germ cell tumours are divided into two major categories: seminoma and non-seminomatous germ cell tumours (NSGCT) [6]. The NSGCT must be further classified as pure or a mixed tumour and all the components present should be reported with the approximate volumes of each component. The NSGCT include embryonal carcinoma, immature or mature teratomas, choriocarcinoma and other rare trophoblastic tumours, endodermal sinus tumour, diffuse embryomas and polyembryomas; these may be found alone or in various combinations as mixed tumours. Tumours containing both seminomatous and NSGCT components and tumours that are histologically pure seminomas but with a significantly elevated serum AFP level are regarded as NSGCTs or mixed tumours for treatment purposes.

Embryonal carcinoma “pure” constitutes 1% to 3% of germ cell tumours when yolk sac differentiation was based on AFP immunoreactivity [7]. Presence of AFP in the cell or serum is an evidence of yolk sac differentiation [8]. Microscopically embryonal carcinoma exhibits an acinar, tubular, papillary or solid pattern with areas of necrosis, haemorrhage and fibrosis. Cells are markedly pleomorphic with eosinophilic cytoplasm. Immunohistochemically cytokeratin cocktail, PLAP and ki-1 (CD30) are positive. AFP may be positive in tumour cells. HCG, CEA and Leu-M1 are negative. Cytogenetic analysis has confirmed the presence of isochromosome 12p in many cases. There is a high cure rate obtained with modern chemotherapy even if metastases have developed. The most frequently used chemotherapy protocol for advanced NSGCTs is the combination of bleomycin, cisplatin and either vinblastine or ectoposide. After treatment a residual mass may require surgery or more aggressive chemotherapy. Adverse prognostic factors of a NSGCT include presence of an embryonal carcinoma of more than 80% of the total volume in the primary tumour, presence of vascular or lymphatic invasion, presence of a teratoma in less than 50% of the tumour [1].

Our patient had an embryonal carcinoma with an elevated serum AFP and HCG. After chemotherapy his tumour volume subsided. The well designed PEB chemotherapy is equally effective for primary and secondary germ cell tumours. The tumour mass and the vertebral deposits were well controlled by chemotherapy, and the residual disease was helped by radiation therapy.

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References

Two families in Sri Lanka with southeast Asian ovalocytosis

HMS Vidyatilake¹ and LV Gooneratne²

(Index words: Autosomal dominant, band 3, hyperstable, malaria, ovalocytes)

Introduction
Inherited defects of the red cell membrane which lead to abnormal red cell morphology, indices and osmotic fragility are not uncommon. Some defects cause clinically significant haemolysis whereas others run a relatively benign course. This paper emphasises the importance of

¹Haematologist and ²Registrar in Haematology, Lady Ridgeway Children’s Hospital, Colombo 8, Sri Lanka.
Correspondence: HMSV, Tel: +94 11 2896643, e-mail: drsdharma@sltnet.lk (Competing interests: none declared).
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being aware of one such benign disorder. Southeast Asian ovalocytosis (SAO), also known as stomatocytic elliptocytosis of Melanesians, has a unique red cell morphology in the blood film [1]. It is most commonly seen among Melanesians in Papua New Guinea, in some population groups in Indonesia and Malaysia, and in Malaysian aboriginals [2,3,4]. We report two families with SAO in Sri Lanka.

Case 1

A 7-year old boy was admitted to the Lady Ridgeway Hospital with a viral fever. A blood picture done as a preliminary investigation showed over 30% of the red cells to be large ovalocytes with some showing a central slit or transverse ridge. These features are characteristic of SAO. Screening of family members revealed similar features in the blood picture of his sister (only sibling), father and paternal grandmother whose father was an immigrant from Malaysia (Figure 1, Table 1). The blood picture of the boy’s mother was normal. There was no evidence of haemolysis in any of the affected members.

**Case 2**

A 30-month old boy had been investigated for a haemolytic anaemia since birth at Base Hospital, Horana. He was pale, icteric and had hepatosplenomegaly with a haemoglobin of 6.5 g/dL, a reticulocyte count of 10.5% and a serum bilirubin of 170 μmol/L. The blood picture showed red cells which were markedly hypochromic and microcytic, a population of large ovalocytes, some with central ridges, polychromasia and normoblastae. The white cells and platelets were normal. Haemoglobin electrophoresis and alkaline denaturation test (HbF - 21.3%) confirmed that he had beta thalassaemia major together with features characteristic of SAO. As he was the only live child, his parents were screened for both conditions. His father, a Sinhalese from Anuradhapura was found to have a beta thalassaemia trait (HbA2 - 5.8%) with SAO. His mother a Sinhalese from Kurunegala had a beta thalassaemia trait (HbA2 - 5.1%) with no evidence of SAO (Figure 2, Table 2).

