Metabolic syndrome in people living with HIV: criteria prevalence and agreement

Síndrome metabólica em pessoas vivendo com HIV: prevalência e concordância de critérios
Síndrome metabólico en personas que viven con el VIH: prevalencia y concordancia de criterios

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Conflicts to interest: nothing to declare.

Abstract

Objective: To identify the prevalence of metabolic syndrome and the agreement between the criteria of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) and the International Diabetes Federation (IDF) in people living with HIV.

Methods: This is a cross-sectional analytical study, carried out in five specialized services in a city in the interior of São Paulo, from 2014 to 2016, with 340 people living with HIV. Sociodemographic and clinical variables necessary for classification of the metabolic syndrome by the NCEP-ATPIII and IDF criteria were collected through interviews. To assess the agreement between MS, NCEP-ATPIII and IDF criteria, the first-order agreement coefficient statistic was used. To verify the relationship between the metabolic syndrome and the study variables, Poisson regression with robust variance was used.

Results: The prevalence of metabolic syndrome was 28.5% by the NCEP-ATPIII criterion and 39.3% IDF. The highest prevalence was associated with females and age groups from 50 years old, while, in the time of diagnosis between 2 and 10 years, lower prevalence. The agreement between the two criteria was considered substantial.

Conclusion: The substantial agreement between the IDF and NCEP-ATPIII criteria suggests the possibility of interchange between them. Moreover, the results signal the need for special attention from services for the assessment of the metabolic profile and identification of people living with HIV who are at high cardiovascular risk.

Keywords
Metabolic syndrome; HIV; Acquired Immunodeficiency Syndrome; Prevalence

Descritores
Síndrome metabólica; HIV; Síndrome de imunodeficiência adquirida; Prevalência

Resumo

Objetivo: Identificar a prevalência da síndrome metabólica e a concordância entre os critérios do National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) e da International Diabetes Federation (IDF) em pessoas vivendo com HIV.

Métodos: Estudo analítico transversal, realizado em cinco serviços especializados em município do interior paulista, de 2014 a 2016, com 340 pessoas vivendo com HIV. Variáveis sociodemográficas e clínicas necessárias para classificação da síndrome metabólica pelos critérios do NCEP-ATPIII e da IDF foram coletadas por meio de entrevistas. Para avaliar a concordância entre os critérios da SM, NCEP-ATPIII e IDF, foi utilizada a estatística first-order agreement coefficient. Para verificar a relação entre a síndrome metabólica e as variáveis do estudo, utilizou-se a regressão de Poisson com variância robusta.

Resultados: A prevalência da síndrome metabólica foi de 28,5% pelo critério NCEP-ATPIII e 39,3% IDF. As maiores prevalências foram associadas ao sexo feminino e faixas etárias a partir dos 50 anos, enquanto que, no tempo de diagnóstico entre 2 a 10 anos, prevalências menores. A concordância entre os dois critérios foi considerada substancial.

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Conflicts to interest: nothing to declare.
Introduction

Advances in combination antiretroviral therapy (ART) and its universal access have had a major impact on people living with HIV (PLHIV). Patients with limited prospects began to experience a new phase of treatment. Adherence to medication has been the main contributor to the decline in AIDS-related deaths, increased survival, improved quality of life, decreased transmission and suppression of viral activity, thus making it possible to live with HIV from the perspective of a chronic condition.\(^{(1,2)}\)

Worldwide, by 2018, it was estimated that about 37.9 million PLHIV and, among those diagnosed, about 62% are being treated with antiretrovirals (ARV). In Brazil, there are about 900,000 PLHIV and according to the Ministry of Health 66% of these have access to medication.\(^{(3-5)}\)

Despite the encouraging possibilities offered by the use of ART, patients may experience metabolic changes and specific toxicities linked to the use of this medication. These metabolic changes often meet the criteria for identifying the presence of metabolic syndrome (MS).\(^{(6)}\) MS is on the rise worldwide and has been considered a complex entity due to the fact that it aggregates well-established cardiovascular risk factors, increasing overall mortality.\(^{(5)}\) There is evidence that MS is more prevalent among people living with HIV than in the general population, which requires adequate assistance from the health team.\(^{(1,6,7)}\)

