Creatinine measurement on dry blood spot sample for chronic kidney disease screening

Detecção de disfunção renal através da dosagem de creatinina em amostra de gota de sangue seca no papel filtro

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Submitted on: 06/24/2015.
Approved on: 09/24/2015.

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DOI: 10.5935/0101-2800.20160004

ABSTRACT

Introduction: Chronic kidney disease (CKD) screening is advisable due to its high morbidity and mortality and is usually performed by sampling blood and urine. **Objective:** Here we present an innovative and simpler method, by measuring creatinine on a dry blood spot on filter paper. **Methods:** One-hundred and six individuals at high risk for CKD were enrolled. The creatinine values obtained using both tests and the demographic data of each participant allowed us to determinate the eGFR. The adopted cutoff for CKD was an eGFR < 60 ml/min. **Results:** Mean age was 57 ± 12 years, 74% were female, 40% white, and 60% non-white. Seventy-six percent were hypertensive, 30% diabetic, 37% had family history of CKD, and 22% of smoking. The BMI was 29.5 ± 6.9 kg/m^2, median systolic blood pressure was 125 mmHg (IQR 120-140 mmHg) and median diastolic blood pressure was 80 mmHg (IQR 70-80 mmHg). According to MDRD equation, sensitivity was 96%, specificity 55%, predictive positive value 96%, predictive negative value 55% and accuracy 92%. By the CKD-EPI equation the sensitivity was 94%, specificity 55%, predictive positive value 94%, predictive negative value 55% and accuracy 90%. A Bland and Altman analysis showed a relatively narrow range of creatinine values differences (+ 0.68mg/dl to -0.55mg/dl) inside the ± 1.96 SD, without systematic differences. **Conclusion:** Measurement of creatinine on dry blood sample is an easily feasible non-invasive diagnostic test with good accuracy that may be useful to screen chronic kidney disease.

Keywords: creatinine; dried blood spot testing; mass screening; renal insufficiency, chronic.

RESUMO

Introdução: A identificação precoce da doença renal crônica (DRC) por meio de amostras de sangue e urina é preconizada em populações de risco devido à elevada morbimortalidade. **Objetivo:** Apresentamos um teste simples e inovador para dosar a creatinina coletada em gota de sangue seca em papel filtro (PF). **Métodos:** Cento e seis pessoas em risco de DRC foram rastreadas com avaliação de dados clínicos, exame físico e coleta de sangue de forma convencional e em PF. Com os dados obtidos, foi estimada a taxa de filtração glomerular (e-TFG). Foi considerado diagnóstico de DRC a e-TFG < 60 ml/min. **Resultados:** A idade dos participantes foi de 57 ± 12 anos, 78 (73,5%) eram mulheres, 43 brancos (40,5%), 36 pardos (34%) e 27 negros (25,5%). O índice de massa corpórea foi de 29,5 ± 6,9 kg/m^2, a pressão arterial sistólica foi de 125 mmHg (120-140 mmHg) e a pressão arterial diastólica de 80 mmHg (70-80 mmHg). A sensibilidade pela equação CKD-EPI foi de 94%, a especificidade 55%, o valor preditivo positivo foi de 94%, o valor preditivo negativo de 55% e a acurácia de 90%. A estatística de Bland-Altman mostrou que as diferenças entre os valores de creatinina dos dois testes estão numa faixa relativamente estreita (+ 0,68 mg/dL e -0,55mg/dL) para um desvio padrão de ± 1,96 mg/dL. **Conclusão:** A dosagem da creatinina coletada em gota de sangue em PF é um teste diagnóstico simples de ser realizado, pouco invasivo e que apresentou uma ótima acurácia, podendo ser útil para rastrear DRC.

Palavras-chave: creatinina; falência renal crônica; programas de rastreamento; teste em amostras de sangue seco.

