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The fifth human malaria species – *Plasmodium knowlesi*

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Abstract

Over 20 species of malaria are known to infect non-human primates but until recently only four were thought to regularly infect humans (*Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*). Over the last decade *Plasmodium knowlesi* has been recognised as a significant cause of malaria infection in Southeast Asia. It occurs naturally in the Macaque monkey population in the forested areas of Malaysia, Borneo, Myanmar, Singapore, the Philippines, Indonesia, Thailand, Taiwan and Vietnam, and is transmitted by the *Anopheles* mosquito species. There have been fatalities caused by *P. knowlesi* infection due to high parasite loads and subsequent hepatorenal dysfunction and/or acute respiratory distress syndrome. The morphological features of the parasite resemble those of *P. malariae* and it has frequently been misdiagnosed as that species. The molecular techniques now available have enabled the correct identification of *P. knowlesi* as one of the major causes of malaria in this region.

Keywords: *Plasmodium knowlesi*, malaria parasites, *Anopheles* mosquito, zoonoses, macaques

Introduction

*Plasmodium knowlesi* is a malaria parasite of the long-tailed (*Macaca fascicularis*) and pig-tailed (*Macaca nemestrina*) Macaque monkeys (Garnham 1966) and causes a chronic infection in these natural hosts. Until recently only sporadic cases of human infections with *P. knowlesi* have been reported. The results from studies undertaken by a group from the University Malaysia Sarawak and Sarawak Health Department have shown this to be incorrect, and this species accounts for between 28% and 100% of cases in Sarawak, Sabah and Pahang districts in Malaysian Borneo and peninsular Malaysia (Cox-Singh et al 2008). The study was undertaken by this group when it was noted that many of the infections in this region were diagnosed as *P. malariae* but the incidence of this species in this area was known to be low (this was confirmed by the study). The other anomaly that triggered the investigation was the high parasite counts in these cases – *P. malariae* is associated with a low parasite count with the count rarely >5000/μL (Garnham 1966; Cox-Singh et al 2008). The erythrocytic cycle of *P. malariae* is 72 hours whereas *P. knowlesi* replicates and completes its blood stage cycle every 24 hours. This short life cycle rapidly increases the parasite load and leads to the development of symptoms and complications (Daneshvar et al 2009).

History

Knowles and Das Gupta first identified *P. knowlesi* in a long tailed macaque in 1931 and a year later showed it to be infectious to humans by inoculation of infected blood (Knowles and Das Gupta 1932). It was found to be lethal in Rhesus monkeys (*Macaca mulatta*) when it attained high counts (Garnham 1966). The parasite was used in the 1930s in patients with neurosyphilis to induce hyperpyrexia as this was regarded as a therapeutic treatment (Nicol 1935). In 1960 there was the report of two laboratory workers bitten by *Anopheles freeborni* mosquitoes who developed malaria – the mosquitoes had fed on *Macachi rhesus monkeys* that had been repeatedly exposed to sporozoite inoculations of *P. cynomolgi* (Eyles et al 1960). The simian to human transmission was also confirmed by Chin et al when they enrolled eight volunteers in a prison located in Atlanta, Georgia. The prisoners were subjected to bites from the *Anopheles balabacensis* mosquito that had previously acquired the malaria from infected monkeys (Chin et al 1968).

The first case of a natural infection in an American traveller to peninsular Malaysia had been previously reported in 1965 by that same group of workers (Chin et al 1965). It was initially diagnosed as a *P. falciparum* infection, but was changed to a diagnosis of *P. malariae* a day later but subsequently confirmed as *P. knowlesi* when the infected blood was inoculated into Rhesus monkeys. The lethality of this species in Rhesus monkeys confirmed *P. knowlesi* and was the diagnostic criterion prior to the advent of molecular techniques.
A subsequent naturally acquired case was reported in 1971, again from peninsular Malaysia, which was diagnosed initially as *P. malariae* but changed to *P. knowlesi* based on travel history and serological tests (Fong *et al* 1971).

There were studies undertaken in peninsular Malaysia to investigate natural infections of *P. knowlesi* in humans in the 1960s but they failed to identify these cases as *P. knowlesi*; it was not until the advent of nested PCR that it was correctly identified as the causative species.

**Transmission**

Nine different types of the *Anopheles* species have been identified as able to transmit *P. knowlesi* but it is *A. latens* (previously named *A. leucocephyrus*) that is the predominant vector (Vythilingam *et al* 2006). The *A. latens* mosquito is attracted to both humans and monkeys, whereas other species such as *A. hackeri*, still capable of transmitting malaria to humans, is not normally attracted to them and will feast preferentially on the Macaque population (Wharton and Eyles 1961; Reid and Weitz 1961). The *A. latens* mosquitoes have been found more at the rain forest edges and farm areas and bite the monkeys when they are in the trees, three to six metres in height. These mosquitoes then attack humans at dusk and overnight, with a peak time around midnight (Reid and Weitz 1961). Man may therefore acquire infections when hunting in the forest or returning home after a working day on the farm.

**Incidence**

For the five year timeframe between 1998 and 2002, the annual incidence of malaria in Sarawak, Malaysian Borneo was between 2,496 and 3,155 registered cases (Singh *et al* 2004). It was reported at the time that the predominant species was *P. vivax* (69.1% of all cases), followed by *P. falciparum* (19.7%), *P. malariae* (9.7%) and mixed species infections 1.8%. There were no reported cases of *P. ovale*. The reported *P. malariae* cases were clustered in the specific regions of Kapit and Miri, the former being an area rich in rain forest. When this cluster of reportedly *P. malariae* cases were investigated by Singh *et al* (2004), they found that nearly all the patients (105 of 108 cases) had severe symptoms, high parasite counts and unusual microscopic features. When nested PCR was performed on the blood of 208 inhabitants of the Kapit region with malaria from 2000 – 2002, 106 (51%) were *P. knowlesi* alone and 14 (7%) were mixed infections of *P. knowlesi* and another *Plasmodium* species (Singh *et al* 2004). The microscopy identification of these 106 single species infections had diagnosed 101 (97.1%) as *P. malariae*, three as *P. falciparum* and two as *P. vivax*.

Since this first study, the same group has identified the species present in 960 patients during 2001 and 2006 in Sarawak, extracted DNA from 54 archival blood films from patients from 15 districts in Sabah and four districts in Pahang that were diagnosed as *P. malariae*, and investigated four cases whose suspected cause of death was due to *P. knowlesi* malaria (Cox-Singh *et al* 2008). The incidence of *P. knowlesi* in the cohort of 960 patients from Sarawak hospitals was 27.7% (266/960). DNA from the archived blood films identified *P. knowlesi* infections in 83.7% (41/49) from Sabah patients and 100% (5/5) from Pahang. All of the four patients that died had *P. knowlesi* infection confirmed (Cox-Singh *et al* 2008).

There has been another study of *P. knowlesi* incidence in Myanmar by Jiang *et al* in 2010. They were examining the frequency of co-infections with other malaria parasites in the southern Myanmar region and found that there was an incidence of mono-infection with *P. knowlesi* of 2.7% (4/146) and mixed infections with either *P. falciparum* or *P. vivax* of 8.9% (26/146) (Jiang *et al* 2010).

