median 6, range 1–17] and excluded in the other 129 patients [54 boys, 75 girls; age 9.0 (4.4) years, median 9 years, range 1–17 years]; the latter patients made up the control group. By the Marsh–Oberhuber classification of celiac lesions (8), 8 children were classified as type 1 (normal villous architecture and increase in IEL), 2 as type 2 (normal villous architecture and increase in IEL and crypt hyperplasia), 50 as type 3a (mild villous flattening), 20 as type 3b (marked villous flattening), and 81 as type 3c (total villous flattening). In all cases, a fasting blood sample was obtained before performing endoscopy. After centrifugation at 3000g for 5 min, sera were stored at −20 °C until biochemical assays were performed. Fully written informed consent was obtained in writing from the parents of all children included in the study, and the study protocol was approved by the institutional review board committee.

Of the 161 children diagnosed as having CD, 47 [19 boys, 28 girls; age 6.0 (3.7) years; median 5; range 1–14] were followed for 30 (18) months, median 24, range 12–84. All of these children were on a gluten-free diet (GFD) and in clinical remission. Compliance with the diet was ascertained through interviews conducted by an expert dietitian. A further group of 13 children underwent a second endoscopy: 5 were CD children without clinical remission and 8 were non-CD children with persistent abdominal discomfort, which was associated with increased serum levels of antibodies in 5 cases (see Table 3 for details).

We performed the following ELISA serum assays: AGA IgA II (Quanta Lite Gliadin IgA II), AGA IgG II (Quanta Lite Gliadin IgG II), and tTG IgA (Quanta Lite h-tTG IgA) (INOVA Diagnostics). Total IgA were measured using an immunonephelometric assay (Dade Behring GmbH). In a subgroup of 16 control and 28 CD children, we also measured tTG IgG (Quanta Lite h-tTG IgG; INOVA Diagnostics).

We carried out the statistical analysis of data using 1-way ANOVA, repeated-measures ANOVA, Student t-test for unpaired data, and ROC curve analysis (SPSS statistical software, version 9.0). From ROC curves, we identified the cutoff values associated with the highest sensitivity and specificity by calculating the differential positive rate (DPR) for each cutoff value from the formula DPR = sensitivity − (1 − specificity). Areas under the ROC curves were compared with χ² test using Stata statistical software, version 9.2 (Stata Corp.). The following formulas were used to calculate posttest probability: positive likelihood ratio (LR+) = sensitivity/(1 − specificity); negative likelihood ratio (LR−) = (1 − sensitivity)/specificity; posttest odds = pretest odds × LR; posttest probability = posttest odds/(1 + posttest odds) × 100.

Results

The individual values of AGA IgA II, AGA IgG II, and tTG IgA in children with (CD) or without (control) a proven histological diagnosis of celiac disease are shown in Fig. 1. The mean values observed in CD children were significantly higher than those of controls for all 3 assays (t = 15.5, P < 0.0001 for AGA IgA II; t = 14.9, P < 0.0001 for AGA IgG II; and t = 20.2, P < 0.0001 for tTG IgA). Two children with normal histology and high antibody levels had a second histological evaluation, which revealed the presence of typical celiac disease lesions (for details, see Table 3). These 2 children with latent celiac disease were excluded in subsequent analyses since their data might introduce a bias in the calculation of test sensitivity and specificity.

The areas under the ROC curves for tTG IgA [mean (SE) 0.976 (0.01) 95% CI 0.959–0.993] and of AGA IgG II [0.960 (0.01); 95% CI 0.939–0.980] were significantly higher than those for AGA IgA II [0.929 (0.02); 95% CI 0.897–0.961] (χ² = 14.95, P < 0.001 and χ² = 4.32, P < 0.05, respectively). The cutoff val-
ues, identified on the basis of the best DPR, were 20 absorbance units (AU) for all 3 indices and corresponded to those established by the manufacturer. Considering AGA IgA II, AGA IgG II, and tTG IgA individually, sensitivity was 80.7%, 80.1%, and 92.5%; specificity was 92.9%, 96.9%, and 97.6%; positive predictive value (PPV) was 93.5%, 97.0%, and 98.0%; and negative predictive value (NPV) was 79.2%, 79.4%, and 91.2%. Table 1 reports the sensitivity, specificity, PPV, and NPV of various combinations of the 3 markers.