Introduction

The 2002 standardization of chronic renal failure into the term chronic kidney disease (CKD), with its classification and stratification in 5 stages[1], enabled us to know the prevalence and the risk of mortality associated with decreased renal function. Thus, the current prevalence of CKD in all its stages, was estimated at 10.4% of the world population, and 78% would be in developing countries.[2] Consequently, CKD started to be recognized as a serious health problem - the subject of international guidelines,[3] advocating its early diagnosis and prevention. The definition for the diagnosis of CKD is based on three components: I. anatomical or structural component (kidney damage markers); II- functional component (based on glomerular filtration rate - GFR) and a time component III. According to established criteria, patients with GFR < 60 mL/min/1.73m^2 or GFR ≥ 60 mL/min/1.73m^2, associated with at least one marker of renal damage (e.g., albuminuria) and maintained for a period longer than three months, is carrying CKD.[4]

For direct measurement of GFR, one needs to use exogenous markers such as insulin, Cr^{51}-EDTA, iothalamate, iohexol, and others. However, the use of exogenous markers is complex, expensive and difficult to implement in clinical practice.[5] Thus, creatinine is the most common endogenous marker. GFR is estimated through equations derived from serum creatinine, with adjustment for the extra-renal factors that influence serum levels such as age, gender and race. The equation considered most appropriate to estimate GFR in the general adult population is the one from the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI),[6] for having been created from a cohort that included healthy subjects with normal GFR.

The conventional way to dose creatinine requires collecting a volume of blood through peripheral venous puncture, store it in refrigerated containers and transport for later biochemical processing, which may hamper the calculation of estimated GFR. We present in this study an alternative and simple way to dose creatinine, by collecting a drop of blood and store on filter paper. The aim of this study is to measure the accuracy of the new test to estimate GFR, comparing it with the conventional method.

Methods

We carried out a cross-sectional observational study in healthy volunteers performing clinical data evaluation, physical examination and blood collection. Dosages of standardized creatinine obtained by the two tests and the demographics of each participant allowed to estimate the glomerular filtration rate (GFR). The study was approved by the Ethics Committee for Clinical Research of the Federal University of Rio de Janeiro and all participants signed an informed consent form.

Population

The participants were recruited to include people with increased susceptibility to CKD among caregivers and staff of the Niterói Hospital Complex (CHN) and three social works carried out by the Pavuna Baptist Church in underserved communities. The inclusion criteria were: people with one or more cardiovascular risk factors such as hypertension, *diabetes mellitus*, smoking, overweight/obesity or age above 50 years. Exclusion criteria were: subjects with a previous diagnosis of CKD already established or those with any feature that evidently could interfere with the correct calculation of estimated kidney function, for example, amputees or those with obvious ascites. A total of 119 people met the inclusion criteria. The recruitment period was April 3, 2012 to December 15, 2012.

Design

After completing a questionnaire containing demographic and clinical data, we measured the participants' weight, height and blood pressure. After the interview and physical examination, we collected venous blood by needle puncture and peripheral blood using a lancet. All venous blood samples were placed in thermal containers to be sent to laboratories.

Demographic and physical examination data were entered into an electronic register specially designed for the study, programmed to calculate the equations used to estimate the glomerular filtration rate (and/or creatinine clearance) from the results of creatinine values. The gold standard of creatinine measurement was performed in an automated manner by the modified Jaffe method (automated equipment: Dimension RxLMax® - Siemens Healthcare, Newark, United States of America) in a branch of Sérgio Franco's laboratory at CHN.

Samples collected from a drop of blood and stored on filter paper (Schleicher & Schuell Bioscience, Inc. - New Hampshire, United States) were sent to Biomarc Laboratory at the Vital Brazil Institute (IVB) - Niterói - RJ. In this lab, the filter paper was submitted to organic elution with alcoholic solution. The eluate is then placed in a microplate (ELISA type), which is the support for the automation of the technological procedure. The creatinine present in it reacts with creatinase to form creatine. Subsequently, in the presence of creatininase, the following products are formed sacacin + urea. Hydrogen peroxide is formed in the presence of superoxide dismutase and finally, with the presence of peroxidase, a quinone colored complex is formed, which is quantified in the optical density of $\lambda = 556$ nm. The optical density values are obtained from an ELISA spectrophotometer. The laboratories had no access to the clinical data from the study participants.

Statistical analysis

The variables with normal distribution were described as mean ± standard deviation and the variables with non-normal distribution were described in median and interquartile range. We used 2 × 2 tables to calculate test characteristics such as sensitivity, specificity, positive predictive value, negative predictive value, odds ratio, accuracy measurement and Kappa index for sample agreement. We also evaluated the correlation between the two ways of dosing creatinine using the Pearson correlation. Persistent errors and dispersion results were evaluated by the Bland-Altman analysis. Values considered statistically significant were $p < 0.05$.

