Screening criteria for β thalassaemia trait in pregnant women

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Abstract

Aims—To establish suitable screening criteria for β thalassaemia trait during pregnancy using an automated blood counter incorporating light scattering technology.

Methods—Pregnant women ($n=857$) at a London antenatal clinic were investigated for β thalassaemia trait if the Technicon H.2 full blood count showed either a mean corpuscular volume (MCV) <85 fl or a mean corpuscular haemoglobin (MCH) <27 pg. Results were then analysed to establish which of these variables was more suitable for screening and to determine suitable cut off points for calculating the haemoglobin A_2 percentage.

Results—The MCH was superior to the MCV for thalassaemia screening as it was a more stable measurement and fewer unnecessary tests were performed. A MCH less than 27 pg is a suitable cut off point for screening. This screening criterion was equally applicable to a Coulter impedance counter.

Conclusions—Pregnant women presenting at an antenatal clinic with a MCH <27 pg should be investigated further to confirm or exclude a diagnosis of β thalassaemia trait.

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Keywords: β thalassaemia trait, prenatal diagnosis, automated blood count, Technicon H.2 counter.

In ethnic groups with a high prevalence of heterozygous β thalassaemia and with a general community awareness of the genetic implications, it is common for the diagnosis of β thalassaemia trait to be established before pregnancy. However, most patients attending our antenatal clinic subsequently found to have β thalassaemia trait are not aware of the diagnosis. This is likely to be the case at most antenatal clinics in Britain. As performing a haemoglobin A_2 estimation for all patients is costly, it is necessary for haematology laboratories to establish screening criteria for further

investigation which are applicable during pregnancy. Various formulae based on the full blood count (FBC) have been proposed to separate thalassaemia trait from iron deficiency, but these are unreliable during pregnancy.¹ Most laboratories therefore use the mean corpuscular volume (MCV) for screening, but the specific cut off point below which further investigation is carried out varies widely. A smaller number of laboratories use the mean corpuscular haemoglobin (MCH) to establish this cut off point. The BCSH General Haematology Task Force has recently proposed guidelines for screening for β thalassaemia trait² but did not give specific criteria for selecting which pregnant women should be investigated.

We have previously published data indicating that antenatal screening for β thalassaemia trait should be carried out whenever the booking MCV, as measured on an impedance counter, is <83 fl.¹ Following this study, our policy was to perform haemoglobin electrophoresis on all antenatal patients attending our clinic for the first time, regardless of ethnic origin or red cell indexes, but to quantitate haemoglobin A_2 only when the MCV was <83 fl. Two years ago, we purchased a new automated counter which estimates MCV from light scattered at two angles by isovolumetrically spherized red cells. As estimates of MCV by impedance technology and by light scattering are not comparable, it was necessary to re-establish screening criteria for β thalassaemia trait. As the MCH has theoretical advantages over the MCV, measurements being less dependent on the technology and method of calibration used, we also assessed the suitability of the MCH for screening.

Methods

Haemoglobin electrophoresis on cellulose acetate (pH 8.2 to 8.6) was performed on all patients attending our clinic for the first time. FBCs were performed on 50 healthy, non-pregnant, white women to establish ranges for MCV and MCH. The 2.5 percentiles for MCV (85 fl) and MCH (27 pg) were then used as screening criteria. The haemoglobin A_2 percentage was estimated by microcolumn chromatography (Helena β -thal Hb A2 Quik Column) in patients with a MCV <85 fl or a MCH <27 pg. A diagnosis of β thalassaemia trait was made if the haemoglobin A_2 was greater than 3.5%. Data were examined to establish whether the MCV or the MCH provided the better screening criterion based on our ability to choose a cut off point which would allow all cases of β thalassaemia trait to

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Table 1 Patients' ethnic origin and the percentage diagnosed with β thalassaemia trait

Ethnic origin	Total number tested	Number (%) with β thalassaemia trait
African or Afro-Caribbean	295	13 (4.4)
Mediterranean	106	10 (9.4)
Chinese and Far East Asian	35	2 (5.7)
Arab and other Middle Eastern	89	12 (13.5)
Indian subcontinent	145	12 (8.3)
White (except Mediterranean)	117	2 (1.7)
Not stated on request form	70	5 (7.1)
Total	857	56 (6.5)

Table 2 Percentage of women with a MCV <85 fl and MCH <27 pg, later diagnosed with β thalassaemia trait

	β thalassaemia trait	Not β thalassaemia trait	Total
MCV <85 fl/MCH <27 pg	56	550	606
MCV <85 fl/MCH not <27 pg	—	224	224
MCH <27 pg/MCV not <85 fl	—	27	27
Total	56	801	857

Figure 1 Histogram showing the number of subjects tested and the number diagnosed with β thalassaemia trait (■) for each MCV value. HbA₂=haemoglobin A₂.

Figure 2 Histogram showing the number of subjects tested and the number diagnosed with β thalassaemia trait (■) for each MCH value. HbA₂=haemoglobin A₂.

be diagnosed with a minimum of unnecessary testing. Having established appropriate criteria for the Technicon H.2 counter, we re-analysed our previous data from a Coulter S Plus IV counter to determine whether the same MCH criterion was transferable to an instrument incorporating different technology.

