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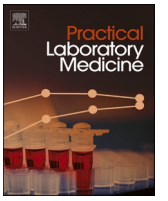

To disinfect or not to disinfect that is the question – Procedure when drawing blood for alcohol measurements in Denmark

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ABSTRACT

Swabbing with ethanol to disinfect the skin before venipuncture does not bias measurements of blood ethanol, as previously suspected. International evidence-based theory may not always be successfully integrated into local practices, where old customs may remain. So how are the local protocols for swabbing in practice – if they even do swab? Not disinfecting may risk patient safety. We aim to put a focus on the venipuncture disinfection procedure in practice when measuring blood alcohol for clinical matters and if their procedure refers to a guideline.

Specialized biomedical laboratory scientists (BLS) are typically responsible for the phlebotomy procedure in Denmark, thus questionnaires were sent to the relevant BLS in 2020 to map disinfection procedures in all Danish hospitals and affiliated blood draw clinics (n = 58).

The response rate was 93% (54/58). We observed an inter-laboratory dissimilarity in swabbing procedures, when measuring blood alcohol: A quarter did not use any disinfectant (26%), while the remaining disinfected with ethanol 55%, isopropanol 13%, and 6% with ethanol/chlorhexidine. Of the five Danish regions, three had a regional guideline (3/5), otherwise the swabbing protocol was locally based. There was a regional difference in disinfecting or not (Chi^2 $p < 0{,}0001$).

Danish protocols do not always parallel international literature and international guidelines. Not applying disinfectant may jeopardize patient safety. Laboratories are encouraged to work with evidence-based practice or follow newest standardized international guidelines.

1. Introduction

Standardization of laboratory procedures aim to optimize analytical quality and patient safety. To promote standardization, various laboratory guidelines are available for numerous clinical biomedical laboratory procedures [1,2]. Laboratory guidelines are developed on a national or international scale; however, clinical laboratories may also develop their own protocols exempt from the more official existing guidelines [1,2]. In Denmark, individual laboratories may choose which combination of official guidelines and local protocols suit their local practices and quality assurance strategies. This autonomy may not be just a Danish phenomenon, as a study showed that only 63 % of European laboratories followed a national guideline regarding the handling and storage of blood samples for coagulation tests [3]. This autonomy may be a concern if locally developed protocols neither follow official guidelines nor the scientific evidence-based literature - as we previously observed for blood tube order of draw in Denmark [4].

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Phlebotomy is an important preanalytical step and requires standardization of procedures. Mostly, biomedical laboratory scientists (BLS) perform phlebotomy in Denmark, but also nurses and other trained phlebotomists [5]. Health care professionals must follow protocols to assure preanalytical quality and patient safety, which includes ethanol swabbing of the skin to prevent infection introduced mainly from the bacterial flora on the skin [6–8]. However, it has previously been disputed if disinfecting the skin with ethanol could cause spourious results and analytical bias for blood alcohol (p-ethanol) measurements. But scientific studies have repeatedly shown that swabbing with ethanol when drawing blood for p-ethanol will improbable affect neither clinical nor even forensic legal decisions [9–12].

At times, it may be difficult for health care professionals to standardize completely and always follow the existing protocols provided by the laboratory, and this may jeopardize patient safety [2,13]. However, what if staff DO follow the local protocols, but the protocols themselves jeopardize patient safety when drawing blood for p-ethanol? That would be if the local protocol requests not to disinfect the phlebotomy site before needle insertion. It may be an issue for patient safety if laboratories do not disinfect the skin before phlebotomy. Perhaps laboratories stick to old customs and do not update their local protocols to newer evidence-based literature and international guidelines for various reasons. This was the case in our own laboratory, and we found this worrisome for our patient safety, and we wondered about the clinical practice at other hospitals. Our laboratory may not stand alone, so therefore, we started to investigate Danish laboratory local protocols in relation to disinfection procedures when drawing blood for p-ethanol. If Danish protocols parallel international literature and international guidelines. Basically, to what extent international theory is successfully integrated into local practices or if old customs still prevail – like in our laboratory. With this study, we map local laboratory disinfection protocols when drawing blood for p-ethanol (for clinical matters and not forensic). If they swab the venipuncture site or not – that is the question for blood draw laboratories in Denmark.