The child is being managed with on demand blood transfusions and parenteral iron chelation therapy.

**Figure 1. Family tree of the first case.**

**Figure 2. Family tree of the second case.**

**Table 1. Comparison of red cell indices of family members of first case**

An increase in the variation of red cell size is indicated by a high red cell distribution width or RDW (normal < 13.2%), which is probably due to the ovalocytes.

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>RBC (x10^12/L)</th>
<th>Hb (g/dL)</th>
<th>RDW (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband</td>
<td>7</td>
<td>4.65</td>
<td>12.9</td>
<td>14.2 (high)</td>
</tr>
<tr>
<td>Sister</td>
<td>3</td>
<td>4.41</td>
<td>12.6</td>
<td>13.8 (high)</td>
</tr>
<tr>
<td>Father</td>
<td>37</td>
<td>4.71</td>
<td>14.9</td>
<td>15.3 (high)</td>
</tr>
<tr>
<td>Mother</td>
<td>36</td>
<td>4.78</td>
<td>13.7</td>
<td>12.0</td>
</tr>
<tr>
<td>Grandmother</td>
<td>65</td>
<td>3.7</td>
<td>12.1</td>
<td>14.6 (high)</td>
</tr>
</tbody>
</table>

Hb = haemoglobin, RBC = red blood (cell) count, RDW = red cell distribution width

**Discussion**

Southeast Asian ovalocytosis has an autosomal dominant inheritance. It results in red cells that are rigid and hyperstable (rather than unstable) giving rise to the unique red cell morphology [2]. The red cell membrane is composed of a phospholipid and cholesterol bilayer and a number of membrane proteins. These proteins have been categorised as integral membrane proteins and peripheral membrane proteins, and assigned specific names. These are based on the mobility of each protein on a sodium dodecyl sulphate polyacrylamide gel electrophoresis.

The protein that is defective in SAO (anion exchanger 1) is identified on band 3 when stained with Coomassie brilliant blue stain [5]. Two mutations that cause abnormalities in the band 3 protein have been identified...
in patients with SAO. Homozygosity for these mutations are thought to lead to embryonic lethality while the heterozygous state results in the formation of abnormal erythrocytes which exhibit increased binding of band 3 protein to Ankyrin (band 2.1 protein), increased tyrosine phosphorylation of band 3 protein, inability to transport sulfate anions and a markedly restricted lateral and rotational mobility [2,3]. Other abnormalities associated with SAO are reduced osmotic fragility of red cells, resistance to shape change by echinocytic agents and a reduced expression of many red cell antigens.

Another remarkable feature of SAO erythrocytes is their resistance to invasion by several strains of malarial parasites, including \textit{Plasmodium falciparum} and \textit{P. knowlesi}, particularly against heavy infections and cerebral malaria [2,3]. Band 3 serves as one of the receptors for the malarial parasite, as evidenced by inhibition of invasion in vitro [2]. The abnormal band 3 protein may not function as a receptor for the malarial parasite.

The diagnosis of SAO can be made accurately on a blood film stained with Romonowsky stain [6,7]. The finding of 30% or more of oval shaped red cells with stomatocytes, with a notable absence of clinical and laboratory evidence of anaemia and haemolysis is highly suggestive [2]. There is also a minor population of macro-ovalocytes, many of which are stomatocytic. The stoma is two in each cell. It may be longitudinal, transverse, V-shaped or Y-shaped [1]. The haemoglobin, mean corpuscular value (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and the reticulocyte count are normal. A useful screening test is the demonstration of the resistance of ovalocytes or their ghosts to changes in shape produced by treatment that produces spiculation in normal cells, such as overnight incubation of red cells [2]. Rapid genetic diagnosis can be made by demonstrating a shorter band (27 base pair deletion) compared to a normal control [2,7].

### Conclusion

Diagnosis of SAO can be made on a blood picture or by DNA analysis. Awareness of the presence of this red cell disorder in Sri Lanka, with a multi-racial population and possible preservation of the gene in areas endemic for malaria, is important.

### References