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Prediction of Maturation of The Fetal Lung by Lamellar Bodies' Count of Amniotic Fluid in PPROM

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ABSTRACT

Background: There are many risk factors for Preterm Premature Rupture of Membranes (PPROM). Infection is the main one (15-25%), especially at earlier gestational ages. Neonatal respiratory distress syndrome of the neonate is an important obstacle in cure of preterm infants.

Objective: To assess the efficacy of the amniotic fluid lamellar body counting (LBC) from vaginal pool in predicting fetal lung maturity in women with preterm premature rupture of membranes**.**

Patients and Methods: This study was conducted on 92 cases. Prediction of fetal lung maturity and the presence of respiratory distress syndrome (RDS) were estimated. LBC was measured for all cases.

Results: There were a significant negative correlation between respiratory distress syndrome and gestational age. Also a significant correlation between level of LBC and fetal lung maturity using 38.0 ($x10^3/\mu L$) as a cut-off point for LBC; as it is can be considered a good predictor for fetal lung maturity with sensitivity 92.9% and specificity 90.6%, positive predictive value of 81.3% and negative predictive value of 96.7%**.**

Conclusion: There is a significant correlation between level of LBC and fetal lung maturity. LBC could be considered a good predictor for fetal lung maturity.

Keywords: Lamellar body counting- Fetal lung maturity-Preterm premature rupture of membranes.

INTRODUCTION

Preterm Premature Rupture of Membranes (PPROM) is defined as spontaneous rupture of the amniotic membranes before 37 weeks gestation and before the time of labor**.** There are many risk factors for PPROM. Infection is the main one (15-25%), especially at earlier gestational ages. Previous history of PPROM, shortness of cervical length, second-trimester and third-trimester hemorrhage, decreased body mass index, bad socioeconomic status, smoking, and drugs intake are also major risk factors for PPROM **(1)**.

Respiratory distress syndrome is a main reason of fetal morbidity and mortality that is caused by a decreased level of lung surfactant in premature fetus. Therefore, laboratory investigations were developed to measure the presence and/or level of pulmonary surfactant and lamellar bodies in amniotic fluid to evaluate maturity of the fetal lung **(2).**

Neonatal respiratory distress syndrome of the neonate is still an important obstacle in cure of preterm infants. It is associated by inflammatory occurrence with free radical generation and oxidative stress **(3).**

Fetal lung maturity testing can give us information to prevent a preterm delivery or to start maternal drug therapy to support lung maturity. The fetal lung maturity tests can be divided into 2 groups, biochemical and biophysical. Biochemical tests estimate components of the surfactant which are the lecithin/sphingomyelin ratio and phosphatidylglycerol. The physical tests estimate the physical characteristics of the phospholipid surfactants which are the surfactant/albumin (S/A) ratio and lamellar body counts. These tests depend on the amniotic fluid which reflects the fetal lung fluid components **(4).**

Lamellar bodies are found in intracellular storage granules in lung cells or pneumocytes. The lamellar bodies are discharged to become a surfactant monolayer in the alveolar space. Surfactant and lamellar bodies are secreted into amniotic fluid due to fetal breath movements starting around 28 to 32 weeks of fetal development. The risk of respiratory distress syndrome due to decreased surfactant concentrations is appeared during gestational weeks 32 to 36, and more accurate evaluation of that risk is facilitated by estimation of surfactant phospholipid levels or, as was shown, by lamellar body counts (LBC) **(5).**

Lamellar body count is a direct assessment of surfactant releasing by type II pneumocyte cells within the pneumocytes in fetal alveolus and released to amniotic fluid during respiratory movement that have the same size of the platelets **(6).**

The diameter of intact lamellar body is about 1 to 5 microns and its volume is about 1.7 to 7.3 fl **(7).**

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Lamellar bodies count (LBC) is a good screening test for predicting fetal lung maturity. Its advantages are fast, reliable, not expensive, low sample volume needed and widely availability [6].

This study is designed to assess the efficacy of the amniotic fluid lamellar body counting from vaginal pool in predicting fetal lung maturity in women with preterm premature rupture of membranes.

PATIENTS AND METHODS

This study was a prospective cohort study which conducted at El–Monera General Hospital.

