



Separation sign: novel ultrasound sign for ruling out diagnosis of placenta accreta spectrum

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CONTRIBUTION

What are the novel findings of this work?

In this study, we describe a novel ultrasound marker of normal placental separation, called the separation sign, which can be used to rule out placenta accreta spectrum (PAS). Our findings indicate that a positive separation sign is a reliable predictor of normal placental separation at delivery.

What are the clinical implications of this work?

The separation sign should be used by fetal medicine specialists and sonographers to exclude the diagnosis of PAS in pregnancies at risk for the condition. This should prevent unnecessary treatment and iatrogenic harm following a false-positive diagnosis.

ABSTRACT

Objective To assess the performance of the 'separation sign' as a predictor of normal placental separation in a large cohort of women at risk for placenta accreta spectrum (PAS) and in a high-risk subgroup with placenta previa or anterior low-lying placenta and at least one previous Cesarean delivery.

Methods This was a prospective study of women at risk for PAS referred to a specialist clinic at between 22 and 38 weeks' gestation. All women underwent ultrasound assessment for the presence of the separation sign, which detects the difference in elasticity between the myometrium and the placenta, characterized by different rates of rebound after an ultrasound probe is used to apply pressure over the uteroplacental interface. When the sign is positive, the placenta appears to move relative to the myometrium, leading to the appearance or enhancement of the clear zone. The predictive performance of

the separation sign for normal spontaneous placental separation at delivery was assessed.

Results Of the 194 included women, 163 had a positive separation sign, all of whom went on to have normal placental separation at delivery. Of the 24 women with a negative separation sign, three (12.5%) had normal placental separation and 21 (87.5%) were diagnosed with PAS. This yielded a sensitivity of 98.2% (95% CI, 94.8–99.6%) and specificity of 100% (95% CI, 83.9–100%). In the high-risk cohort (n = 35), a positive separation sign remained a reliable predictor of normal placental separation, with a positive predictive value of 100%, sensitivity of 88.9% (95% CI, 65.3–98.6%) and specificity of 100% (95% CI, 80.5–100%).

Conclusions The separation sign could be a useful tool in women considered to be at risk for PAS, as it can facilitate the prediction of normal placental separation at delivery. This may prevent overtreatment, the associated iatrogenic morbidity and unnecessary allocation of clinical resources. © 2022 The Authors. *Ultrasound in Obstetrics & Gynecology* published by John Wiley & Sons Ltd on behalf of International Society of Ultrasound in Obstetrics and Gynecology.

INTRODUCTION

Placenta accreta spectrum (PAS) is an umbrella term used to describe a range of diseases characterized by pathological attachment of the placenta to the myometrium, which prevents separation at delivery^{1,2}. The greatest risk factor for PAS is a history of previous Cesarean delivery (CD) combined with placenta previa or anterior low-lying placenta (placental margin < 2 cm from the internal os)³ in the current pregnancy. This risk increases with each CD, rising from 3.3% after one CD to 11% after two CDs and to 40% after three CDs⁴.

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Prenatal diagnosis is crucial to reduce maternal morbidity and mortality^{5–8}; however, there is a danger of iatrogenic harm following a false-positive diagnosis. Midline abdominal incision, fundal uterine incision and Cesarean hysterectomy^{2,9} are otherwise seldom performed in routine obstetrics, and there is a high rate of procedure-related complications associated with interventional radiology, which is commonly employed when PAS is suspected¹⁰. Moreover, the antenatal diagnosis of PAS and subsequent maternal anxiety have been associated with an increased risk of post-traumatic stress syndrome¹¹.

Hitherto, there has been a lack of standardized descriptors for ultrasound markers of PAS¹². Recently proposed markers of PAS have shown promising performance. However, the high predictive performance values may not be reproducible owing to the wide variation reported for sensitivity and specificity values^{13–16}. Furthermore, several studies detected ‘classic’ markers of PAS in high- and low-risk patients who subsequently had normal placental delivery^{17,18}. This highlights the need for more robust signs of normal placentation in women at risk of PAS to prevent potential overdiagnosis.

