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New concept for control material in glucose point-of-care-testing for external quality assessment schemes

https://doi.org/10.1515/cclm-2024-0822
Received July 17, 2024; accepted September 10, 2024;
published online September 24, 2024

Abstract

Objectives: Until now, the external quality assessment (EQA) of glucose point-of-care testing (POCT) has lacked a high quality, suitable and commutable control material to assess measurement accuracy. Here we present a concept for determining the accuracy of glucose measurements, which uses human whole blood and does not require stabilising agents.

Methods: This new generation of quality control samples uses a bead that contains a specific amount of glucose. The bead is then dissolved in a whole blood matrix by the EQA participant immediately before the POCT. We analysed its suitability as an EQA material with respect to its reproducibility, homogeneity and stability, and applied it in an EQA pilot study. The glucose target value was determined using the reference measurement procedure and served as an evaluation criterion for the accuracy of the EQA survey results.

Results: The homogeneity and stability of the new control material fulfilled the quality requirements of ISO 17043. Based on the reference measurement value for glucose, the results of the pilot EQA scheme showed a pass rate of 84.6 % for the participating POCT devices. The acceptance limit was a 15 % permitted deviation from the target value according to Rili-BAEK. All of the device collectives deviated from the target value by 0–4.4 % with the exception of one device type, which deviated by 21 %.

Conclusions: The new concept offers, for the first-time, whole blood-based trueness controls for glucose POCT analysis for external quality assurance. The concept does not require the addition of any stabilising reagent and is easy to use.

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Keywords: glucose bead; glucometer; proficiency testing; reference measurement procedure; traceability; whole blood

Introduction

Glucose measurements are performed either in laboratories using clinical chemistry analysers, or close to the patient using point-of-care testing (POCT) devices. For measurements conducted in clinical laboratories, blood samples are commonly stabilized with citrate and fluoride salts in order to inhibit *in vitro* glycolysis in the blood cells [1, 2]. Without glycolysis inhibitors, glucose concentrations in patient samples would decrease by 5–7 % per hour after blood sampling [3]. Glucose POCT is performed immediately after blood sampling in fresh human whole blood. Whereas clinical laboratories require specially trained employees, POCT devices can be handled by non-laboratory personnel without the need for further laboratory equipment. Overall, glucose POCT is of great medical and economic importance [2, 4].

Internal and external quality assurance for glucose measurements in plasma, serum, whole blood, urine and cerebrospinal fluid are mandatory in Germany. The frequency of participation as well as the evaluation criteria are defined in the guideline of the German Medical Association for the Quality Assurance of Laboratory Medical Examinations (Rili-BAEK) [5]. For internal quality controls, the permissible deviation is 8 % of the manufacturer's target value. For external quality controls, it is 15 % of the reference measurement value (RMV). Other limits and evaluation criteria might be applicable for other countries or EQA organizations. Providing sample material for POCT analysis controls poses a particular challenge due to the rapid glycolysis in whole blood. A patient-like sample of unprocessed human whole blood without additives and stabilisers cannot be used. With regard to the external quality assessment (EQA) of POCT, several studies propose preparing the POCT EQA samples by adding stabilisers and further processing the whole blood [6–8]. Another approach is to separate the plasma from the glycolysis-driving blood cells:

The plasma fraction is spiked with glucose and both blood fractions are then mixed again prior to performing the measurement [9]. However, most EQA providers worldwide circulate serum or plasma which is not a suitable matrix for POCT quality control. Due to matrix effects, the RMV often cannot be used to evaluate the EQA results. Instead, the results must be evaluated within collectives using the same measurement procedure [10].

We propose a novel EQA material for POCT which is comparable to a patient whole blood sample, and which can also be adeptly handled by non-laboratory personnel without the need for laboratory equipment such as pipettes. The concept is based on a two-component sample where the matrix and the analyte are kept apart until the measurement is performed. The matrix contains human venous whole blood from a healthy donor with lithium heparin as a natural anticoagulant. After blood sampling from a healthy donor, no stabilisers, processing steps or further additives are applied to the matrix. For the analyte, a defined amount of glucose is prepared with an inert supporting material and formed into a solid bead. Prior to the measurement, the glucose bead is completely dissolved in the whole blood matrix. This whole blood-bead (WBB) material is therefore comparable to a patient sample. Its measurement results can be compared to the RMV, regardless of which measurement procedure the POCT device uses. For the first time, an evaluation can be made based on accuracy. In this study, the novel EQA material was validated under controlled laboratory conditions. It was used in parallel with commercially available control material as part of an EQA pilot study, and the performance of the two materials was compared.