There has been cases reported in the literature of *P. knowlesi* naturally acquired in peninsular Malaysia, Malaysian Borneo, the Philippines, Singapore and by travellers from Sweden, Finland, USA and Australia (Singh *et al* 2004; Jiang *et al* 2010; Figtree *et al* 2010; Ng *et al* 2008; Bronner *et al* 2009; Luchavez *et al* 2008; Kantele *et al* 2008; CDC 2009). The travellers had been in a variety of locations - Indonesian Borneo, peninsular Malaysia, Malaysian Borneo and on Palawan island in the Philippines and all visited rural or forest areas which were natural habitats of the macaques. The naturally acquired cases demonstrate that the *P. knowlesi* is indeed widespread in Southeast Asia (Fig. 1) and raises the probability that more cases will be confirmed as man encroaches into the forested areas. Van den Eede *et al* (2009) failed to discover any symptomatic cases of *P. knowlesi* in the human population in Vietnam even though the vectors are present.
Clinical features

The symptoms of patients infected with *P. knowlesi* are typically non-specific but fever and chills are present in nearly all cases. Other frequent symptoms include abdominal pain, a productive cough, breathlessness, anorexia and myalgia (Daneshvar et al 2010). In the retrospective study conducted by Daneshvar et al from 2006 to 2008, most of the 107 patients (93.5%) had uncomplicated malaria but seven (6.5%) of these had severe malaria at hospital admission (as defined by the WHO criteria). Another three patients developed severe malaria. Seven of these had respiratory distress, three developed acute renal failure, two had hypotension and one suffered from hypoglycaemia. Two patients died giving an overall mortality rate of 1.8% (Daneshvar et al 2010). Both of the deceased patients had hyperparasitemia with platelet counts >200,000/μL. One had multi-organ failure, lactic acidosis and hypoglycaemia on presentation and died six hours after admission. The other presented with signs and symptoms of right hemiparesis and a history of uncontrolled hypertension and died on day eight after neurological deterioration. In the article by Cox-Singh et al (2008) where four fatal cases are described, the clinical presentation of all the patients included fever, rigors, abdominal pain and diarrhoea with three out of four suffering also from vomiting or headache or both. In a recent study by William et al (2011), six patients who later died presented with fever, headache and diarrhoea. These ten cases died from acute renal failure, acute respiratory distress, and shock (Cox-Singh et al 2008; William et al 2011).

The symptoms in the travellers who acquired malaria in Southeast Asia were similar to those described above. The Swedish patient presented with a two day history of fever, sweats, headache and fatigue (Bronner et al 2009), the Australian tourist had symptoms of morning fevers and mild headaches which had started 13 days after leaving Indonesian Borneo (Figtree et al 2010), the Finnish traveller had fever and had experienced some mild abdominal symptoms which had resolved (Kantele et al 2008), and the US citizen had headaches, fever and chills (CDC 2009).

Laboratory investigations

The baseline laboratory investigations that were performed for the 107 patients in the Daneshvar et al (2010) study showed that thrombocytopenia (platelet count <150 x 10⁹/L) was present in 98% (104/107) of the patients, with 29% (31/107) having a count <50 x 10⁹/L. The three patients that had normal platelet counts had low parasitemias ranging from 5 - 170 parasites/μL. Anaemia (Hb < 100 g/L) and lymphopenia was found in only 4.6% (5/107) and 6.5% (7/107) of the patients respectively, with none of the patients fitting the criteria for severe anaemia. An elevated serum alanine aminotransferase (ALT) level and a low serum albumin level, indications of mild hepatic dysfunction, was relatively common and mild to moderate hyponatremia 122-135 mmol/L (RR 136-152) was found in 29% of cases (Daneshvar et al 2010). The four fatal cases reported by Cox-Singh et al (2008) all had severe thrombocytopenia with counts ≤ 25 x10⁹/L, increased ALT, decreased albumin levels and elevated bilirubin results.

The parasite counts for all the reported infected cases ranged from 5 - 764,720 parasites/μL blood (Singh et al 2004, Jiang et al 2010, Figtree et al 2010, Ng et al 2008; Bronner et al 2009; Luchavez et al 2008; Kantele et al 2008; Cox-Singh et al 2008; Daneshvar et al 2010).

The malarial parasites were first detected on thick and thin films in all reported cases but confirmed with PCR performed with specific primers for *P. knowlesi*. The rapid diagnostic tests (RDT) for malaria will usually give a positive result for the pan-malarial aldolase antigen and negative result for the *P. falciparum* histidine rich protein 2 except when there is a low parasitemia (Bronner et al 2009). The RDTs which utilise the *P. falciparum*-specific LDH and the pan-malarial LDH, may show cross reactivity and a positive result for both lines (McCutchan et al 2008).

Morphological features

There are other forms of simian malaria which also resemble the four previously identified human malarias. Examples of these are *P. coatneyi* resembling *P. falciparum*, *P. inui* and *P. brasilianum* resembling *P.
malariae. *P. fieldi* and *P. simiovale* resembling *P. ovale*, whilst *P. simium* and *P. eylesi* resembles *P. vivax* (CDC 2009). The morphological features of *P. knowlesi* have been likened to *P. falciparum* when there are early or young trophozoites present and *P. malariae* when more mature stages are evident (Singh et al 2004) as shown in Figure 2.

![Figure 2](http://www.cdc.gov/ncidod/EID/vol10no12/04-0293-G1.htm)

The early trophozoites are ring shaped and may have double chromatin dots and marginal (accolé forms). They are small to medium sized parasites and there can be multiple infections of the red cells (Fig. 3).

![Figure 3](http://www.cdc.gov/ncidod/EID/vol10no12/04-0293-G1.htm)

In late or mature trophozoites, band forms may appear that resemble *P. malariae* with the red chromatin increasing in size and the pigment as dark grains (Fig. 4 and 5).

![Figure 4](http://www.cdc.gov/ncidod/EID/vol10no12/04-0293-G1.htm)  
![Figure 5](http://www.cdc.gov/ncidod/EID/vol10no12/04-0293-G1.htm)

The schizonts have between eight and 16 merozoites and the dark brown/black pigment can collect into one or a few masses. The host red cell is not enlarged and the schizont nearly fills the whole cell (Fig. 6).

![Figure 6](http://www.cdc.gov/ncidod/EID/vol10no12/04-0293-G1.htm)

The gametocytes are usually spherical and fill the host red blood cells. The cytoplasm stains blue and the eccentric nucleus stains red. Pigment is coarse and black, and is scattered irregularly in the cytoplasm (Fig. 7 and 8).

![Figure 7](http://www.cdc.gov/ncidod/EID/vol10no12/04-0293-G1.htm)  
![Figure 8](http://www.cdc.gov/ncidod/EID/vol10no12/04-0293-G1.htm)

All authors agree that it is very difficult to morphologically distinguish *P. knowlesi* from *P. malariae*. The absence of pigment in the trophozoite stages, the distinctive shape of the merozoites and the coarse black irregularly scattered pigment in the gametocyte are not the usual features seen in *P. malariae*, and along with possible high parasite counts and the area where the infection was acquired, should alert the microscopist to consider *P. knowlesi* as the causative species.