2. Materials and methods

Most phlebotomists in Denmark are BLS, but also nurses, laboratory technicians and other professions may be trained to perform phlebotomy [5]. In the Danish hospitals, BLS specialists from the clinical biochemistry departments are typically part of the team responsible for the phlebotomy procedure in the hospitals. Therefore, we approached BLS specialists with a questionnaire: BLS specialists from all public Danish hospitals (n = 44) and affiliated blood draw clinics (n = 14). In this study, our main focus was blood sampling of p-ethanol for clinical matters and not blood sampling for forensic/police matters. From our previous work [4] and updated via the search engine Google (California, USA), we systematically collected telephone numbers, and in April 2020, we rang all the laboratories and gathered oral consent from the responsible respondents and an email address for future correspondence. Each respondent was informed that participation was voluntary, anonymous, and confidential. The questionnaire was in Danish (native language of Denmark) and distributed via email to each respondent. The e-mail covered information about our research followed by a short questionnaire. The questionnaire included four questions concerning the venipuncture swabbing procedures at their laboratory when measuring p-ethanol and a request to attach their local protocol, which is shown in Table 1. Two of the questions were configured as closed dichotomous, and two were open-ended regarding type of disinfectant and guideline. Some participants also added an extra qualitative comment. We also gathered data from which region the participant represented. There are five regions in Denmark: Capital Region of Denmark; Region Zealand; Region of Southern Denmark; Central Denmark Region; and North Denmark Region. Participants were instructed to reply in the actual e-mail with their answers and hence send it back to the sender (the investigators), and email reminders were used if necessary. All information from the emails were transferred to Excel for data management and statistics and the emails were deleted after transfer. The categorical test Chi^2 were applied with α set to 0,05. GraphPad Prism 8.4.3 software (GraphPad Software, San Diego, CA, USA) was used to illustrate data.

3. Results

From the 58 Danish clinical blood draw laboratories, two did not answer our first phone call, one did not answer our email with the questionnaire, and one was excluded because of not filling out the survey correctly. Hence, overall response rate to the questionnaire was 93% (54/58; 40 hospitals plus 14 affiliated laboratories). Of those, 83% (45/54) had attached their local protocol along with their answers to the questions. The protocols/guidelines are shown in Table 2. Respondents referred to a regional web-link for the regional guideline when drawing blood for p-ethanol for region Zealand [14]; Central Denmark Region [15]; and North Denmark Region [16]. The regional guideline from Region Zealand stated: *When disinfecting the skin with ethanol, you must ensure that the skin is dry before*

Table 1
The questionnaire that was distributed to all blood draw laboratories in Denmark.[a]

Questions concerning the disinfection procedure before blood sampling of p-ethanol	
Do you use disinfection before venipuncture, when drawing blood for p-ethanol?	Yes/No
If yes, what type of disinfection product, do you use?	(Open ended)
If you use ethanol, must the disinfectant site evaporate before performing phlebotomy?	Yes/No
If you have additional guideline procedures, please describe	(Open ended)
Please attach your local guideline for phlebotomy of p-ethanol.	(Request)

[a] Biomedical laboratory scientists (BLS) were approached. At clinical biochemistry departments or at clinical biochemistry outpatient clinics, BLS are typically responsible for the phlebotomy procedure in Denmark.

Table 2
Regional guidelines * and local protocols for disinfection procedures of the phlebotomy site before drawing blood for p-ethanol.