Results

Of the 119 people who agreed to participate in the study, thirteen were excluded. Three volunteers recruited in one of the Social Works did not have their blood drawn for the two tests. Ten other people did not have their samples on filter paper processed because they were lost on the way to the laboratory. One hundred and six people were included in this pilot study. Twenty-eight were recruited among the employees and companions to CHN patients, 18 from the social works of July 7, 2012; 32 from the social works of September 22, 2012 and 28 from the social works of December 15, 2012.

The age of participants was 57 ± 12 years, 78 (73.5%) were female; 43 were white (40.5%); 36 were brown (34%) and 27 blacks (25.5%). Their body mass index was 29.5 ± 6.9 kg/m^2, the systolic blood pressure was 125 mm Hg (interquartile range [IQR] 120-140 mmHg) and diastolic blood pressure of 80 mmHg (IQR 70-80 mmHg). The general data of the study population are depicted on Table 1.

Table 1 Demographic and clinical characteristics of the population

N	106
Age (years)	57 ± 12
Female gender (%)	78 (73.5)
White race (%)	43 (40.5)
Black race (%)	27 (25.5)
Brown race (%)	36 (34)
BMI (kg/m^2)	29.5 ± 6.9
Systolic BP in screening (mmHg)	125 (120-140)
Diastolic BP in screening (mmHg)	80 (70-80)
Other diseases reported:	
Arterial hypertension or heart disease (%)	80 (76)
Diabetes mellitus (%)	32 (30)
Family history of CKD (%)	39(37)
smoking (%)	23 (22)

Data expressed as mean SD, median (interquartile range), or by frequency, BMI: Body mass index; BP: Blood Pressure; CKD: Chronic kidney disease.

Among the 106 study participants, 80 (76%) had a previous diagnosis of hypertension and 32 (30%) had received a diagnosis of *diabetes mellitus*. Of those interviewed, 39 (37%) claimed to have a family history of CKD and 23 (22%) reported being smokers. The clinical data are presented on Table 1.

The validity of the test done with FP was calculated considering as a variable of interest to identify individuals with estimated GFR lower than 60 ml/min/1.73 m^2, determined by the CKD-EPI equation.

As mentioned in the contingency tables, the test measuring serum creatinine was conventionally considered the "gold standard". Thus, the sensitivity of the PF was 94% in the CKD-EPI for the diagnosis of GFR lower than 60 ml/min/1.73m^2. Similarly, the specificity to the FP test was 55%. These results are depicted on Table 2.

As a categorical variable, the ability to identify as "sick" those who had an estimated GFR lower than 60 ml/min/1.73 m^2, the proportion of non-random correlation calculated by the Kappa index was 0.45 for the CKD-EPI.

The Pearson correlation coefficient of the nominal values of creatinine, obtained between the two tests showed a correlation of 0.30 (p = 0.002). As for the estimation of GFR by the CKD-EPI equation using creatinine values obtained by the two tests, the Pearson correlation coefficient was 0.48 (p = 0.001). These results are presented in Figure 1.

The correlation analysis between the tests according to the method proposed by Bland and Altman showed that the vast majority of the differences between the measures within the ± 1.96 SD range is within the creatinine values in a relatively narrow range (+ 0.68 mg/dl and - 0.55 mg/dl) and a systematic difference was not observed within the range of creatinine values found (mean of the two methods measuring at least 0.45 mg/dl and at most 1.9 mg/dl). These results are depicted on Figure 2. The analysis of the correlation of the GFR estimated by the CKD-EPI equation can also be seen on Figure 2.

Discussion

The technique of storing and analyzing a blood sample on filter paper was described 100 years ago by Ivar Bang.[7,8] During the 60s, this technique gained notoriety due to Robert Guthrie. Motivated by having a niece and then a child with PKU,[9] Guthrie created a simple and effective method to track this disease, by collecting a drop of blood from the heels of babies.[10] Today, the famous "heel prick test" is widely used and has been expanded and can identify 31 disorders a newborn can have.[11] However, in the light of our knowledge, the level of creatinine collected from a drop of blood and stored on filter paper has not yet been used to track CKD.