**Treatment**

The standard antimalarial treatment rapidly clears the parasites from the blood after three days (Singh et al 2004). Except for two of the patients in this cohort chloroquine was given for three days followed by primaquine for either two to three days or two weeks. The two patients that had alternate treatment were both given quinine – this was chosen due to the species identified by microscopy, the severity of the symptoms, the age of the patient and the attending physician. William et al (2011) report that some of the patients admitted in a hospital in Sabah, Malaysia were successfully treated with artemether-lumefantrine.
and artesunate also. The artemether-lumefantrine was used for those patients with uncomplicated malaria and intravenous artesunate was given to those with severe malaria. The treatment for the travellers that contracted malaria varied; atovaquone/proguanil was used for the Australian, atovaquone/proguanil followed by primaquine for the US patient, mefloquine for the Swede and IV quinine and oral doxycycline for the Finn (Kantele et al 2008; CDC 2009; Bronner et al 2009; Figtree et al 2010). As there are no hypnozoites (liver stages) there is no evidence of relapse in patients that have been inadequately treated (Carlton et al 2008).

Conclusion

*Plasmodium knowlesi* was thought to be transmitted to man on rare occasions but is now known to be a major cause of infection in Southeast Asia, particularly in Malaysia, and both Malaysian and Indonesian Borneo. It is a chronic infection of the long- and pig-tailed Macaque monkeys in the region, but with the increased activity of humans in and around the forests and jungles, it has the potential to be a significant cause of morbidity and mortality. It had been misidentified sometimes as *P. falciparum* but mainly *P. malariae* because of the morphological similarities, but the course of the infection and the high parasite counts can help distinguish it from the other species. Molecular testing can confirm the positive identification and has lead to the realisation that this parasite is one of the three main species causing malaria in this region.

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ORIGINAL ARTICLE

The use of the automated parameter RDW-SD as a discriminator of both alpha and beta thalassaemia trait from iron deficiency

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Abstract

The use of the automated parameter red cell distribution width SD (RDW-SD) to assist in the discrimination of both alpha and beta thalassaemia trait from iron deficiency was evaluated in a large community laboratory in Brisbane, Australia. Differentiation between these common causes of red cell microcytosis is important clinically, as failure to identify a thalassaemia trait may lead to an unpredicted thalassaemia major which can affect the quality of life or, in the case of alpha thalassaemia, lead to death in utero. A total of 313 samples were divided into three groups - alpha thalassaemia (103), beta thalassaemia (102) and iron deficiency (108). The red cell parameters red cell count (RBC), mean corpuscular haemoglobin (MCH), red cell distribution width CV (RDW-CV) and RDW-SD, as well the discriminative formulas Mentzer Index, England and Fraser, Srivastava Index, Ricerca Index, Green and King Index and RDW Index, were evaluated. Receiver operating characteristic curves, sensitivity, specificity, area under the curve, Youden index and positive and negative predictive values were calculated. While all parameters and formulas managed to discriminate a varying proportion of cases of both alpha and beta thalassaemia trait from iron deficiency, the RDW-SD showed most promise as a simple, automated parameter that could be used as a sensitive and specific predictor of both alpha and beta thalassaemia trait because of the high sensitivity (97% alpha thalassaemia and 96% beta thalassaemia) and specificity (95% for both alpha and beta thalassaemia). Further evaluation of the parameter was also performed, incorporating reproducibility, stability studies and correlation to other analyser platforms, proving the basic laboratory performance of the RDW-SD.

Keywords: RDW-SD, alpha thalassaemia, beta thalassaemia, iron deficiency, microcytosis

Introduction

The thalassaemias are characterized by reduced synthesis of globin chains. Those with reduced alpha globin chains are termed alpha thalassaemias, and those with reduced beta globin chains are termed beta thalassaemias. Both thalassaemia trait and iron deficiency are characterised by red cell microcytosis, making it sometimes difficult to distinguish between the two. This differentiation is clinically important as failure to identify a thalassaemia trait may lead to an unpredicted thalassaemia major which can affect the quality of life of the individual or, in the case of alpha thalassaemia, lead to death in utero. The prevalence of thalassaemia is almost certainly the result of the protection they provided against malaria in the past. The old world malaria endemic regions - the Mediterranean, regions of Africa, the Middle East, the Indian sub-continent and South-East Asia - are areas of high frequency of thalassaemias and haemoglobinopathies (Clegg and Weatherall 1999; Kwiatkowski 2005). While Australia is not considered an endemic region, migration from endemic areas has meant this differentiation is becoming more important in non-endemic areas (Weatherall and Clegg 2001; Henderson et al 2009).

Beta thalassaemia trait is characterised by a raised HbA2 (Weatherall and Clegg 2001; Bain 2006; Mosca et al 2009). However there are exceptions, including silent beta thalassaemia and normal HbA2 beta thalassaemia (Tzetis et al 1994; Galanello et al 1994). In these cases the HbA2 levels are not elevated which complicates the diagnosis. Alpha thalassaemia trait, on the other hand, is not characterised by any diagnostic change in the levels of the common haemoglobins or by the presence of a measurable haemoglobin variant (Weatherall and Clegg 2001; Bain 2006). In the majority of laboratories the diagnosis is based on the presence of HbH bodies. This is commonly performed using a supra vital staining technique such as brilliant cresyl blue, however this is not a very sensitive test. Lafferty

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et al (2008) reported that HbH bodies were detected in only a minority of heterozygous and homozygous α⁺ deletions and in less than 100% of heterozygous α⁻ deletions. As a result, a significant number of alpha thalassaemia traits can go undiagnosed in the routine laboratory. Definitive diagnosis in these cases requires genetic testing which is complex, expensive and may not be readily available in some routine laboratories. Finding a simple automated predictor of both alpha and beta thalassaemia trait with high sensitivity and specificity would therefore be beneficial.

Numerous studies have evaluated the usefulness of automated parameters in assisting in the differentiation of microcytosis, including the early work of JD Bessman (Bessman and Feinstein 1979; Bessman et al 1983). Parameters commonly studied include red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and red cell distribution width CV (RDW-CV). Newer automated parameters have also been studied. The percentage of microcytic red cells to hypochromic red cells was evaluated on the H*1 analyser (Technicon Instrument Corp, Tarrytown, NY) by d’Onofrio et al (1992) and Robertson et al (1992), and more recently on the XE5000 analyser (Sysmex Corporation, Kobe, Japan) by Urrechaga et al (2011). Both studies showed that the M/H ratio on the H*1 and the M-H index on the XE5000 were useful discriminators. In addition to these automated parameters, a number of formulas incorporating at least two red cell parameters have been proposed to assist in differentiation, some dating back to the early 1970s (Mentzer 1973; England and Fraser 1973; Srivastava and Bevington 1973; Shine and Lal 1977; Ricerca et al 1987; Green and King 1989; Jayabose et al 1999).

