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CORRESPONDENCE

A pilot study of placental membrane lucency

To the Editor,

A tool to identify placental membrane translucency more easily may allow a quantitative measure and assist in selection of placentas for histological and microbiological study, particularly in the diagnosis of amnionitis. This would be useful in limited resource settings. Most experts in placental studies contend that all placentas should be examined initially by birth attendants.[1,2] However, assessment of placental membranes is subjective and difficult, especially in placentas with otherwise normal gross morphology.[3]

Methods of macroscopic assessment of the degree of membrane lucency have not previously been studied or reported. In a 2020 Hamamatsu University study, a clear correlation between membrane opacity and histological chorioamnionitis was demonstrated.[4]

The present observational study involves using printed images of scales calibrated to assess the degree of opacity (gradient tools) with imprinted numerical scores. The scores were used to determine the lucency of placental membranes and predict histological abnormalities including possible chorioamnionitis. This study allows cut-offs to be developed to determine utility of the gradient tools. The use included five gradient tools developed from colour palette ideas and included two modified forms of the Ringelmann smoke chart (Fig. 1D,E).[5] Initial testing of the tools involved using two control placentas which were negative for chorioamnionitis. The tools were laminated, then placed directly below the chorion layer and viewed through the amnion. Two other inert materials were used in testing to simulate differing levels of opacity: a surgical glove and a nappy liner, which demonstrated a higher score in correlation with increased opacity of the material stretched over it.

Ethics approval for this study was granted from Gold Coast Hospital and Health Service Human Research Ethics Committee (HREC) (ERC00160) and permission from the developer of the Ringelmann Charts was obtained for this study.

Thirty sequential placentas with a known clinical indication for referral for pathological examination were included in the clinical trial.[5] During the clinical study, prior to formal histological testing, the clinical history on the request form was not available to the observers using the five gradient tools.

The score was determined by the lowest score noted on the Opacity Chart that could be visualised behind the membrane. The devices were placed directly on the chorion and viewed through the area of greatest apparent obscurity in the membrane in all cases (Fig. 1), and a numerical score assigned.

Each placenta was then processed in the usual manner[1] with membranes selected for histological examination by staff not involved in the observational study. Placental membrane histology was assessed by a single pathologist (TW) who applied the Amsterdam criteria for membrane inflammation.[1] In all cases, the presence or absence of haemosiderin was noted. The results of the pilot study of histopathology were recorded separately. The single specialist histopathologist was blinded to the pilot membrane translucency results until the conclusion of the study.

To obtain sensitivities and specificities, binary categories were created from different opacity cut-off scores for each tool. A plot of individual sensitivity/specificity pairs in the receiver operating characteristic (ROC) space was created to visually compare the diagnostic performance of the tools. Jamovi version 1.6.23,[6] and R version 4.0.2[7] were used for statistical analysis.

The Amsterdam criteria were used for histopathological diagnosis.[1] Sensitivity and specificity were calculated to compare diagnostic ability of the tools. Different combinations of the results for each tool were also used to determine accuracy of diagnosis. Thirty placental membranes were included in the data analysis and two were excluded due to loss to follow-up. Chorioamnionitis (Stage 2−3) was found for four placentas (13.3%). A single stage 1 (sub-chorionitis) placenta was reported. No haemosiderin was noted in any of the placentas.

Gradient Tool 1 (Trial A) showed the best diagnostic performance (Table 1, Fig. 2). This was evident in Trial D with the binary cut-offs 0 versus 1−5, resulting in a sensitivity of 75.0% and specificity of 69.2%. This indicates that a score of zero could effectively rule out chorioamnionitis and a score between one and five could reasonably predict chorioamnionitis.

Of the gradient tools used, Gradient Tool 1 provided the best results, with our findings suggesting placental membrane translucency can be assessed with a gradient tool. There is promising potential for producing a reusable small-sized tool such as a ruler that could be incorporated into routine clinical care, particularly for limited resource settings. However, further research is warranted with a larger study in a more diverse patient population to assess the viability of this gradient tool.