Sample size: A sample of 92 women was included in the study. Sample size was calculated in Community Department in Zagazig University: Assuming that total number of female coming with preterm premature rupture of membranes (PPROM) in El-Monera General Hospital in 6 months expected to be 120 cases, PPV of (lamellar bodies counting) 58.13%, so total sample size 92 at CI 95% and power of study 80%.

Inclusion criteria: Pregnant women with preterm premature rupture of membranes. Singleton pregnancies. Gestational age from 28-36 weeks. The fetus is alive with regular heart beats by ultrasound. Delivery within 2 days after sample collection.

Exclusion criteria: Gestational age 37 weeks or more. Uncertain gestational age. Oligohydramnios before preterm premature rupture of membranes. Infants with major congenital or chromosomal abnormalities. Amniotic fluid samples containing blood or meconium. Diabetes mellitus (diabetic mother). Presence of chorioamnionitis.

Study outcome: Prediction of fetal lung maturity and the presence of RDS "Diagnosis of RDS was confirmed by findings on chest radiographs".

Ethical approval:

An approval of the study was obtained from Zagazig University academic and ethical committee. Every patient signed an informed written consent for acceptance of the operation.

All women participating were subjected to the following:

1. History: Careful history taking regarding personal, last menstrual period, obstetric, medical, surgical histories and history of present pregnancy.
2. Examination:
   - General examination: including blood pressure, pulse and temperature.
   - Laboratory investigations: U/S, CBC and RBS.
   - Complete physical examination to exclude any disorder may interfere with the results.
3. Gestational age was calculated from the last menstrual period and confirmed by ultrasound measurement during the first trimester of pregnancy.
4. Amniotic fluid samples were collected by a sterile speculum inserted in the posterior fornix of the vagina.
5. Samples containing 2 mL of amniotic fluid were immediately transported to the clinical laboratory in a test tube and analyzed according to an established protocol.
6. LBC was estimated in uncentrifugated amniotic fluid samples using The Sysmex K – 800 hematological analyzer and its platelet channel.
7. Management according to Prelabor Rupture of Membranes (PROM) protocol :-

- **Drugs which were given:**

  Antibiotics: The regimen of prophylactic antibiotics was given for seven days to pregnancies <34 weeks of gestation at the time of membrane rupture as follow: IV ampicillin (2 grams/6 hours) and erythromycin (250 mg/6 hours) for 48 hours followed by oral amoxicillin (250 mg/8 hours) and erythromycin base (333 mg/8 hours) for 5 days.

  Corticosteroids: Course of corticosteroids was given to pregnancies between 23 and 34 weeks of gestation as follow: Betamethasone (celestone) 12 mg IM/24 hours x 2 doses or dexamethasone 6 mg IV or IM/12 hours x 4 doses

- **Induction:** If testing showed a low risk of neonatal respiratory problems, we initiated delivery because we believe the risks of prematurity were small in this setting compared to the risk of developing maternal or fetal complications during expectant management.
- **Spontaneous labor:**

8. The neonatal respiratory status and existence of RDS was reviewed by attending neonatologists, who was not informed about the concentration of LBs.

9. Neonatal outcome:

The fetal outcome was reviewed after birth as follows:

a. Apgar score at 1 and 5 minutes.

b. Diagnosis of RDS was based on the presence of the following items:-

- Physical signs (nasal flaring, chest retractions, grunting and tachypnea).
- Supplementary oxygen requirement longer than 24 hours.
- Radiographic findings (reticulogranular opacification of lung fields with superimposed air bronchogram).

Statistical Analysis

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for the Social Sciences) version **25.0**. Qualitative data were represented as frequencies and relative percentages. Continuous data were presented as mean±SD if normally distributed or Median (Range) if not normally distributed. Categorical data were presented by the frequency and percentage. Normality was checked by Kolmogorov-Smirnov test. Homogeneity of variances was checked by Leven's test. Kruskal-Wallis H test. Independent-samples t-test Mann-Whitney u test. The chi-squared test: Fisher's Exact Test: Spearman's correlation: Pearson's correlation. P-value<0.05 indicates significance. Receiving operating characteristics (ROC) was used. Diagnostic characteristics (Sensitivity, specificity, predictive positive value, predictive negative value, diagnostic accuracy, Youden's index, and Kappa) were calculated.

