THE ROLE OF DISCRIMINANT FUNCTIONS IN SCREENING FOR BETA-THALASSAEMIA TRAITS DURING PREGNANCY

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ABSTRACT

Introduction: The mean red cell volume (MCV) has been shown to be useful in a 2-stage screening process for β-thalassaemia traits among pregnant women though associated with a large number of false positive results. We tested prospectively the ability of 5 discriminant functions (DF), (England & Fraser, Shine & Lal, Mentzer, Srivistava and Klee et al) to reduce the number of false positives when used as additional screening determinants for β-thalassaemia in antenatal patients with red cell microcytosis.

Methods: The diagnostic performance of each DF was compared in 493 patients with microcytosis and known β-thalassaemia status. Truth table analysis and Receiver Operation Characteristic curves for each function were determined.

Results: 11.4% of the patients with microcytosis were diagnosed to have β-thalassaemia traits. DFs incorporating the red cell indices: haemoglobin or total red cell count are unsuitable during pregnancy. Shine & Lal's index {(0.01xMCHx(<MCV)²} reduced the number of people recalled for confirmatory testing by 31.1% and increased the diagnostic yield to 38.7% while maintaining a negative predictive value for the test of 0.993.

Conclusion: We conclude that a 3-stage screening process for β-thalassaemia among pregnant women in Singapore involving the MCV, Shine & Lal's index and a confirmatory test to be both valid and cost-effective.

Keywords: thalassaemia, discriminant function, pregnancy, screening, mean red cell volume (MCV)

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INTRODUCTION

β-thalassaemia trait is characterised by red cell microcytosis and the mean cell volume (MCV) is often the first parameter to be tested in screening programmes. We previously showed that there was little change in the MCV throughout pregnancy[1]. It thus remains a valid test parameter in screening programmes targeted at the antenatal population.

While the use of the MCV as a single parameter for screening is helpful, it is limited by its low specificity. In an attempt to increase the initial identification rate, we investigated the use of additional red cell discriminant functions (DF). These have been shown to be useful in the differentiation of thalassaemia from iron deficiency in the general population[2-6]. The application of DFs to pregnant patients however has to be rigorously tested as the physiological changes associated with pregnancy, eg haemodilution, may possibly invalidate the use of the indices.

METHODS

Three thousand six hundred ninety-six (3,696) consecutive antenatal patients received full blood counts (FBC) as part of their evaluation when they first registered at our hospital for antenatal follow-up. Patients with MCV < 80fl were recalled and tested for the presence of β-thalassaemia trait by haemoglobin electrophoresis and HbA_2 quantitation (HbEP). DFs were applied on all microcytic patients to re-assign them into β-thalassaemia trait and non β-thalassaemia trait groupings. The assignment by DFs was compared against that by HbEP. The use of a MCV cut-off value at 80fl ensured the inclusion of 99.7% of the identifiable β-thalassaemia trait population for a further, more definitive testing[7]. The original patient group was not selected for any particular obstetric complications.

The FBC was determined on an impedance-based Contraves 801 automated haematology analyser. The machine was maintained regularly and preserved red cell samples were run as normal controls on the machine at least twice daily. Haemoglobin electrophoresis was carried out on cellulose acetate membrane at alkaline pH. HbA_2 quantitation was performed by mini-column chromatography (Helena Laboratories). β-thalassaemia trait was diagnosed if in addition to microcytosis, there were no variant haemoglobin bands and the HbA_2 level was in the range 3.4-10% of total haemoglobin content. Cao et al has shown earlier that there is no overlap between the HbA_2 range of normal individuals and those with identifiable β-thalassaemia traits[8].

Five different discriminant functions were applied to the data set of microcytic patients accrued: England & Fraser[2], Shine & Lal[3], Mentzer[4], Srivistava[5] and Klee et al[6]. Disease classification by each DF was made according to decision levels described[9] previously. Receiver Operating Characteristic (ROC) curves were plotted for each DF and compared to the ROC curve with the MCV as a single screening parameter. The percentage of cases correctly classified and the positive (PPV) and negative (NPV) predictive values were calculated for each DF.