There are some limitations to this study. The judgement of the grading was performed by two separate researchers. We did not assess interobserver examination variation in observation in this pilot study and a larger study would provide more information. Due to the red colour of Gradient Tool 1, there was some difficulty in distinguishing opacities from intense blood staining on the membranes. This may have affected their perception of visibility. A contrasting colour could be considered when modifying the tools for use in the future for easy decision-making. There were no placentas with meconium staining and a larger study would allow us to consider this. This important relationship of the maximum alteration recorded in the histological examination of the membranes could more effectively be studied in future research. We did not have the issue of thickened adherent decidua, which was not apparent in this study. This type of tool may limit the location of testing on the membrane, due to the lamination and size of the gradient tool but is worthy of further study. While 30 placentas provide a reasonable indication of the potential diagnostic performance of a tool, and is adequate for a pilot study, a larger sample size is required to confirm these findings. Additional investigations of gradient tools may provide better devices. The nature of the ethics

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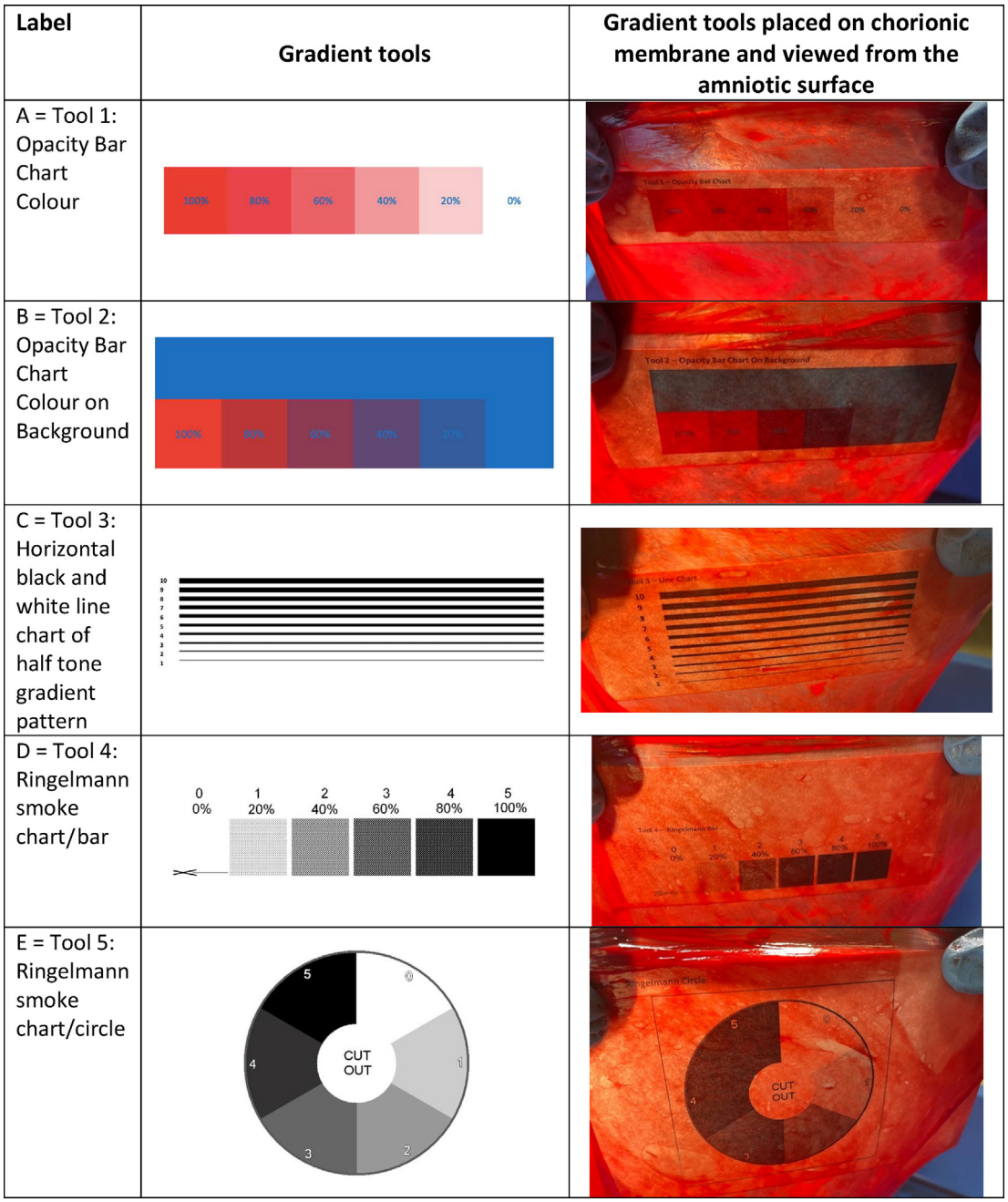

Fig. 1 Method of visualising tools for assessment of membrane lucency.