RESULTS

This table shows demographic characteristics among the studied cases **Table (1).**

Table (1): Maternal characteristics among the studied cases

Variables		Mean±SD	Range
Age (years)		27.0±4.1	18.0–37.0
BMI (kg/m^2)		26.2±2.1	21.8–31.3
		N	**%**
Parity	**Primipara**	32	34.8
	Multipara	60	65.2
Mode of delivery	**Vaginal**	59	64.1
	Cesarean Section	33	35.9

Total=92

Figure (1) show that **respiratory distress** was present in less than one third of the studied cases.

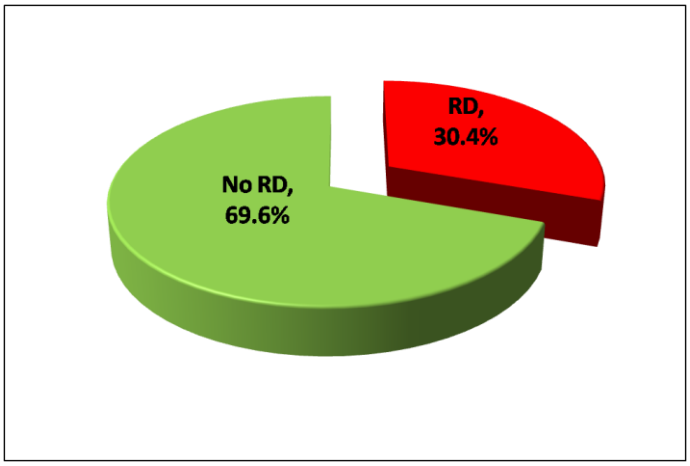

Figure (1): Respiratory distress among the studied cases.

Figures (2,3,4,5,6) show that there were significant positive correlations between LB and GA, birth weight and APGAR scores as well as significant negative correlation with respiratory distress grade**.**