LR+ values for AGA IgA II, AGA IgG II, and tTG IgA were 11.37, 25.84, and 38.54, respectively, and the LR– values were 0.208, 0.205, and 0.077. We assumed the pretest probability of CD in the pediatric population of our geographic area to be 0.26%, corresponding to the reported disease prevalence (34). The posttest probability of CD for a positive test result was 2.88% for AGA IgA II, 6.31% for AGA IgG II, and 9.13% for tTG IgA. The posttest probability of CD for a negative test result was 0.05% for AGA IgA II and AGA IgG II and 0.02% for tTG IgA.

Three children with CD in our series had an absolute IgA deficiency (<0.06 g/L). All 3 children had low AGA IgA II (1.28, 2.70, and 1.10 AU) and low tTG IgA (1.3, 3.0, and 3.0 AU), but high AGA IgG II (147.42, 45.35, and 193.80 AU) or tTG IgG (45.65, 27.65, and 44.27 AU) levels. This latter assay, performed in a total

Table 1. Sensitivity, specificity, PPV, and NPV of the combined evaluation of AGA IgA II, AGA IgG II, and tTG IgA.

<table>
<thead>
<tr>
<th>Positive result</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGA IgA II and tTG IgA</td>
<td>80.7 (74.0–86.1)</td>
<td>100 (97.1–100)</td>
<td>100 (97.1–100)</td>
<td>80.4 (73.5–85.8)</td>
</tr>
<tr>
<td>AGA IgA II or tTG IgA</td>
<td>92.5 (87.4–95.7)</td>
<td>90.6 (94.2–94.5)</td>
<td>92.5 (87.4–95.7)</td>
<td>90.6 (84.2–94.5)</td>
</tr>
<tr>
<td>AGA IgG II and tTG IgA</td>
<td>77.6 (70.6–83.4)</td>
<td>99.2 (95.7–99.9)</td>
<td>99.2 (95.6–99.9)</td>
<td>77.8 (70.8–83.5)</td>
</tr>
<tr>
<td>AGA IgG II or tTG IgA</td>
<td>95.0 (90.5–97.5)</td>
<td>95.3 (90.0–97.8)</td>
<td>96.2 (92.0–98.3)</td>
<td>93.8 (88.2–96.8)</td>
</tr>
<tr>
<td>AGA IgA II and AGA IgG II and tTG IgA</td>
<td>72.0 (64.7–78.4)</td>
<td>100 (97.1–100)</td>
<td>100 (96.8–100)</td>
<td>73.8 (66.8–79.8)</td>
</tr>
<tr>
<td>AGA IgA II or AGA IgG II or tTG IgA</td>
<td>95.0 (90.5–97.5)</td>
<td>89.8 (83.3–93.9)</td>
<td>92.2 (87.7–95.8)</td>
<td>93.4 (87.6–96.6)</td>
</tr>
</tbody>
</table>

* Data are % (95% CI). Each test was considered positive if >20 AU and negative if ≤20 AU.
of 16 control children and 28 CD children, was >20 AU in 2 other CD patients, both of whom also had high tTG IgA and normal total IgA levels. All controls and the remaining 23 CD children had tTG IgG levels <10 AU. A reevaluation was made by considering only patients who were not IgA deficient, and this left 127 control and 158 CD children. Fig. 2 illustrates the ROC curves for AGA IgA II, AGA IgG II, and anti tTG IgA calculated in this series of patients. tTG IgA discriminated CD from control significantly better than AGA IgA II ($\chi^2 = 13.01, P < 0.001$) or AGA IgG II ($\chi^2 = 4.82, P < 0.05$).

