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# Performance of the quantification of adenosine deaminase and determination of the lactate dehydrogenase/adenosine deaminase ratio for the diagnosis of pleural tuberculosis in children and adolescents

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Study carried out at the Hospital de Clínicas de Porto Alegre and in the Departamento de Pediatria, Universidade Federal do Rio Grande do Sul, Porto Alegre (RS) Brasil.

## ABSTRACT

**Objective:** To evaluate the accuracy of determining the adenosine deaminase (ADA) level, the 2'-deoxyadenosine/ADA ratio, and the LDH/ADA ratio in pleural fluid for the diagnosis of pleural tuberculosis (PT) in children and adolescents. **Methods:** This was a retrospective cross-sectional study conducted at a tertiary hospital in a high-tuberculosis-incidence area, between 2001 and 2018. All patients with ADA in pleural fluid and a confirmed diagnosis of PT (cPT) or parapneumonic effusion (PPE) were included. **Results:** The cPT and PPE groups comprised 25 and 68 individuals, respectively. At a cutoff of 40 U/L, ADA measurement showed the following: sensitivity, 88%; specificity, 31%; positive predictive value (PPV), 32%; negative predictive value (NPV), 88%; and overall accuracy, 46%. The best cutoffs were an ADA level of 125 U/L, a 2'-deoxyadenosine/ADA ratio of 0.5, and an LDH/ADA ratio of 8.3, with AUC of 0.67, 0.75, and 0.82, respectively. The sensitivity, specificity, PPV, NPV, and overall accuracy of the 125 U/L ADA cutoff were 84%, 65%, 47%, 92%, and 70%, respectively, compared with 79%, 79%, 59%, 91%, and 79%, respectively, for the 8.3 LDH/ADA ratio cutoff. Changing the LDH/ADA ratio cutoff to 3.0 increased the specificity to 98%. **Conclusions:** The ADA level and the 2'-deoxyadenosine/ADA ratio are not good biomarkers for the diagnosis of PT in pediatric patients. Determination of the LDH/ADA ratio provides the best overall accuracy for the diagnosis of PT in such patients.

**Keywords:** Adenosine deaminase; Pleural effusion; Tuberculosis, pleural/diagnosis; Child; Adolescent.

## INTRODUCTION

Tuberculosis is a disease caused by infection with *Mycobacterium tuberculosis*.[1] Despite the success of pharmacotherapy over the past seven decades, tuberculosis is the leading cause of death from infectious disease worldwide.[2] Brazil is on the list of the 30 countries with the highest tuberculosis burdens.[1] In 2017, the incidence of tuberculosis in the city of Porto Alegre, capital of the southern Brazilian state of Rio Grande do Sul, was 90 cases per 100,000 population.[3]

Severe manifestations of tuberculosis are more common in the pediatric age group. Extrapulmonary tuberculosis occurs in approximately 20% of childhood cases of tuberculosis.[1] Studies conducted in low- and middle-income countries have found pleural tuberculosis (PT) to be the most common type of extrapulmonary tuberculosis among children and adolescents.[4-6]

A definitive diagnosis of PT is made via the identification of *M. tuberculosis* bacilli in sputum, pleural fluid, or pleural biopsy specimens.[7] It is difficult to diagnose tuberculosis in pediatric patients, and the use of diagnostic strategies that were developed for adults can result in many pediatric cases being missed.[8] The analysis of gastric aspirate and sputum samples reportedly has a low diagnostic yield[9]: 40-50% and 20-30%, respectively. Therefore, the diagnosis of PT in pediatric patients is usually based on a history of contact with an adult with pulmonary tuberculosis, together with clinical findings suggestive of the diagnosis, a positive reaction on a tuberculin skin test (TST), and pleural fluid analysis (showing unilateral exudative pleural effusion [PE] with a predominance of lymphocytes, as well as high protein content). Children with PT typically present with a low number of bacilli. Therefore, microscopy of pleural fluid samples rarely identifies AFB, and cultures are often negative. Although pleural biopsy offers a better sensitivity profile, it is invasive and not feasible in some settings.[9,10] Consequently, the quantification of pleural fluid biomarkers has become an attractive alternative to pleural biopsy.[11]

The analysis of adenosine deaminase (ADA) in pleural fluid, using a cutoff of 40 U/L, has been shown to