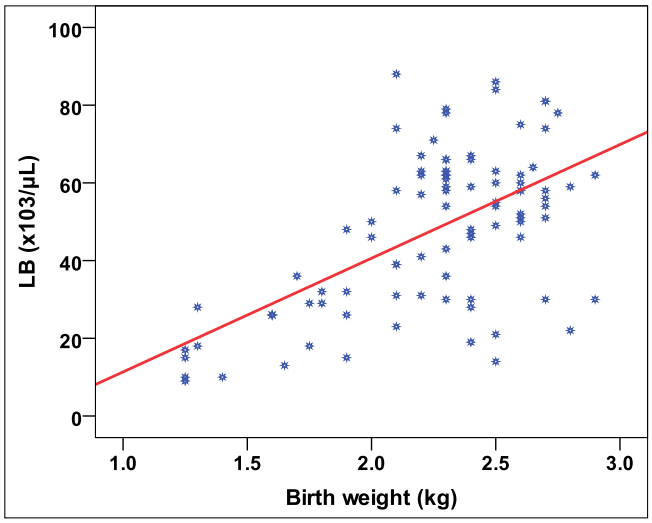

Figure (2): significant positive correlation between LB and birth weight

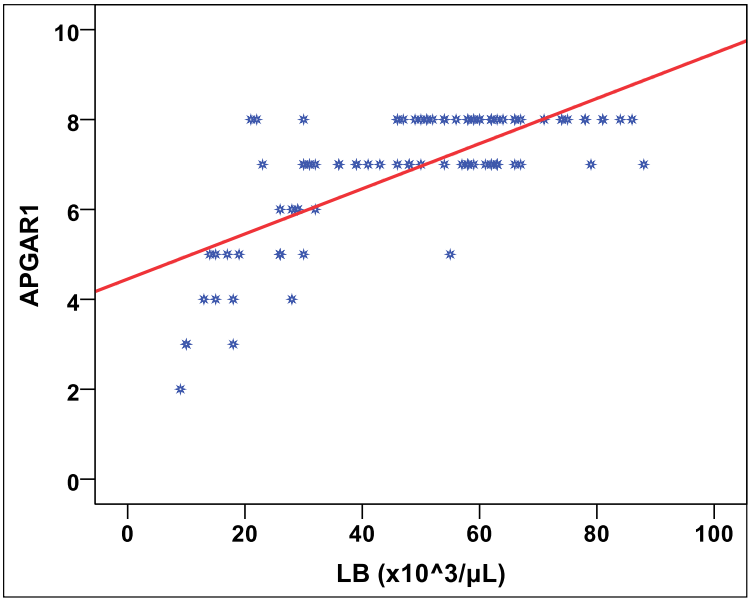

Figure (3): Significant positive correlation between LB and APGAR1

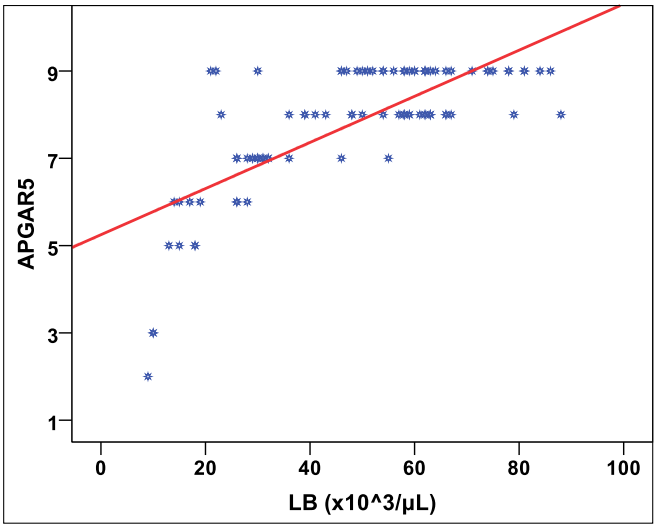

Figure (4): Significant positive correlation between LB and APGAR5

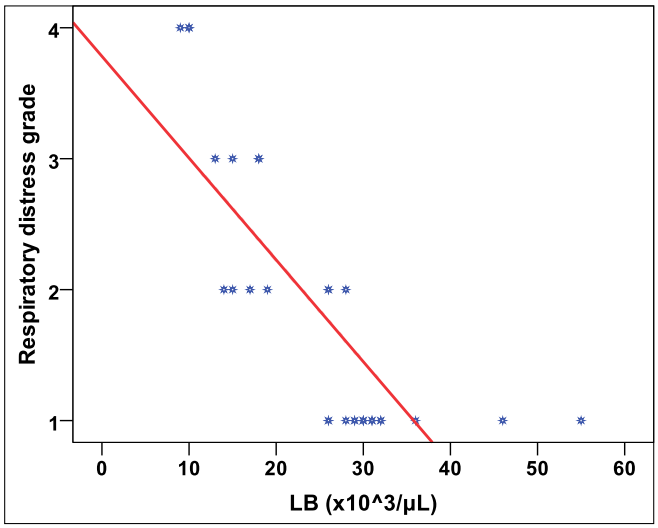

Figure (5): Significant negative correlation between LB and respiratory distress grade

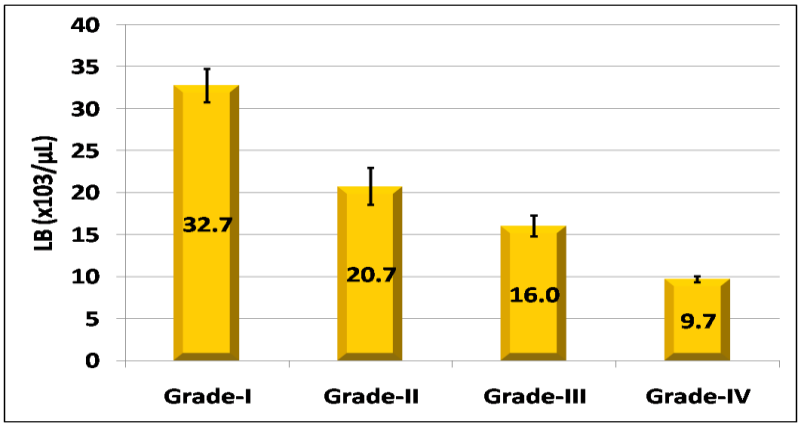

Figure (6): Comparison according to respiratory distress grade regarding LB

Figure (7) shows that: No significant difference according to respiratory distress regarding maternal age, BMI, parity and mode of delivery. Cases with respiratory distress significantly had lower LB, GA and birth weight.