This study describes the ‘separation sign’, a novel ultrasound marker. We hypothesized that a positive separation sign would predict normal placental separation at delivery. We aimed to assess its validity in a large cohort of women at risk for PAS, including those with placenta previa or anterior low-lying placenta and a history of one or more previous CDs, referred to a tertiary clinic specializing in placenta-related disorders.

METHODS

This was a prospective study of women at risk for PAS referred to the specialist placenta clinic at the John Radcliffe Hospital, Oxford, UK, between March 2018 and September 2020. Women were eligible if there was a suspicion of PAS prior to their scan and if they were over 16 years of age and able to provide informed consent. A high-risk cohort of women was defined as those who had placenta previa or an anterior low-lying placenta, in addition to at least one previous CD. All women provided written informed consent to participate in the study and there were no exclusions. Local research ethics approval was obtained (14/NS/0069).

Relevant obstetric and personal history was obtained, and a detailed transabdominal sonographic examination of the placenta was performed, assessing standard ultrasound markers of PAS described previously¹². Two experienced sonographic operators (S.C. and A.S.) performed all examinations using a Voluson E8 ultrasound system (GE Healthcare, Zipf, Austria) with a RAB4-8-D 3D/4D curved-array transabdominal transducer (4–8.5 MHz). The XBeam CRI (compound resolution imaging) was switched off, as it ‘smooths’ the image and reduces the clarity of different placental features. All examinations were performed with a full bladder, defined as a volume between 250 mL and

500 mL quantified using the inbuilt ellipsoid volume calculation. All women were examined for the presence of the separation sign, and the result was recorded simultaneously using a standardized pro forma.

To assess for the separation sign, pressure was applied using an ultrasound probe so that the hypoechoic retroplacental clear zone normally observed between the placenta and myometrium disappeared. The pressure was then rapidly released in order to generate the force required to see movement. On release, in cases with normal placentation, the non-elastic placenta keeps moving away from the probe after the highly elastic myometrium has ‘snapped’ back into place. This sometimes causes the placenta either to keep moving briefly or even to ‘bounce’ and leads to the appearance or enhancement of the clear zone. With a negative separation sign, no separate movement of the placenta from the myometrium can be seen. Videoclip S1 and Figure 1 demonstrate a positive separation sign, and Videoclip S2 presents a negative separation sign.

The separation sign was recorded as positive if separation of the myometrium from the placenta was observed in all areas of the placenta. The sign was recorded as negative if the myometrium and placenta moved as one structure and no clear zone could be seen over any part of the placenta after release, even if separation was noted at the margins of the placenta. Classification into positive or negative separation sign was highly stringent and, in case of any doubt, the result was recorded as uncertain. The latter occurred most often when the images obtained were suboptimal, for example, owing to tissue attenuation, high body mass index (BMI) or significant scarring. If women were scanned on multiple occasions, the separation sign result obtained closest to 28 weeks’ gestation was used in order to minimize differences in scanning over the course of gestation.

Details of gestational age at the time of scanning, maternal age and risk factors for PAS (previous CD, history of dilatation and curettage (D&C), *in-vitro* fertilization (IVF) and other previous uterine surgery) were assessed. Patient information was collected from antenatal ultrasound reports from the placenta clinic and from delivery notes. Patients who did not deliver at the John Radcliffe Hospital were followed up at their hospital and their notes were processed in the same way.

The presence and severity of PAS was assessed at delivery according to the International Federation of Gynecology and Obstetrics (FIGO)¹⁹ classification system by one of the authors of the classification system (S.C.) and based on histopathology results in cases in which hysterectomy was performed. A diagnosis of PAS was excluded if the placenta separated spontaneously at delivery, was delivered by controlled cord traction or was delivered by simple manual removal, even with subsequent identification of retained products of conception (see FIGO classification¹⁹). Histopathological analysis was carried out by a pathologist with expertise in PAS, with the specific sampling site marked by the surgical team according to intrapartum findings, as has