The majority of the studies evaluating parameters and discriminative formulas have focused on differentiating only beta thalassaemia from iron deficiency. One exception was the review of nine discriminative formulas by AlFadhli et al (2006) which included both alpha and beta thalassaemia groups, and showed consistency between both groups in relation to their ability to be discriminated from iron deficiency. To date, few studies have incorporated the automated parameter red cell distribution width SD (RDW-SD). Lin et al (1992) compared the performance of the RDW-SD against RDW-CV and haemoglobin distribution width (HDW). Both alpha and beta thalassaemia were included in the study population which concluded that RDW-SD was a promising discriminator compared to the other parameters studies. A previous study by Lin et al (1991) had noted a low RDW-SD in patients during their evaluation of methods for alpha thalassaemia screening. Akai et al (1998) compared the RDW-SD to several parameters and well studied formulas using a beta thalassaemia group only and demonstrated that the RDW-SD was a sensitive and specific discriminator within the scope of their study.

The aim of this study was to evaluate significant numbers of both alpha and beta thalassaemia trait against automated parameters and discriminative formulas and determine whether the RDW-SD is a more sensitive and specific discriminator for both forms of thalassaemia from iron deficiency. It was also important to confirm the ability of the various discriminators previously studied to be valid in our geographical location, as the prevalence and genotype of thalassaemia will vary from that of the areas where previous studies were performed.

Materials and Methods

The samples evaluated were from adult patients (>14 years) presenting for full blood count, haemoglobin electrophoresis and iron studies over a twelve month period. The majority of patients were presenting for the investigation of microcytosis, for refugee screening or for family/partner studies. As the aim of the study was to evaluate microcytosis, samples with an MCV greater than 79fL were excluded. Samples that did not fit into the three study groups – iron deficiency, alpha thalassaemia trait or beta thalassaemia trait – were also excluded. This included several cases of beta thalassaemia major and HbH disease, as it was considered the blood film morphology of these cases was sufficient to allow discrimination. Haemoglobinopathies were also excluded, as were thalassaemic samples with concurrent iron deficiency.

The alpha thalassaemia group was defined by the presence of HbH bodies using a brilliant cresyl blue stain, or demonstration of a known alpha thalassaemia deletion by molecular techniques. The beta thalassaemia group was defined as having a HbA₂ >3.6% on a Biorad Variant II analyser (Biorad Laboratories, Hercules, California) in the setting of thalassaemic red cell indices. The iron deficient group was defined as having a ferritin <30ug/L and a transferrin saturation <20% performed on a Roche Modular System (Roche Diagnostics, Basel, Switzerland). A previously documented MCV >84fL was also considered a prerequisite for the iron deficient group to ensure undiagnosed thalassaemias were not included, as HbA₂ may be reduced in the presence of iron deficiency (Harthoorn-Lasthuizen et al 1999; El-Agouza 2002). The RBC, MCV, MCH, RDW-CV
and RDW-SD for all samples were determined using a Sysmex XE2100 analyser (Sysmex Corporation, Kobe, Japan).

The RDW-SD is determined on the Sysmex XE2100 from the red cell histogram. With the peak height of the histogram assumed to be 100%, the distribution width at the 20% level is the RDW-SD (expressed in fL). In comparison, the RDW-CV classically is determined by dividing one standard deviation of the red cell volume by the MCV (expressed as %). One important difference between these parameters is that the RDW-SD is measured from a lower position on the red cell histogram than the RDW-CV. Theoretically this makes it more sensitive to small populations of microcytic cells (Walters and Garrity 1993, Akai et al 1998, Hillman et al 2005).

The discriminative formulas Mentzer Index (MI), England and Fraser (E&F), Srivastava Index (SI), Ricerca Index (RI), Green and King (G&K) Index, RDW Index (RDWI) were determined for each sample (Table 1).

**Table 1. Discriminative Formulas**

<table>
<thead>
<tr>
<th>Discriminative Formula</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mentzer Index (MI)</td>
<td>MCV/RBC</td>
</tr>
<tr>
<td>England and Fraser (E&amp;F)</td>
<td>MCV-RBC-(5xHB)-3.4</td>
</tr>
<tr>
<td>Srivastava Index (SI)</td>
<td>MCH/RBC</td>
</tr>
<tr>
<td>Ricerca Index (RI)</td>
<td>RDW-CV/RBC</td>
</tr>
<tr>
<td>Green and King (G&amp;K) Index</td>
<td>MCVxRDW-CV/(Hbxs100)</td>
</tr>
<tr>
<td>RDW Index (RDWI)</td>
<td>MCVxRDW-CV/RBC</td>
</tr>
</tbody>
</table>

Statistical Formulas *

<table>
<thead>
<tr>
<th>Statistical Formula</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>TP / (TP + FN)</td>
</tr>
<tr>
<td>Specificity</td>
<td>TN / (TN + FP)</td>
</tr>
<tr>
<td>Youden Index</td>
<td>SENS + SPEC−100</td>
</tr>
<tr>
<td>PPV</td>
<td>TP / (TP + FP)</td>
</tr>
<tr>
<td>NPV</td>
<td>TN / (TN + FN)</td>
</tr>
</tbody>
</table>

*TP, true positive; TN, true negative; FP, false positive; FN, false negative; SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value.

The alpha and beta thalassaemia groups were compared to the iron deficiency group using statistical software (Analyse-it Software Ltd, Leeds, United Kingdom). Receiver operating characteristic (ROC) curves were used to assess cut-offs, determine sensitivity and specificity and to aid in determination of the best discriminator.

Comparison studies, reproducibility studies and stability studies were also performed to ensure the performance characteristics of the RDW-SD parameter. Comparison studies involved correlation between RDW-SD results obtained on the XE2100 and results obtained on both the DxH 800 (Beckman Coulter, Miami, USA) and the pocH-100i (Sysmex Corporation, Kobe, Japan), allowing for comparison with both another high-volume analyser from a separate manufacturer and a smaller point-of-care style analyser. Reproducibility was established by processing specimens ten times consecutively. The stability of the RDW-SD parameter was assessed in both short term and long term stability studies, with specimens stored at both room temperature and 4°C run hourly over several hours as well as daily over a four day period.

**Results**

ROC curves comparing the iron deficiency group to both the alpha and beta thalassaemia groups for the parameters RBC, MCH, RDW-CV and RDW-SD, and for the discriminative formulas MI, E&F, SI, RI, G&K and RDWI were assessed and the best cut-off for each parameter and formula was determined. Where cut-offs have previously been suggested, these were assessed, and new alternative cut-offs used in accordance with our data. Sensitivity, specificity, area under the curve, Youden's Index and positive and negative predictive values (Table 2) were determined. Due to the automation available during the period of this study, newer automated parameters such as the M-H ratio/M-H index were not able to be included.

**Table 2. Statistical Formulas**

General performance studies showed the RDW-SD to be a reliable parameter under appropriate conditions. Comparison studies showed good correlation between the XE2100 analyser and the DxH 800 (Fig. 1) and pocH-100i (Fig. 2) analysers, indicating that results of this study would be evaluable.

Evaluation of the data (Table 3) confirmed the RDW-SD as the discriminator with the best specificity and sensitivity when comparing the alpha and the beta thalassaemia trait groups individually with the iron deficiency group. Using the cut-off proposed by the ROC curves of 42 fL for RDW-SD, the alpha thalassaemia group showed a sensitivity of 97% and specificity of 96%, while the beta thalassaemia group showed a sensitivity of 96% and specificity of 95%.