Since the 1980s, when MS was recognized, initially called Syndrome X, several criteria have been used to define it. Currently, the most used criteria nationally and internationally among PLHIV are the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) and the International Diabetes Federation (IDF). These organizations, despite applying the criteria differently, agree that the basic components of MS are obesity, insulin resistance, dyslipidemia and hypertension.\(^{(8,9)}\) As there are different criteria for the diagnosis of MS, there are also varying estimates of its prevalence. In a meta-analysis that analyzed studies on the prevalence of MS in PLHIV, the results ranged from 17 to 31%.\(^{(9)}\)

Despite the establishment of improved immunity, the prolonged use of this drug therapy may increase the risk of developing MS in PLHIV, identifying this problem provides support for the management of cardiovascular risk in this population. Given the above, the present study aims to identify the prevalence of MS and agreement between the NCEP-ATPIII and IDF criteria in PLHIV.

Methods

This is a cross-sectional analytical study, carried out in the five specialized care clinics for people living with HIV in the countryside of São Paulo from October 2014 to October 2016.
Participating in the PLHIV study were users of services that met the inclusion criteria: being 18 years of age or older; having been on ART for at least six months; being in clinical-outpatient follow-up at the chosen services. Individuals in confinement situations (institutionalized and living in support homes), pregnant women and with a previous history of cardiovascular disease were excluded.

To obtain the number of participants, a sample calculation was performed based on the number of individuals using ART in each reference service in the municipality studied in 2014, which totaled 1,920 patients. To calculate the sample size, the following formula was used:

$$n = \frac{Z^2 \cdot (P \cdot Q)}{d^2},$$

where $n$ is the sample size, $Z$ is the reduced variable to $\alpha=5\%$, $P=50\%$ and an accuracy level $d=5\%$. Correction was made for a finite population, which resulted in 43 in the outpatient clinic in the North, 119 in the Central, 50 in the East, 78 in the West and 50 in the South district, totaling a sample size of 340. The sample was non-probabilistic and for convenience. Thus, participants were invited to participate as they appeared in the service.

Data were collected through individual interviews, with an average duration of 30 minutes, before or after medical and/or nursing consultations, in private rooms of the clinic itself. Data collection was performed by trained and certified staff. A semi-structured questionnaire designed for study was used. The questionnaire was submitted to face and content validation by four researchers, who assessed it for acceptance of the questioning, easy understanding, relevance of items, wording clarity, presence of ambiguities, in addition to suggestions for changes. There was only qualitative appreciation regarding the merits of researchers’ opinions, without prior publication to this study.

Sociodemographic variables and personal medical history were questioned to the patient. Abdominal circumference (WC) and blood pressure (BP) were measured after the interview, and data on ART time, therapeutic regimen and laboratory tests were collected from the medical record. Of the exams, those with the closest date of entry for patients in the study were recorded and exams performed within a year were discarded.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were assessed using auscultatory technique, recorded on the arm, with an aneroid sphygmomanometer, calibrated manometer and cuff of the appropriate size. The assessment took place after five minutes at rest, in a sitting position, the trunk resting on the back of the chair, legs uncrossed and resting on the floor.\(^{(10)}\)

WC was measured with an inextensible tape measure, in an orthostatic position, with the sole of the foot resting on the floor, oriented so that the abdomen was relaxed, moving the clothes away from the measurement region for better measurement accuracy. The measurement was taken at the midpoint between the last rib and the iliac crest, at the end of the expiratory breathing movement.\(^{(11)}\)

Biochemical measurements carried out in SAE regarding fasting venous glucose (FVG), High Density Lipoproteins Cholesterol (HDL-c) and triglycerides (TG) were performed by enzymatic method in automated equipment (Rxl Max\(^{®}\)) in a central laboratory linked to the municipal secretariat with samples of venous serum under fasting guidance for 12 hours.