The mean age of children with CD [mean (SE) 6.5 (0.4)] was slightly lower than that of children without CD [9.0 (0.4)]. To verify whether the 3 CD markers exert a different performance in the different age classes, we divided our series into the following age groups: ≤2 years (16 controls and 30 CD); >2 and ≤4 years (10 controls, 33 CD); >4 and ≤10 years (47 controls, 66 CD); and >10 years (54 controls, 32 CD). ROC curves were made for each biochemical parameter in each age group; the results obtained are shown in Table 2. The best cutoff for AGA IgA II and tTG IgA was confirmed to be 20 AU for each age class; for AGA IgG II the best cutoff was 10 AU for children of <2 years and 20 AU for the other age groups. Because 2 of the 3 CD children with total IgA deficiency were <2 years old, we reevaluated the diagnostic performance of the 3 studied indices in this age class after these 2 cases were excluded from the analysis. The results are reported in Table 2. The areas under the ROC curves did not statistically differ considering children <4 years old, with or without total IgA deficiency. tTG IgA allowed distinguishing CD from control better than AGA IgA II ($\chi^2 = 3.84, P < 0.05$) or AGA IgG II ($\chi^2 = 4.54, P < 0.05$) in children 4–10 years old. This was confirmed only in part in children older than 10 ($\chi^2 = 6.46, P < 0.05$ and $\chi^2 = 2.44, P = 0.12$, respectively).

Subsequently, we evaluated the association between serum measurements and histological grade. The 2 children with Marsh–Oberhuber type 2 lesions were grouped with the 8 children with type 1 lesions. In this enlarged group, lower levels of both AGA IgG II and tTG IgA were found compared with children with type 3a, 3b, or 3c lesions (1-way ANOVA: $F = 3.85, P < 0.05$ and $F = 6.11, P < 0.01$, respectively). The highest AGA IgA II levels were found in children with type 3c lesions compared to those with type 1–2 or 3a lesions ($F = 7.79, P < 0.001$). Fig. 3 shows median values and interquartile ranges for children with Marsh–Oberhuber type 1–2, 3a, 3b, and 3c lesions.

All CD patients were given a GFD after diagnosis. Among children who were in compliance with the diet, tTG IgA levels were available at 1-year follow-up in 47 cases; in 33 of these cases, values were also available at 6-month follow-up. Data on AGA IgA II and AGA IgG II were available in 43 CD children at 1-year follow-up and in 30 after 6-month follow-up. The levels of all 3 indices significantly diminished over time (repeated-measures ANOVA: $F = 93, P < 0.0001; F = 45, P < 0.0001$; and $F = 54, P < 0.0001$, respectively) (Supplemental Fig. 1, which accompanies the online version of this article at www.clinchem.org/content/vol55/issue1).

After 1 year of GFD, levels <20 AU were reached in 51% of the cases for tTG IgA, 87% for AGA IgA II, and 70% for AGA IgG II, but the percentage of reduction in titers did not differ between the 3 tests (1-way ANOVA: $F = 0.23$, NS).

Biochemical and histological details of 13 children who were subjected to repeated endoscopies are given in Table 3. Of these 13 children, 5 had CD without clinical remission and persistent histological lesions paralleled by persistently increased serum levels of AGA IgA II, AGA IgG II, and tTG IgA (Table 3, patients 1–5). Two children with persistently increased serum levels of antibodies had histological evidence of CD only at the second endoscopy (Table 3, patients 6 and 7). The remaining 6 children always had a negative histology for CD, despite an uncertain serology in 3 cases (Table 3, patients 10–12).