Materials and methods

Instruments

This study used two commercially available POCT devices for plasma-based glucose measurements: (1) StatStrip Xpress2 (Nova Biomedical GmbH, Moerfelden-Walldorf, Germany) and (2) Accu-Chek Aviva Nano (Roche Diagnostics GmbH, Mannheim, Germany). For comparison, a medical laboratory device, the AU480 Clinical Chemistry Analyser (Beckman Coulter GmbH, Krefeld, Germany), was used whose analytical method is based on the hexokinase reaction. For trueness verification, RMVs were determined using the gas chromatography isotope dilution mass spectrometry (GC-IDMS) reference measurement procedure listed in the JCTLM database for higher order reference methods (https://www.jctlmdb.org/). The procedure was carried out in INSTAND's calibration laboratory, which is accredited according to DIN EN ISO/IEC 17025:2018 [11] and DIN EN ISO 15195:2019 [12].

Production of the glucose beads

The glucose beads covered a clinically relevant sample concentration range (3.1–19.9 mmol/L) and were obtained from Hart Biologicals (Hartlepool, UK). Individual beads were produced from a precisely adjusted standard solution of pure glucose (D-(+)-Glucose, Sigma Aldrich, G5767-500G) and 10 % polyvinylpyrrolidone (PVP, PVP40, Sigma Aldrich, PVP40-50G). A high-precision pump was used to eject droplets of a very specific volume into liquid nitrogen. These droplets formed near-perfect spheres when placed into the liquid nitrogen. In order to prevent multiple beads from sticking together, a patented manufacturing process [13] was used to keep them separated while they were frozen solid. The beads were then freeze-dried following a specific protocol. Once they were dry, the beads were separated into individual glass vials, evacuated and stored at ambient temperature where, according to the manufacturer, they can remain stable for at least 12 months. The homogeneity of the bead lots was tested and approved by INSTAND's calibration laboratory in accordance with DIN EN ISO/IEC 17043:2010 [14].

Preparation of the whole blood matrix

The whole blood matrix was obtained from a professional manufacturer. Whole blood was drawn from a healthy donor by venous puncture using a collection bag with a preset Li heparin concentration of 17–19 IU/mL. Immediately after blood sampling, aliquots of 0.5 mL were prepared in polypropylene (PP) screw-on dropper vials (obtained from Changsha Renji Medical Equipments Co., Ltd.). The aliquots were first stored at 2–8 °C for 96 h and then at ambient temperature for 24 h. Careful blood collection and gentle handling of the samples prevents haemolysis of the whole blood. The absence of glucose in the whole blood aliquots was verified. The whole blood aliquots were stored at 2–8 °C and used for the measurements within three days. The donated blood was tested by the manufacturer and found to be negative for HIV, hepatitis B and C virus. Homogeneity was also tested and approved by the manufacturer.

Determination of dissolving conditions

The optimum dissolving time was then established for four different glucose concentrations within the clinically

relevant range of 3.1–19.9 mmol/L. To do this, a glucose bead was transferred to the whole blood aliquot for each glucose measurement. This was mixed 10 times overhead, left to rest for 15, 20, 30 and 60 min, and mixed again 10 times overhead at ambient temperature. The glucose concentrations were measured in 20 µL aliquots with all instruments.

Reliability of the WBB concept

To verify the reliability of the WBB concept, the four different glucose concentrations in a clinically relevant range of 3.1–19.9 mmol/L were determined nine times under the predetermined dissolving conditions. Glucose beads with a concentration of 5.2 mmol/L were tested six times on three consecutive days. Beads that contained no glucose were tested to verify the inertness of the PVP supporting material. The glucose measurements were performed on all instruments and the RMVs were determined.

The homogeneity of the beads was tested in INSTAND's calibration laboratory in batches of three different glucose concentrations. This was done by dissolving 10 beads in 0.5 mL pure water (grade 1) each and measuring the glucose concentration in duplicate with the routine analyser AU480.

