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Early predictors of preterm labor and preeclampsia: A prospective study

Ali Babacan (*), Özgür Dündar (*), Murat Muhcu (*), Ercüment Müngen (*), Vedat Atay (*), Cem Kızılaslan (**), Yaşam Kemal Akpak (***)

SUMMARY

Aims: This study aimed to investigate various clinical/biochemical parameters as potential predictors of preeclampsia and preterm labor. MMP-2, MMP-9, pregnancy-associated plasma protein A (PAPP-A), fms-like tyrosine kinase 1, and beta human chorionic gonadotropin (β-HCG) levels were measured and Doppler ultrasonography measurements were made in pregnant women at 11-14 weeks of gestation. Findings were recorded and patients were followed until delivery.

Results: Patients consisted of preeclampsia and/or preterm labor cases (n=40) and selected controls were pregnant women with a normal pregnancy period who delivered between 37 and 40 weeks of gestation (n=55). Multivariate analysis identified high fms-like tyrosine kinase 1 level (OR, 1.004; 95% CI: 1.001-1.007, p= 0.005), high UtA PI (OR, 30.6; 95% CI: 1.1-852, p= 0.044) and presence of any notch (OR, 36.4; 95% CI: 1.6-826, p= 0.024) as independent predictors of preeclampsia; whereas only maternal MMP-9 level emerged as a significant predictor of preterm birth, (OR, 1.001; 95% CI: 1.000-1.001, p< 0.001).

Conclusions: These parameters deserve further investigation for their potential use (either alone or in combination) in the prediction of preeclampsia and preterm birth in the clinical setting.

Key Words: *preterm labor, preeclampsia, Doppler ultrasonography, matrix metalloproteinase (MMP), pregnancy-associated plasma protein A (PAPP-A), fms-like tyrosine kinase 1, beta human chorionic gonadotropin (β-HCG).*

ÖZET

Erken doğum ve preeklampsinin erken belirteçleri: prospektif bir çalışma

Bu çalışmanın amacı, değişik klinik ve biyokimyasal parametreleri, preeklampsi ve erken doğumun olası belirteçleri olarak değerlendirmektir. Gebeliğinin 11-14 haftalarında olan kadınlarda MMP-2, MMP-9, gebelik ile ilişkili plazma protein A (PAPP-A), fms-benzeri tirozin kinaz 1, insan beta koriyonik gonadotropini (β-HCG) düzeyleri ölçülmüş ve Doppler ultrason ölçümleri yapılmıştır. Bulgular kaydedilmiş ve hastalar doğuma kadar izlenmiştir. Preeklampsi ve/veya erken doğum gelişen 40 hasta çalışma grubunu, gebeliğinin 37 ile 40. haftalarında normal doğum yapmış seçilmiş 55 hasta kontrol grubunu oluşturmuştur. Çok değişkenli analiz, yüksek fms-benzeri tirozin kinaz 1 düzeyini (OR, 1.004; 95% CI: 1.001-1.007, p= 0.005), yüksek UtA PI değerini (OR, 30.6; 95% CI: 1.1-852, p= 0.044) ve herhangi bir notch olması durumunu (OR, 36.4; 95% CI: 1.6-826, p= 0.024) preeklampsinin bağımsız belirteçleri olarak belirlemiştir. Erken doğumun anlamlı belirteci olarak ise sadece annenin MMP-9 düzeyi bulunmuştur (OR, 1.001; 95% CI: 1.000-1.001, p< 0.001). Klinik ortamda bu parametrelerin preeklampsi ve erken doğumu tahmin etmedeki değerinin (tek başına ya da kombinasyon olarak) daha ileri çalışmalarda araştırılması gerekmektedir.