RESULTS

Among the various FBC indices, we have previously

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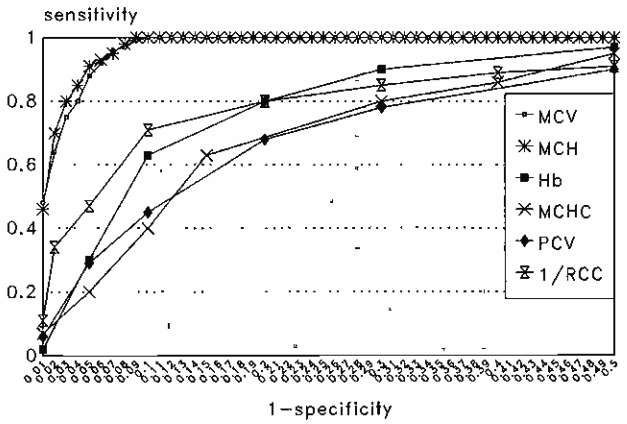
demonstrated that the MCV is a good discriminator at 80fl for β-thalassaemia[2] (Fig. 1). Four hundred ninety-three (493) (13.4%) of the initial 3,696 patients screened had a MCV ≤ 80fl and were further tested by HbEP. Fifty-six (11.4%) of these 493 were subsequently ascertained to be β-thalassaemia traits. The prevalence of β-thalassaemia trait in the general antenatal population thus determined is 1.52%.

Fig 1 – ROC curves of MCV, MCH, Hb, MCHC, PCV & 1/RCC (β-thalassaemia)



Application of the 5 DFs at the decision levels indicated generated the truth table (Table I). In general the use of a DF led to an improvement in the efficiency of disease classification. Thalassaemia classification by 4 of the DFs used (England & Fraser, Mentzer, Srivistava and Klee et al) improved the accuracy of the classification from 11.4% to more than 80%. This was generally achieved by having an increased specificity, trading in turn for a lower sensitivity.

In a multi-staged screening process as is intended here, it may be argued that the sensitivity of the first stage screening test is considerably more important than its specificity. Therefore for the function to be useful in screening for thalassaemia, any gain in specificity has to be made without sacrificing sensitivity. With this additional requirement, the DF proposed by Shine & Lal is the only index out of the 5 tested which is able to contribute to the diagnostic screening process. Shine & Lal's DF increased the accuracy of classification from 11.4% to 38.7% while maintaining a NPV of 0.993. This translates to a reduction in the number of false positives by 136 (31.1%). There was one false negative. The NPV of the other DFs ranged from 0.89 to 0.92; values which would have allowed a number of carriers to slip through the screening net.

DISCUSSION

Discriminant functions have been shown to be useful for the differentiation of thalassaemia trait from iron deficiency in microcytic patients. The discriminants generally centre around the observation that microcytosis is more prominent in thalassaemia trait than in iron deficiency at a given haemoglobin level. This relationship may however be altered by the haemo-dilution and other physiologic changes which normally take place during pregnancy, making the applicability of DFs in such situations uncertain.

Changes in the MCV are however relatively minor, allowing its un-modified use on a quantitative basis throughout pregnancy[1,10]. The low specificity of the MCV however gives rise to a significant number of patients being sent unnecessarily for further testing. Our data re-affirms that DFs are able to improve on the accuracy of classification for β-thalassaemia trait.

Despite the improvement in the accuracy of classification by DFs, our results show that the lower sensitivity of these functions precludes their use in screening. Of the 5 functions tested, Shine & Lal's index was the only function which had a sensitivity high enough to be included in the screening process. The failing of the other 4 DFs in this respect is perhaps their inclusion of parameters that are more greatly affected by the expanded plasma volume in pregnancy (haemoglobin, Total Red Cell Count), altering their discriminatory ability. Choosing a different decision level alters the balance between test sensitivity and specificity. It is however obvious from the ROC curve of the 4 DFs that the decision levels cannot be adjusted to give the sensitivity that is required here. (Fig 2).

Unlike the other functions, Shine & Lal's index makes no attempt to relate the microcytosis of the red cells to the haemoglobin level (or red cell count), choosing instead to magnify the difference in the MCV and mean cell haemoglobin (MCH) in the thalassaemia patient. It therefore is the DF which is least likely to be altered by pregnancy. This same characteristic, however, also makes it difficult to predict whether the index will be just as useful in another antenatal population where the balance of thalassaemia traits and other diseases giving rise to microcytosis is possibly different. When implemented in our population, however, the index can result in a 30% reduction in the number of HbEP requested.