**Table 3. Statistical Formulas**

<table>
<thead>
<tr>
<th>Statistical Formula</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>TP / (TP + FN)</td>
</tr>
<tr>
<td>Specificity</td>
<td>TN / (TN + FP)</td>
</tr>
<tr>
<td>Youden Index</td>
<td>SENS + SPEC−100</td>
</tr>
<tr>
<td>PPV</td>
<td>TP / (TP + FP)</td>
</tr>
<tr>
<td>NPV</td>
<td>TN / (TN + FN)</td>
</tr>
</tbody>
</table>

*TP, true positive; TN, true negative; FP, false positive; FN, false negative; SENS, sensitivity; SPEC, specificity; PPV, positive predictive value; NPV, negative predictive value.
transferable across all platforms. Cut-offs would need to be confirmed on each platform.

**Table 3. Sensitivity, Specificity, Area Under the Curve (ROC), Youden’s Index, Positive and Negative Predictive Values of Various Parameters and Formulas for Alpha and Beta Thalassaemia Groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alpha Thalassaemia</th>
<th>Beta Thalassaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proposed Sensitivity</td>
<td>94</td>
<td>85</td>
</tr>
<tr>
<td>Specificity</td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td>AUC</td>
<td>0.93</td>
<td>0.90</td>
</tr>
<tr>
<td>Youden’s Index</td>
<td>75</td>
<td>76</td>
</tr>
<tr>
<td>PPV</td>
<td>77</td>
<td>86</td>
</tr>
<tr>
<td>NPV</td>
<td>95</td>
<td>84</td>
</tr>
<tr>
<td>MCH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alpha-thal</td>
<td>&gt;5.0</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>beta-thal</td>
<td>&gt;5.0</td>
<td>&gt;5.0</td>
</tr>
<tr>
<td>Proposed Sensitivity</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>Specificity</td>
<td>81</td>
<td>81</td>
</tr>
<tr>
<td>AUC</td>
<td>0.90</td>
<td>0.90</td>
</tr>
<tr>
<td>Youden’s Index</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>PPV</td>
<td>66</td>
<td>82</td>
</tr>
<tr>
<td>NPV</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td>RDW-CV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alpha-thal</td>
<td>&lt;17</td>
<td>&lt;17</td>
</tr>
<tr>
<td>beta-thal</td>
<td>&lt;17</td>
<td>&lt;17</td>
</tr>
<tr>
<td>Proposed Sensitivity</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>Specificity</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>AUC</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Youden’s Index</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>PPV</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>NPV</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>RDW-SD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alpha-thal</td>
<td>&lt;42</td>
<td>&lt;42</td>
</tr>
<tr>
<td>beta-thal</td>
<td>&lt;42</td>
<td>&lt;42</td>
</tr>
<tr>
<td>Proposed Sensitivity</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>Specificity</td>
<td>95</td>
<td>95</td>
</tr>
<tr>
<td>AUC</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Youden’s Index</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>PPV</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>NPV</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>MI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alpha-thal</td>
<td>&lt;14</td>
<td>&lt;14</td>
</tr>
<tr>
<td>beta-thal</td>
<td>&lt;14</td>
<td>&lt;14</td>
</tr>
<tr>
<td>Proposed Sensitivity</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td>Specificity</td>
<td>84</td>
<td>84</td>
</tr>
<tr>
<td>AUC</td>
<td>0.84</td>
<td>0.84</td>
</tr>
<tr>
<td>Youden’s Index</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>PPV</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>NPV</td>
<td>88</td>
<td>88</td>
</tr>
</tbody>
</table>
| RBC, red blood cells (10^12/L); MCH, mean corpuscular haemoglobin (pg); RDW-CV, red cell distribution width CV (%); RDW-SD, red cell distribution width SD (fL); MI, Mentzer Index; E&F, England and Fraser; SI, Srivastave Index; RI, Ricerca Index; G&K, Green and King Index; RDWI, red cell distribution width Index; alpha-thal, alpha thalassaemia; beta-thal, beta thalassaemia; AUC, area under the curve (ROC); PPV, positive predictive value; NPV, negative predictive value.

Results for reproducibility studies are shown in Table 4. The RDW-SD showed good reproducibility, confirming the manufacturer’s specifications (less than 2%).

**Table 4. Within-Run Precision**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>RDW-SD (fL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>44.4</td>
</tr>
<tr>
<td>SD</td>
<td>0.44</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.01</td>
</tr>
<tr>
<td>Specification</td>
<td>&lt;2.0%</td>
</tr>
</tbody>
</table>

* RDW-SD, red cell distribution width SD.

Short term stability studies (Fig. 3) showed there was no significant variation in RDW-SD results over a six hour period at both room temperature and 4°C (<1% variation on average from the baseline result over this period). Long term stability studies (Fig. 4) showed no significant variation for specimens stored at 4°C over a 48 hour period. However, after 72 hours, there was on average a 2.5% reduction in results, and this continued to show further reduction at 96 hours. In comparison, results at room temperature showed on average a 10% increase in the RDW-SD after only 24 hours, and this continued to increase over the evaluation period.
Discussion

A suitable discriminator of thalassaemia from iron deficiency needs to detect maximum numbers of thalassaemias to provide the highest sensitivity, while not including non-thalassaemic samples to ensure specificity. This study confirmed the RDW-SD as such a parameter, providing the best specificity and sensitivity when comparing the alpha and the beta thalassaemia trait groups individually with the iron deficiency group. Being an automated parameter, its day to day performance can be monitored with the commercial control material provided by the manufacturer which is an advantage over discriminative formulas.

No other automated parameter showed both a sensitivity and specificity greater than 90%. The RBC appeared to be the next best performing automated parameter. It was difficult to make a direct comparison of automated parameters between this study and other studies, as the exact parameters evaluated varied between studies and none included RDW-SD, but Demir et al (2002), AlFadhli et al (2006) and Ntaios et al (2007) all proposed the RBC as the best automated parameter confirming our findings. However Rathod et al (2007) proposed that MCV and MCH performed better than the RBC.

While the discriminative formulas generally performed better than the other automated parameters, they did not outperform the RDW-SD. This study identified the RDWI and G&K Index as the next best discriminators after the RDW-SD. Again there were no consistent findings throughout previous studies, and direct comparison was also difficult due to variations in formulas studied, however our findings mirrored those of Sirdah et al (2008) who also identified RDWI and G&K as the best performing discriminative formulas. Other studies have also confirmed the performance of the RDWI or G&K, including Demir et al (2002), Ntaios et al (2007), Shen et al (2010), and Urrechaga et al (2011). However AlFadhli et al (2006) and Rathod et al (2007) did not consider RDWI or the G&K Index as one of the best discriminators.

This highlights that studies assessing the performance of discriminators of thalassaemia have been unable to produce consistent conclusions with different parameters and formulas proposed as the best discriminators. Conceivably, performance would be influenced by factors such as differences in analyser technology, variations in the data type included in evaluations and the different geographical locations from which the data was obtained. This emphasises the importance of individual laboratories evaluating the performance of any discriminators that they are considering incorporating into their work processes.