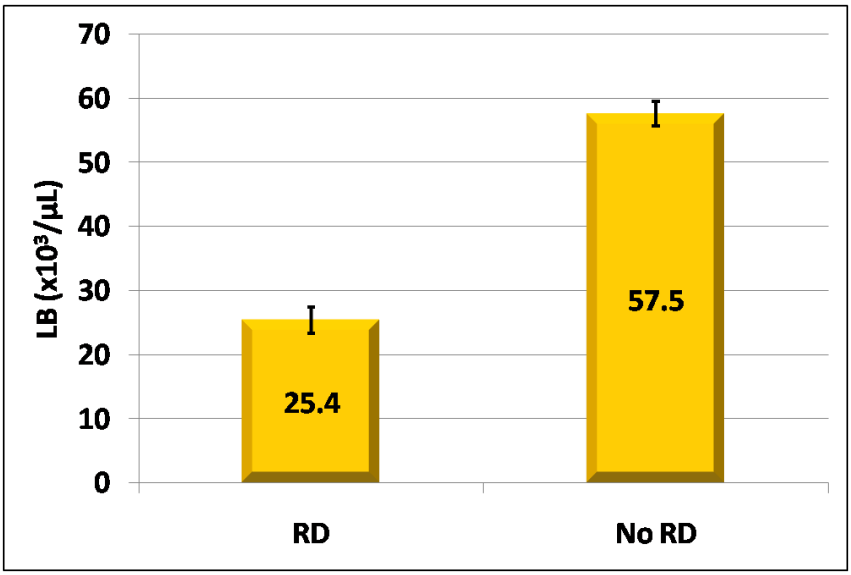

Figure (7): Comparison according to respiratory distress regarding LB

Figure (8) shows that LB had significant high diagnostic performance in predicting respiratory distress**.**

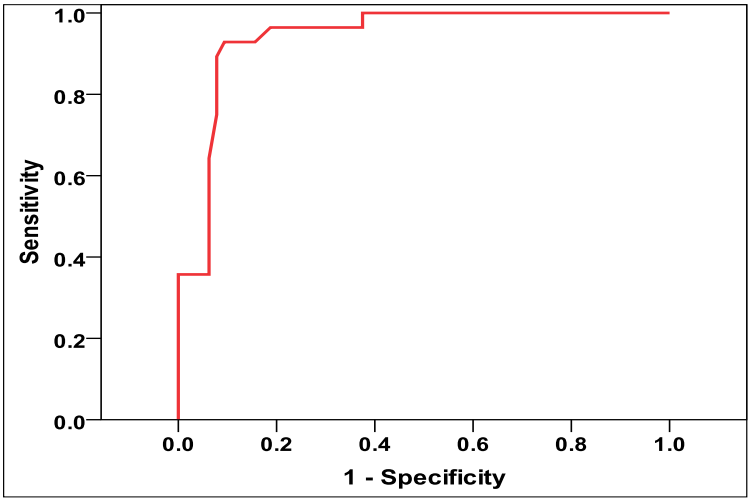

Figure (8): ROC curve for LB in predicting respiratory distress

Figure (9) shows LB ≤38.0 (x10^3/µL) had high diagnostic characteristics in predicting respiratory distress**.**