Restricting the calculation of DFs to microcytic patients rather than the main population reduces slightly the number of false positives encountered. It also saves on unnecessary work as most patients with MCV > 80fl are classified by the DFs as not having β-thalassaemia trait.

Table I - Truth table for discriminant functions and their predictive values

Function	Reference	Decision level	True +ve	True -ve	False +ve	False -ve	Correct Classification	PPV*	NPV*
MCV	Pearson[7]	80	56	0	437	0	11.4%	0.114	1
MCV-RCC-(5xHb)-3.4	England & Fraser[2]	0	1	430	7	55	87.4%	0.125	0.887
0.01xMCHx(MCV)^2	Shine & Lal[3]	1530	55	136	301	1	38.7%	0.154	0.993
MCV/RCC	Mentzer[4]	14.0	23	379	58	33	81.5%	0.284	0.920
MCH/RCC	Srivistava[5]	4.4	22	375	62	34	80.5%	0.262	0.917
RCC	Klee, e al[6]	5.0	11	388	49	45	80.9%	0.183	0.896

*PPV = Positive predictive value, NPV = Negative predictive value

Fig 2 – ROC curves of various discriminant functions (β– thalassaemia)

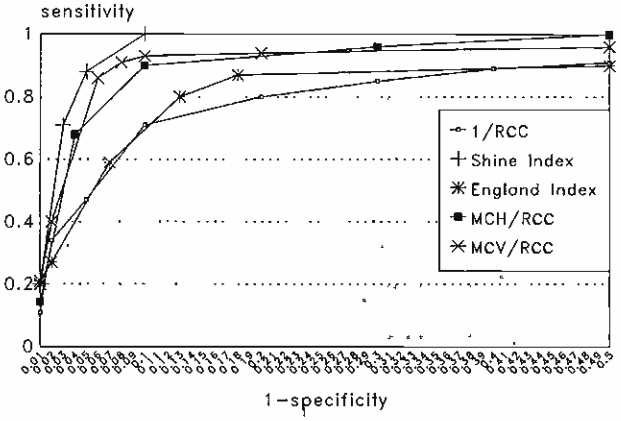
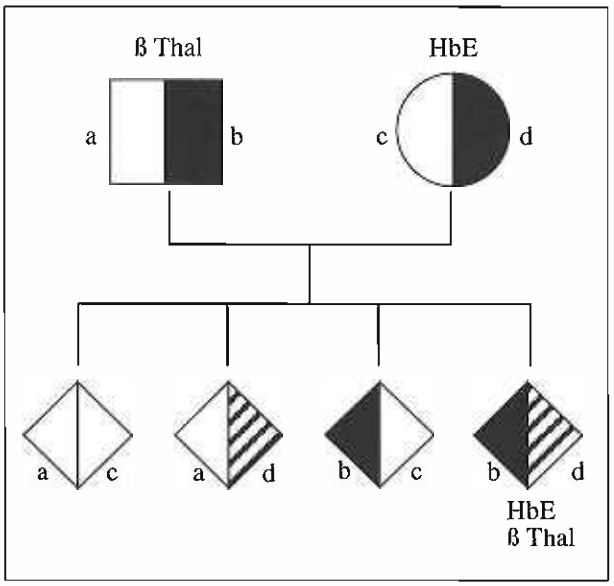


Fig 3 – Mendelian inheritance pattern of ß-thalassaemia and HbE carries



There are 2 other issues of relevance in deciding whether our data would be applicable to other antenatal populations.

The first concerns possible machine differences. Quality control is an integral part of any programme. With different technologies being used in automated haematology analysers, there is some concern that the measured MCV may not be similar between competing platforms[11]. The MCH has been suggested as an equivalent and perhaps more robust alternative to the MCV[12]. Our data show the ROC curves of the 2 parameters to be almost identical, suggesting that the MCH may be as useful as the MCV for screening (Fig 1). We have chosen the MCV because of its finer incremental values.

There are also concerns whether significant biological differences might exist across geographical regions, affecting red cell physiology.

Bull presented data showing red cell indices of healthy individuals taken from different geographical regions to be similar[13]. The population indices however would be affected by the differing disease prevalence. Different screening strategies will have to be developed to suit the conditions present in different patient populations. Cao et al reported a significant overlap in the MCV and MCH of β-thalassaemia traits and the general population in Sardinia[8]. As a result, HbEP were required as first line investigations in all of his patients. We are fortunate to be able to use the MCV to stratify our patients. The consequent cost savings are quite considerable. Other populations would be advised to verify that our strategy is applicable to their particular patient group before proceeding on to a full scale screening programme.