Total (n = 54)	Capital Region of Denmark (n = 13)	Region Zealand (n = 11)	Region of Southern Denmark (n = 11)	Central Denmark Region (n = 12)	North Denmark Region (n = 7)
No special guideline for ethanol samples, so no local guideline was attached	5		4		
"Regarding sampling for Ethanol determination, the usual alcohol swabs must not be used. This especially applies to samples requested by the police, where the supplied disinfectant wipes without ethanol must be used. Follow the instructions included with the police sampling kit"	3				
"Do not use alcohol or other volatile disinfectants at the venipuncture site"	1				
"The sampling site is disinfected with an alcohol wipe. Allow the area to dry. The skin must not be disinfected if P-Ethanol has been requested"	1				
"When disinfecting the skin with ethanol, you must ensure that the skin is dry before venipuncture. The sample is drawn into a closed system, which must not be decapped before analysis. For alcohol intoxication. Cannot be used forensically. May, however, have implications for insurance matters" *		10			
"No disinfection of the phlebotomy site of any kind before taking p-ethanol samples"	3	1			
"When taking samples, do not clean with ethanol. The skin may be cleaned with Medi-Swab, which contains isopropanol 70%, if you make sure that the skin is completely dry again before insert"			3		
"If a sample is to be taken to measure P-Ethanol, special swabs containing 70% isopropyl alcohol (purple) are used. If this is not available, the skin is not cleaned"			1		
"Use 70% isopropyl alcohol swaps for disinfection before sampling p-ethanol"			2		
"Disinfect injection site with minimum 70% isopropyl alcohol or ethanol, unless otherwise specified, and allow to evaporate. At the same time, rinse your fingers and let it evaporate. It is only permitted to feel for the vein in the disinfected area with the alcohol disinfected fingers that have not touched other things"			1		
"Disinfection is performed according to normal procedures. The analysis may only be used for diagnostic and therapeutic purposes. Investigations with legal consequences must be carried out at the Institute of Forensic Medicine, and samples must be taken by a doctor"*				12	
"Do not use skin disinfection of any kind when taking the sample. The analysis should only be used for diagnostic purposes. Alcohol tests of legal importance are carried out at the Institute of Forensic Medicine" *					7

venipuncture. The sample is drawn into a closed system, which must not be decapped before analysis. For alcohol intoxication. Cannot be used forensically. May, however, have implications for insurance matters [14]. Table 2 shows that one affiliated laboratory did not follow the regional guideline from Region Zealand. The reason behind this is not known based on the findings in this study.

In two regions, Central and North Denmark Region, cases with legal consequences are handled by Institute of Forensic Medicine (Table 2). In the other three regions, a special sampling kit is used as a disinfectant when P-Ethanol ordered by the Institute of Forensic Medicine or the Police. This kit contains a butterfly needle, two sampling tubes, and swabs (containing 75% V/V isopropanol) [17–19].

The results presented in Fig. 1 and in Table 3 only include sampling for clinical purposes and exclude sampling for police or forensics. As shown in Fig. 1, disinfection procedures differed when drawing blood for p-ethanol, with 26 % (14/54) of facilities not disinfecting at all before venipuncture. Table 3 also shows a regional statistically significant difference in disinfecting or not (Chi 2 test; $p < 0{,}0001$ when pooling ethanol/ethanol + chlorhexidine/isopropanol versus no disinfectant) where North Denmark Region does not disinfect, and Capital Region of Denmark is mixed. The other three regions disinfect apart from the one affiliated blood draw clinic in Region Zealand.

All laboratories that swabbed with ethanol (*i.e.* with ethanol only (n = 30) and ethanol with chlorhexidine (n = 3)); the ethanol had to evaporate completely before venipuncture.

Some laboratories included a qualitative comment to our study:

Capital Region of Denmark: *"We do not use disinfectant before a phlebotomy when measuring p-ethanol. This is an old procedure. Our department is planning to make a risk assessment to see if disinfection influence our test results or not, so we can start to disinfect"*. Off note, this