EQA survey

INSTAND offers the EQA scheme "Dry Chemistry 01 – POCT: Glucose (800)" six times a year. The scheme is conducted in accordance with the requirements of DIN EN ISO/IEC 17043:2010 [14]. Our study includes results from October 2023 and March 2024 in which two commercially available, ready-for-use plasma samples with different clinically relevant concentrations were circulated.

EQA pilot study

In March 2024, each participant received an additional sample of the novel EQA material as part of the regular EQA survey for glucose POCT. Detailed instructions for handling the novel EQA material were enclosed with the dispatched sample (see Supplementary Material).

Results were submitted online via the platform RV-online (https://rv-online.instandev.de). The participants were given 17 days to report the results for the plasma samples and three days for the bead sample after dispatch. Participation in the EQA pilot study was voluntary and not part of the external quality control certification process.

The homogeneity and stability of all EQA samples were within the acceptance criterion of ≤4.5 % according to INSTAND's quality policy.

Reference measurement values

The RMV was determined in plasma since the manufacturers of the POCT devices had already followed the recommendation of the International Federation on Clinical Chemistry and Laboratory Medicine (IFCC) to report the concentration in plasma irrespective of sample type and measurement technique [15].

In the case of the commercial plasma samples, the target values were determined before the EQA material was dispatched. For this purpose, two samples from each batch were analysed on three consecutive days using the reference measurement procedure.

In order to assign a target value to the novel EQA material, two aliquots were prepared daily over the three days of the EQA pilot study in accordance with the preparation instructions. The prepared samples were centrifuged, and the plasma was immediately frozen and stored at −40 °C until it could be analysed by the reference measurement procedure.

The GC-IDMS reference measurement procedure is listed as a calibration procedure in the JCTLM database for higher order reference methods. Metrological traceability of glucose measurement values was established by using the primary reference material D-Glucose NIST 917c. For quality control intercomparison, samples from the RELA-IFCC External Quality Assessment Scheme for Reference Laboratories in Laboratory Medicine or certified reference materials from NIST (National Institute of Standards and Technology, Gaithersburg, USA) with assigned target values were analysed as part of each measuring sequence.

Data analysis and statistics

The results of the glucose measurements of the EQA scheme from October 2023 are presented as a Youden Plot for the sample pairs of each participant. Collectives were created for devices with the most participants and represented as coloured ellipses, indicating 95 % of the data of the corresponding split. The results for the pilot study in March 2024 are presented as a box plot diagram. For all boxes, the whiskers stretch from the 1st quartile − 1.5*(interquartile range) to the 3rd quartile + 1.5*(interquartile range). Statistical analyses were performed using JMP 17.0.0 from SAS Institute (Cary, NC, USA).

To calculate the RMV, a mean value was determined from the six individual values of each EQA sample batch. The corresponding expanded measurement uncertainty was calculated using the GUM Workbench (Metrodata GmbH, Grenzach-Wyhlen, Germany) with a coverage factor of k=2 in each case; all issued with 1 %.

Results and discussion

The new concept of EQA material for glucose testing using POCT systems enables the use of whole blood samples without the addition of stabilisers. The challenge of very limited glucose stability in the whole blood matrix can be overcome by having the user mix the analyte with the matrix immediately prior to analysis. The new concept is designed to be used without any dosing aids so that an accuracy control material that is manufacturer independent can also be provided outside of laboratory facilities, for example in care settings.

Reliability of the WBB concept

As part of the development of the application instructions, an investigation was conducted into the length of time the beads needed to soak until they were completely dissolved in the whole blood matrix. Glucose concentration was found to reach maximum values 20 min after addition of the beads for all tested bead glucose levels, which then remained stable for at least 1 h.

An investigation was also conducted on how to ensure the sample was completely mixed with the dissolved glucose. We found it was important to carefully mix the viscous matrix whole blood with the dissolved glucose at least 10 times overhead in the screw-on dropper vials to ensure reproducible mixing.

Under the optimised sample processing conditions, whole blood samples were prepared with beads of different glucose levels.