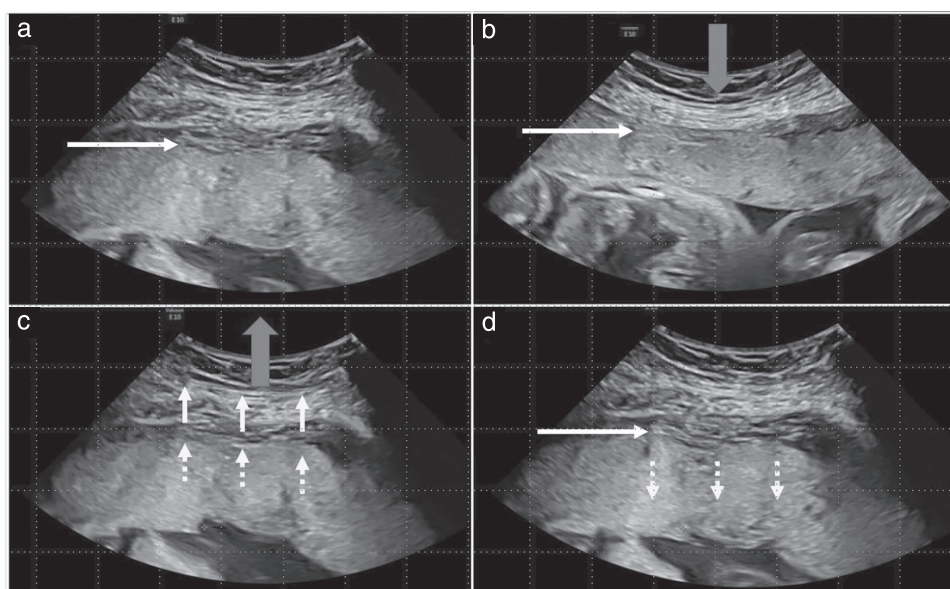


Figure 1 Grayscale ultrasound images demonstrating the positive separation sign. (a) When minimal ultrasound probe pressure is applied, the uteroplacental interface (thin arrow) is seen. (b) The hypoechoic clear zone at the uteroplacental interface disappears as pressure is applied using the probe (thick arrow indicates direction of pressure). (c) As pressure is released rapidly (thick arrow), the placenta (dashed arrows) and the myometrium (solid arrows) spring rapidly back to their initial positions. (d) The placenta then rebounds away from the myometrium in the opposite direction (dashed arrows), appearing to either ‘bounce’ or move slowly, depending on the speed of the release of the probe, revealing the uteroplacental interface and accentuating the clear zone.

been recommended previously¹⁹. The pathologists were blinded to the separation sign classification but not to surgical findings.

Statistical analysis was carried out using SPSS v 26.0 (IBM Corp., Armonk, NY, USA). The Shapiro–Wilk test was used to test for normality for scale variables, and the Mann–Whitney *U*-test and the unpaired *t*-test were used to compare differences in maternal demographics and number of scans performed. Categorical variables were compared using the chi-square or Fisher’s exact test, as appropriate. Sensitivity and specificity were presented with the ‘exact’ Clopper–Pearson CIs, while positive (PPV) and negative (NPV) predictive values were presented with the standard logit CIs²⁰. For all tests, $P < 0.05$ was used to indicate statistical significance.

RESULTS

A total of 194 women were included in the study and 224 ultrasound assessments were performed. Figure 2 shows the study population, including separation sign results at the scan performed closest to 28 weeks’ gestation and delivery outcome. Women with a negative separation sign underwent significantly more CDs ($P < 0.001$) and D&C ($P = 0.006$) than did those with a positive separation sign. There was no significant difference in maternal age ($P = 0.137$), rate of IVF ($P = 0.585$) or rate of other previous uterine surgery ($P = 0.751$) between the two groups (Table 1). BMI at booking was not available for all women; however, across the cohort BMI ranged from 19.2 to 48.6 kg/m².

Assessment for the separation sign was performed between 22 and 38 weeks’ gestation. Mean gestational

age at the first visit to the placenta clinic was significantly higher among women with a negative separation sign than among women with a positive separation sign (31.7 *vs* 29.8 weeks; $P = 0.002$) and non-significantly higher in women who delivered outside Oxford compared with those who delivered in Oxford (31.0 *vs* 29.9 weeks; $P = 0.123$). Women with a negative or uncertain separation sign underwent significantly more scans than did women with a positive separation sign; 94.5% of women with a positive separation sign underwent one scan compared with only 41.7% with a negative separation sign and 57.1% with an uncertain separation sign.