This study was also able to demonstrate that the performance of discriminative formulas that incorporate RDW-CV could be improved by replacing the RDW-CV in their formulas with RDW-SD. Using the alpha thalassaemia group, these modified formulas were compared to the iron deficiency group and further ROC curves generated. Using a cut-off of <190, the sensitivity for a modified G&K Index improved from 91% to 94%, and the specificity from 90% to 93%. Using a cut-off of <7.9, the sensitivity for a modified Ricerca Index remained 97%, but the specificity improved from 80% to 95%. Using a cut-off of <575, the sensitivity for a modified RDWI improved from 96% to 99%, and the specificity from 92% to 94%, making it a slightly better discriminator than the RDW-SD alone.
One subgroup of interest was patients who were both iron deficient and had either alpha or beta thalassaemia. While these samples were excluded from the initial data, they pose an important diagnostic challenge. Patients with beta thalassaemia and coexisting iron deficiency can show a reduction in HbA2 levels. As this elevation is the diagnostic criteria used by routine laboratories for beta thalassaemia trait, a reduction in the HbA2 level can lead to misdiagnosis. The reduction in HbA2 is the result of intracellular lack of iron reducing alpha globin chain synthesis relative to that of non-alpha globin chains. When the supply of alpha globin chains is limited, beta globin chains compete more effectively for alpha globin chains than delta globin chains, resulting in reduced levels of HbA2 (Harthoorn-Lasthuizen et al 1999; El-Agouza 2002). Fifteen patients were identified that fitted into this subgroup. While this is not a statistically large group, thirteen of the fifteen samples were able to be differentiated from the iron deficient group using an RDW-SD cut-off of 42fL, with only two samples consistent with the iron deficient group. This is not an unexpected finding, as thalassaemic patients already have a reduced MCV. When they develop iron deficiency, there will be a lesser distribution of red cell size than would be expected in a normal individual. These non-thalassaemic individuals would, at least initially, have a mix of red cells with a normal MCV, as well as microcytic cells. Closer review of the two thalassaemic samples with an RDW-SD >42fL revealed both of these samples had an RDW-CV of >20%. This significant difference in the RDW-CV from the remaining thirteen patients could possibly be the result of treatment for iron deficiency, and the emergence of some polychromatic cells. It may be that an RDW-CV >20% will need to be considered (and possibly excluded) when establishing discriminative cut-offs for RDW-SD. While further data is required to confirm this finding, it appears possible that the RDW-SD could differentiate alpha and beta thalassaemia with concurrent iron deficiency from the purely iron deficient group in a significant number of samples.

While alpha and beta thalassaemia are by far the most common thalassaemias, there are other forms that can be encountered including delta beta thalassaemia. The distinction between delta beta thalassaemia trait and another disorder also affecting the delta and beta globin genes, hereditary persistence of fetal haemoglobin (HPFH) is subtle and where molecular testing is not available, is made primarily on haematological findings. Heterozygotes for delta beta thalassaemia tend to have only a modest elevation of Hb F (5–20%), while HPFH heterozygotes can be characterized by higher levels of Hb F of up to 30%. Further distinction requires review of haematological parameters, with the red cell indices and blood film in delta beta thalassaemia trait consistent with those of beta thalassaemia trait. Heterozygotes for HPFH are generally not anaemic with normal or mildly microcytic red cell indices (Bain 2006). The diagnosis is generally straightforward when characteristic findings are present. However there is some overlap between the haematological parameters in these conditions, and there are certain situations in which the diagnosis is more difficult. For example, patients with HPFH who are iron deficient will likely have haematological parameters that mimic delta beta thalassaemia trait. Old (2003) states that it is important to differentiate delta beta thalassaemia from HPFH for genetic counselling because compound heterozygotes for HPFH and beta thalassaemia can have a milder phenotype compared to delta beta thalassaemia and beta thalassaemia. While no cases where identified during this study, it may be that the RDW-SD could form part of the haematological assessment of such cases to assist in the differentiation of heterozygous delta beta thalassaemia from HPFH with concurrent iron deficiency.

Evaluation of the performance characteristics of the RDW-SD identified inappropriate storage as a potential source of error when using it as a discriminator of thalassaemia. If specimens are not stored appropriately at 4°C prior to processing, stability studies indicated that there is significant elevation of the RDW-SD over 24 hours. After 48 hours, specimens stored appropriately at 4°C consistently demonstrated a slight reduction in results. These variations in results could result in patients that would otherwise been discriminated as thalassaemia being misclassified as iron deficiency, and vice versa. Incorporation of this parameter into laboratory processes would therefore require the implementation of stability guidelines for laboratory staff detailing when the RDW-SD should be reported.

Conclusion

This study evaluated the performance of the parameter RDW-SD against both automated parameters and formulas in discriminating both alpha and beta thalassaemia trait from iron deficiency. In our geographic location, it was important to expand on previous studies and include a significant alpha thalassaemia trait group, as a significant proportion of the thalassaemias diagnosed in our geographic location are alpha thalassaemia. While no parameter provided
an absolute predictor for thalassaemia trait, this study confirmed that in cases of microcytosis, the parameter RDW-SD served as a very good discriminator of both alpha and beta thalassaemia trait, especially when compared to other previously documented parameters and discriminative formulas. The identification of RBC as the next best automated predictor, and RDWI and G&K as the best predictive formulas confirmed the findings of a number of other studies and validated our data.

This study also demonstrated an improvement in discriminative formulas by replacing RDW-CV with RDW-SD. However none of these modified formulas significantly out performed the RDW-SD parameter (the modified RDW1 provided slightly better specificity), and therefore this study concludes the use of formulas may not be required when compared to a simple automated parameter available as part of a routine full blood count on most modern haematology analysers.

In cases of microcytosis, the assessment of the RDW-SD could therefore play an important role in full blood count reporting. By determining in-house limits for the RDW-SD, full blood count commenting could suggest to the clinician the need for thalassaemia testing in one group and in another group iron studies, with follow up thalassaemia testing only where microcytosis persists after the patient is iron replete, there will conceivably be less cases of concurrent iron deficiency that may mask beta thalassaemia. In conclusion, this study found the RDW-SD to be a suitable predictor of thalassaemia. It should be noted that no single predictor provides 100% sensitivity and specificity, and therefore can not be relied on as a stand-alone screening test for thalassaemia. The role of these predictors appear to lie in assisting laboratory staff in areas such as blood film commenting and suggestion of follow up testing, and may be possible predictors of thalassaemia PCR testing in cases where basic thalassaemia screening tests are not diagnostic.

The work in this article was originally submitted as an AIMS Fellowship Dissertation

References


The reports provide participants’ own results along with graphical representation of the results of their peers allowing for easy comparison and analysis by supervising staff.

Absolute confidentiality of results is assured.