The results presented in Table 1 show that four different glucose levels ranging from 3.1 to 19.9 mmol/L could be generated with good reproducibility. The glucose concentrations in the freshly prepared WBB samples were analysed in parallel using two different POCT systems. The imprecision of these measurement results is due to the overall error generated by the test strips and the preparation of the samples from the glucose beads. Therefore, these samples were additionally measured with the high-precision routine device AU480 which produces measurements with minimal imprecision. In addition, the glucose concentration in the WBB samples was analysed for each of the four bead glucose levels using the reference measurement procedure. Applying the RMV as the target value, the deviation of the measurement results for the POCT systems and AU480 routine values was calculated as a measure of accuracy. The bead model was shown to have good precision and accuracy for the clinically relevant concentration range.

The reliability of the bead production was tested in a homogeneity test on three batches as part of a validation procedure (see Table 2). The glucose concentration in the beads with a CV between 0.39 and 1.62 % was shown to be highly reproducible. The beads were prepared by adding 10 % PVP. The glucose-free ‘empty beads’ demonstrated that 10 % PVP in the bead neither interferes with measurements of the POCT systems tested, nor with the AU480 and the reference measurement procedure.

The novel EQA material was found to meet the stability requirements of DIN EN ISO/IEC 17043:2010 [14] with respect to the processing time of three days for all investigated devices and measurement procedures (see Figure 1). A processing time of three days is sufficient to conduct an EQA survey. The novel EQA material should undergo further investigation regarding maximum stability over time in order to potentially extend the EQA submission deadline.

Table 2: Homogeneity test by AU 480 for three different glucose bead batches dissolved in H_2O (number of samples per batch n=10, CI, confidence interval).

Batch	Mean, mmol/L	CV, %	95 % CI, mmol/L
1	4.92	0.59	4.91–4.94
2	9.27	0.39	9.24–9.29
3	10.17	1.62	10.07–10.27

Table 1: Mean glucose measurement results of beads with four different glucose levels in whole blood (number of samples n=9).

RMV[a], mmol/L	3.1			5.3			10.5			19.9		
	Accu-Chek Aviva Nano	StatStrip Xpress2	AU 480	Accu-Chek Aviva Nano	StatStrip Xpress2	AU 480	Accu-Chek Aviva Nano	StatStrip Xpress2	AU 480	Accu-Chek Aviva Nano	StatStrip Xpress2	AU 480
Mean, mmol/L	3.5	3.0	3.3	5.5	5.1	5.6	10.8	10.9	10.6	21.0	20.7	21.0
CV, %	2.4	7.1	2.6	3.4	5.6	2.7	3.8	3.1	1.1	4.1	4.0	0.7
Bias, %	10.9	−3.3	6.5	3.8	−4.0	6.6	2.4	4.1	0.8	5.2	4.1	5.5

[a]RMV, reference measurement value, determined as a mean of six single values with expanded measurement uncertainty U=1 %, k=2.

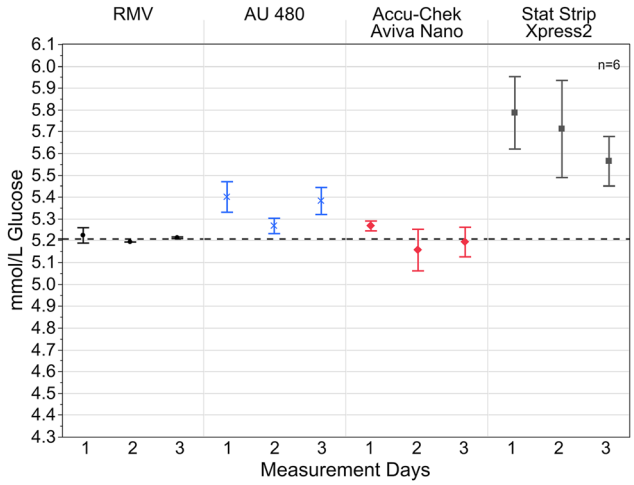

Figure 1: Stability test for the glucose beads in whole blood from the EQA pilot study conducted in March 2024. Displayed are the mean values with the standard deviation on three consecutive measurement days separately for the reference measurement procedure (RMP) and each investigated instrument. The reference measurement value (RMV) was calculated from the mean of the six single values (2 single values were measured per day by the RMP). The expanded measurement uncertainty of U=1 %, k=2 was estimated using the GUM Workbench.