Anahtar Kelimeler: *erken doğum, preeklampsi, Doppler ultrason, matriks metaloproteinaz (MMP), gebelikle ilişkili plazma protein A (PAPP-A), tirozin kinaz, insan beta koriyonik gonadotropini (β-HCG)*

*Haydarpaşa Sultan Abdülhamid Eğitim ve Araştırma Hastanesi, Kadın Hastalıkları ve Doğum AD, İstanbul,Türkiye.
**Şehit Murat Erdi Eker Devlet Hastanesi, Kadın Hastalıkları ve Doğum AD, Ankara, Türkiye (eski kurum).
***Dışkapı Yıldırım Beyazıt Eğitim ve Araştırma Hastanesi Mevki Binası, Kadın Hastalıkları ve Doğum AD, Ankara,

Corresponding Author: Ali BABACAN
Haydarpaşa Sultan Abdülhamid Eğitim ve Araştırma Hastanesi, Kadın Hastalıkları ve Doğum AD, İstanbul,Türkiye
ababacan_@hotmail.com

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Introduction

Preeclampsia is an important cause of mortality and morbidity affecting 1 to 2 % of pregnancies (1). Preterm labor is even more prevalent complicating approximately 12% of all pregnancies (2, 3). Although the exact mechanism of preeclampsia has not been definitely understood, vascular and inflammatory factors have been proposed for its pathogenesis (4); and a multitude of risk factors have been proposed for preterm birth (2).

Risk assessment for the development of these conditions may help to identify the individuals at higher risk and to develop preventive strategies. To date, several markers have been tested as potential predictors of pregnancy related hypertensive conditions and preterm labor, including uterine artery Doppler measurements and several biochemical markers (5-15). Doppler examination is a non-invasive and useful method that gives valuable information on the development of the fetus and the placenta. Several ultrasonography parameters, particularly uterine artery indexes and presence of uterine artery notch has been associated with preeclampsia (15-18).

Pregnancy-associated plasma protein A (PAPP-A), is a peptidase which can be detected during pregnancy in maternal circulation. It is suggested to be involved in local proliferative processes (19, 20). Decreased plasma levels of PAPP-A have been reported in association with preeclampsia (21, 22).

Placental soluble fms-like tyrosine kinase 1 (sFlt1) is an antagonist of vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). High levels have been found in maternal blood during preeclampsia. In addition, increased levels of this molecule is associated with decreased circulating levels of VEGF and PlGF (23).

Human chorionic gonadotropin (HCG) is a hormone that play role in the maintenance of the corpus luteum during early pregnancy. Several recent studies pointed out to a relation between human chorionic gonadotropin levels and adverse pregnancy outcomes (24-26).

Matrix metalloproteinases (MMPs), gelatinase A (MMP-2) and gelatinase B (MMP-9) in particular, are thought to play role in adverse pregnancy outcomes since they seem to have role in uterine and placental artery remodeling (27, 28).

This study aimed to investigate clinical and biochemical parameters as predictors of two different adverse pregnancy outcomes, namely preeclampsia and preterm labor.

Materials and methods

Patients

This prospective study included pregnant women admitting to Obstetrics and Gynecology Outpatient Clinic of GATA Haydarpasa Research and Training Hospital between October 2011 and October 2012 for routine obstetrical follow-up visits at their 11 to 14 weeks of gestation. Exclusion criteria were as follows: multiple pregnancy, early or late abortion, systemic disease and patients who are not planning to deliver in our institution. During the study period, about 6000 out-patient admissions occurred in our clinic, among them 520 were pregnant women admitting for their routine follow-up examination at 11-14 weeks and fulfilling eligibility criteria. All patients provided informed consent prior to study entry and study protocol was approved by local ethics committee. The study was conducted in accordance with Declaration of Helsinki. Patients underwent a thorough obstetrical work-up including history and physical examination, and ultrasonography examination at 11-14 week visit, blood samples were obtained and stored until delivery. Findings were recorded and patients were followed until delivery. Biochemical analyses of stored blood samples were done for patients who developed preeclampsia and/or preterm birth and for 55 randomly selected controls among uncomplicated pregnancies. Figure 1 shows the study diagram.