All women with a positive separation sign ($n = 163$) had normal placental separation at delivery, and none was diagnosed with PAS. Of women with a negative separation sign, 21/24 were diagnosed with PAS.

Table 2 shows the sensitivity and specificity analysis of the separation sign in 187 women in whom the separation sign outcome was certain (seven women with an uncertain result were excluded). The sensitivity and specificity values of a positive separation sign in the prediction of normal placental separation were 98.2% (95% CI, 94.8–99.6%) and 100% (95% CI, 83.9–100%), respectively, with a PPV of 100% and a NPV of 87.5% (95% CI, 69.5–95.6%). A further sub-group analysis was performed on 35 women who met the criteria for inclusion in the high-risk cohort. Sixteen women in the high-risk cohort had a positive separation sign, all of whom had normal placental separation at delivery. Of those with a negative separation sign, two had normal placental separation. The remaining 17 did not have normal placental separation and were diagnosed with PAS. Within the high-risk cohort, the sensitivity and

specificity values of the positive separation sign for normal placental separation were 88.9% (95% CI, 65.3–98.6%) and 100% (95% CI, 80.5–100%), respectively, with a PPV of 100% and a NPV of 89.5% (95% CI, 69.7–96.9%).

DISCUSSION

Main findings

The separation sign is an excellent predictor of normal placental separation at delivery. None of the women

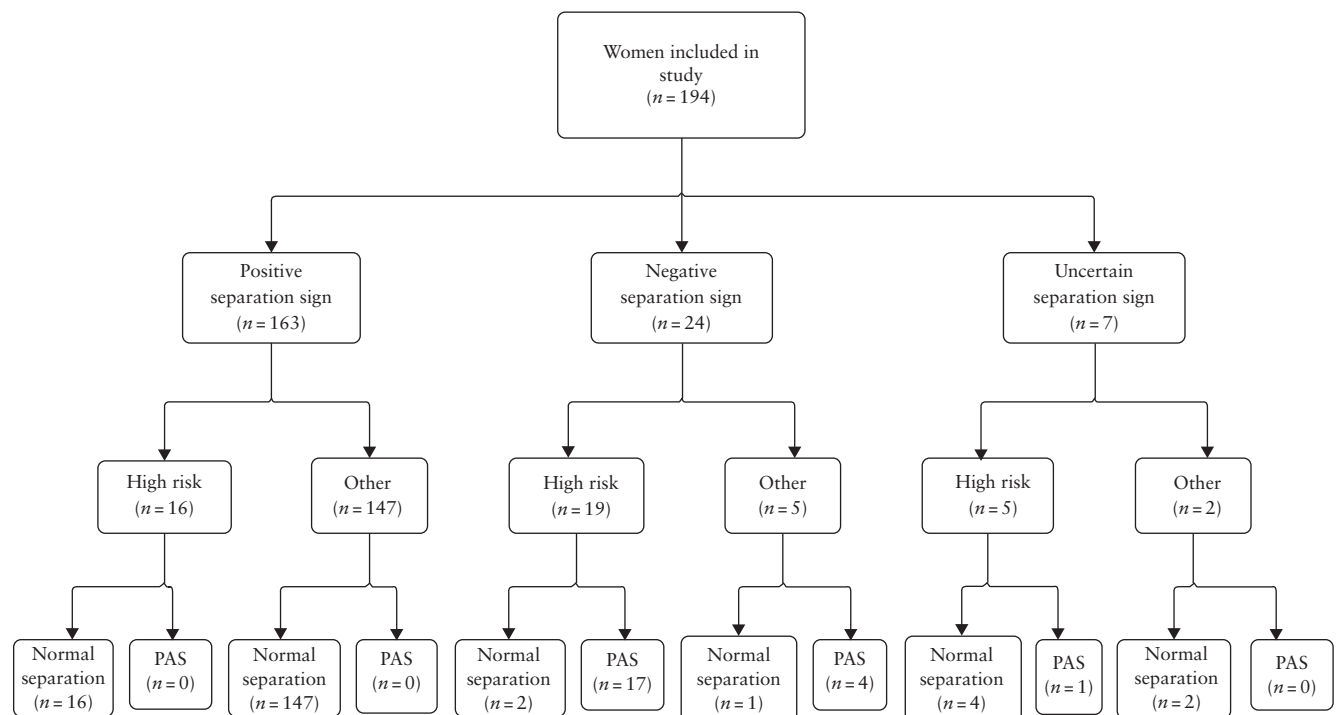


Figure 2 Flowchart summarizing separation sign status at the scan closest to 28 weeks' gestation and the outcome at delivery of 194 women at risk for placenta accreta spectrum (PAS). High-risk women were those who had placenta previa or anterior low-lying placenta at the time of the scan and at least one previous Cesarean delivery.