Performance of the plasma samples in the regular EQA scheme

The current situation of using plasma samples as EQA material for glucose POCT is shown in Figure 2. The results from October 2023 serve as a representative example. The results of the individual device collectives of the POCT test systems show a strong scatter within the collectives and the means of the collectives at times differ strongly from each other. The mean value of the collective is in good agreement with the RMV for both samples in the case of only a few manufacturers. The deviation of the mean value from the target value is over 20 % for several collectives; for one manufacturer it is even over 60 %. According to Rili-BAEK, the acceptance criteria for glucose in an EQA scheme is a ±15 % permitted deviation from the RMV. Hence, the pass rate for Sample 1 would have been 43 %, for Sample 2 it would have been 34 %. As a result, in the INSTAND EQA scheme, an evaluation of the participant results against the RMV for plasma samples was only applied to those collectives whose mean value did not deviate by more than 10 % from the RMV.

Performance of the WBB concept in an EQA pilot study

In the March 2024 EQA survey, participants received two plasma samples and one WBB sample (see Figure 3). Based on the acceptance criterium of a ±15 % permitted deviation from the RMV for the plasma samples, the pass rates of the participants' results were under 50 % – comparable to those from October 2023. The WBB sample, on the other hand, had a pass rate of 84.6 % under the same acceptance criteria. The mean of the individual device collectives deviated from the RMV by between 0 and 4.4 %. Interestingly, the device collective that deviated most from the RMV in the plasma samples with a positive bias, also exhibited a 21 % deviation in the WBB sample, but with a negative deviation from the RMV. As the WBB samples are not ready to use upon delivery, participants must take some care and attention when preparing the material. However, this challenge also applies to other EQA

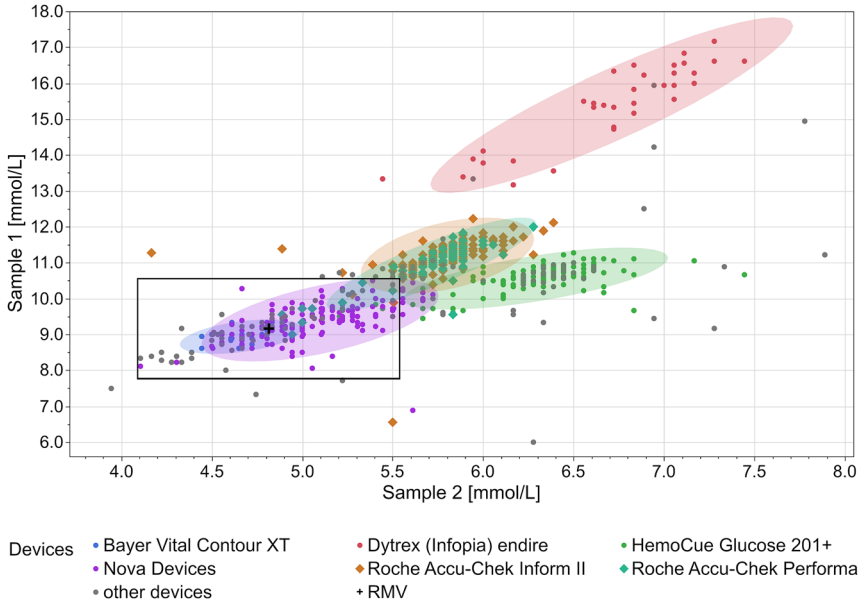

Figure 2: EQA scheme results for glucose POCT in October 2023. The Youden plot presents the glucose POCT results for sample 1 and sample 2 from 683 participants. Different device collectives are marked in coloured ellipses which contain approximately 95 % of the points for each device. The RMVs (sample 1: 9.2 mmol/L, sample 2: 4.8 mmol/L) are marked by a black cross and served as target values for the EQA evaluation. The black frame indicates the acceptance range of ±15 %, which was the permitted deviation from the target value.