Table 1 Results of Gradient Tool 1 at different binary cut-offs

Trial	Cut-off values	True positive *n*	True negative *n*	Sensitivity %	Specificity %	Positive predictive value %	Negative predictive value %
A	0–3 vs 4–5	1	25	25	96.2	50.0	89.3
B	0–2 vs 3–5	2	24	50	92.3	50.0	92.3
C	0–1 vs 2–5	2	19	50	73.1	22.2	90.5
D	0 vs 1–5	3	18	75	69.2	27.3	94.7

Letters represented by different trials (A, B, C and D) correspond to the same trials plotted in Fig. 2.

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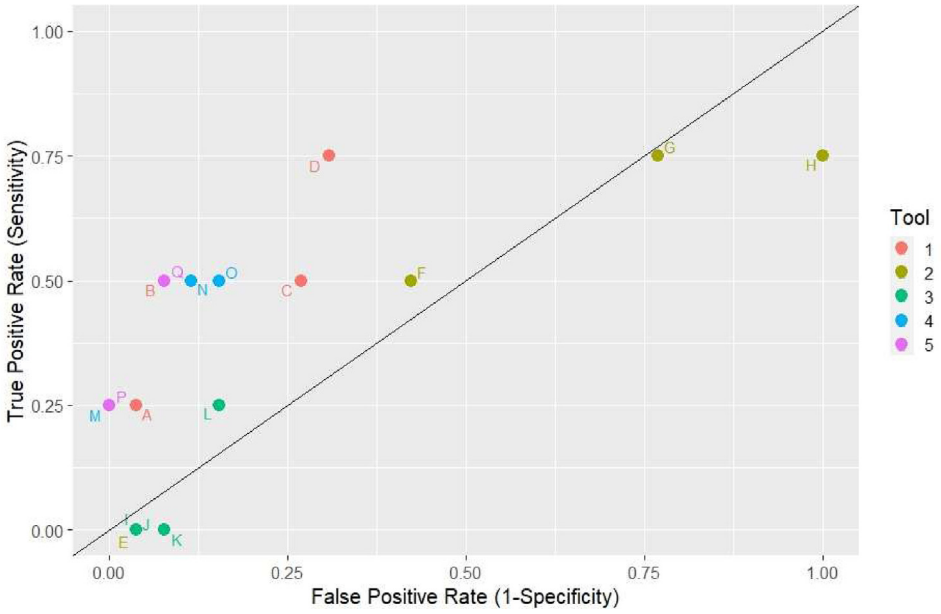

Fig. 2 Plot of individual sensitivity/specificity pairs in the ROC space. Points that are closer to the top-left corner indicate a better diagnostic performance.

approval limited the clinical information available to the investigators and the observers were blinded from the clinical details. A larger study requires correlation of the clinical history with the macroscopic testing of membrane lucency using these tools.

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1. Khong TY, Mooney EE, Ariel I, *et al*. Sampling and definitions of placental lesions: Amsterdam placental workshop group consensus statement. *Arch Pathol Lab Med* 2016; 140: 698−713.
2. Queensland Clinical Guidelines. *Normal birth*. Herston, Qld: Queensland Health, 2017; updated 2018 https://www.health.qld.gov.au/__data/assets/pdf_file/0014/142007/g-normalbirth.pdf
3. Baergen RN. *Manual of Pathology of the Human Placenta*. Boston: Springer, 2011; Macroscopic evaluation of the second- and third-trimester placenta.
4. Horijoshi Y, Yaguchi C, Furuta-Isomura N, *et al*. Gross appearance of fetal membrane on placental surface is associated with histological chorioamnionitis and neonatal respiratory disorders. *PLoS One* 2020; 15: e0242579.
5. The Ringelmann Smoke Chart. UK: Soliftec, 2010; cited 10 Jun 2021 http://www.soliftec.com/Ringelmann%20Smoke%20Chart.pdf
6. The Jamovi Project. Jamovi (Version 1.6.23). https://www.jamovi.org.
7. R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2022. https://www.R-project.org/

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