Table 1 Maternal characteristics and risk factors in a cohort of women referred due to risk of placenta accreta spectrum (PAS) and in a subgroup of high-risk women, according to separation sign outcome

Variable	Separation sign outcome									
	Full cohort (n = 194)					High-risk cohort (n = 40)				
	Positive (n = 163)	Negative (n = 24)	P	Uncertain (n = 7)	P	Positive (n = 16)	Negative (n = 19)	P	Uncertain (n = 5)	P
Maternal age (years)	34.1 (22–47)	35.6 (28–43)	0.137*	35.4 (30–43)	0.466*	33.7 (24–42)	35.8 (28–43)	0.176*	34.4 (30–39)	0.765*
Gestational age (weeks)	29.8 (20.6–38.6)	31.7 (24.6–37.7)	0.002†	28.9 (25.6–31.1)	0.490†	31.3 (26.1–36.3)	31.9 (24.6–37.7)	0.611*	29.7 (28.9–31.1)	0.093*
Previous CD			< 0.001		0.018			0.126		0.141
0	11 (6.7)	2 (8.3)		2 (28.6)		—	—		—	
1	117 (71.8)	8 (33.3)		2 (28.6)		12 (75.0)	7 (36.8)		2 (40.0)	
2	31 (19.0)	9 (37.5)		2 (28.6)		4 (25.0)	7 (36.8)		2 (40.0)	
3	2 (1.2)	2 (8.3)		1 (14.3)		0	2 (10.5)		1 (20.0)	
4	2 (1.2)	2 (8.3)		0		0	2 (10.5)		0	
5	0	1 (4.2)		0		0	1 (5.3)		0	
Previous D&C			0.006		< 0.001			0.181		0.069
≤ 2	163 (100)	21 (87.5)		6 (85.7)		16 (100)	17 (89.5)		4 (80.0)	
≥ 3	0	1 (4.2)		1 (14.3)		0	2 (10.5)		1 (20.0)	
Previous IVF	9 (5.5)	2 (8.3)	0.585	1 (14.3)	0.335	1 (6.3)	1 (5.3)	0.900	1 (20.0)	0.361
Other previous uterine surgery	17 (10.4)	2 (8.3)	0.751	1 (14.3)	0.745	0	0		0	—

Data are given as mean (range) or *n* (%). *P*-values are given for comparisons with the group with positive separation sign. The *t*-test (*) or Mann–Whitney *U*-test (†) were used for normally distributed and non-normally distributed continuous variables, respectively, and chi-square or Fisher's exact test was used for categorical variables. CD, Cesarean delivery; D&C, dilatation and curettage; IVF, *in-vitro* fertilization.

Table 2 Normal placental separation according to positive or negative separation sign in the full cohort of women at risk of placenta accreta spectrum ($n = 187$) and in the high-risk subgroup ($n = 35$)

Group	Separation sign	Normal placental separation		P	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)
		Yes	No			
Full cohort	Positive ($n = 163$)	163 (100)	0 (0)	< 0.001	98.19 (94.8–99.6)	100 (83.9–100)
	Negative ($n = 24$)	3 (12.5)	21 (87.5)			
High-risk cohort	Positive ($n = 16$)	16 (100)	0 (0)	< 0.001	88.9 (65.3–98.6)	100 (80.5–100)
	Negative ($n = 19$)	2 (10.5)	17 (89.5)			

Data are given as n (%), unless stated otherwise. Seven cases with an uncertain separation sign were excluded from this analysis. Chi-square or Fisher's exact test was used to compare the groups.

with a positive separation sign was diagnosed with PAS, while 87.5% of those with a negative separation sign were diagnosed with PAS. This novel diagnostic tool was also assessed in women at high risk for PAS. Excellent sensitivity and specificity were also identified in this cohort, with a 100% PPV for normal placental separation in the presence of a positive separation sign, making it a clinically relevant diagnostic tool.