HbE patients do not exhibit the degree of microcytosis similar to β-thalassaemia carriers. Our experience, some 20%-50% of HbE carriers have mild microcytosis which are not screened out by the discrimination tests based on FBC. To perform the antenatal FBC screening for the wife alone may lead to a situation where a female HbE carrier may produce a blood transfusion dependent offspring with a β-thalassaemia carrier spouse (Fig 3). The protocol for screening of β-thalassaemia in Singapore would best be summarised in Fig 4 and Fig 5.

Fig 4 – Screening protocol for beta-thalassaemia traits among pregnant women in Singapore (Not including HbE)

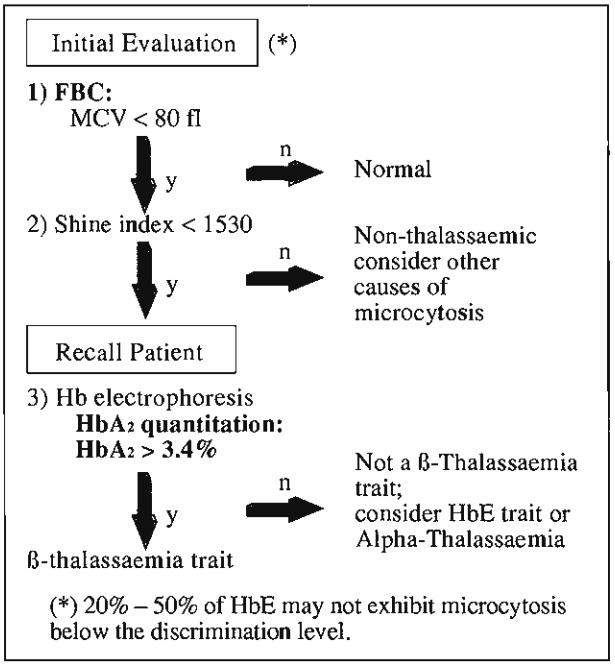
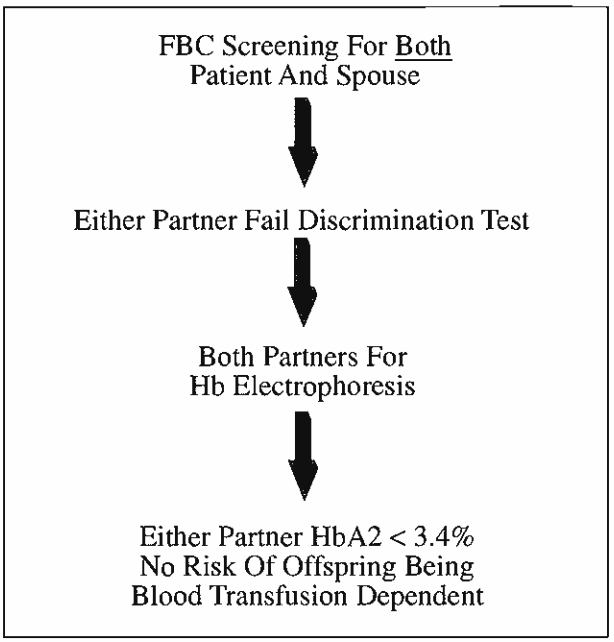


Fig 5 – Antenatal screening proposal for Singapore



CONCLUSION

We conclude that the use of DFs should be rigorously tested before being adopted as part of a screening process for thalassaemia. For pregnant patients in Singapore, it is reasonable and efficacious to stratify the screening process of β-thalassaemia into a 3-step process (Fig 4). Steps 1 and 2 and possibly step 3 as well may be completed at the initial consultation. The patient will need at most one recall before the diagnosis of β-thalassaemia is reached.

In Singapore, antenatal screening should take into consideration the high incidence of HbE. In the initial screening of the pregnant woman, the spouse should be simultaneously called and tested with a FBC screening (Fig 5). This protocol aims to ensure adequate time for foetal testing and the timely termination of affected pregnancies, if necessary. The reliability of foetal testing and social acceptance of the screening programme are other essential components which remain to be addressed.

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