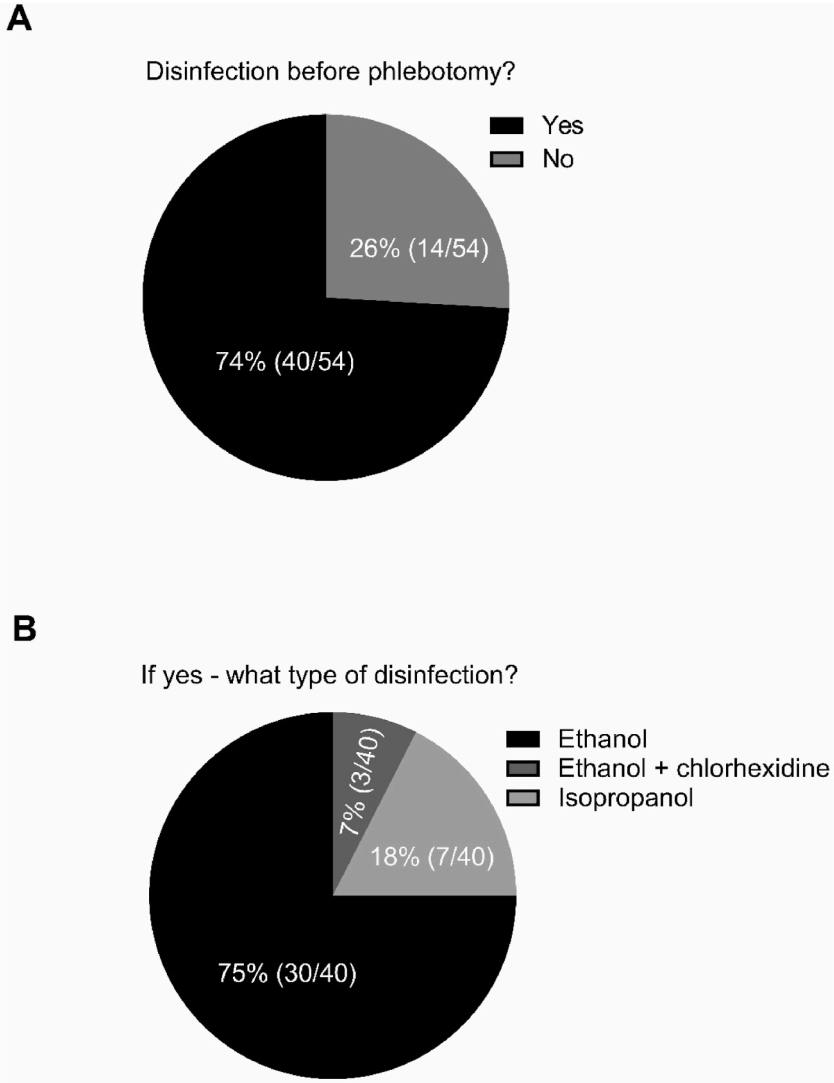

Fig. 1. Panel A: Shows how many of the Danish hospitals and affiliated blood draw clinics decide to disinfect the skin - or not - before venipuncture when drawing blood for p-ethanol (blood alcohol measurements) for clinical matters like intoxication or abuse (not forensic/police matters). Panel B: For the laboratories that do disinfect at phlebotomy, the circle shows the distribution of the type of disinfection.

Table 3
Disinfection types in Denmark and regional distribution.

Region of Denmark	Ethanol	Ethanol + chlorhexidine[a]	Isopropyl alcohol	No disinfectant at all	Total
Capital Region of Denmark	7	–	–	6	13
Region Zealand	10	–	–	1	11
Region of Southern Denmark	4	1	6	–	11
Central Denmark Region	9	2	1	–	12
North Denmark Region	–	–	–	7	7
TOTAL	30 (55%)	3 (6%)	7 (13%)	14 (25%)	**54**

[a] Swabs with 82% ethanol and 0,5% chlorhexidine.

quote was from our hospital.

From another hospital in Capital Region of Denmark: "*When sampling for p-ethanol, the usual ethanol swabs must not be used. This especially applies to samples requested by the police, where the supplied disinfectant wipes without ethanol must be used. The instructions included with the police sampling kit must be followed*". From this, it is not clear, what the phlebotomist should use instead.

Region Zealand: *"Our ethanol sampling guideline was amended back in 2010. It has always been that we were not allowed to disinfect. I*

know that there was a talk in our region; and now the region recommends disinfecting with ethanol which need to be completely evaporated before performing phlebotomy for p-ethanol measurement for clinical samples, so basically it follows the general sampling procedure".

Region of Southern Denmark: *"The 70% isopropanol swabs are used for measuring p-ethanol for clinical purposes. Exceptionally, if any are not available, the skin will not be disinfected".*

"Our p-ethanol must only be used diagnostic and therapeutic. For drunk driving and forensic, it's a very special kit we get handed where it states exactly what to do, and we simply just follow the instructions".