Discussion

Celiac disease, diagnosed on the basis of histological findings in duodenal or jejunal biopsies, presents with classic symptoms in the majority of cases, but in some cases, its presentation may be insidious. Some atypical
clinical manifestations, such as abdominal pain and/or abdominal distension, are extremely common in adults and children with or without celiac disease (1, 2, 4, 5). To avoid overlooking CD, endoscopy could be performed in all patients with a suspicious clinical picture, but this would place a heavy diagnostic burden on healthcare facilities. Serologic markers are an economic and noninvasive tool in helping clinicians identify patients for whom a duodenal biopsy is required (13, 33). On the basis of tTG IgA determination alone, celiac disease may be suspected or reliably ruled out, the sensitivity and specificity of this test being 95% to 98% in subjects without total IgA deficiency. The measurement of IgA and IgG class antibodies against gliadin-derived peptides has recently been reported to be a reliable tool for celiac disease diagnosis, even better than tTG IgA in adult patients (26). In the present study, we evaluated these new assays were evaluated in a pediatric population and compared the results with those obtained with tTG IgA. The serum levels of all 3 tests, as expected, were significantly higher in patients with CD than in controls, thus confirming the association between serology and disease. Interestingly, in 2 children with normal histology and high antibody levels (open squares in Fig. 1), typical celiac histology was revealed at a second endoscopy. These 2 children, classified as having latent CD, were therefore excluded from the data set to avoid any bias in the calculation of sensitivity, specificity, and predictive values of these tests. Based on the best DPR, obtained from ROC curves, the cutoff level for each test was 20 AU, and this was equal to the cutoff set by the manufacturer, derived from large series of controls. By using this cutoff value, among the 3 tests, the most sensitive and specific CD marker was confirmed to be tTG IgA. Because the sensitivity of AGAs was lower than that of tTG IgA, their negative predictive values were also lower (>80%). Positive and negative predictive values calculated in the present series indicate, respectively, the probability of disease presence or absence when the test is employed in selected patient cohorts, with a high pretest probability of disease (prevalence 55.5%). For screening purposes, CD prevalence (i.e., pretest probability of disease) in the population to be investigated must be

<table>
<thead>
<tr>
<th>Age class and test</th>
<th>Mean AUC (SE) (95% CI)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>…2 years</td>
<td>AGA IgA II 0.852 (0.066) (0.723–0.981) 80.0 93.8 96.0 71.4</td>
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<tr>
<td>…2 years with no IgA deficit</td>
<td>AGA IgA II 0.895 (0.061) (0.776–1.014) 85.7 93.8 96.0 78.9</td>
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<tr>
<td>…2–4 years</td>
<td>AGA IgA II 0.988 (0.012) (0.964–1.012) 87.9 100 100 71.4</td>
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<tr>
<td>…4–10 years</td>
<td>AGA IgA II 0.970 (0.013) (0.946–0.995) 84.8 93.6 94.9 81.5</td>
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<tr>
<td>…&gt;10 years</td>
<td>AGA IgA II 0.881 (0.040) (0.802–0.960) 65.6 90.7 80.8 81.7</td>
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a ROC curves are based on distinguishing children with CD from control children subdivided according to their age classes. Because 2 of 3 CD children with total IgA deficiency were <2 years old, the diagnostic performance of the 3 studied indices was reevaluated in this age class after these 2 cases were excluded from the analysis. The best cutoff value for any test and for any age class calculated on the basis of the best differential positive rate for AGA IgG II in children <2 years old was 10 AU; in all the other conditions it was 20 AU.