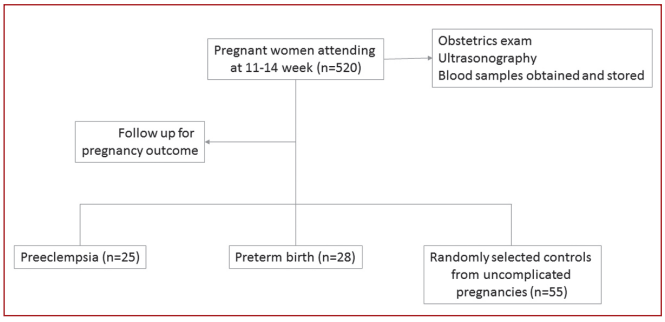

Figure 1. Study diagram

Study assessments

Maternal venous blood samples were obtained for the assessments of MMP-2, MMP-9, PAPP-A, fms-like tyrosine kinase 1, and beta human chorionic gonadotropin (β-HCG) levels during 11-14 week visit and the samples were stored until delivery. Blood samples were obtained in the morning when fasting and stored at -80 C in deep freezer (New Brunswick Scientific Model - U410, UK). All maternal serum biochemical markers analyses were done using BOSTER Human Biomarker ELISA device and kits. In addition, following transabdominal ultrasonography assessments were made using Voluson E8 Expert Scanner (GE Healthcare, Wauwatosa, WI, USA) ultrasonography device and 2-7 MHz convex probe: crown-rump length (CRL), nuchal thickness, presence of uterine artery notch, uterine artery pulsatility index (UtA PI), uterine artery resistance index (UtA RI), uterine artery systolic/diastolic ratio (UtA S/D). Ultrasonography measurements were done as previously described (29).

Definition of pregnancy outcomes

Preeclampsia was defined as the onset of hypertension (>140/90 mmHg at two successive measurements at least 6 hours apart) at the second half of the pregnancy which disappears after delivery plus proteinuria (300 mg/d or >1+) (30). Preterm delivery was defined as delivery before 37 weeks of gestational age (2).

Statistical analysis

SPSS version 21 was used for the analysis of data. Descriptive statistics are presented as mean±SD or number (percentage), where appropriate. Normality was tested using Shaphiro-Wilk test and graphical methods. For the comparison of normally distributed continuous variables, student t test for independent samples was used. Mann Whitney U test was used for the comparison of continuous variables without normal distribution. Categorical variables were compared using Pearson chi square test or Fisher's exact test, where appropriate. Stepwise logistic regression was used for multivariate analysis to identify the independent predictors of preeclampsia or preterm delivery. Diagnostic parameters including sensitivity and specificity of independent predictors in predicting adverse pregnancy outcomes were calculated. Receiver operator characteristic curve (ROC) was generated to examine the accuracy of estimations and potential cut-off values. A p value smaller than 0.05 was considered an indication for statistical significance.

Results

Patient characteristics

The mean age of the whole study population (n=95) was 30.5 ± 4.3 years. The median gestational age at the time of first trimester visit was 12 weeks 2 days (range, 11 weeks 1 day to 13 weeks 6 days). Patients consisted of preeclampsia and/or preterm labor cases (n=40). Controls were pregnant women randomly selected from uncomplicated pregnancies (n=55). Table I and II shows demographical and clinical characteristics of subjects with regard to preeclampsia and preterm birth, respectively. Six pregnancies had intrauterine growth retardation (IUGR) and one intrauterine exitus occurred. Among 6 IUGR, 3 had preeclampsia and 2 of these preeclampsia cases had HELLP syndrome.

Preeclampsia

Preeclampsia developed in a total of 25 pregnancies. In the group of patients that developed preeclampsia, the mean age, maternal MMP-2, MMP-9, and fms-like tyrosine kinase 1 levels, UtA PI, UtA RI, and UtA S/D was significantly higher and maternal PAPP-A levels were significantly lower at first trimester visit when compared to patients that did not develop preeclampsia during their pregnancy (Table I). In addition, presence of notch (either unilateral or bilateral) was significantly more frequent in the preeclampsia group (Table I). Multivariate analysis identified a high fms-like tyrosine kinase 1 level (p= 0.005), a high UtA PI (p= 0.044) and presence of notch (either unilateral or bilateral) (p= 0.024) as independent predictors of preeclampsia (Table III). Area under the receiver operator characteristics (ROC) curve for predicting preeclampsia is

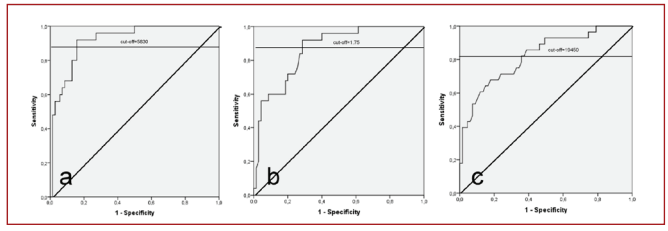

Figure 2. Receiver operating characteristic (ROC) curves for fms-like tyrosine kinase 1(a) and UtA PI (b) for the prediction of preeclampsia. (c) ROC curve for MMP-9 for the prediction of preterm labor.