Only two women had a change in their separation sign status. Both changed from a negative to an uncertain result between scans. One of the two women had placenta previa; assessment at 30 + 5 weeks revealed a negative separation sign, bulky uterus consistent with adenomyosis and no other signs of PAS. The separation sign was detected in some areas but was not uniform at the second scan at 32 + 5 weeks and was thus considered uncertain. The second woman underwent three scans: her first (at 28 + 6 weeks) and third (at 35 + 6 weeks) visits revealed an uncertain separation sign, while her second scan (at 32 + 6 weeks) was negative. Both women had normal placental separation and no diagnosis of PAS at delivery.

Only one of the seven cases with an uncertain separation sign was subsequently diagnosed with PAS. This woman had a positive separation sign at the superior edge of the placenta but did not demonstrate it throughout the rest of the placenta. She had a high *a-priori* risk at the time of the scan, with three previous CDs and one D&C. At delivery, placenta increta (FIGO Grade 2) was diagnosed. This demonstrates that only a positive separation sign throughout the entire placental bed should be considered a reassuring feature.

Women with a negative separation sign had a later average gestational age at the first scan in the placenta clinic, probably owing to later out-of-area referrals. Women with a negative separation sign had a greater number of previous CDs and D&Cs, both of which are risk factors for PAS. It is unsurprising that women screened to be at higher risk for PAS using the separation sign had been exposed to more risk factors than had those with a positive separation sign. They also had more follow-up scans in the placenta clinic to monitor progress and formulate the most appropriate management plan for delivery.

Addressing the diagnostic gap

The pathogenesis of PAS is not well understood but it is thought to be due to a defect in trophoblastic

function leading to abnormal attachment or inadequate decidualization of the myometrium in the area of the scar²¹. Because of this, the difference between the inherent elasticity of the placenta and myometrium is not observed in PAS. Ruling out PAS on the basis of observable relative elasticity has the advantage over many other ultrasound markers of PAS because, with the former, an abnormal result is present only in pathological circumstances. Many other signs of PAS are present in variants of normal placentation¹⁸ or can be missed owing to poor scanning technique. The separation sign has considerable potential to be used as a binary sign to rule out PAS, particularly in high-risk cases, in which there is a danger of missing PAS but also a significant possibility of iatrogenic harm.

As a rule-out sign, the separation sign predicts normal placental separation with a PPV of 100% and a NPV of 87.5% (95% CI, 69.5–95.6%) in all cases and a PPV of 100% and NPV of 89.5% (95% CI, 69.7–96.9%) in the high-risk cohort. We recommend using the separation sign alongside other ultrasound markers of PAS, outlined in the International Society of Ultrasound in Obstetrics & Gynecology (ISUOG) consensus statement for reporting ultrasound assessment of abnormally invasive placenta¹². In our prospective study, the separation sign was used alongside other signs and there were no false positives.

Strengths and limitations

There are some potential limitations associated with the separation sign. Anecdotally, it is more challenging to elicit in patients with a high BMI, as the uterus cannot be distended easily with the probe. Our study was not powered to assess the impact of BMI on the separation sign, but our data included a wide range of BMI values. The presence of the separation sign cannot be assessed in close proximity to the cervix or in cases with posterior placenta because the probe cannot be used to induce movement of the placenta. However, in cases with posterior placenta, fetal movements can sometimes cause the placenta to move and the separation sign is demonstrated. Furthermore, the separation sign must be assessed throughout the entire placental bed to confirm normal separation in all areas. If this is not done, a small area of focal placenta accreta cannot be ruled out. It is therefore important to use other markers of PAS in combination with the separation sign, with the latter employed to target placental areas of particular concern in terms of separation at delivery.