The use of filter paper has several advantages. Obtaining blood by a sting is simpler and does not require specialized training. The amount of blood required for analysis is small, around 10-25 µl.[12] The inputs cost much less than those used in the conventional method.[13] In addition, ease of transport, storage, handling, processing, analysis and sample destruction, the entire logistics operation is simpler and less expensive.[14] The disadvantages of dosing substances by the filter paper technique are due to the nature of the sample itself. The blood stored and dried on filter paper suffers hemolysis. Thus, the amount of hemoglobin and release of intracellular components can interfere on the concentrations of some analyses.[15] Recently, it has been proven that the stability in the analysis of some metabolites may be influenced by room temperature.[16]

This type of test with filter paper has been used in clinical screenings.[17] Thus, to know whether a screening will bring some benefit to the people examined, it is necessary to answer the three classic questions from Geoffrey Rose and DJ Parker:[18] Will early treatment improve prognosis? What is the validity and reliability of the screening test used? And, finally, what is the screening result?

Our study sought to track people at higher risk of developing CKD. Confirming this expectation, the average age found was 57 ± 12 years; 75.5% of participants had a BMI above 25 kg/m^2; 76% were known to be hypertensive, and 30% had *diabetes mellitus*.

The prevalence of CKD using the criterion of a GFR < 60 mL/min/1.73 m^2 ranged from 8.4 to 10.3% in the two equations used to estimate GFR in both tests. There are few publications on the prevalence of CKD in Brazil to do comparisons. Bastos *et al.*,[19] estimated the GFR by MDRD, < 60 ml/min/1.73m^2 in 24,248 individuals over the age of 18, who collected blood in a Juiz de Fora (MG, Brazil) laboratory between January 2004 and December 2005, and

Table 2 Values of sensitivity, specificity, positive predictive values, negative predictive values and accuracy of the filter paper creatinine test to estimate a GFR < 60 ml/min/1,73m^2

Equation	Sensitivity % (IC 95%)	Specificity % (IC 95%)	PPV % (IC 95%)	NPV % (IC 95%)	Accuracy %
MDRD	96 (89-98)	55 (21-86)	96 (89-98)	55 (21-86)	92
CKD-EPI	94 (87-97)	55 (21-86)	94 (87-97)	55 (21-86)	90

CI: Confidence Interval, MDRD: Modification of Diet in Renal Disease, CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration, PPV: Positive Predictive Value, NPV: Negative Predictive Value

Figure 1. Correlation of the creatinine values and the filtration rate estimated by the CKD-EPI equation. CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; GFR: Glomerular Filtration Rate