Central Denmark Region*: "We were not allowed to swab when measuring p-ethanol for clinical matters in the past. However, that has been changed after a risk assessment that showed swabbing with ethanol does not have an influence on the p-ethanol analysis. Also, because we only insert the needle when the alcohol is evaporated. So if any contamination, it would be minimal, and this is the risk we have taken plus this p-ethanol is not used for forensics, like drunk driving".*

North Denmark Region: *"Skin disinfection of any kind is not used when sampling p-ethanol".*

4. Discussion

When blood is drawn for alcohol testing, p-ethanol measurements may document previous oral administration of alcohol. This test is clinically applied, for instance, to monitor alcohol abuse or to detect intoxication in acutely hospitalized patients. The volume of alcohol intake is directly related to the concentrations measured in the blood [20,21]. In Denmark, it is customary to apply ethanol for skin disinfection before phlebotomy. Ethanol reduces microbial organisms on the skin and must evaporate to enhance its disinfection properties [22–24] Ethanol evaporates rapidly from skin, reducing the quantity applied to 50% in 12 s [25]. But when drawing blood for p-ethanol, our study showed that many (39%) avoid ethanol-based solutions for swabbing, and a quarter decide not to disinfect at all. Most Danish laboratories did disinfect before phlebotomy, using ethanol (55%); ethanol with chlorhexidine (6%); or isopropanol (13 %). Studies have shown that these are all efficient disinfectants, and that isopropanol is an effective alternative to the ethanol-based solutions [23,24,26–28]. However, a quarter (26%) of Danish laboratories decided not to disinfect at all, despite the availability of effective alternatives to ethanol, and even swabbing with ethanol should be a safe procedure [9,10,29].

4.1. Scientific literature and international guidelines

Studies have consistently shown that applying ethanol to the skin and allowing it to evaporate before venipuncture does not contaminate the blood sample or lead to spurious conclusions [9–12]. Lippi et al. found that taking the blood sample within 5 seconds after applying ethanol and not allowing the skin to dry does not alter the concentration of p-ethanol. In this study, the alcohol concentration was undetectable (i.e., <0.22 mmol/L; 0.01 g/L) in EDTA plasma and whole blood using reference head-space gas chromatography, and p-ethanol was also undetectable using a tested commercial enzymatic assay [9]. Similar findings were reported in a newer study by Nakao et al., which found that ethanol was undetectable (<0.001 mg/mL) in 80 blood specimens obtained from 40 participants who had their skin wiped with ethanol - including those wiped less than 5 seconds before venipuncture [10]. Studies have also shown that isopropanol also does not cause spurious results in p-ethanol concentrations [29,30,31].

A new international guideline from 2018 favors alcohol swabbing to ensure patient safety - even for forensic matters. This European & Latin American working group recommends using ethanol, which must evaporate on the skin before performing phlebotomy [8]. Examples of other international bodies creating guidelines for laboratory practice procedures also include World Health Organization (WHO) and the Clinical and Laboratory Standard Institute (CLSI) [6,7]. The WHO recommends alcohol swaps and does not specifically mention p-ethanol testing. However, they state that alcohol is preferable to iodine because povidone iodine may falsely increase levels of potassium, phosphorus, or uric acid in laboratory test results [6]. Surprisingly, the recent CLSI guideline from 2017 advices not to use ethanol-based solutions for disinfection to avoid potential contamination and analytical bias [7]. When international guidelines remain somewhat divergent this may add to uncertainty in the laboratories.

4.2. Danish protocols/guidelines

Our national study shows that some Danish laboratories do not adhere to contemporary international guidelines, nor to the latest evidence on ethanol swabbing and p-ethanol. However, discordancy in international guidelines might be confusing for laboratories when developing their protocols. These factors, combined with long-standing local traditions and habits, may influence laboratory practices. However, work pressure may also contribute to unawareness regarding new evidence-based knowledge on the subject. Denmark does not have its own standardized national recommendations for the disinfection procedure when drawing blood for p-ethanol. We have previously shown that Danish laboratories typically develop their own local protocols and that national laboratory guidelines are not custom in our country [4]. If interlaboratory guidelines exist, they are regional based, as shown in this study. We found that three of the five regions had developed a guideline, two where drawing blood for p-ethanol follow normal phlebotomy procedures and one that recommends not to disinfect at all. Some laboratories in the regions did not adhere to the regional guideline. For the last two regions, the approach to disinfection or not was more mixed. Our previous study found that standardization between laboratories was poor (order of draw) [4] Our current study shows that there is a mismatch between local practices and international scientific literature and guidelines. Perhaps now, with the Danish bachelor's degree for BLS being more rooted in research and science since 2013 [32], new generations of BLS that we educate may find it more natural to update local practices with the newest scientific literature. We believe that further focus on interlaboratory standardization should also be considered nationwide. This focus may not only be relevant in Denmark because a European survey revealed that only 25% of included countries had a national guideline for

phlebotomy [33].