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have high sensitivity and specificity (89-99% and 88-99%, respectively) for the diagnosis of PT in adults. In children and adolescents, the differential diagnosis of elevated ADA activity is usually between PT and empyema, because other causes are rare in that population. The few studies involving pediatric patients have shown that the measurement of ADA is not very accurate in such patients.[12,13] In addition, measuring the activity of the ADA isoenzymes ADA1 and ADA2 could be valuable in cases of suspected false-negative or false-positive results.[10] Local anecdotal observations have suggested that the level of LDH in pleural fluid is generally lower in PT than in other types of inflammatory PE in which ADA is elevated, and that the assessment of this relationship has diagnostic utility.[14]

The aim of this study was to evaluate the determination of the ADA level, ADA isoenzyme levels, and the LDH/ADA ratio in pleural fluid as parameters to discriminate between tuberculous PE and parapneumonic effusion (PPE) in children and adolescents. We hypothesized that the determination of ADA isoenzyme levels and the LDH/ADA ratio would have greater accuracy for the diagnosis of PT than would the quantification of ADA.

## METHODS

This was a retrospective cross-sectional study of all individuals ≤ 18 years of age for whom ADA levels were measured in pleural fluid samples at the Porto Alegre *Hospital de Clínicas*, a tertiary university hospital in the city of Porto Alegre, Brazil, between January of 2001 and June of 2018. Eligible participants were identified by reviewing the electronic medical records.

The study population consisted of two subgroups: patients with a confirmed diagnosis of PT; and patients diagnosed with PPE. Individuals with transudative effusion, as defined by Light's criteria,[15] were excluded, as were those with malignant PE, rheumatologic diseases, probable tuberculous PE, or PE of unknown etiology.

The etiology of PE was defined as follows:

- Confirmed PT (cPT)—*M. tuberculosis* identified by culture, PCR, or smear microscopy (Ziehl-Neelsen staining) of pleural fluid, pleural biopsy, sputum, BAL fluid, or gastric lavage fluid samples; or pleural biopsy sample showing a granuloma, with or without necrosis, and a clinical picture suggestive of PT
- Probable PT—clinical diagnosis of tuberculosis and good clinical response to treatment with antituberculosis drugs
- PPE—exudative PE associated with an infection in the pulmonary parenchyma related to any pathogen except *M. tuberculosis*, as well as the absence of an alternative cause identified over the clinical course
- Empyema—purulent PE, as evidenced by the macroscopic presence of pus, or a positive gram stain result or culture result in pleural fluid
- PE of unknown etiology—no clinical or pathological diagnosis determined from the medical record
- Malignant PE—positive pleural fluid cytology, positive pleural tissue histology, or confirmed malignancy at another site with radiological evidence of metastatic thoracic involvement

During the study period, LDH was analyzed by photometry; ADA was determined by the colorimetric method described by Giusti and Galanti[16]; and ADA isoenzyme activity was quantified by calculating the 2'-deoxyadenosine/ADA ratio.[17] Laboratory personnel were not blinded to patient clinical data, because the tests were ordered according to the treatment routine. In this context, it is mandatory for the attending physician to state why a specific examination is important for the investigation of the case.

The following clinical and biochemical data were retrieved from electronic medical records: in pleural fluid samples—pH, total protein, glucose, LDH, total leukocyte count with differential cytology, malignant cell screening result, ADA, 2'-deoxyadenosine/ADA ratio, LDH/ADA ratio, PCR result for *M. tuberculosis*, smear microscopy result, and the result of culture for aerobic bacteria and *M. tuberculosis*; in pleural biopsy samples—histopathological examination finding and results of smear microscopy/culture for *M. tuberculosis*; and other—TST result, as well as smear microscopy, culture (for *M. tuberculosis*), and PCR (for *M. tuberculosis*) results in gastric lavage fluid, sputum, and BAL fluid samples.

Statistical analyses were performed with the IBM SPSS Statistics software package, version 23.0 (IBM Corporation, Armonk, NY, USA). Categorical variables are expressed as absolute and relative frequencies. Continuous variables are expressed as median and interquartile range if their distribution was non-normal, and continuous variables with symmetric distribution, as mean and standard deviation or 95% confidence interval if their distribution was normal. The Kolmogorov-Smirnov test was used in order to determine whether the variables were normally distributed. The chi-square test was applied in order to compare categorical variables, and the Mann-Whitney test was used in order to detect significant differences between medians, whereas independent two-sample t-test was used in order to detect significant differences between means.