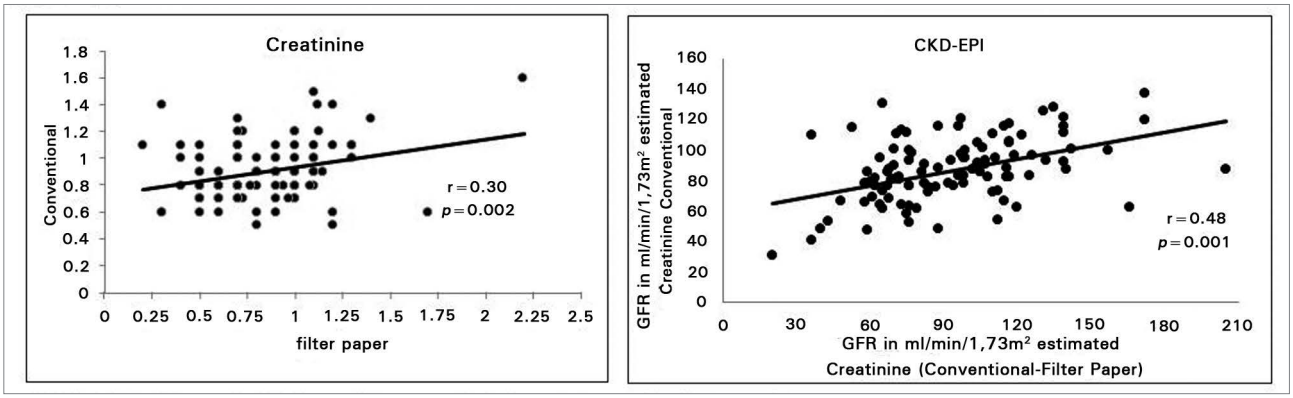
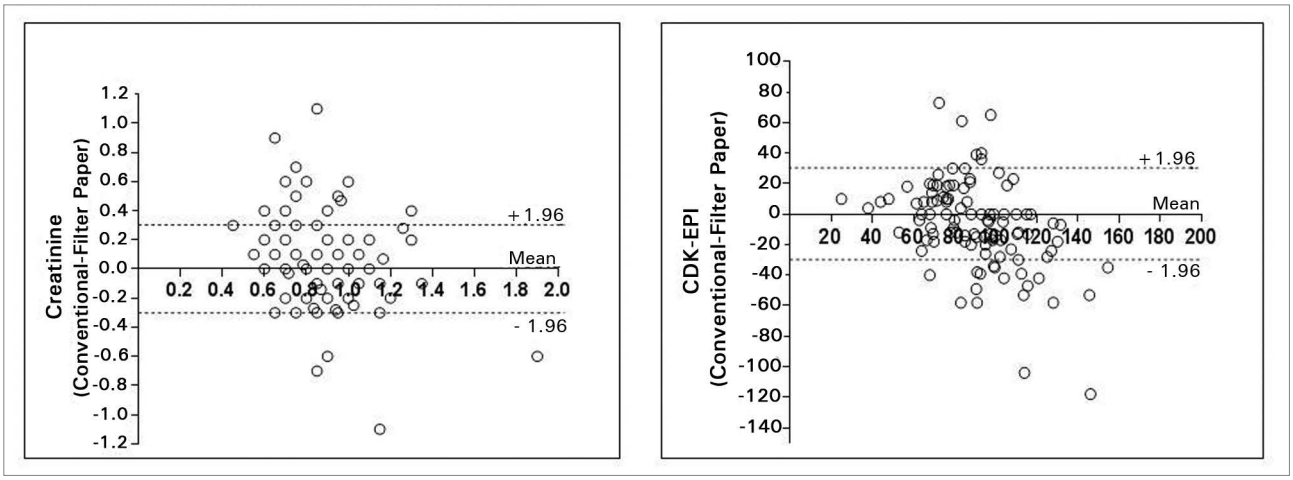


Figure 2. Agreement by the Bland-Altman method of the creatinine values and the estimated filtration rate by the CKD-EPI equation; CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration SD: Standard Deviation



found a CKD prevalence of 9.6% in patients younger than 60 years and 25.2% in those over 60 years. Passos *et al*.[20] conducted one of the first studies on the prevalence of CKD in developing countries in Bambuí (Minas Gerais, Brazil). It was an observational study carried out between January and August of 1997. The criteria for CKD diagnosis was a creatinine greater than 1.3 mg/dL in men and 1.2 mg/dL in women. The prevalence of CKD in the population over 60 years who participated in the study was 5.09%. Our study found a higher prevalence of CKD, which can be explained by the fact that we tracked a risky population.

Upon employing the filter paper to track CKD, the test was highly sensitive, which yielded a positive predictive value of over 95% in the two equations that estimated the GFR in the screened population. It is important to note that these are the most important features of a test used in screening.

The use of filter paper is not intended to replace the collection of conventional creatinine for diagnosis and monitoring of CKD. Upon comparing the values of the two tests, we found a "weak" correlation by the Pearson Index." This finding is due in part to the narrow range of results, since most of the volunteers were healthy individuals. However, the Pearson correlation for the CKD-EPI equation was "moderate." We did not study the Pearson correlation or agreement by the Bland-Altman plots for the CG and MDRD equations, for they were not validated for GFR > 60 ml/min/1.73m^2. The agreement was assessed by the Kappa index to discriminate who had a GFR < 60 ml/min/1.73m^2. The performance of the filter paper test was considered "moderate" in the CKD-EPI, according to a commonly used scale to assess the results of the Kappa statistics.[21]

However, the Kappa index, lends itself primarily to comparing measurements between two different

observers, for example, two pathologists and methods in which there are still questions about what would be the gold standard method. By contrast, the Bland-Altman, showed that the methods (gold standard and filter paper) do not differ systematically in creatinine values and, most importantly, the difference between the two methods did not yield a very wide dispersion. When we employed the Bland-Altman method to calculate the renal function equations we found, as expected and mentioned above, that the CKD-EPI equation corrects distortions of other equations for higher values of glomerular filtration. Therefore, the results from the correlation coefficient and the K test support the test usefulness to evaluate GFR in people at risk for CKD. According to this statement, we found an accuracy of 90% in the CKD-EPI for the diagnosis of estimated GFR < 60 ml/min/1.73m^2.