Table I. Comparison of the patients with or without preeclampsia with regard to demographical and clinical characteristics

Characteristic	No preeclampsia (n=70)	Preeclampsia (n=25)	P value
Age, y	29.9 ± 4.1	32.2 ± 4.6	0.021*
BMI, kg/m^2	25.4 ± 1.6	25.4 ± 3.0	0.995*
Primigravida, n (%)	56 (80.0%)	17 (68.0%)	0.222‡
Biochemical assessments			
MMP-2, ng/ml	2483 ± 1350	3979 ± 3397	0.011†
MMP-9, ng/ml	17908 ± 2570	21610 ± 1638	<0.001*
Fms-like tyrosine kinase 1, pg/ml	5059 ± 950	6447 ± 492	<0.001*
PAPP-A, MoM	1.22 ± 0.62	1.02 ± 0.81	0.020†
B-HCG, IU/ml	1.34 ± 0.88	1.15 ± 0.62	0.532†
USG assessments			
CRL, mm	59.2 ± 7.8	62.0 ± 7.8	0.114*
Nuchal thickness, mm	1.46 ± 0.59	1.39 ± 0.29	0.765†
Presence of any notch n (%)	27 (38.6%)	24 (96.0%)	<0.001‡
Presence of bilateral notch n (%)	6 (8.6%)	11 (44.0%)	<0.001§
UtA PI	1.53 ± 0.45	2.18 ± 0.36	<0.001†
UtA RI	0.66 ± 0.12	0.72 ± 0.10	0.020†
UtA S/D	3.49 ± 1.05	3.89 ± 0.88	0.023†

Unless otherwise stated, data are presented as mean ± standard deviation.
Test for intergroup comparison: * student t test for independent samples, †Mann-Whitney U test, ‡Pearson chi square test, § Fisher's exact test
Abbreviations: BMI, body mass index; MMP, matrix metalloproteinase; PAPP-A, pregnancy-associated plasma protein A; MoM, multiple of the median; B-HCG, beta human chorionic gonadotropin; USG, ultrasonography; CRL, crown-rump length; UtA PI, uterine artery pulsatility index; UtA RI, uterine artery resistance index; UtA S/D, uterine artery systolic/diastolic ratio.

Table II. Comparison of the patients with or without preterm labor with regard to demographical and clinical characteristics

Characteristic	Term labor (n=67)	Preterm labor (n=28)	P value
Age, y	30.0 ± 4.3	31.6 ±4.2	0.107*
BMI, kg/m^2	25.5 ± 2.3	25.3 ± 1.9	0.835*
Primigravida, n (%)	52 (77.6%)	21 (75.0%)	0.783‡
Preeclampsia, n (%)	12 (17.9%)	13 (46.4%)	**0.004**‡
Biochemical assessments			
MMP-2, ng/ml	2693 ± 1685	3314 ± 3037	0.294†
MMP-9, ng/ml	17962 ± 2615	21081 ± 2187	**<0.001***
Fms-like tyrosine kinase 1, pg/ml	5299 ± 1004	5723 ± 1115	**0.049**†
PAPP-A, MoM	1.23 ± 0.63	1.03 ± 0.77	**0.030**†
B-HCG, IU/ml	1.25 ± 0.80	1.38 ± 0.89	0.462†
USG assessments			
CRL, mm	59.2 ± 8.0	61.7 ± 7.3	0.166*
Nuchal thickness, mm	1.42 ± 0.52	1.49 ± 0.56	0.714†
Presence of any notch n (%)	33 (49.3%)	18 (64.3%)	0.180‡
Presence of bilateral notch n (%)	11 (16.4%)	6 (21.4%)	0.561‡
UtA PI	1.58 ± 0.50	1.99 ± 0.43	**<0.001***
UtA RI	0.67 ± 0.13	0.69 ± 0.10	0.298†
UtA S/D	3.56 ± 1.04	3.68 ± 0.97	0.470†

Unless otherwise stated, data are presented as mean ± standard deviation.
Test for intergroup comparison: * student t test for independent samples, †Mann-Whitney U test, ‡Pearson chi square test
Abbreviations: BMI, body mass index; MMP, matrix metalloproteinase; PAPP-A, pregnancy-associated plasma protein A; MoM, multiple of the median; B-HCG, beta human chorionic gonadotropin; USG, ultrasonography; CRL, crown-rump length; UtA PI, uterine artery pulsatility index; UtA RI, uterine artery resistance index; UtA S/D, uterine artery systolic/diastolic ratio.

