

### Thalassemia screening based on red cell indices in the Chinese

By correlating red cell indices with genotypic data, we show that mean corpuscular volume (MCV) < 80 fL or mean corpuscular hemoglobin (MCH) < 27 pg is able to detect all heterozygous carriers of ( $\alpha$ -thalassemia (SEA) deletion and common  $\beta$ -thalassemia alleles in the Chinese population. They should be recommended as cut-off values for thalassemia screening.

Public health concern about carrier detection and prenatal diagnosis of thalassemia is not only confined to geographic areas with high disease prevalence, but becomes a global issue due to population emigration. Screening for thalassemia in community-based and antenatal programs entail determination of red cell indices, namely MCV and MCH, as the initial step.<sup>1</sup> However, clinical guidelines and practices are variable. The British Committee for Standards in Haematology recommends testing for  $\beta$ -thalassemia and  $\alpha$ -thalassemia traits when MCH is < 27 pg and < 25 pg respectively.<sup>2</sup> In Sardinia, while hemoglobin (Hb) chromatography is performed for all subjects, further characterization of thalassemia status is carried out for MCV < 78 fL and MCH < 27 pg.<sup>3</sup> Screening strategies based on MCV < 75 fL<sup>4</sup> and MCV < 80 fL<sup>5,6</sup> have been described in Hong Kong. Further investigations by Hb analysis or genotyping are indicated for definitive diagnosis of thalassemia carrier status in subjects with MCV or MCH below these cut-off values.

We critically appraise thalassemia screening based on red cell indices by analyzing the distribution of MCV and MCH in four thalassemia carrier states most commonly encountered in our locality, namely the SEA deletion (n = 110),  $\beta$ -thalassemia (n = 66), single  $\alpha$ -globin gene deletion and mutations (n = 70) and Hb E (n = 9). Data for the present report were generated from archive results and stored samples from the previous population study,<sup>5,6</sup> but with

determination of genotype extended to a MCV of 85 fL instead of 80 fL. Parents of known Hb H disease patients were also included. The MCV and MCH values were determined by a Technicon H\*2 automated cell counter (Technicon, Tarrytown, N.Y. USA), and the globin genotypes were determined by standard mutation detection techniques.<sup>5,6</sup> In all subjects, the configuration of the  $\alpha$ -globin gene was documented by Southern blot analysis, and concurrent iron deficiency was excluded. The  $\beta$ -thalassemia alleles comprise codons 41/42 (-CTTT), IVSII-654(C→T), nt-28 (A→G), codon 17 (A→T), codons 71-72(+A), codons 14-15(+G) and codon 43 (G→T). The single  $\alpha$ -globin gene deletion and mutations include  $-\alpha^{3,7}$ ,  $-\alpha^{4,2}$ , Hb Constant Spring, Hb Quong Sze and  $\alpha 2$  codon 30 DGAG.

Based on results that are shown graphically in Figure 1, an MCV cut-off of 80 fL or an MCH cut-off of 27 pg was able to detect all heterozygous carriers of SEA deletion and  $\beta$ -thalassemia alleles commonly encountered in our locality, and hence should be recommended for the Chinese population. An MCV cut-off of 75 fL will miss 4/110 (3.6%) carriers of SEA deletion and 2/66 (3%) carriers of  $\beta$ -thalassemia, whereas an MCV cut-off of 78 fL will miss 1/110 (0.9%) carriers of SEA deletion. An MCH cut-off of 25 pg for  $\alpha$ -thalassemia will miss 2/110 (1.8%) carriers of SEA deletion.

Less common thalassemia carrier states harboring the SEA deletion, such as concurrent SEA deletion in heterozygous  $\beta$ -thalassemia,<sup>7</sup> and SEA deletion in association with triplicated  $\alpha$ -globin genes ( $\alpha\alpha\alpha/-SEA$ ),<sup>8</sup> may potentially be associated with high MCV and MCH values. No SEA deletion or  $\beta$ -thalassemia allele was, however, identified in those subjects with MCV from 80 – 85 fL, implying that the two aforementioned rare thalassemia carrier states harboring the SEA deletion are amenable to being detected with an MCV cut-off of 80 fL. Moreover, in two previous reports,<sup>7,9</sup> heterozygous carriers of both  $\beta$ -thalassemia trait and SEA deletion in the Chinese who were described all showed MCV values of below 80 fL, further supporting that this cut-off limit is applicable to the detection of double heterozygosity for  $\beta$ -thalassemia trait and SEA deletion. Finally, it is imperative that detection of single  $\alpha$ -globin gene deletion and mutations

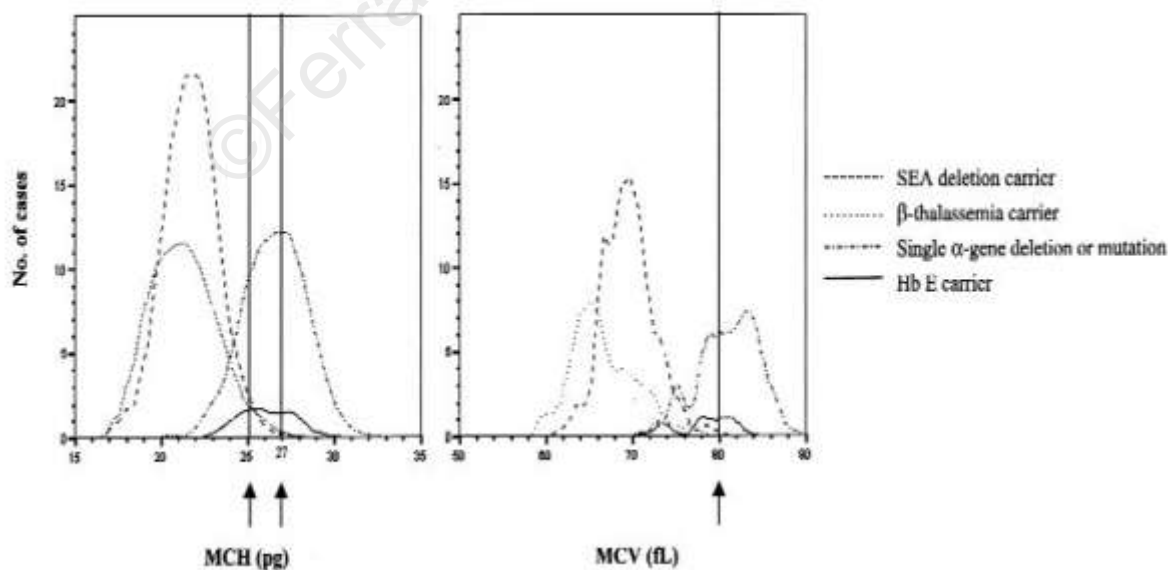


Figure 1. Smoothed curve showing the distribution of four different thalassemia carrier states with respect to MCH and MCV.

and Hb E carrier state should be performed in appropriate clinical situations irrespective of red cell indices. These situations may include detection of Hb E in partners of patients with known  $\beta$ -thalassaemia trait to predict for Hb E/ $\beta$ -thalassaemia,<sup>10</sup> and detection of non-deletional  $\alpha$ -globin gene mutations in partners of known SEA deletion carriers to predict for more severe forms of Hb H disease.<sup>11</sup>

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Key words: thalassaemia screening, red cell indices, MCV, MCH, Chinese.

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