The use of filter paper technology for dosing substances in humans and animals need, in our view, to be expanded. By requiring, in the pre-analytical phase, a small amount of blood collected by lancet, the patient may, in the future, make the collection himself and send the filter paper by mail. This will enable monitoring without requiring long displacements to go to a collection station. In addition, the filter paper may be a great option for collecting biological material from animals, aligned with the current policy of protection and non-aggression.

Regardless of the benefit to the population that the creatinine dosing technique on filter paper may have, our study had some limitations. The first was to have selected a population with low creatinine values, who agreed to participate in "social works". These screenings were performed in communities. The largest participation was from females who are more receptive to this type of activity. It should also be noted the relatively small number of participants. However, for the purpose of this study, which is the initial validation of the filter paper assay method for screening renal disease, this number proved to be adequate. Evidently, the next step is to test the method in a more robust population sample. A relevant and specific issue refers to the ability of the equations used to estimate kidney function in the Brazilian population. Very few publications have addressed this issue in Brazil. Silveiro *et al.*[22] compared the MDRD and CKD-EPI equations with the EDTA clearance in 105 type 2 diabetic patients at the Porto Alegre University Hospital (RS). The inclusion criteria were: blood glucose < 200 mg/dL and GFR > 60 ml/min/1.73m^2. The results were: GFR by EDTA was 103 ± 23, by CKD-EPI83 ± 15 and by MDRD GFR 78 ± 17 mL/min/1.73 m^2. The conclusion was that the equations underestimated GFR in this population of Brazilian diabetics. Another Brazilian study, Soares *et al.*,[23] compared GFR measured by EDTA with the MDRD and CKD-EPI equations in 96 healthy subjects of Rio Grande do Sul. The values found were 94 ± 19 18 mL/min/1.73 m^2 for MDRD and 102 ± 18 ml/min/1.73 m^2 for the CKD-EPI. According to the authors, the CKD-EPI performance in this population was better. Finally, the creatinine dosages were carried out in different laboratories (Sergio Franco and Vital Brazil). Furthermore, although the collections were carried out at the same time their processing was carried out at different periods.

The study aimed to compare two estimates of GFR by conventional dosing and filter paper. Subjects were screened at in social works. In this sense, the results are valid only for those with the same characteristics. Other studies, involving a larger number of people selected at random, are needed to ensure external validity. Similarly, we recognize that a resampling within the same study population as a means of internal validation, would reinforce the conclusions reached. The low specificity of the test tells us that we should use it with caution to diagnose persons. Likewise, any patient who has an abnormal GFR should have its examination confirmed by conventional method before being labeled as a carrier of the disease.

It is important to mention that, regardless of the technique used to measure glomerular filtration, we must be cautious in diagnosing moderate reductions as CKD, particularly among older individuals, since in this population in particular, such a "diagnosis" may not represent a higher risk for renal replacement therapy or even death.[24]

Conclusion

In spite of the aforementioned limitations, this study showed that the filter paper test may be useful in the screening of kidney disease. In fact, this study showed that the creatinine dosage collected from a drop of blood on filter paper is a simple diagnostic test to be performed, it is minimally invasive and has a great accuracy for estimating GFR < 60 ml/min/1.73m^2. Therefore, it can be used to estimate GFR, especially

in mass campaigns, and when combined with other tests, such as the measurement of albuminuria. Other studies involving a larger number of patients should be performed to confirm our findings.

Acknowledgements

The authors would like to thank the Sérgio Franco laboratory, the Niteroi Hospital Complex, the Vital Brazil Institute, the Biomarc Laboratory and the First Baptist Church of Niteroi for their invaluable collaboration for carrying out this study.

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