Table III. Results of multivariate analysis of the parameters in predicting preeclampsia and preterm labor

Parameter	Odds ratio	95% CI	P value
Predictors of preeclampsia			
MMP-9, ng/ml	1.001	1.000-1.002	0.055
Fms-like tyrosine kinase 1, pg/ml	1.004	1.001-1.007	0.005
Presence of any notch	36.4	1.6-826	0.024
UtA PI	30.6	1.1-852	0.044
Predictors of preterm labor			
MMP-9, ng/ml	1.001	1.000-1.001	<0.001

Stepwise logistic regression (forward conditional) was used for multivariate analysis. For continuous variables, odds ratios and confidence intervals are for per unit change in the parameter.

Abbreviations: MMP, matrix metalloproteinase; UtA PI, uterine artery pulsatility index

0.917 (95% CI, 0.859-0.976, p<0.001) for fms-like tyrosine kinase 1 indicating excellent accuracy and 0.865 (95% CI, 0.788-0.941, p<0.001) for UtA PI indicating good accuracy (Figures 1a and 1b). An optimal cut-off value of 5830 for fms-like tyrosine kinase 1 would result in sensitivity and specificity levels of 92% and 84% respectively. Corresponding figures for an optimal cut-off value of 1.75 for UtA PI are 92% and 71%, respectively.

Preterm birth

Preterm birth developed in a total of 28 pregnancies. In the group of patients with preterm birth, maternal MMP-9 and fms-like tyrosine kinase 1 levels, and UtA PI were significantly higher and maternal PAPP-A levels were significantly lower at the first trimester visit when compared to patients that gave birth at term (Table II). In addition, presence of preeclampsia was significantly more frequent in the preterm birth group (Table II). Only maternal MMP-9 level emerged as a significant predictor of preterm birth on multivariate analysis (p< 0.001) (Table III). Area under the receiver operator characteristics (ROC) curve for predicting preterm birth was 0.821 (95% CI, 0.727-0.915, p<0.001) for MMP-9 indicating good accuracy (Figure 1c). An optimal cut-off value of 19450 would result in sensitivity and specificity levels of 81% and 64% respectively.

Discussion

This study examined a spectrum of clinical and radiological parameters as potential predictors of preeclampsia or preterm birth and found significant relations. Multivariate analysis identified high fms-like tyrosine kinase 1, high UtA PI and presence of notch as independent predictors of preeclampsia. Baumann et al. (12) found increased fms-like tyrosine kinase-1 levels at the first trimester in women who subsequently developed preeclampsia; however, Akolekar et al. (11) did not find an association between fms-like tyrosine kinase and preeclampsia. Both studies obtained samples between 11 to 13 weeks of gestation. Similarly, this study identified fms-like tyrosine kinase 1 levels as a significant predictor of preeclampsia. In addition, our findings related to ultrasonographic measurements are in line with previous observations (8, 9, 15, 31).