For the classification of MS, the NCEP-ATP III and IDF criteria were used. In the reviewed NCEP-ATP III, participants must have at least three of the five components: BP (≥ 130/85 mm/Hg or use of antihypertensive), WC (≥102 cm for men and ≥88 cm for women), TG (≥ 150mg/dl or use of hypolipidemic agents), FVG (≥ 100mg/dl or use of hypoglycemic agent), and/or HDL-c (<40 mg/dl for men and <50 mg/dl for women or use of hypolipidemic agents).\(^{(12)}\)

The IDF criterion assumes the same values for BP, TG, FVG and HDL-c, it differs only in WC (women ≥80 cm and men ≥90 cm).\(^{(12)}\) According to the IDF criterion, the presence of alterations in WC is mandatory, added to two other criteria, and ethnic parameters must be respected. Since there are no important studies that establish parameters in Central and South America, it is recommended to use the reference of South Asians, already described.\(^{(13,14)}\)
Thus, the sociodemographic variables studied were sex (male, female), age group in years (20-29, 30-39, 40-49, 50-59, ≥60), education in years (≤8, > 8). The clinical variables studied were time of diagnosis in years (≤1, 2-4.5-10, > 10), time of ART use in years (≤1, 2-4.5-10, > 10), load viral in copies/ml (> 40, ≤40), SBP and DBP (mmHg), WC (cm), TG (mg/dl), FVG (mg/dl), HDL-c (mg/dl), Hypertension (HP) (yes, no), Diabetes Mellitus (DM) (yes, no), MS (yes, no), use of antihypertensive drugs (yes, no), use of hypolipidemic (yes, no), use of hypoglycemic agent (yes, no).

For data analysis, descriptive statistics (mean and standard deviation) were used for continuous variables. Student’s t-test for independent samples was used to compare components of SD (continuous variables) between men and women. To estimate the adjusted Prevalence Ratio, Poisson regression with robust variance was used. When assessing the measure of statistical significance, the Wald test was used. In these analyzes, the software Statistical Package for Social Science (SPSS), version 22.0 was used. To assess the agreement between MS, NCEP-ATPIII and IDF criteria, the AC1 (first-order agreement coefficient) statistics was used. The AC1 statistic has advantages over Kappa concordance index, the resistance with respect to marginal homogeneity and the prevalence trait, in addition to having the same interpretation as the Kappa statistic [poor/without agreement (<0.0), mild (0.0-0.2), reasonable (0.21-0.40), moderate (0.41-0.60), substantial (0.61-0.80), or almost perfect (0.81-1.00)]. The software version R version 3.5.1 was used for the last analyzes mentioned. In all analyzes performed in the study, a 95% confidence interval and a significance level of 0.05 were adopted. The study was approved by a Research Ethics Committee. PLHIV who agreed to participate in the study signed an Informed Consent Form.

Results

Thus, 340 PLHIV taking ART on an outpatient basis participated in the study. Regarding interviewers’ characterization, it was found that 197 (57.9%) were male, with average age of 44.35 ± 11.7, ranging from 20 to 75 years. The highest concentration was in the 40-49 year age group, with 34.1% and had more than eight years of study (50.9%).

As for clinical variables, it was identified that 53.8% were diagnosed with HIV less than ten years ago. This same period - less than ten years - was predominant when the duration of antiretroviral treatment was assessed, 65% of respondents. Viral load was predominantly undetectable (<40 copies/ml), 80.9%. From the DM criteria used, a prevalence of 28.5% (NCEP-ATPIII) and 39.3% (IDF) was obtained.

The variables that maintained an association after Poisson regression with the highest prevalence for SD (Table 1) were, according to the IDF criterion, the age range of 50-59 (PR=2.46; CI: 1.17-5.21) and 60 and older (PR=3.04; CI: 1.43-6.47). Diagnosis time between 2-4 years (PR=0.33; CI: 0.12-0.88) and 5-10 years (PR=0.40; CI: 0.14-1.10) represented lower prevalence for the syndrome. In the NCEP-ATPIII criterion, female figured prominently (PR=1.89; CI: 1.32-2.70) (Table 1).