FOR ENROLMENT ENQUIRIES CONTACT
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Severe anaemia in a 2-month-old child

Gillian Rozenberg
South Eastern Sydney and Illawarra Area Health Services, Prince of Wales Hospital, Sydney, New South Wales

A 2-month-old term child was noted to have become jaundiced over a two day period. The child was brought to the Children's Casualty Department. A full blood count and reticulocyte count were performed. The results were as follows:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>42 g/L</td>
<td>RR = 102-130 g/L</td>
</tr>
<tr>
<td>Hct</td>
<td>0.128</td>
<td>RR = 0.30-0.38</td>
</tr>
<tr>
<td>MCV</td>
<td>77.6 fL</td>
<td>RR = 84-98 fL</td>
</tr>
<tr>
<td>MCH</td>
<td>25.5 pg</td>
<td>RR = 29.0-33.8 pg</td>
</tr>
<tr>
<td>Retic count</td>
<td>8.7 %</td>
<td>RR = 0.0-1.0 %</td>
</tr>
<tr>
<td>Retic absolute</td>
<td>150.2 x 10⁹/L</td>
<td>RR = 20-80 x 10⁹/L</td>
</tr>
<tr>
<td>LDH</td>
<td>426 IU/L</td>
<td>RR = &lt;250 IU/L</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>104 μmol/L</td>
<td>RR = 0-15 μmol/L</td>
</tr>
</tbody>
</table>

The child was noted to have a profound anaemia. The red cells were microcytic and hypochromic. The reticulocyte count was significantly raised at 8.7 %. The blood film showed the presence of spherocytes with 4 NRBC / 100 WBC.

A Direct Antiglobulin Test (DAT) was performed with a negative result.

A Hb EPG and parvovirus B19 titre were suggested.

The Hb EPG results were as follows:

- Hb EPG Cellulose Acetate (pH 8.6) An abnormal band that did not separate from HbA was detected.
- Hb EPG Agar Gel (pH 6.0) An abnormal band that did not separate from HbA was detected
- Hb A2: 2.0 % RR = 2.0-3.5 %
- Hb F: 11.5 % RR = 29.4-60.8 %
- Hb H inclusions: not detected
- Hb EPG result: Abnormal Hb variant detected
- Hb variant confirmed by HPLC
- Hb variant 18.4 % at retention time of 1.51 sec.

Parvovirus B19 results were as follows:
- Parvovirus B19 IgG Antibody: Negative
- Parvovirus B19 IgM Antibody: Positive
- Comment: Consistent with acute Parvovirus B19 infection
The next day a full blood count and Hb EPG were performed on the parents of the child. The results were as follows:

**Father:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>159 g/L</td>
<td>130-180 g/L</td>
</tr>
<tr>
<td>Hct</td>
<td>0.466</td>
<td>0.40-0.54</td>
</tr>
<tr>
<td>MCV</td>
<td>87.9 fL</td>
<td>80-100 fL</td>
</tr>
<tr>
<td>MCH</td>
<td>30.0 pg</td>
<td>26.5-33.0 pg</td>
</tr>
</tbody>
</table>

The blood film was normal.

Figure 3: Father’s blood film showing normal red morphology

Figure 4: HPLC result on father

The Hb EPG results were as follows:

- Hb EPG Cellulose Acetate (pH 8.6): An abnormal band that did not separate from HbA was detected.
- Hb EPG Agar Gel (pH 6.0): An abnormal band that did not separate from HbA was detected.
- Hb A2: 2.4 %, RR = 2.0-3.5 %
- Hb F: <1 %, RR = <1 %

Hb EPG Result: Abnormal Hb variant detected
Hb variant confirmed by HPLC
Hb variant 23.3 % at retention time of 1.51 sec.

**Mother:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>131 g/L</td>
<td>115-165 g/L</td>
</tr>
<tr>
<td>Hct</td>
<td>0.374</td>
<td>0.37-0.47</td>
</tr>
<tr>
<td>MCV</td>
<td>89.5 fL</td>
<td>80-100 fL</td>
</tr>
<tr>
<td>MCH</td>
<td>31.3 pg</td>
<td>26.5-33.0 pg</td>
</tr>
</tbody>
</table>

The blood film showed features of a splenectomy, namely acanthocytes and Howell Jolly bodies. The presence of spherocytes was also noted.

Figure 5: Mother’s blood film showing acanthocytes, occasional spherocyte and Howell Jolly body

The Hb EPG performed on the mother was normal.
In view of the mother’s abnormal blood film, a red cell membrane screening assay was performed on the child’s red cells using the eosin-5´-maleimide dye flow cytometric method. (King et al. 2000). The result was as follows:

EMA Ratio = 0.69 \quad RR >0.80

The child was diagnosed with hereditary spherocytosis.

The Hb EPG performed on the child demonstrated the same Hb variant that was detected in the father. In view of the fact that the father had a haemoglobin of 159 g/L and an MCV of 87.9 fl, and the mother had a normal Hb EPG, the consultant caring for the child chose not to further investigate as to the nature of the variant Hb as it was not considered to be the cause of the profound anaemia.

The EMA flow cytometry test is an excellent screening assay for a range of red cell membrane abnormalities including hereditary spherocytosis. In many laboratories it has replaced the osmotic fragility test as the front-line screening assay.

The parents were interviewed by the treating consultant when they brought the child back for a follow-up visit one week after diagnosis. The mother was questioned as to the reason for her splenectomy. She replied that her spleen had been removed when she was seven years old for a blood dyscrasia. The mother’s blood film confirmed that she did indeed have hereditary spherocytosis.

The parvovirus B19 results were consistent with acute infection. Parvovirus B19 infects the proerythroblasts in the bone marrow, leading to a transient erythroblastopenia, especially in patients with a chronic haemolytic anaemia such as hereditary spherocytosis. Parvovirus B19 infection can induce a haemolytic anaemia with a haemoglobin often as low as 50 g/L as well as a reticulocytosis. Recovery is usually spontaneous however in cases of severe anaemia, blood transfusion may be required.

The child was transfused and continues to do well.

The lesson from this case is the importance of investigating the parents of a child with a severe red cell dyscrasia.

Reference

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Validation of calculated ionised calcium for intensive care patients

SDC Thomas, BD Rumbelow, GH White
Chemical Pathology, S.A Pathology, South Australia

Abstract

A previously published formula for calculating ionised calcium (iCa) is currently used by our institution. This study assessed the validity of this formula for critically ill patients. The plasma iCa in 365 samples from 108 critically ill adults were calculated using a published formula, and compared with iCa measured using an ion selective electrode. The calculated and measured iCa ranges were 0.86-1.77 mmol/L and 0.87-1.72 mmol/L respectively. Bias between the methods was 0.004 mmol/L (Bland Altman). Linear regression demonstrated a high correlation \( r^2 = 0.89 \); calculated \( \text{iCa} = (0.902 \times \text{measured } \text{iCa}) + 0.1299 \). For patients with albumin <20 g/L bias was 0.005 mmol/L \( r^2 = 0.95 \); calculated \( \text{iCa} = (1.0792 \times \text{measured } \text{iCa}) + 0.091 \). Differences at medical decision points were substantially greater than the desirable bias quoted in quality specifications. This study found that a significant bias exists between calculated and measured iCa in critically ill patients at the medical decision limits. This highlights the importance of measuring rather than calculating iCa in this group.

Keywords: ionised calcium, calculated ionised calcium, measured calcium

Introduction

Calcium is present in three physicochemical states in the circulation. Approximately 50% of plasma calcium occurs as divalent cations, 40% is bound to plasma proteins (pH dependent binding to albumin, globulin etc) and about 10% is complexed to anions such as bicarbonate, lactate, phosphate and citrate (Bushinsky 1998). The ionized fraction of calcium (iCa) is the physiologically active form (McLean and Hastings 1934). The availability of ion selective electrodes have made the measurement of ionised calcium routine with whole blood electrolytes and blood gases in the acute clinical setting. It is recommended that iCa should be used in determining the calcium status (Slomp et al 2003) because total calcium measurements can fluctuate according to the total plasma protein concentration (Payne et al 1979) while iCa fraction is tightly regulated by homeostatic mechanisms (White et al 1986).