The performance of the determination of the ADA level, the LDH/ADA ratio, and the 2'-deoxyadenosine/ADA ratio in differentiating cPT from PPE was assessed by constructing ROC curves. We used the MedCalc statistical package, version 19.2.6 (MedCalc, Mariakerke, Belgium) to estimate the sample size. For an alpha level of 0.05, with a power of 90%, three times as many negative cases as positive cases, and a target AUC of 0.8 for the LDH/ADA ratio, significantly different from the null hypothesis value (0.5), the required sample sizes in the positive (cPT) group and the negative (PPE) group were 12 and 36, respectively. The best cutoff points were determined by calculating the Youden index. The

following test characteristics were calculated: overall accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). In the analysis of the accuracy of determining the 2′-deoxyadenosine/ADA and LDH/ADA ratios, we excluded patients for whom there were missing data.

The correlation between ADA level and age was determined by Spearman's correlation coefficient (r), which was classified as weak (r ≤ 0.3), moderate (r = 0.4-0.6), or strong (r > 0.6). Values of p ≤ 0.05 were considered statistically significant.

This study was approved by the Research Ethics Committee of the Porto Alegre *Hospital de Clínicas* (Reference no. 19814619.8.0000.5327). Because of the retrospective nature of the study, the requirement for written informed consent was waived.

## RESULTS

We identified 131 patients for whom data regarding ADA levels were available. Of those 131 patients, 38 (29%) were excluded for the following reasons: having PE of unknown etiology (n = 18); having probable PT (n = 10); having malignant PE (n = 2); and having transudative PE (n = 7). One case (from the PPE group) was excluded due to an abnormally high ADA level (771 U/L), which was considered a laboratory error. Therefore, we reviewed data for a total of 93 patients (25 with cPT and 68 with PPE). Of the 25 patients in the cPT group, 1 (4.0%) was HIV-infected, as were 2 (2.9%) of the 68 patients in the PPE group. Other significant comorbidities among the patients in the PPE group were neoplastic disease (n = 5), sickle cell disease (n = 3), Down syndrome (n = 2), cystic fibrosis (n = 1), Fanconi anemia (n = 1), mitochondrial disease (n = 1), and congenital disorder of glycosylation (n = 1). The criteria for a clinical diagnosis of tuberculosis included fever (observed in all of the patients), cough (in 78%), chest pain (in 82%), dyspnea (in 43%), night sweats (in 8%), weight loss (in 17%), and hemoptysis (in none). Table 1 shows the main characteristics of each group. The methods used in order to confirm the diagnosis of PT are shown in Table 2. The PE was classified as empyema in 9 (13.2%) of the patients in the PPE group. The pleural fluid was cultured in 66 (97.1%) of the PPE group patients, and bacterial growth was detected in only 9 (13.6%), as follows: *Streptococcus pneumoniae* (n = 4); *Staphylococcus aureus* (n = 2); *Haemophilus* sp. (n = 1); *Stenotrophomonas maltophilia* (n = 1); and *Enterobacter* sp. (n = 1).

When we applied the most widely used ADA cutoff (40 U/L), we found the following in relation to its ability to diagnose PT: sensitivity of 88% (95% CI: 69-98); specificity of 31% (95% CI: 20-43); PPV of 32% (95% CI: 27-37); NPV of 88% (95% CI: 70-96); and overall accuracy of 46% (95% CI: 36-57). Because empyema is known to result in elevated ADA levels, which could worsen the diagnostic performance of their measurement, the analysis was repeated after the exclusion of cases of purulent PE. At the 40 U/L cutoff, the sensitivity, specificity, PPV, NPV, and overall accuracy were 88% (95% CI: 69-98), 36% (95% CI: 24-49), 37% (95% CI: 31-42), 88% (95% CI: 70-96), and 51% (95% CI: 40-62), respectively.