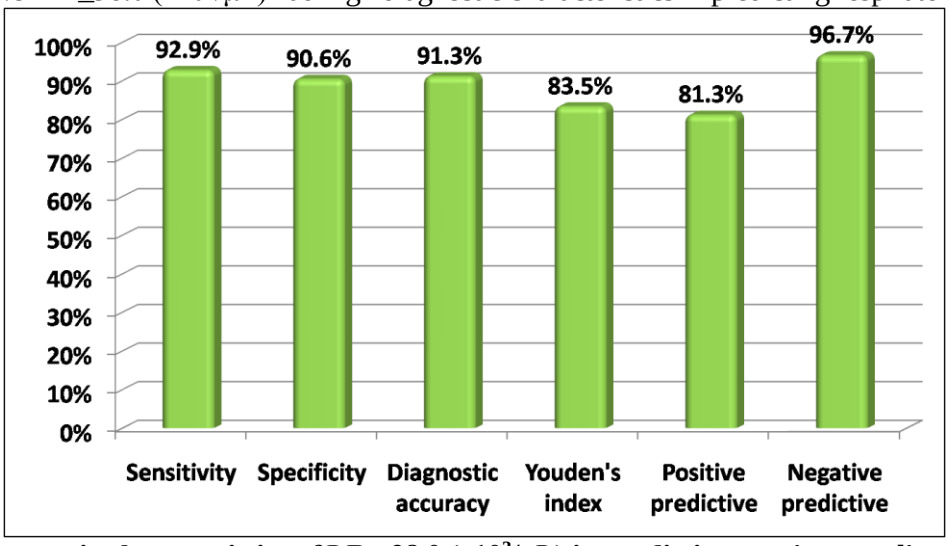

Figure (9): Diagnostic characteristics of LB ≤38.0 (x10^3/µL) in predicting respiratory distress

DISCUSSION

In the current study the neonates with diagnosis of prenatal lung immaturity had significantly younger gestational age.

The current study revealed a highly significant increase in the lamellar body count with a mean of 57.5±15.5 in cases giving birth to neonates without RDS compared to 25.4±10.7 in cases giving birth to neonates with RDS. This result was similar to the findings of **Štimac *et al.*** [8] who aimed to determine the lamellar body count (LBC) cutoff value for fetal lung maturity and to evaluate the clinical usefulness of LBC in predicting the severity of neonatal respiratory distress syndrome (RDS). A prospective study was conducted from 2002 until 2010. LBC was estimated in uncentrifugated amniotic fluid samples using Cell-Dyn 1800 analyzer. Amniotic fluid samples were obtained by amniocentesis or by puncturing embryonic membranes during cesarean section. The presence of mild, moderate, and severe RDS was assessed by neonatologist. They found that, antenatal amniotic fluid LBC method was able to differentiate between the neonates without RDS and the neonates who were expected to develop moderate and/or severe forms of acute RDS. And hence, based on their results, more severe forms of RDS were accompanied by lower median LBC.

Results of the present study showed a significant negative correlation between respiratory distress syndrome and gestational age 34.8±0.9 weeks in neonates with no RDS compared to 31.9±2.4 weeks in neonates suffering from RDS. This finding goes in accordance with **Reuter *et al.*** [9] who found statistically significant association between incidence of respiratory distress syndrome and gestational age

This disagrees with **Rimar *et al.*** [10] who reported that the incidence of RDS in newborns born after week 32 of gestation did not significantly change. What did change were the causes. They might be due to leading causes of RDS (e.g. sepsis and influence of which diminished due to better prenatal care).

Our study showed that a significant correlation between level of LBC and fetal lung maturity using 38.0 ($x10^3/\mu L$) as a cut-off point for LBC; as it is can be considered a good predictor for fetal lung maturity with sensitivity 92.9% and specificity 90.6%, positive predictive value of 81.3% and negative predictive value of 96.7%

This finding was in disagreement with **Zarean *et al.*** [11] who found mean of lamellar body was 31266 ± 15831 µl in matured lung infants compared to 63081 ± 16966 µl in immature lung infants ($p < 0.001$). The optimal cut-off point was evaluated as 47500 µl in predicted pulmonary maturity with sensitivity of 85.1%, specificity of 91.2%, positive predictive value of 92.6% and negative predictive value of 82.5%.

KulKarni and Jayamma [12] found that among 50 cases, LBC was <30,000/µl in 15 cases, between 30,000-35,000/µl in five cases and >35,000/µl in 30 cases. Those who developed RDS had LBC < 30,000/µl. Sensitivity and specificity of LBC to predict RDS with cut-off values of 30,000/µl were 100% and 97.2% respectively.

Wijnberger *et al.* [13] reported that a LBC 32000/µL guaranteed fetal lung maturity. They showed that the performance of the LBC in the prediction of RDS was equal to the L/S ratio. In their Meta-analysis they concluded the LBC may be considered as the test of first choice in the assessment of fetal lung maturity.

CONCLUSION

There is a significant correlation between level of LBC and fetal lung maturity using 38.0 ($x10^3/\mu L$) as a cut-off point for LBC. This could be considered a good predictor for fetal lung maturity with sensitivity 92.9% and specificity 90.6%, positive predictive value of 81.3% and negative predictive value of 96.7%.

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