Regarding the average of the components of MS assessed by sex (Table 2), values of fasting venous glucose (p=0.02) and HDL-c (p=0.001) were higher in women, while SBP was higher in men (p=0.01).

According to the distribution of the prevalence of MS components, according to the NCEP-ATPIII criteria, the altered metabolic components that reached the highest percentages were HDL-c (56.1%) and TG (45.5%), followed by BP (40.0%), WC (32.9%), and FVG (15.5%). In the IDF criterion, WC reached 62.9% of changes in the assessed PLHIV, following the same percentages as NCEP-ATPIII in the other components. FVG represented the least prevalent metabolic alteration in both criteria.

Regarding the distribution of patients according to the number of components of MS, it was found that according to IDF, 86.7% of participants have at least one component that characterizes this metabolic disorder [no component (13.3%), one (17.6%), two (28.0%), three (22.6%), four (13.8%), five (4.7%)]. In NCEP-ATPIII, 83.2% has at least one criterion for MS [no component
Table 1. Prevalence, prevalence ratio and confidence intervals between the metabolic syndrome in and the study variables, in people living with HIV (n=340)

<table>
<thead>
<tr>
<th>Variables</th>
<th>NCEP-ATPIII criterion</th>
<th>IDF criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence n(%)</td>
<td>PR (95%CI)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35(36.1)</td>
<td>1.0</td>
</tr>
<tr>
<td>Female</td>
<td>62(63.9)</td>
<td>1.89 (1.32-2.70)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>3(3.1)</td>
<td>1.0</td>
</tr>
<tr>
<td>30-39</td>
<td>14(14.4)</td>
<td>1.90 (0.60-5.99)</td>
</tr>
<tr>
<td>40-49</td>
<td>28(28.9)</td>
<td>1.50 (0.46-4.86)</td>
</tr>
<tr>
<td>50-59</td>
<td>36(37.1)</td>
<td>2.9 (0.91-9.27)</td>
</tr>
<tr>
<td>60 and older</td>
<td>16(16.5)</td>
<td>9.8 (0.88-9.13)</td>
</tr>
<tr>
<td>Education (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤8</td>
<td>60(61.9)</td>
<td>1.0</td>
</tr>
<tr>
<td>&gt;8</td>
<td>37(38.1)</td>
<td>1.09 (0.78-1.53)</td>
</tr>
<tr>
<td>Diagnostic time (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>5(5.1)</td>
<td>1.0</td>
</tr>
<tr>
<td>2-4</td>
<td>9(9.3)</td>
<td>0.57 (0.12-2.65)</td>
</tr>
<tr>
<td>5-10</td>
<td>18(18.6)</td>
<td>0.62 (0.15-2.46)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>65(67.0)</td>
<td>1.05 (0.26-4.22)</td>
</tr>
<tr>
<td>ART time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>8(8.3)</td>
<td>1.0</td>
</tr>
<tr>
<td>2-4</td>
<td>14(14.4)</td>
<td>1.85 (0.58-5.92)</td>
</tr>
<tr>
<td>5-10</td>
<td>27(27.8)</td>
<td>2.34 (0.81-6.77)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>48(49.5)</td>
<td>1.84 (0.63-5.36)</td>
</tr>
<tr>
<td>Viral load (copies/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤40</td>
<td>85(87.6)</td>
<td>1.0</td>
</tr>
<tr>
<td>&gt;40</td>
<td>12(12.4)</td>
<td>1.59 (0.93-2.72)</td>
</tr>
</tbody>
</table>

NCEP-ATPIII - National Cholesterol Education Program Adult Treatment Panel III; IDF - International Diabetes Federation; PR - Prevalence Ratio; CI - Confidence Interval; *: Wald test; ART - antiretroviral therapy

Table 2. Metabolic syndrome components in people living with HIV, according to sex (n=340)