b AUC, area under the ROC curve.
considered. With this in mind, we compared AGA IgA II, AGA IgG II, and tTG IgA in suggesting CD in an unselected pediatric population coming from our geographic area, which prevalence was previously estimated to be 2.6 per 1000 (34). Once again, the best performance was that of tTG IgA: a positive result enhances CD probability from 0.26% to 9.13%, whereas a negative result reduces the probability of CD to 0.02%. Overall, these results clearly show that tTG IgA should be preferred to AGAs in the diagnosis of CD. However, since tTG IgA determination alone did not allow a correct classification of all patients, we evaluated whether tTG IgA used in combination with AGAs might provide more satisfactory results. Only AGA IgG II was shown to add information to tTG IgA: when children were considered positive by either of the 2 tests, the NPV of their combination was higher (93.8%) than that obtained with any single test. The improvement in results obtained by combining AGA IgG II with tTG IgA may depend on the fact that different Ig class antibodies are measured with the 2 assays. In particular, AGA IgG II may be helpful in identifying CD children with a total IgA deficiency (35–37), whose prevalence was 1.8% in the present series. In effect, 3 of 12 CD children with false-negative tTG IgA findings had total IgA deficiency. These 3 children also had, as expected, false-negative AGA IgA II results. In contrast, the AGA IgG II levels were above the cutoff, as were tTG IgG levels. Therefore, as observed by other authors (35–37), the measurement of tTG IgG, or of AGA IgG II, appears to be of real utility for the diagnosis of CD in the presence of total IgA deficiency. Although efficient in detecting CD children with total IgA deficiency, tTG IgG had an overall low sensitivity (17.9%), in agreement with previous finding by Agardh (29). These results indicate that tTG Ig determination should be confined to patients with total IgA deficiency, and any other application (i.e., in screening programs) should be considered inappropriate. The 9 children with false-negative tTG IgA but without total IgA deficiency also had normal AGA IgA II levels and, in 8 of 9 cases, normal AGA IgG II levels. It remains to be elucidated whether these cases might be identified by using serum markers other than tTG IgA or AGAs. Thus, in the absence of total IgA deficiency, tTG IgA remains the most accurate available CD serum marker, as shown in Fig. 2. AGA IgG II or tTG IgG should be used as an alternative to enhance sensitivity in patients with total IgA levels <0.06 mg/L.

tTG IgA is reported to be of limited diagnostic utility in infants <2 years old, for whom antigliadin measurement is preferred (21). Our findings on comparing the diagnostic performances of the studied markers in children of different age classes confirm that tTG IgA is the best available marker in subjects >4 years old, whereas AGA IgG II allowed the correct classification of almost all infants <2 years old. This better performance of AGA IgG II, however, was consequent to the fact that in this age class 2 children had total IgA deficiency. When they were excluded from the analysis, tTG IgA performed as well as AGA IgG II.

Inconclusive data (25, 38, 39) are available in the literature regarding the correlation between the degree of intestinal mucosal atrophy and the levels of circulating antibodies. In the present study tTG IgA or AGAs levels were higher in patients with (Marsh–Oberhuber type 3a-3c) than in patients without (type 1–2) villous atrophy (Fig. 3). Only AGA IgA II showed a mild correlation with the degree of villous atrophy. Although this issue is open to debate, the lack of association found is not surprising given that many patients with positive serology have no signs of histological lesions (potential celiac disease) and can develop them later, as borne out by the demonstration that tTG deposits in a morphologically normal jejunum are predictive of forthcoming overt celiac disease with villous atrophy (40). The present finding thus further stresses the clinical significance of tTG IgA measurement, since it can suggest the presence of CD before any severe damage occurs to the intestinal mucosa.
All the studied markers were found to be associated with a GFD, titers declining progressively after 6 months and 1 year of therapy. At 1 year of a gluten-free diet, 24 of 47, 41 of 47, and 33 of 47 children had levels /H11021 20 AU for tTG IgA, AGA IgA II, and AGA IgG II, respectively. Although not statistically significant, overall tTG IgA disappeared more slowly than AGA IgA II or AGA IgG II in celiac disease children on GFD, and this finding is in agreement with previous results from Liu et al. (27). Notably, persistently increased serum levels of any marker correlated with low compliance to the GFD and with the persistence of histological lesions, further stressing their utility in follow-up.

In conclusion, the findings of the present study demonstrate that, in the absence of total IgA deficiency, the best available marker for making a diagnosis of celiac disease in children of > 2 years of age is tTG IgA. In infants < 2 years old, tTG IgA and AGA IgG II are equally reliable. In cases of total IgA deficiency, the measurement of tTG IgG or AGA IgG II is equally effective in identifying children with celiac disease. Gluten-free diet monitoring can be achieved using any of the studied serum markers. In clinical practice, it must be borne in mind that tTG IgA levels decline slowly and may still be above the cutoff level even after 1 year on a gluten-free diet.

**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.

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