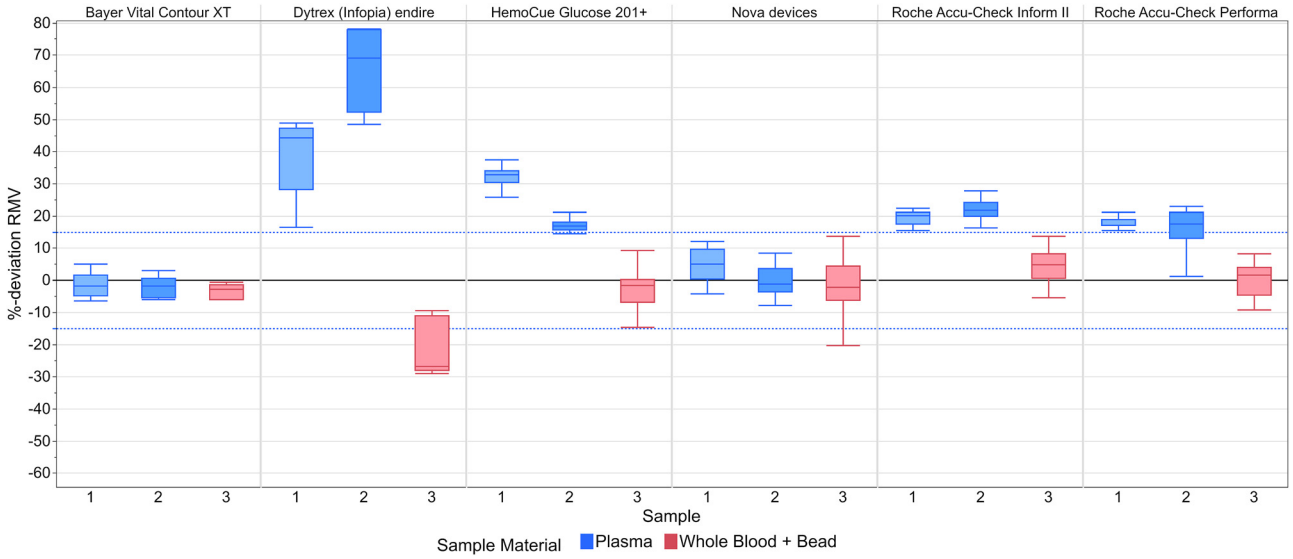

Figure 3: Results for glucose POCT from the regular EQA survey and the pilot study conducted in March 2024. The boxplot shows the glucose POCT measurement results for three samples. The EQA samples containing plasma are marked blue, and the whole blood bead sample is marked red. Analysis of the device-dependent deviation from the RMV in per cent, based on the EQA sample material used. The blue dotted lines mark the evaluation range of ±15 % around the RMV. For all boxes, the whiskers stretch from the 1st quartile − 1.5*(interquartile range) to the 3rd quartile + 1.5*(interquartile range).

sample materials, such as lyophilised material, which must be reconstituted before use. The detailed instructions provided to the participant and training measures were helpful.

Commutability of the WBB control material

The WBB concept presents a control material which is as close to a patient sample as possible. Stabilising additives and further processing of the whole blood matrix are completely avoided, the small amount of PVP showed no interferences with the different devices and measurement procedures applied. Unfractionated heparin a naturally occuring glycosaminoglycan is used as anticoagulant. Therefore, a high degree of commutability of the WBB concept could be assumed but further commutability investigations with a focus on the whole blood matrix are required. However, the design of commutability studies for POCT, which requires comparison measurements with completely unprocessed whole blood material and a fast degrading measurand as glucose are challenging and will be addressed in further studies of the WBB concept.

Conclusions

The new concept for preparing whole blood material for the glucose POCT EQA scheme is a promising approach to providing quality control material without the addition of stabilising additives. The glucose WBB model demonstrates very good reliability according to the validation data and meets the performance criteria for use as EQA material according to Rili-BAEK [5] and DIN EN ISO/IEC 17043:2010 [14]. By completely avoiding the use of stabilising additives, the new model makes it possible to provide an EQA material that is as close as possible to patient material. Preparing the material for use as sample material in quality assurance is simple and can be performed by the user without any additional laboratory equipment. For the first time, our model allows glucose POCT results to be measured against a target value that is metrologically traceable to a true value. The concept can also be used as control material outside of large laboratories, such as in care facilities or pharmacies.

Research ethics: Not applicable.
Informed consent: Not applicable.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Use of Large Language Models, AI and Machine Learning Tools: None declared.
Conflict of interest: The authors state no conflict of interest.
Research funding: None declared.
Data availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Supplementary Material: This article contains supplementary material (https://doi.org/10.1515/cclm-2024-0822).