The strengths of our study are its prospective design, 100% follow-up, clearly defined outcomes and strong statistical significance of all findings. The John Radcliffe Hospital is a tertiary referral center for a large region covering more than 31 275 births per year. A relatively large number of cases of PAS (21 cases) were diagnosed within the short period of our study. The high-risk group contained a sufficient number of cases with a positive separation sign to evaluate the predictive performance of the sign among women with a high index of suspicion for PAS at the time of scanning based on history alone. Although this was a single-center study in which assessment for the separation sign was performed by a highly experienced operator, which may limit generalizability of our findings, it provides an opportunity for further research. Furthermore, no additional resources were needed to perform assessment for the separation sign. This makes the test translatable to many environments, without requiring expensive new equipment. This is advantageous considering the rising incidence and risk of PAS in low-resource countries^{22,23}.

Implications for clinical practice and research

The next stage of assessment of the separation sign should involve validation, including inter- and intraobserver variability assessment, of this marker by multiple operators at different centers with experience in the diagnosis of PAS using ultrasound. A larger cohort of women examined prospectively would provide further evidence for its applicability and allow full follow-up of variables such as booking BMI, which was missing for some of the participants in our cohort.

Conclusions


We have demonstrated that a positive separation sign may be a reliable tool for the prediction of normal placental separation in women at risk for PAS who are referred to a specialist clinic. It also maintained an excellent PPV among women at particularly high risk for PAS. The separation sign should be validated further in a multicenter study, alongside other ultrasound markers for PAS, because it has the potential to reduce false-positive diagnoses and thereby reduce psychological and physical iatrogenic harm.