As shown in Figure 1, the ROC curve analysis of the determination of the ADA level, 2′-deoxyadenosine/ADA ratio, and LDH/ADA ratio showed that the best cutoffs, respectively, were as follows: 125 U/L (AUC = 0.67; 95% CI: 0.57-0.77; p = 0.003), 0.5 (AUC = 0.75; 95% CI: 0.62-0.85; p = 0.0001), and 8.3 (AUC = 0.82; 95% CI: 0.73-0.90; p < 0.0001). The characteristics of the tests at their best cutoffs are displayed in Table 3. After purulent PE had been excluded from the analysis, the best ADA cutoff (125 U/L) showed a slightly better diagnostic performance,

**Table 1.** Characteristics of the patients and of the pleural fluid samples.

| Variable | PPE (n = 68) | cPT (n = 25) | p |
|---|---|---|---|
| Male patients, n (%) | 44 (64.8) | 12 (48.0) | 0.22* |
| Patient age (years); median (IQR) | 5.4 (2.4-13.9) | 13.4 (7.4-14.7) | 0.02† |
| TST induration ≥ 10 mm, n (%)[a] | 1 (8.3) | 11 (73.3) | <0.001* |
| pH, median (IQR)[b] | 7.30 (7.11-7.42) | 7.34 (7.26-7.38) | 0.72† |
| Glucose (mg/dL), mean ± SD[c] | 68.2 ± 47.6 | 68.9 ± 26.2 | 0.93‡ |
| Proteins (g/dL), mean ± SD[d] | 4.3 ± 1.3 | 5.3 ± 0.8 | < 0.01‡ |
| ≥ 50% mononuclear cells, n (%)[e] | 2 (13.3) | 11 (78.6) | < 0.01* |
| LDH, median (IQR)[f] | 883.5 (407.8-6097.3) | 679.5 (484.8-1193.8) | 0.29‡ |
| ADA (U/L), mean ± SD | 117.6 ± 107.5 | 159.7 ± 66.3 | 0.03‡ |
| LDH/ADA ratio, median (IQR) | 22.8 (10.4-43.5) | 4.9 (2.7-8.3) | < 0.01† |
| 2′-deoxyadenosine/ADA ratio, mean ± SD[g] | 0.51 ± 0.18 | 0.35 ± 0.08 | < 0.01‡ |

PPE: parapneumonic effusion; cPT: confirmed pleural tuberculosis; TST: tuberculin skin test; and ADA: adenosine deaminase. [a]Data available for only 23 of the PPE group patients and only 15 of the cPT group patients. [b]Data available for only 46 of the PPE group patients and only 16 of the cPT group patients. [c]Data available for only 61 of the PPE group patients and only 24 of the cPT group patients. [d]Data available for only 63 of the PPE group patients and only 24 of the cPT group patients. [e]Data available for only 15 of the PPE group patients and only 14 of the cPT group patients. [f]Data available for only 62 of the PPE group patients and only 24 of the cPT group patients. [g]Data available for only 40 of the PPE group patients and only 22 of the cPT group patients. *Chi-square test. †Mann-Whitney test. ‡t-test.

**Table 2.** Laboratory methods used in order to confirm the diagnosis of tuberculous pleural effusion (n = 25).

| Method | Sample type | n (%) |
|---|---|---|
| Smear microscopy | Gastric lavage fluid | 1 (4.0) |
| Culture | Pleural fluid | 10 (40.0) |
| Culture | Gastric lavage fluid | 2 (8.0) |
| Culture | BAL fluid | 1 (4.0) |
| Culture | Pleural biopsy | 6 (24.0) |
| PCR | Pleural fluid | 2 (8.0) |
| PCR | BAL fluid | 1 (4.0) |
| Histopathology | Pleural biopsy | 5 (20.0) |

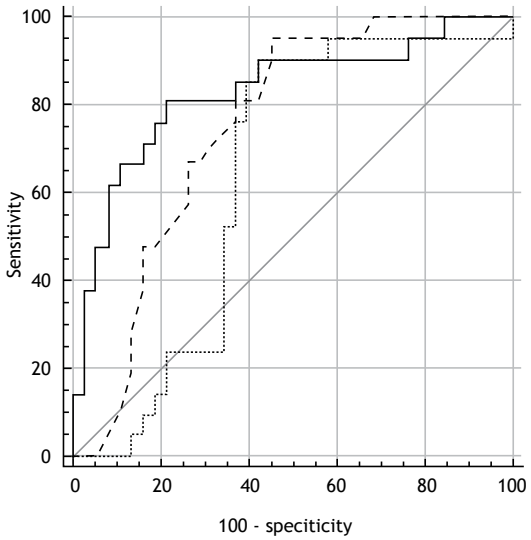


**Figure 1.** ROC curve analysis of the determination of the adenosine deaminase level (dotted line), the 2'-deoxyadenosine/adenosine deaminase ratio (dashed line), and the LDH/adenosine deaminase ratio (solid line) in pleural fluid samples.

