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Investigation and quantification of composition variability in urinary stone analysis

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Purpose: To investigate the variability in urinary stone composition analysis due to sampling and suggest potential solutions.

Materials and Methods: We collected 1,135 stone fragments from 149 instances that had undergone a stone removal at Hanoi Medical University Hospital from January 2022 to August 2022. Each fragment was ground into fine powder and divided into separate specimens if the amount was abundant. For composition analyzing every specimen, Fourier transform infrared spectroscopy was performed. The composition of a given fragment was the average of its belonging specimens. The variability in composition was assessed on the fragment level (i.e., between fragments of an instance). We defined an instance as "significantly variable" if the maximum difference in any composition across its belonging fragments was equal to or greater than a given threshold.

Results: On average, there were 7.6±3.3 stone fragments per instance and 2.3±0.5 specimens per fragment. We found that the variability could be substantial on the fragment level. Eighty-nine (69.5%) and 70 (54.7%) out of 128 multiple-component instances were significantly variable if the threshold was set at 20% and 30%, respectively. The variability of an instance on the fragment level was correlated with the size of fragment and the number of components.

Conclusions: Our study demonstrated the significant variability in urinary stone composition and showed that it correlated with the size and the impurity of samples. Mapping denotation while sampling and analyzing as well as reporting the composition of individual fragments could be valuable to reduce potential variability.

Keywords: Fourier transform infrared spectroscopy; Sampling error; Urinary stones

INTRODUCTION

Urinary stone is a common health burden with the estimated prevalence of approximately 10% and 5% in the US and Germany, respectively [1,2]. Meanwhile in Asia, it was estimated that 1%–19.1% of the population suffer from urolithiasis depending on the location [3]. The lifetime prevalence of stone disease in Korea is 11.5%, with a steady increase [4]. Stone may induce no symptoms [5], which can lead to a delayed management. Once stone bearers are diagnosed with stone disease, composition analysis should be performed at least once for them since it provides insight into potentially

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Table 1. Distributions of stone positions and treatment methods

Position of stone	No. of instances	Treatment method	No. of instances
Kidney	140	Percutaneous nephrolithotomy	134
Ureter	2	Ureteroscopy	7
Bladder	5	Retroperitoneal laparoscopy	1
Urethra	2	Cystoscopy	7

abnormal metabolisms, which can aid in the determination of underlying causes and the prevention of recurrence [6-8].

The preferred methods of stone composition analysis include infrared spectroscopy and X-ray diffraction (XRD) [7]. Although Fourier transform infrared spectroscopy (FTIR) has been widely used due to its superior characteristics, namely short running time and the ability to detect drug-induced components [9], the lack of quality control and the inconsistent reports from commercial laboratories remain to be of concern [10-12]. The selection of reference spectra, standard nomenclature, and the qualification of the performer can contribute to inconsistent results.

Previous studies suggested that stone sampling can be another contributor to the conflicting results [13,14]. There are two stages where stone sampling can affect the results for composition analysis: at the hospital following stone surgeries and at the analytical laboratory. In the first stage, the entire stone(s) may not be collected due to the dusting technique that causes retrieving the whole samples unfeasible, or only a part of stone(s) may be selected for analysis by a surgical staff. In addition, patients often request a part of stone as surgical proof. In the second stage, sampling error may occur if only a part of, but not the entire stone fragments are included in the analysis.

The effect of stone sampling on composition analysis has been investigated in a previous study where authors used the simulation of a virtual two-dimensional model of ideally concentric stones [15]. In clinical practice, however, patients often have multiple stones and rarely possess concentric ones. Since stone fragments are usually collected when the stone removal procedure is complete, fragments from different stones or different areas of a stone (e.g., core or perimeter) are collected as a whole and often become indistinguishable. This practice can result in a large variability in composition reports.

In this study, we demonstrated how a typical clinical setting can lead to inconsistent results in stone composition analysis and assessed the level of composition variability across the fragments of a stone sample. In addition, we provided suggestions regarding the practical approaches to mitigate the risk of misclassification.