4.3. Not disinfecting

Despite scientific evidence that swabbing with ethanol does not jeopardize analytical results, another question remains: Could not disinfecting the skin before venipuncture jeopardize patient safety? Because microorganisms from the skin may be introduced to the subcutaneous area of phlebotomy site or perhaps even circulation and cause infection or bacteremia. However, studies have suggested that not disinfecting the skin before subcutaneous, intramuscular, or intravenous injections does not pose a risk of infection [22,34]. Speculatively, this may be the reason why laboratories have chosen not to disinfect and have not felt the need to change their local protocol– perhaps because they have not experienced any side effects from of not swabbing before phlebotomy. However, disinfecting the venipuncture site for blood transfusions is done carefully and in two steps to avoid case of bacteremia/sepsis, which are risks [35,36]. Also, when drawing blood for blood cultures to detect bacteremia, disinfection is important to avoid contamination and false positive blood cultures [28,37]. So at phlebotomy, microorganisms like bacteria may be present, but possibly not posing a hazard during the short phlebotomy session. To our knowledge, it remains uncertain whether immunosuppressed individuals may be at higher risk when the skin is not disinfected before blood sampling. However, disinfecting the site does anyway not remove all microorganisms including bacteria [35–38]. The risk of not disinfecting the venipuncture site before phlebotomy needs further new investigation.

4.4. Limitations

In our study, it was not clarified what knowledge the regional guidelines and local protocols were based upon and how old they were. It would also have been valuable to know the annual number of p-ethanol samples requested for clinical purposes from the different included laboratories.

Another limitation was the extent to which staff followed the existing guideline/protocol at their laboratory, as other studies found this problematic [2,13]. Samples for p-ethanol may be taking from different departments around the hospital, such as emergency wards, hospital wards and ambulatory care, by phlebotomists with various backgrounds. And how well they adhere to the local protocol/guideline for this issue was not investigated. Our study does also not include how general practitioners prepare the venipuncture site before drawing blood for p-ethanol.

5. Conclusions

From our observational study, we asked the question of disinfection or not to disinfect and we mapped disinfection procedures in Denmark when drawing blood for p-ethanol. Some laboratories had their own local protocol, and some followed a regional guideline, but there is no national guideline. Some had a regional guideline but did not adhere to this. More than a third of laboratory protocols avoided ethanol swabbing- despite evidence showing it does not affect analytical results - and of these a quarter did not disinfect at all. However, according to the literature, it is suggested that not disinfecting does not affect patient safety, but this needs further investigation. For now, we suggest as a mitigation protocol that the normal skin disinfection procedure for overall blood sampling procedures should also include blood sampling for p-ethanol measurements *i.e.,* ethanol swabbing, which needs to evaporate before venipuncture. We also suggest further interlaboratory standardization to ensure adherence to updated evidence-based practices. Contemporary evidence-based science and current international guidelines would be more likely to be incorporated into local protocols through increased interlaboratory standardization, whether regional or national. This would alleviate the burden on individual laboratories to continuously update their practices. This will safeguard both patients and their test results.

Research funding

None declared.

Informed consent

Informed consent was obtained from all individuals participating in the study.

Ethical approval

Approval from ethics committees was not required for the survey (Danish ethical committees deem this study type exempt from review).

CRediT authorship contribution statement

Rebekka Lynge: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Christina I. Kirkvaag:** Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Writing – review & editing. **Ida H. Eilenberger:** Investigation, Methodology, Project administration, Writing – review & editing. **Anne M.D. Hansen:** Conceptualization, Project administration,

Supervision, Writing – review & editing. **Julie Smith:** Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

Authors state no conflict of interest.

Data availability

Data will be made available on request.

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