REFERENCES

1. Fitzpatrick KE, Sellers S, Spark P, Kurinczuk JJ, Brocklehurst P, Knight M. The management and outcomes of placenta accreta, increta, and percreta in the UK: A population-based descriptive study. *BJOG* 2014; **121**: 62–71.
2. van den Akker T, Brobbel C, Dekkers OM, Bloemenkamp KWM. Prevalence, Indications, Risk Indicators, and Outcomes of Emergency Peripartum Hysterectomy Worldwide. *Obstet Gynecol* 2016; **128**: 1281–1294.
3. Jauniaux E, Bhide A. Prenatal ultrasound diagnosis and outcome of placenta previa accreta after cesarean delivery: a systematic review and meta-analysis. *Am J Obstet Gynecol* 2017; **217**: 27–36.
4. Silver RM, Landon MB, Rouse DJ, Leveno KJ, Spong CY, Thom EA, Moawad AH, Caritas SN, Harper M, Wapner RJ, Sorokin Y, Menachen M, Carpenter M, Peacemaker AM, O'Sullivan MJ, Sibai B, Langer O, Thorp JM, Ramin SM, Mercer BM. Maternal morbidity associated with multiple repeat cesarean deliveries. *Obstet Gynecol* 2006; **107**: 1226–1232.
5. Tikkanen M, Paavonen J, Loukovaara M, Stefanovic V. Antenatal diagnosis of placenta accreta leads to reduced blood loss. *Acta Obstet Gynecol Scand* 2011; **90**: 1140–1146.
6. Buca D, Liberati M, Cali G, Forlani F, Caisutti C, Flacco ME, Manzoli L, Familiari A, Scambia G, D'Antonio F. Influence of prenatal diagnosis of abnormally invasive placenta on maternal outcome: systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2018; **52**: 304–309.
7. Melcer Y, Jauniaux E, Maymon S, Tsviban A, Pekar-Zlotin M, Betser M, Maynon R. Impact of targeted scanning protocols on perinatal outcomes in pregnancies at risk of placenta accreta spectrum or vasa previa. *Am J Obstet Gynecol* 2018; **218**: 443.e1–443.e8.
8. Bartels HC, Rogers AC, O'Brien D, McVey R, Walsh J, Brennan DJ. Association of implementing a multidisciplinary team approach in the management of morbidly adherent placenta with maternal morbidity and mortality. *Obstet Gynecol* 2018; **132**: 1167–1176.
9. Grantcharov TP, Rosenberg J. Vertical compared with transverse incisions in abdominal surgery. *Eur J Surg* 2001; **167**: 260–267.
10. Salim R, Chulski A, Romano S, Garmi G, Rudin M, Shalev E. Precesarean prophylactic balloon catheters for suspected placenta accreta: a randomized controlled trial. *Obstet Gynecol* 2015; **126**: 1022–1028.
11. Tol ID, Yousif M, Collins SL. Post traumatic stress disorder (PTSD): The psychological sequelae of abnormally invasive placenta (AIP). *Placenta* 2019; **81**: 42–45.
12. Alfirevic Z, Tang A-W, Collins SL, Robson SC, Palacios-Jaraquemada J. Pro forma for ultrasound reporting in suspected abnormally invasive placenta (AIP): an international consensus. *Ultrasound Obstet Gynecol* 2016; **47**: 276–278.
13. D'Antonio F, Iacovella C, Bhide A. Prenatal identification of invasive placentation using ultrasound: systematic review and meta-analysis. *Ultrasound Obstet Gynecol* 2013; **42**: 509–517.
14. Comstock CH, Love JJ, Bronsteen RA, Lee W, Vetrano IM, Huang RR, Lorenz RP. Sonographic detection of placenta accreta in the second and third trimesters of pregnancy. *Am J Obstet Gynecol* 2004; **190**: 1135–1140.
15. Cali G, Forlani F, Timor-Trisch I, Palacios-Jaraquemada J, Foti F, Minneci G, Flacco ME, Manzoli L, Familiari A, Pagani G, Scambia G, D'Antonio F. Diagnostic accuracy of ultrasound in detecting the depth of invasion in women at risk of abnormally invasive placenta: A prospective longitudinal study. *Acta Obstet Gynecol Scand* 2018; **97**: 1219–1227.
16. Skupski DW, Duzjy CM, Scholl J, Perez-Delboy A, Ruhstaller K, Plante LA, Hart LA, Palomares KTS, Ajemian B, Rosen T, Kinzler WL, Ananth C. Evaluation of classic and novel ultrasound signs of placenta accreta spectrum. *Ultrasound Obstet Gynecol* 2022; **59**: 465–473.
17. Hamada S, Hasegawa J, Nakamura M, Matsuoka R, Ichizuka K, Sekizawa A, Okai T. Ultrasonographic findings of placenta lacunae and a lack of a clear zone in cases with placenta previa and normal placenta. *Prenat Diagn* 2011; **31**: 1062–1065.
18. Philips J, Gurganus M, DeShields S, Cunningham T, Sinkovskaya E, Kanaan C, Porche L, Abuhamad A. Prevalence of Sonographic Markers of Placenta Accreta Spectrum in Low-Risk Pregnancies. *Am J Perinatol* 2019; **36**: 773–780.
19. Jauniaux E, Ayres-de-Campos D, Langhoff-Roos J, Fox KA, Collins S, Duncombe G, Klaritsch P, Chantraine F, Kingdom J, Grønbeck L, Rull K, Tikkanen M, Sentilhes L, Asatiani T, Leung W, Alhaidari T, Brennan D, Seoud M, Hussein AM, Jegasothy R, Shah KN, Bombardieri D, Hubinont C, Soma-Pillay P, Mandic NT, Lindqvist P, Arnadottir B, Hoesli I, Cortez R. FIGO classification for the clinical diagnosis of placenta accreta spectrum disorders. *Int J Gynaecol Obstet* 2019; **146**: 20–24.
20. Mercaldo ND, Lau KF, Zhou XH. Confidence intervals for predictive values with an emphasis to case-control studies. *Stat Med* 2007; **26**: 2170–2183.
21. Jauniaux E, Jurkovic D. Placenta accreta: pathogenesis of a 20th century iatrogenic uterine disease. *Placenta* 2012; **33**: 244–251.
22. Escobar MF, Gallego JC, Nasner D, Gunawardana K. Management of abnormal invasive placenta in a low- and medium-resource setting. *Best Pract Res Clin Obstet Gynaecol* 2021; **72**: 117–128.
23. Barss P, Misch KA. Endemic placenta accreta in a population of remote villagers in Papua New Guinea. *BJOG* 1990; **97**: 167–174.

SUPPORTING INFORMATION ON THE INTERNET

The following supporting information may be found in the online version of this article:

-  **Videoclip S1** Grayscale ultrasound imaging demonstrating an example of a positive separation sign.
- Videoclip S2** Grayscale ultrasound imaging demonstrating an example of a negative separation sign.