MATERIALS AND METHODS

1. Research population

Institutional Review Board approval of Dinh Tien Hoang Institute of Medicine (IRBDTHIM number: IRB-2308) was obtained for this retrospective study, and informed consents were waived. We collected stone samples from the storage of Department of Urology, Hanoi Medical University Hospital. These samples are from patients who had undergone stone surgeries from January 2022 to August 2022. Various stone surgeries were performed according to stone location such as percutaneous nephrolithotomy, ureteroscopy, retroperitoneal laparoscopy and cystoscopic removal (Table 1). Following the procedures to remove bio-residues, the samples were dried and stored in sealed containers at room temperature.

Since a patient may have undergone multiple surgeries with the resulting samples being stored separately, we used the term "instance" to indicate a stored sample from a separate surgery. Instances with information that did not match the entry in electronic medical records were excluded.

2. Sample preparation

For each instance, stone fragments were washed with distilled water, dried, and separated into test tubes. Each fragment was then ground into fine powder by using a set of pestle and mortar. Between sessions, the pestle and mortar were well cleaned with a 70% alcohol solution and dried by applying heat. If there was a large volume of powder, we divided it into multiple specimens with a roughly same amount, such that each specimen could adequately cover the scanning crystal (see Fig. 1 for the experimental scheme).

3. FTIR analysis

Stone compositions were analyzed by a Spectrum Two FT-IR Spectrometer (PerkinElmer Inc.) equipped with a Universal Attenuated Total Reflectance Accessory (UATR) and a Spectrum IR software v10.7.2.1630 (PerkinElmer Inc.). The spectral range was set from 450 to 2,000 cm^{-1} with the resolution of 4 cm^{-1}.

The powder of each stone fragment was carefully mixed prior to being placed over the crystal of UATR. The back-

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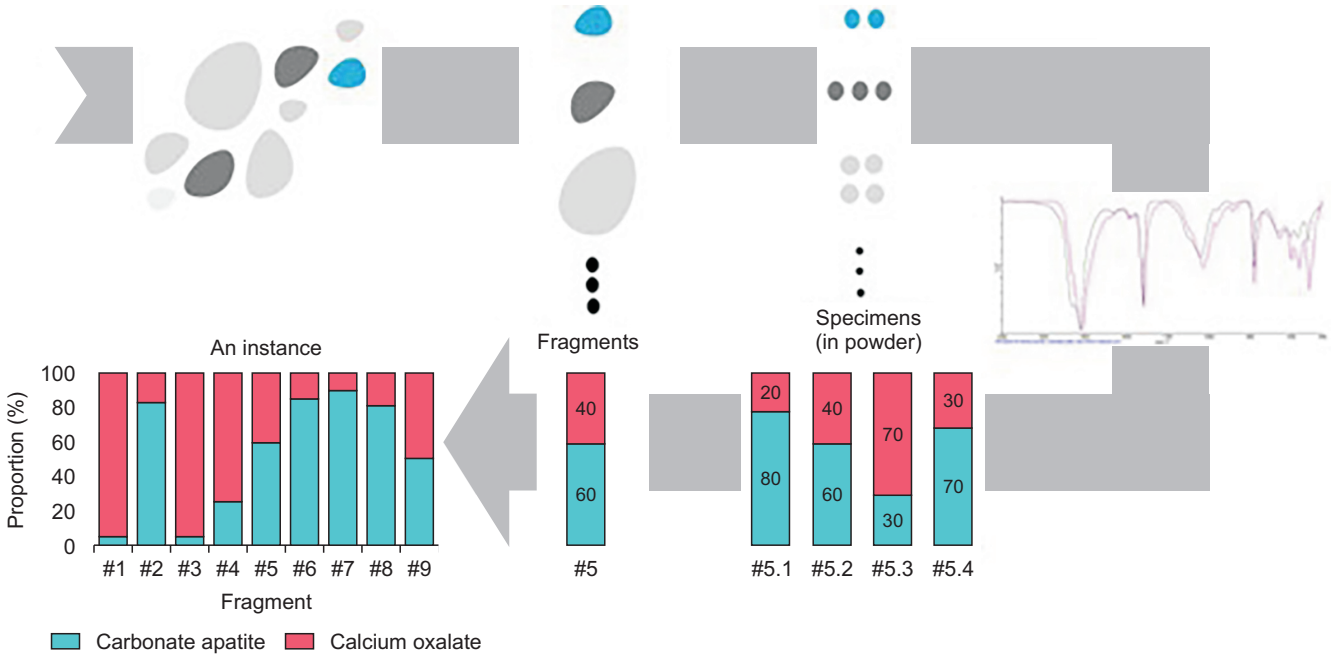

