New Identification Equations Based on Erythrocyte and Reticulocyte Characteristics for Screening Thalassaemia Trait in Pregnancy

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Abstract

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Protocol

Expected Outcomes

Step 1.

Both iron deficiency anemia (IDA) and thalassaemia trait (TT) are the most common microcytic hypochromic anemias. In pregnancy, a woman can be brought into an iron deficiency (ID) states more easily due to the increased iron utilization, so that the identification of TT is more difficultly in the complex situation. Despite several hematological indices calculated by certain parameters obtained from an automated cell counter have been introduced to discriminate quickly the two similar conditions, but the obstetricians and gynecologists would rather select mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) to screen pregnant women with TT. The purpose of the study was to establish identification equations for pregnancy associated with TT, and discuss their clinical application value.

Study population

Step 2.

During the period from January 2015 to September 2016, all pregnant women were enrolled when they attended for their first antenatal care visit. The recruiting procedure of the patients with IDA or α-TT or β-TT is as following:

 At first, all pregnant women would be asked to fill in a antenatal care questionnaire including food intake, underlying diseases, history of blood loss, current medications, family history of anemia and other demographic data, such as age, sex, height and weight.

 Then all pregnant women would be collected their whole blood samples to measure RBC and reticulocyte parameters by XN-9000 automatic machine (Sysmex, Japan). The RBC parameters include 6 indicators: RBC#, Hb, MCV, MCH, mean corpuscular hemoglobin concentration (MCHC) and RBC distributing width (RDW). The reticulocyte parameters include 7 indicators: reticulocyte counts, percentage of reticulocytes (Ret%), low fluorescence ratio (LFR), medium fluorescence ratio (MFR), high fluorescence ratio (HFR), immature reticulocytes fluorescence ratio (IRF) and hemoglobin content of reticulocytes (Ret-He).

 According to hemoglobin level < 110 g/L with decreased MCV and/or MCH, we would screen out pregnant women with microcytic hypochromic anemia. In order to get rid of the effects of infectious anemia or anemia caused by chronic diseases, the subjects with a history of infection or inflammation or underlying medical conditions within one month would be excluded.

 All the pregnant women with microcytic hypochromic anemia would be collected their whole blood samples to make a hemoglobin electrophoresis test. Those of HbA2 <2% (in practical work we selected the critical value as 2.5%) or >4% (in practical work we selected the critical value as 3.5%) would be performed gene analysis.

 All the pregnant women with microcytic hypochromic anemia would be collected their serum samples to make a ferritin measurement.

Inclusion criteria:

① All subjects must be with haemoglobin (Hb) <110g/L and decreased MCV and/or MCH.

② TT carriers must be with any gene deficiency of hemoglobin by gene analysis.

③ The patients with IDA must be with ferritin <14μg/L, and without any gene deficiency of hemoglobin.

Exclusion criteria:

The subjects with a history of infection or inflammation or underlying medical conditions within one month were excluded.

In fact, total 4826 pregnant women were enrolled in this study. 925 subjects were diagnosed as anemia, and furthermore only 564 subjects were diagnosed as microcytic hypochromic anemia without a history of infection or inflammation or underlying medical conditions within one month. By the recruiting procedure, 105 α-TT carriers and 143 β-TT carriers and 177 pregnant women with IDA were recruited.

Development of the new identification equations

Step 3.

At first, a random sampling would be performed in the patients with α-TT or β-TT or IDA, respectively. So the 425 subjects were divided into the development group of 174 cases (of which, 75 cases with IDA, 42cases with α-TT, and 57cases with β-TT) and the validation group of 251 cases (of which, 102 cases with IDA, 63cases with α-TT, and 86 cases with β-TT).

Next then, all subjects of the development group were further classified into two categories as follows: (1) IDA as one whereas α-TT or β-TT as another, for developing the identification equation named logit-P1, which used to distinguish between IDA and TT; (2) Despite of IDA, α-TT as one whereas β-TT as another, for developing another identification equation named logit-P2, which used

to distinguish between α-TT and β-TT carriers.

In finally, logistic regressions and receiver operating characteristic (ROC) curve analyses were employed to create the two identification equations.

In fact, based upon logistic regression, two equations were designed to screen the patients with α-TT (logit-P1) and β-TT (logit-P2), respectively. The superiority parameters of logit-P1 take into account the RBC#, Ret% and IRF parameters, and logit-P2 take into account MCHC, Ret% and MRF parameters.

According to the ROC curve analyses, the logit-P1 presented a cut-off point with a value of 0.84. If the index is <0.84, the individual is classified as an IDA one, whereas as a TT carrier. Similarly, the logit-P2 presented a cut-off point with a value of 0.41. If the individual with the logit-P2 <0.41 is classified as a α-TT carrier, whereas as a β-TT carrier.

Supplementary criteria of the new identification equations

Step 4.

When we validate the two identification equations, it was found that the logit-P1 was less perfect for screening α-TT carrier, misdiagnosing 17 (27.0%) of 63 α-TT carriers in the validation group as IDA patients. In view of RBC# increasing observably in TT carriers[19], the ROC curve analysis was performed again in the development group. Based on the analysis results, the RBC# with a cut-off value of 4.1×10^{12}/L was determined as a supplementary criteria for further distinguishing TT from those first diagnosis as IDA. If their RBC#s are less than 4.1×10^{12}/L, they are classified as IDA, whereas classified as TT.

Comparison with other hematological indices

Step 5.

In this study, 12 hematological indices would be selected to compare with the two identification equations in the validation group, which had been published in various academic journals from 1973 to 2015 [13-16].

For all 14 aforementioned formulas, the ROC curve analyses would be constructed to calculate the area under the curve (AUC), Youden index (YI), specificity (Spe), sensitivity (Sen), likelihood ratio positive (LRP), and likelihood ratio negative (LRN), so as to comprehensively decided their advantages and disadvantages. In addition, the diagnostic value under the proposed criterion would be compared to one another under the optimum criterion (OC) obtained from the validation group.

Then, between using identification equations alone and combination with the supplementary criteria, the rates of the missed diagnosis and the correct diagnosis for the pregnant women with IDA, α-TT and β-TT would be contrastively observed, as well as the kappa coefficient between the identification results and the clinical diagnosis.

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Step 6.

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