with an AUC of 0.73, a sensitivity of 84%, and a specificity of 73%, whereas the performance of the best 2'-deoxyadenosine/ADA ratio cutoff (0.5) and the best LDH/ADA ratio cutoff (8.3) did not improve, with AUCs of 0.72 and 0.81, respectively.

Because biomarker-based, non-sputum diagnostic tuberculosis tests should have high specificity (≥ 98%), with no minimum target sensitivity,[18] we performed an additional analysis of determination of the LDH/ADA ratio at a cutoff of 3.0, which was found to have a sensitivity of 33% (95% CI: 16-55), a specificity of 98% (95% CI: 91-100), a PPV of 89% (95% CI: 51-98), an NPV of 79% (95% CI: 74-84), and an overall accuracy of 80% (95% CI: 70-88). In contrast, a change in the cutoff did not improve the specificity of ADA measurement. Finally, we found an inverse correlation between age and the level of ADA in pleural fluid ($r = -0.24$; $p = 0.02$).

## DISCUSSION

The main results of this study suggest that determination of the ADA level and of the 2'-deoxyadenosine/ADA ratio in pleural fluid samples are not sufficiently accurate to be useful in the diagnosis of PT in children and adolescents. However, determination of the LDH/ADA ratio was found to have good accuracy for discriminating between tuberculous PE and PPE, especially when the cutoff was adjusted to favor specificity.

It is well established that measuring ADA in pleural fluid (with a cutoff of 40 U/L) performs well in the detection of PT in adults, with sensitivity and specificity values above 86%, as well as predictive values above 88%.[1,9,19] However, many studies in adults have included patients with transudative PE, and have not excluded patients with a probable diagnosis of tuberculosis.[19] That could explain the fact that, in our study sample, ADA measurement performed poorly, even after the cutoff was adjusted to 125 U/L, according to the Youden index. Another potential explanation for that fact is that we excluded patients with malignant PE, given that ADA levels are known to be considerably lower in patients with malignant PE than in those with tuberculous PE.[20,21]

Wu et al.[13] suggested that the quantification of ADA in pleural fluid is not an accurate method for the diagnosis of PT in pediatric patients. However, their study sample included patients with a probable diagnosis of tuberculosis. In addition, the authors did not provide data on the sensitivity, specificity, PPV, or NPV of the test.[13] In contrast, Mishra et al.[12] demonstrated that the accuracy of ADA quantification in pleural fluid is greater in patients with confirmed PT than in those with probable PT (85% vs. 75%), although their confirmed PT group comprised only 8 individuals.

Researchers and clinicians have emphasized the need for additional tests for the diagnosis of tuberculosis, especially for patients in whom the disease can be difficult to diagnose (such as children). The WHO recommends that new diagnostic tests for tuberculosis in pediatric patients have a minimum specificity of 98%, as well as recommending target sensitivity and specificity values of 90% and 70%, respectively, for screening tests.[22] In the present study, determination of the LDH/ADA ratio was the test that produced the best results, being particularly useful for excluding a diagnosis of PT, for which it was found to have an NPV of 91% and a sensitivity of 79% when a cutoff of 8.3 was used, as well as a specificity of 98% when a cutoff of 3.0 was used. In agreement with our findings, two retrospective studies showed that LDH/ADA ratios