Fig. 1. Illustration of the experimental scheme.

ground spectrum was collected at the interval of every ten specimen analyses. Each specimen was pressurized to a force gauge of 60 and scanned ten times. The acquired spectra were then baseline-corrected and matched against the reference library. The identification of components and their proportions (in percentage) in spectra were derived using a reference library, NICODOM IR Kidney Stones Library (NICODOM Ltd.).

The proportion of a component in each stone fragment was averaged over the corresponding proportions in its associated specimens. For example, in Fig. 1, the proportion of calcium oxalate in fragment #5 was averaged over the proportions of calcium oxalate from specimen #5.1 to #5.4. To demonstrate the potential variation, we defined an instance as being "significantly variable" when the proportion of any component across its associated fragments differed by equal to or greater than a given cut-off percentage.

RESULTS

1. Research cohort

A total of 146 patients (101 men and 45 women) were included in this study. The mean age of patients was 54.3±12.7 years with the minimum and maximum age of 9 years and 85 years, respectively. Since some patients underwent multiple surgeries, samples from 149 instances with a total of 1,135 stone fragments were collected. In average, there were 7.6±3.3 (range, 1–21) stone fragments per instance and 2.3±0.5 (range, 1–6) specimens per fragment. Other characteristics of the research cohort are presented in Table 1.

Most of the instances in our study were comprised of multiple components, and only 14.1% of the instances consisted of a single component. Calcium oxalate was the most common component among the single-component instances, accounting for 10.1% of the instances. The majority of instances (74.5%) were mixtures of two components. The mixture of Carbonate apatite and Calcium oxalate was the most dominant composition, accounting for 66.9% of all instances. Notably, 11.4% of the instances contained three or more components (Fig. 2).

2. Composition variability across fragments

The compositions of fragments that originated from the same instance showed a high level of variability. The representative results of composition analysis for the instances with multiple components are demonstrated in Fig. 3. Fig. 3 represent the instances that consist of two, three and four components, respectively, and demonstrate how different samplings can lead to inconsistent results. In the example of the instance in Fig. 3B, the sampling and subsequent analyses of fragments #11 or #12 would make Struvite as a dominant component. In contrast, the sampling of fragments #3 and #4 would label Calcium oxalate and Carbonate apatite as a dominant component, respectively.

Fig. 4 shows box plots illustrating the degree of composition variation across instances. The x-axis lists the names of

components in instances and the y-axis indicates the maximum difference in the proportion of component among fragments that belonged to a given instance. The variation was also demonstrated in the number of "significantly variable" instances. Of the total of 128 multiple-component instances, there were 89 (69.5%) and 70 (54.7%) significant variable ones if the cut-off value of the maximum difference was 20% and 30%, respectively (Supplementary Fig. 1). In other words, there were more than half of all the multiple-component instances that their fragments' composition changed at least by 30%.

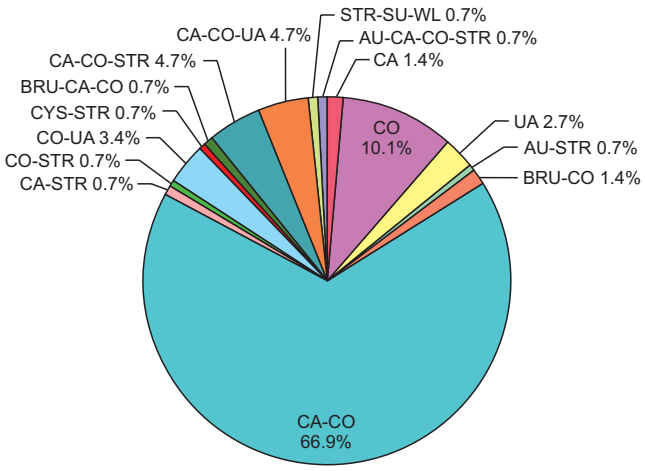

Fig. 2. Distribution of composition in instances. CA, carbonate apatite; CO, calcium oxalate; UA, uric acid; STR, struvite; BRU, brushite; CYS, cystine; SU, sodium urate monohydrate; WL, whitlockite; AU, ammonium urate.