<table>
<thead>
<tr>
<th>MS components</th>
<th>Mean ± SD Male (n=197)</th>
<th>Mean ± SD Female (n=143)</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist circumference</td>
<td>89.5±13.06</td>
<td>88.9±18.71</td>
<td>0.76</td>
</tr>
<tr>
<td>Fasting venous glycemia</td>
<td>84.5±26.40</td>
<td>96.7±57.96</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>187.7±208.15</td>
<td>154.6±113.36</td>
<td>0.06</td>
</tr>
<tr>
<td>HDL-c</td>
<td>42.0±13.76</td>
<td>47.5±16.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic Blood Pressure</td>
<td>119.1±14.46</td>
<td>115.0±15.96</td>
<td>0.01</td>
</tr>
<tr>
<td>Diastolic Blood Pressure</td>
<td>78.3±9.47</td>
<td>76.8±12.62</td>
<td>0.25</td>
</tr>
</tbody>
</table>

SD=Standard Deviation; *: Student’s t; HDL-c: High Density Lipoproteins Cholesterol

Table 3. Agreement between the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) and the International Diabetes Federation (IDF) criteria for metabolic syndrome in people living with HIV (n=340)

<table>
<thead>
<tr>
<th>Criterion</th>
<th>IDF (-)</th>
<th>IDF (+)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCEP-ATPIII (-)</td>
<td>201</td>
<td>42</td>
<td>243</td>
</tr>
<tr>
<td>NCEP-ATPIII (+)</td>
<td>05</td>
<td>92</td>
<td>97</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>134</td>
<td>340</td>
</tr>
<tr>
<td>Agreement</td>
<td>AC1</td>
<td>EP</td>
<td>p*</td>
</tr>
<tr>
<td>NCEP-ATPIII vs IDF</td>
<td>0.74</td>
<td>0.0356</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

(-): absence of metabolic syndrome; (+): presence of metabolic syndrome; AC1: first-order agreement coefficient; EP: Standard error; p*: <0.05; vs: versus

Analysis of the agreement between the criteria for assessment of MS, IDF and NCEP-ATIII, obtained a WC coefficient=0.7493 (p <0.0001), considering the agreement as substantial (Table 3).

Discussion

The results of this study show a high prevalence of MS in PLHIV and an association with females and age groups from 50 years old. The lowest prevalence was associated with the time of diagnosis between 2 and 10 years. There was substantial agreement between the IDF and NCEP-ATPIII criteria.

The study enabled the identification of the prevalence and agreement of criteria for MS, contributing to the reflection on the need to assess the syndrome components during PLHIV treatment. In addition to considering MS as a clinical tool in the recognition of individuals most vulnerable to the occurrence of cardiovascular diseases. Furthermore, the study contributes to the development of scien-
tific knowledge and to the debate on the needs of multidisciplinary care for this population as well as the role of nurses in cardiometabolic assessment from primary to tertiary care.

MS has received increasing attention both for the impact of each of its diagnostic components and for adding cardiovascular risk factors. In this context, several risk factors for the development of MS have been identified, but there is great heterogeneity in the results of the studies. This fact is associated with the variety of criteria used to assess MS and the sociodemographic, behavioral and clinical conditions of the investigated populations.

The high prevalence found in the present study among PLHIV using ART, corroborates the findings of a meta-analysis that investigated 65 publications on MS in PLHIV in the European, American, Asian, and African continents. The study found that a third of the population worldwide living with HIV has MS. In research carried out in the African continent, there was a prevalence between 7.8-50.3%, by the IDF criterion, and 7.2-61.6% by the NCEP-ATPIII criterion. The substantial agreement between the IDF and NCEP-ATPIII criteria of our findings supports what has been shown in literature in studies with PLHIV and other groups. The result of this agreement implies that these criteria can classify the same individual with MS, which can be justified by the use of four metabolic components with the same reference parameter. The divergence of the criteria is in WC, which has a different cutoff point, in IDF, is smaller compared to that of NCEP-ATPIII. Another difference is that WC is a mandatory element in IDF for the syndrome classification.