As antenatal blood specimens are sometimes delivered to hospitals from outlying clinics, we also carried out room temperature storage experiments to establish the stability of the MCV and MCH. For this purpose, we studied 14 blood specimens derived from patients or volunteers. Eleven specimens were from haematologically normal subjects with MCVs spanning the normal range and three were from volunteers with thalassaemia trait (two with β and one with α thalassaemia trait). Samples from these specimens were analysed 30 minutes after venesection and at four, six, eight, 12, and 24 hours on the Technicon H.2 and on a Coulter S Junior analyzer. A smaller number of samples were assessed using the same protocol after storage at 4°C.

Results

Over two years, from September 1992 to September 1994, 857 patients attending the clinic did not have a haemoglobinopathy but had either a MCV <85 fl or a MCH <27 pg; 784 patients had microcytic red cells. The patients' ethnic origin and the percentage of women in each ethnic group diagnosed as having β thalassaemia trait are presented in table 1. Of these 857 patients, 606 had both a MCV <85 fl and a MCH <27 pg. Of these women, 6.5% had β thalassaemia trait (table 2). Two hundred and twenty four patients had a MCV <85 fl but a MCH of at least 27 pg, whereas 27 patients had a MCH <27 pg and a MCV of at least 85 fl. None of the women in either of these groups had β thalassaemia trait. The total number of women tested at each MCV and MCH level and the number found to have β thalassaemia trait is shown in figs 1 and 2. We found that if screening had been based on the MCV, it would have been necessary to test all women with a MCV of 84 fl or less in order to detect all cases of β thalassaemia trait. If screening had been based on the MCH, it would have been necessary to test all women with an MCH <27 pg in order to detect all cases. Selection of the MCH rather than the MCV for screening purposes would have resulted in a 25% reduction in the number of women requiring haemoglobin A₂ estimation. Re-analysis of data derived from the Coulter S Plus IV¹ showed that screening all women with a MCH <27 pg would have detected all 62 women with β thalassaemia trait who were identified in that study on the basis of microcytosis.

Storage experiments showed that MCH was stable at room temperature for up to 24 hours with both instruments (fig 3). On storage at room temperature, MCVs measured on the Technicon H.2 counter showed a progressive increase from eight hours onwards with a mean increase of 4 fl (observed range 2.6 to 5.3

Figure 3 Change in MCH (mean $\pm$ 2 SD) as measured on the Technicon H.2 (+) and the Coulter S Junior (#) after storage at room temperature for 30 up to 24 hours.

Figure 4 Change in MCV (mean $\pm$ 2 SD) as measured on the Technicon H.2 (+) and the Coulter S Junior (#) after storage at room temperature for 30 up to 24 hours.

fl) by 24 hours (fig 4). On storage at room temperature, MCVs measured on the Coulter S Junior showed a mean rise of less than 0.5 fl (observed range 0 to 0.9 fl), by 24 hours. On storage at 4°C, both MCV and MCH were stable on both instruments (data not shown).

Discussion

It is not possible to detect all subjects with β thalassaemia trait by screening on the basis of

the FBC as some subjects have normal red cell indexes (silent β thalassaemia trait).² However, any screening programme being carried out for the purpose of genetic counselling must detect the great majority of cases.

Our data show that, with the Technicon H.2 counter, the MCH is superior to the MCV for screening purposes as fewer unnecessary tests are performed. This makes screening more cost-effective. In addition, our experiments with room temperature storage suggest that laboratories using this instrument which receive antenatal blood specimens from outlying hospitals or clinics should be cautious in using the MCV for screening as artefactual changes may result in the MCVs of some patients being above the selected cut off point unless specimens are refrigerated during transport. The MCH is much more stable at room temperature. This may explain why we were able to choose a cut off point which led to fewer unnecessary tests when the screening criterion was based on this variable. With impedance counters, either the MCV or MCH appears to be suitable for screening, although we are not aware of any study which directly compares the cost-effectiveness of these two instruments. Both the MCV and the MCH rise during pregnancy, but our data indicate that the advice of the WHO Working Group on Haemoglobinopathies³ that patients should be screened for β thalassaemia trait if the MCH is less than 27 pg is applicable to pregnant women. Our findings also illustrate the importance, in multiracial communities, of carrying out such investigations irrespective of ethnic origin. Although we have not considered investigation for α thalassaemia trait in this paper, the adequate investigation of a pregnant woman also requires that if the haemoglobin A₂ percentage is within the normal range, subjects from relevant ethnic groups with a MCH <26 pg should be investigated further for α^0 thalassaemia trait.²

1 Bain BJ. Screening of antenatal patients in a multiethnic community for β thalassaemia trait. *J Clin Pathol* 1988;41: 481-5.

2 The Thalassaemia Working Party of the BCSH General Haematology Task Force. Guidelines for investigation of the α and β thalassaemia traits. *J Clin Pathol* 1994;47: 289-95.

3 WHO Working Group on Haemoglobinopathies. Guidelines for the control of haemoglobin disorders. Geneva: World Health Organisation, 1994.