The inconsistency of composition analysis results increased as the number of components in an instance increased (Figs. 3, 4). We categorized the instances into three groups depending on the number of components: group A (one component), group B (two components), and group C (three or more components). The median (interquartile range) values of the maximum difference in the proportion of any components were 30% (14%–45%) and 80% (37%–100%) for the group B and C, respectively. The group C exhibited a significantly higher maximum difference in the proportion of component than the group B ($p<0.001$ using Mann–Whitney U test).

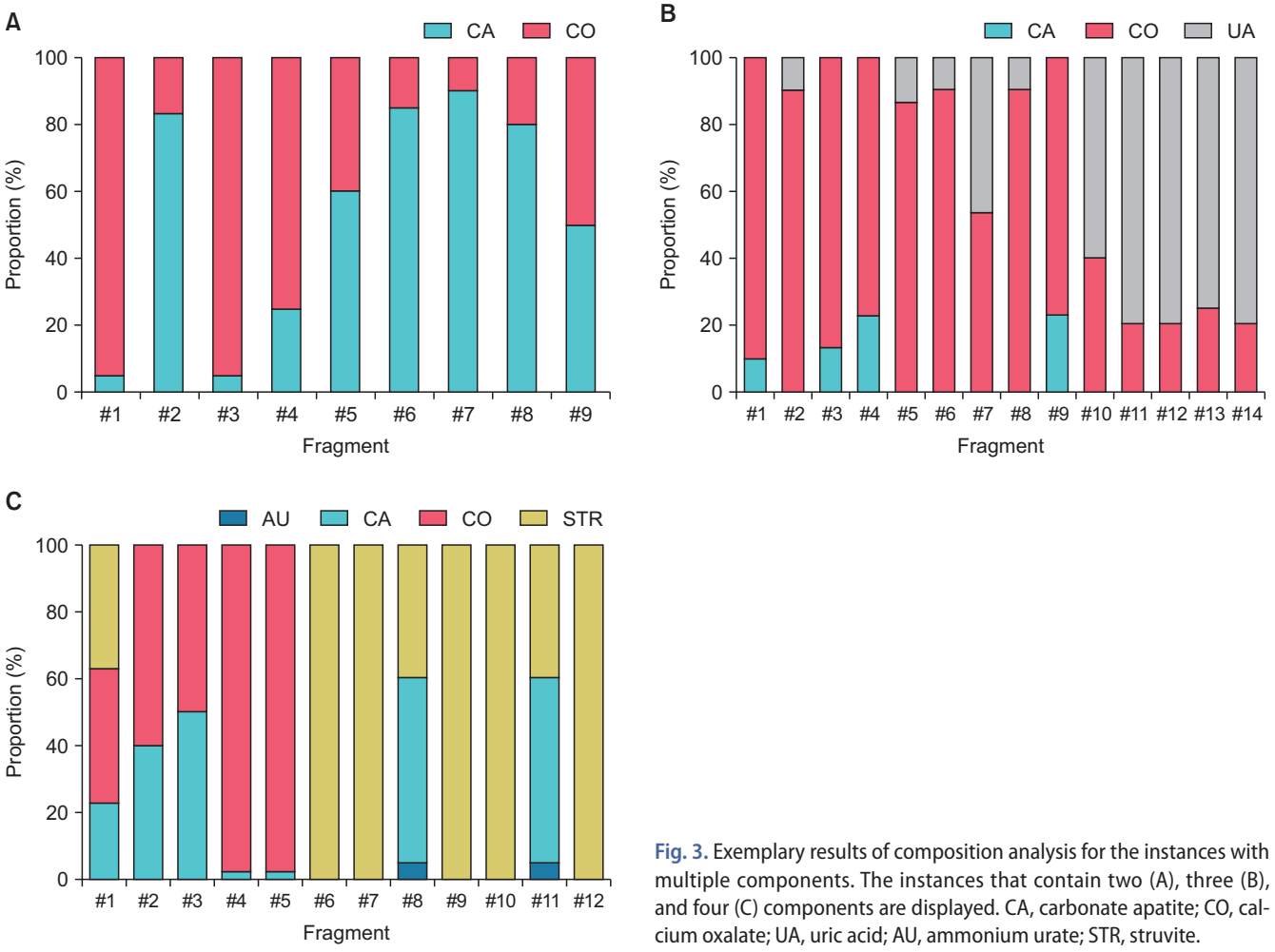

Fig. 3. Exemplary results of composition analysis for the instances with multiple components. The instances that contain two (A), three (B), and four (C) components are displayed. CA, carbonate apatite; CO, calcium oxalate; UA, uric acid; AU, ammonium urate; STR, struvite.

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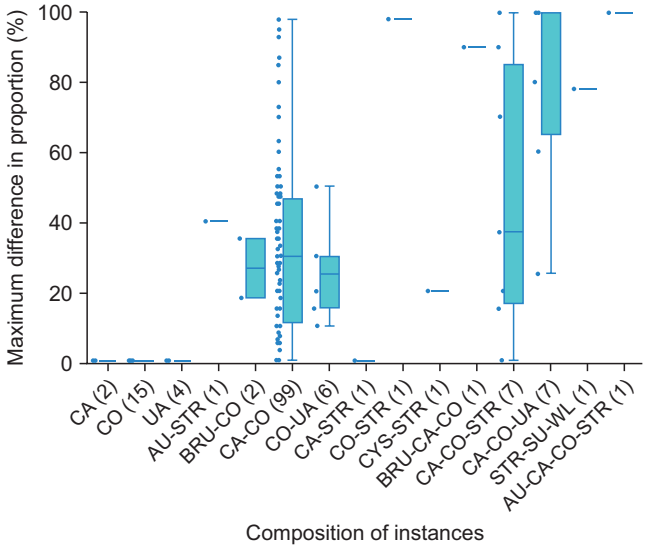

Fig. 4. Box plots showing the maximum difference in the proportion of component across fragments that belong to a given instance. Each dot represents an instance. Numbers in parentheses denote the numbers of instances with the given composition. CA, carbonate apatite; CO, calcium oxalate; UA, uric acid; AU, ammonium urate; STR, struvite; BRU, brushite; CYS, cystine; SU, sodium urate monohydrate; WL, whitlockite.

The number of fragments per instance, which is related to the size of stone, was another contributing factor to the composition variability. Supplementary Fig. 2 is a scatter plot between the number of fragments per instance and the maximum differences in the proportion of component, which shows a statistically significant correlation between the two variables (Pearson's correlation coefficient of 0.4, $p<0.001$).

DISCUSSION

Composition analysis is an important piece of urinary stone management. The knowledge of stone composition may reveal the underlying mechanisms of stone formation, which can lead to more personalized diagnostic and preventive measures. Unfortunately, previous reports have demonstrated that stone composition results obtained from commercial analytical laboratories can be inaccurate and inconsistent [10-12].

Sampling error is one of the major factors that can contribute to the inaccuracy and inconsistency of the stone composition analysis. Using virtual models of ideally concentric stones with two layers of different components, Laube et al. [15] evaluated the proportion of each component in five randomly drawn fragments. The authors showed that the difference in the proportion of component between two fragments can be as high as 60.5%. Our study examined the composition variability in real stone samples and revealed that the difference was more profound. The compositions of fragments that arose from the same instance could be completely different. As shown in Figs. 3 and 4, the difference in proportion was as high as 100% in some components. In addition, we found that the number of components in an instance and the size of instance were positively correlated with the level of composition variability.