**Table 3.** Characteristics of the tests and their best cutoffs, as determined by calculating the Youden index.

| Parameter tested | Cutoff | AUC (95% CI) | Sensitivity % (95% CI) | Specificity % (95% CI) | PPV % (95% CI) | NPV % (95% CI) | Accuracy % (95% CI) |
|---|---|---|---|---|---|---|---|
| ADA (U/L) | > 125 | 0.67 (0.57-0.77) | 84 (64-95) | 65 (52-76) | 47 (38-56) | 92 (82-97) | 70 (60-79) |
| 2′-deoxyadenosine/ ADA ratio | ≤ 0.5 | 0.75 (0.62-0.85) | 95 (77-100) | 50 (34-66) | 51 (43-59) | 95 (74-99) | 66 (53-78) |
| LDH/ADA ratio | ≤ 8.3 | 0.82 (0.73-0.90) | 79 (58-93) | 79 (67-88) | 59 (46-71) | 91 (82-96) | 79 (69-87) |

PPV: positive predictive value; NPV: negative predictive value; and ADA: adenosine deaminase.

were significantly lower in adults with PT than in those with PE of other etiologies.[14,23]

Other authors have reported an inverse correlation between age and the ADA level in pleural fluid.[20,24] On the basis of those data, it has been hypothesized that a higher cutoff would be needed in order to improve the diagnostic accuracy of ADA in pediatric patients. Similarly, in our study sample, we found that the ADA level in pleural fluid decreased significantly in parallel with increasing age. However, the correlation was weak, which indicates that this variation is not large enough to influence the interpretation of the test or to explain the fact that the phenomenon was observed only when the highest cutoff was used.

Our study has some limitations. The retrospective nature of the study and the small sample size resulted in wide confidence intervals. Although tuberculosis is quite prevalent in the region in which our study was conducted, PT is less common. According to the official epidemiological surveillance system for the city of Porto Alegre,[25] there were 352 cases of PT in subjects < 19 years of age in the city between 2001 and 2018. Only 24 (6.8%) of those cases were laboratory-confirmed. It should be borne in mind that our sample was restricted to individuals for whom ADA values were available and in whom the diagnosis of PT had been confirmed by extensive microbiological testing. Therefore, some patients who were classified as having PT in the surveillance system could not be included in our study. We understand the intrinsic limitation imposed by the retrospective nature of the study. However, given that PT requiring hospitalization is an uncommon event in pediatric patients, a prospective design would have entailed a very long follow-up period and still might not have resulted in an adequate number of patients for the analysis of accuracy. In addition, because our study encompassed a period of 17 years, there may be concern about the variability of the diagnostic methods used. In fact, there was no variation in the method of ADA assessment, which was the main outcome measure of the study. As for the analysis of LDH, there was variability in the equipment used (a Roche Cobas analyzer was replaced by a Siemens Advia analyzer, the latter being used for four years), although not in the method of analysis (ultraviolet spectrophotometry). Therefore, there was no difference in the interpretation of the results. Another limitation is that only a small number of patients underwent TST and cytological analysis of the pleural fluid. Consequently, it was not possible to study the accuracy of ADA measurement in a subgroup of patients with positive TST results and lymphocyte-predominant pleural fluid. In addition, we did not have a representative sample of HIV-infected patients.

Our study also has some strengths, such as the careful selection of the cPT group, in which we included only patients in whom the diagnosis had been confirmed by extensive microbiological testing of a variety of biological samples (sputum, gastric lavage fluid, pleural fluid, BAL fluid, and pleural biopsy).[18] In addition, we emphasize the original aspect of the study with regard to diagnostic methods not previously employed in pediatrics, such as the analysis of the LDH/ADA and 2′-deoxyadenosine/ADA ratios.

We are aware that our results do not represent the final word on this subject. Instead, we think that its importance lies in the fact that it raises new questions regarding the interpretation of ADA levels in the diagnosis of PT in pediatric patients, especially because there have been few studies on this subject in such patients. We believe that our findings will encourage the development of prospective studies, with larger patient samples, aimed at assessing the reproducibility of our findings in other populations.

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## AUTHOR CONTRIBUTIONS

JLV: data curation; investigation; validation; visualization; drafting and revision of the preliminary and final versions; and approval of the final version. LF: data curation; formal analysis; investigation; validation; visualization; drafting and revision of the preliminary and final versions; and approval of the final version. ICSF: data curation; investigation; sourcing; validation; visualization; drafting and revision of the preliminary and final versions; and approval of the final version. VCBGC: conceptualization; data curation; formal analysis; investigation; methodology; project administration; sourcing; supervision; validation; visualization; drafting and revision of the preliminary and final versions; and approval of the final version.

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