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases and play role in the breakdown of extracellular matrix components and they are involved in inflammation and tissue modelling. Main subtypes of this group of enzymes are gelatinases, collagenases, and stromelysins. Among them, gelatinase A (MMP-2) and gelatinase B (MMP-9) are particularly related with pregnancy since they have role in remodeling of uterine and placental arteries (32). To date, several studies have tested the association of these enzymes with pregnancy associated hypertensive disease and preeclampsia. Montagnana et al. (27) compared serum levels of non-pregnant, normal pregnant and preeclamptic women. MMP-2 levels were significantly higher in preeclemptic women when compared to both normal pregnant and non-pregnant women. However, that study did not find a difference between normal pregnant and preeclamptic women with regard to serum MMP-9 levels. In that study, blood samples were not drawn at a standard time point. Similarly, Narumiye et al. (28) found significantly higher levels of MMP-2 in preeclamptic women. In contrast, Palei et al (33) found higher levels of MMP-9, but not MMP-2, in women with gestational hypertension when compared to normal pregnant women; however, MMP-9 levels were not higher in preeclemptic women. Similar to this study, two studies tested MMP-9 and MMP-2 as a predictor of pregnancy outcomes. Myers et al. (34) obtained samples from preeclemptic and normal women at 22 weeks, 26 weeks and at delivery/diagnosis. They found significantly increased MMP-2 activity at 22 weeks and at diagnosis but not at 26 weeks in patients that developed preeclampsia; nevertheless, MMP-2 to and tissue inhibitor ratio was disturbed throughout pregnancy in preeclemptic women. In that study, no difference was found with regard to MMP-9 activity. Similarly, in the study by Poon et al. (35), where maternal sera were obtained in the first trimester MMP-9 did not emerge as an important predictor of preeclampsia. In this study however, MMP-9 levels did not emerge as an independent predictor of preeclampsia. Although MMP-2 levels were significantly higher in preeclamptic women on univariate analysis, multivariate analysis was unable to identify it as an independent predictor of preeclampsia.

In this study, two ultrasonography parameters -presence of uterine notch and UtA PI- have emerged as independent predictors of preeclampsia. Our findings are in line with previous findings. In a meta-analysis of large number of studies, pulsatility index combined with notching was the most predictive Doppler index for pre-eclampsia (18). In addition, authors concluded that uterine artery Doppler ultrasonography predicts more accurately when performed in the second trimester than in the first-trimester. Our findings support that these two parameters may still be valuable in the first trimester. Although B-HCG and PAPP-A levels have been associated to some degree with preeclampsia risk in several previous studies (22, 25, 26, 36-38), these two parameters did not emerge as independent predictors in this study, probably due to small sample size or timing of the measurements.

Studies examining the association between preterm delivery

and MMP activity are relatively scarce. Koucky et al. (39) found lower MMP-2 levels in maternal serum of preterm deliveries and Poon et al. (35) found higher MMP-9 levels in these patients, although MMP-9 levels was not found to be useful in predicting preterm birth in the latter study. However, this study identified high MMP-9 levels as the only independent predictor among all parameters tested. These findings overall suggest that matrix metalloproteinases, MMP-9 in particular, predicts preterm labor rather than preeclampsia; partly explaining the variable findings of several previous studies in which preeclampsia and premature labor coexist in a large proportion of patients. This issue deserves further investigation in large-scale studies.

In this study, several important markers such as fms-like thyrosine kinase 1, Ut A PI, presence of notch, PAPP-A and B HCG did not emerge as independent predictors of preterm birth. Similar to our findings, a meta-analysis investigating the biochemical predictors of preterm birth did not identify PAPP-A and B-HCG as significant predictors (40). However, Patiel et al. identified low PAPP-A levels as a useful indicator for preterm delivery (37). A recent study identified maternal fms-like tyrosine kinase/placental growth factor (PlGF) ratio as a useful predictor of induced preterm birth (<34+0 weeks) (41). Maternal fms-like thyrosine kinase 1 and ultrasonography parameters including Ut A PI and presence of notch were mostly studied in the context of preeclampsia risk. Further studies are warranted for the potential role of these parameters in predicting preterm birth.

Main limitation of this study is the low sample size, particularly considering that multivariate analysis has been carried out. Several parameters might not have been able to reach statistical significance on multivariate analysis due to low power. Thus, the findings deserve testing on larger samples. In addition, some of these patients might have been using treatments including progesterone, which may be a potential reason of disturbing uniformity of the study population. This may be considered another study limitation.

In conclusion, several clinical and radiological parameters of the first trimester seem to be associated with adverse pregnancy outcomes. The role of MMP-9 in predicting preterm birth in particular, is relatively a novel finding and deserves further investigation. One remarkable finding of this study is the difference between the significant predictors of preeclampsia and preterm birth, which may indicate different pathogenesis for these two conditions. Although, sample size may be considered somewhat small for a detailed multivariate analysis, in its present form the findings of this study provides some insight for the early prediction of the two clinically important conditions, which might help management in clinical setting. Further studies may test these variables, either alone or in combination, in larger sample sizes to support additional evidence for their clinical use.

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