Our findings suggest that the sampling procedure and technique of urinary stone can have a crucial impact on the result of composition analysis. Precautionary measures can be taken to reduce the chance of erroneous results when collecting and analyzing samples as well as interpreting composition reports. At the first stage, surgical staff who collect stone samples need to be aware that a big stone has a relatively high chance of producing composition variability. Ideally, all stone fragments should be collected and analyzed; however, this is nearly unrealistic in routine clinical practice. Alternatively, mapping and denoting the position of stone fragments while they are sampled and collected in biopsy can at least provide useful information. Since urinary stones are formed from the inner core to the surface, the knowledge of stone fragments' relative location can help urologists comprehend the metabolic history of patients, which will ultimately render an improved decision-making. At the second stage, caution must be taken in sample analysis and composition interpretation. As the fragments from the same instance can have completely distinct compositions, analysis should be conducted for individual fragments and reported separately. In practice, however, the analysis of every fragment from excessively large stones may be unfeasible. These cases also highlight the role of mapping and denoting the position of stone fragments. The practice of keeping track of stone fragments' position can provide urologists with in-depth understanding about the phases of stone formation and thus an extended opportunity to tailor proper therapeutic strategies. Moreover, the positional knowledge of the analyzed fragments may be beneficial to the post-operative treatment. To illustrate this point, let's assume an imaginary scenario where there are residual fragments from the core of the stone following surgery. By mapping, we could learn that fragments from the core mainly comprised of uric acid (similar to fragment #10–#14 in Fig. 3B). In this case, the size of residual fragments would likely be reduced by taking medications. Therefore, the patient would avoid a second invasive surgery since these fragments could successfully pass through urinary tract.

The results of this study have an important implication toward research on predicting urinary composition via computer vision, in which obtaining ground truth is a crucial element. In a recent study, for example, Black et al. [16] took

pictures of stone fragments for model development and validation. The authors did not provide details about how the compositions of the stone were obtained and whether composition analysis was performed for each fragment. As shown by our findings, fragments from the same instance can have completely different compositions; therefore, there may be a possibility of mislabeling the composition of stone fragments if the composition analysis was not performed on the individual fragments. Recently, research interest was directed towards predicting stone composition using intra-operative endoscopic digital images [17-19]. For example, Zhu et al. [17] trained a deep learning model on images derived from intra-operative endoscopic videos. These images captured either the surface or sectional part of stone, which may not represent the composition of the whole or other part of stone. Moreover, if the part of stone that was captured became pulverized during the endoscopic procedure, the captured images would most likely have a wrong label. Labeling images with the composition analysis from the fragments that were captured is more likely to enhance the prediction.

The present study has several limitations. Firstly, our findings are limited to the use of FTIR for composition analysis. Although the composition variability in other composition analysis techniques, such as XRD and chemical analysis, is unknown, our results are of relevance since FTIR is a popular modality of choice in clinical practice. Secondly, we matched the obtained spectra to a commercial reference library. As a result, our results relied on how the reference spectra were obtained and quantified in the commercial reference library. This information was neither disclosed nor found elsewhere. Lastly, all analyzing steps were conducted by one performer, which may not represent the real situation where multiple personnels can be involved in the analysis procedure. In such cases, the composition variability is more likely to be exacerbated.

CONCLUSIONS

In a comprehensive analysis of stone fragments, we demonstrated that the urinary stone composition could be significantly variable across the specimens of a given fragment as well as the fragments of a given instance. The variability was strongly correlated with the number of components that the instance was consisted of and the size of samples. Precautionary measures should be carefully considered when performing the composition analysis as well as applying the results to decision-making processes.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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AUTHORS' CONTRIBUTIONS

Research conception and design: Binh Duy Le, Kyung-Jin Oh, Long Hoang, and Ilwoo Park. Data acquisition: Binh Duy Le and Anh Tuan Le. Statistical analysis: Binh Duy Le. Data analysis and interpretation: Binh Duy Le and Kyung-Jin Oh. Drafting of the manuscript: Binh Duy Le, Kyung-Jin Oh, and Anh Tuan Le. Critical revision of the manuscript: Binh Duy Le and Ilwoo Park. Obtaining funding: Kyung-Jin Oh and Ilwoo Park. Administrative, technical, or material support: Anh Tuan Le, Long Hoang, and Ilwoo Park. Supervision: Long Hoang and Ilwoo Park. Approval of the final manuscript: Binh Duy Le, Kyung-Jin Oh, Long Hoang, and Ilwoo Park.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.4111/icu.20240275.

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