The components of MS usually vary in their rates of occurrence. In this study, about 80% of PLHIV have at least one altered component. Lipid alterations and WC are among the metabolic components that are most prevalent altered, considering the different criteria used. In international research, these components are among the first risk factors for the development of MS. The high number of components is also a worrying factor as it increases the risk of cardiovascular diseases. It is interesting to highlight in our study that, despite the differences between men and women found in some metabolic components, the averages are within the normal parameters of the responsible Brazilian societies.

In addition, there was an association between MS and women living with HIV, which can be attributed to the difference in the social role they take on, with the double work shift, which negatively influences quality of life and affects eating habits and in the practice of physical activity. Furthermore, physiological factors such as the loss of the protective effect of female hormones with the onset of menopause are also important factors that reflect sex and age issues. In this perspective, the prevalence of MS increases with age. Other studies carried out in the same region of Brazil show that women with HIV also have a higher abnormal redistribution of body fat (lipodystrophy) and a higher frequency of cases of abdominal obesity, when compared to men with HIV. The same authors, although they consider the reasons for sexual difference in metabolic responses to be uncertain, reinforce the hormonal contribution, either by polymorphism in the estrogen receptor gene or by the body’s response to the release of growth hormone.

However, the result of the prevalence of MS among those who had a diagnosis time of 2 to 10 years for HIV can be justified by the fact that this classification is among the minority of respondents and, most have more than 10 years of HIV diagnosis (45.5%).

Part of the associations observed between viral load and time of diagnosis, can be explained by mechanisms of immune dysfunction and chronic inflammation caused by HIV infection, older age and drug-related toxicity secondary to prolonged use, especially protease inhibitors. It is worth mentioning that HIV infection itself acts as a catalyst for lipid changes. The increase in inflammation caused by the presence of the virus, through the secretion of inflammatory cytokines, damages TCD4+ cells.

Given the above, in the context of PLHIV, metabolic changes can be attributed not only to the use of different diagnostic criteria, but to the time of ex-
exposure to ART, time of HIV infection, ethnic characteristics, family history (genetic contribution), environmental factors, in addition to eating habits and lifestyle before and after treatment.\(^{(9)}\)

The information generated shows the most used criteria for the MS classification, the agreement between them, the sociodemographic and clinical factors that can be associated with the syndrome, in addition to the metabolic components that are frequently altered in PLHIV. Consequently, the data obtained in this study may support the establishment of care protocols with measures aimed at the identification, prevention, treatment and control of MS in this population. Thus, it is necessary to develop educational and therapeutic strategies and programs with a focus on promoting healthy habits. In addition to conducting longitudinal studies that investigate the behavior of metabolic changes in PLHIV and intervention studies that measure the effect of strategies on improving these changes.

It is noteworthy as a limitation that the study design did not allow to know how long ago the individuals had presented the criteria for MS and that it was due to drug treatment or before it. Moreover, the lack of information on cumulative exposure of patients to each ARV medication and the diversity of their clinical and therapeutic history stands out.

Recognizing the associated risk factors and assessment of the metabolic profile become necessary, since they enable the identification of those with high cardiovascular risk, playing an important role as a marker of metabolic disorders, in order to treat them more quickly, in addition to highlighting the importance of interventions in adopting a healthy lifestyle. In this context, nurses also have a fundamental role with the health team to develop educational strategies that favor health promotion.

**Conclusion**

Thus, based on the results presented, it was found that the prevalence of MS in PLHIV was high by both criteria, more especially by IDF. The highest prevalence of SD was associated with females, and age groups from 50 years old, while, at the time of diagnosis between 2 and 10 years, the prevalence was lower. There was substantial agreement between IDF and NCEP-ATPIII criteria, which implies that these criteria can classify the same individual with MS and thus, there is a possibility of interchange between them.

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**Collaborations**

Costa CRB, Melo ES, Oliveira LB, Moreira RSL, Gir E and Reis RK collaborated with the study design, data analysis and interpretation, article writing, intellectual content review and approval of the final version to be published.

**References**