Most routine biochemical analysers measure total calcium in serum or plasma, but sample and analytical requirements generally preclude iCa being included in this automated analysis. An empirical formula for iCa was proposed using routinely measured variables, ie albumin, globulin, bicarbonate, anion gap and total calcium (Nordin et al 1989), and calculated iCa may be reported as part of the routine biochemistry. This empirical formula was based on values obtained from over 500 postmenopausal ambulatory women and validated against values obtained from healthy adult men and premenopausal women and ambulatory patients with disorders of calcium metabolism. This formula is used in our institution for calculation of iCa routinely. This study assessed the validity of the formula for critically ill patients.

Methods

The cohort of patients was from a tertiary referral hospital adult critical care unit. Plasma biochemical variables were measured using a Roche modular automated analyser (Roche Diagnostics, USA) according to the manufacturer’s instructions. Ionised calcium concentrations in whole blood were measured using a Radiometer ABL735 blood gas analyser (Radiometer Pacific Pty Ltd, Melbourne). Pairs of plasma and whole blood, collected with a maximum time delay of 40 mins of each other, from each patient were used for statistical analysis. Ionised calcium concentrations were calculated using an iterative formula (Nordin et al 1999) where \([\text{Ca}]\) is the calculated iCa activity:

\[
[\text{Ca}] = T\text{Ca} – \{0.01257[\text{Ca}][\text{albumin}] / [1 + 0.01257[\text{Ca}]] – \{0.0049[\text{Ca}][\text{globulin}] / [1 + 0.0049[\text{Ca}]] – \{0.00835[\text{Ca}][\text{anion gap}] / [1 + 0.00835[\text{Ca}]] – \{0.00759[\text{Ca}][\text{bicarbonate}] / [1 + 0.00759[\text{Ca}]]
\]

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The calculated iCa, was compared with the corresponding measured iCa in whole blood. The two sets were compared using Bland Altman plots and least square regression analysis. The bias between the methods at the clinical decision levels (the lower and upper limit of the reference interval used by our institution) was assessed using Passing and Bablock analysis.

Results

Within a two month period, 365 pairs of plasma and serum samples collected within 40 mins from each other were received for routine biochemistry and blood gas analysis from 108 patients (63 males and 45 females). Patient demographics, plasma albumin and bicarbonate concentrations are given in Table 1.

Table 1. Mean, SD and range of patient age, albumin and bicarbonate of the analysed specimens

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (all patients, n = 108)</td>
<td>60</td>
<td>1.9</td>
<td>18 – 92</td>
</tr>
<tr>
<td>Males (n = 63)</td>
<td>60</td>
<td>2.4</td>
<td>18-87</td>
</tr>
<tr>
<td>Females (n = 45)</td>
<td>60</td>
<td>3.1</td>
<td>20-92</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>28.3</td>
<td>6.3</td>
<td>12-48</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>23.6</td>
<td>4.5</td>
<td>8 - 38</td>
</tr>
</tbody>
</table>

Regression analysis of calculated and measured iCa revealed $r^2 = 0.8959$ (calculated iCa = 0.9928 x measured iCa + 0.005) for all data points (Fig. 1). Bland Altman analysis demonstrated good agreement with no significant difference between the methods ($P = 0.77$) across the range of values (0.87 to 1.72 mmol/L; bias = 0.004) (Fig. 2). For samples with albumin <20 g/L, $r^2 = 0.95$ (calculated iCa = 1.0792 x measured iCa + 0.09). Figures 3 and 4 represent the data for specimens with albumin <20g/L and >20g/L respectively. Figures 5 and 6 are Bland Altman plots for specimens with albumin <20g/L and >20g/L respectively. A Passing and Bablock analysis showed that at the clinical decision levels of 1.17 and 1.31 mmol/L (the lower and upper limits of the reference interval for iCa in our institution), the bias was -0.019 (1.62%) mmol/L and -0.012 (0.92%) mmol/L respectively.
Discussion

To our knowledge, the empirical formula for the calculated iCa has not been validated for intensive care patients, in whom significant disturbances in plasma total calcium, albumin, bicarbonate and phosphate are relatively common. Clinical decisions are often made based on calculated iCa thus its validation in this group of patients is important. In addition, the calculated value has a higher uncertainty of measurement due to the cumulative uncertainty of measurements of the measured variables in the formula, compared to the measured iCa value.

Although the study demonstrates a good agreement between the measured and calculated iCa in ICU patients, the bias between the methods at the clinical decision limits (1.17 and 1.31 mmol/L) were substantially higher than the desirable bias quoted for quality specifications in iCa taking biological variation into account. At both concentrations, the bias between the methods was greater than the desirable bias for iCa of 0.6% based on inter-individual and intra-individual variation (Ricos et al 1999).

Assessment of plasma calcium homeostasis by direct measurement of iCa is often clinically important in patients with abnormal concentrations of analytes that can affect the equilibrium between iCa and bound calcium. In such patients ready access to direct iCa measurement is required in order to produce diagnostically reliable iCa measurements. This study demonstrated unacceptable biases between measured and calculated iCa in critically ill hospitalised patients.

Acknowledgements

Dr Penelope Coates for critical reading of the manuscript.

References


The first editor of the journal, then known as “The Laboratory Journal of Australasia” was a scientist from the School of Public Health and Tropical Medicine at the University of Sydney, Arthur Joseph Bearup.

Arthur Bearup was editor of the journal from the first issue in July 1936. He lived and worked in an era prior to diagnostic pathology as we know it today. His main interests were in parasitology and there is a good record of his visit to the highlands of New Guinea near Mt Hagen with Dr George Heydon in 1934 (National Library of Australia, Accessed May 24th 2010). The Canberra Times reported the trip on page 4 of the newspaper on 8th November 1934 (Fig. 1). The objectives of the field trip were to investigate the extent of infection with protozoan and helminth parasites and to determine, by use of skin tests, the degree of exposure of the population to bacterial infections such as tuberculosis, diphtheria and scarlet fever. Europeans had first entered the Ramu Valley at Kainantu only two years before and the Wahgi Valley at Mount Hagen in 1933.

Bearup obviously enjoyed his time in New Guinea and was known to interact with the indigenous population and a favourite trick was to remove his dentures (Fig. 4). From 1940 to 1945 Bearup and his colleagues investigated the prevalence of parasitic infection in Australian Army recruits.

Other field trips were closer to home along the estuaries and coastal lagoons of New South Wales. Bearup studied the life cycle of *Austrobilharzia terrigalensis* Johnston 1917. His original observations from the seagull *Larus Novaehollandiae*, led to the observation that the cercariae will cause dermatitis, and that cases of dermatitis resembling cercarial dermatitis were reported after bathing in Narrabeen Lagoon, near Sydney (Bearup et al 1949). His work on *Austrobilharzia terrigalensis* was continued by John Walker, who later became well known to AIMS members for his talks on malarial parasites. Bearup published extensively and this includes a review of the parasitic infections in New South Wales for the “Medical Journal of Australia (Bearup 1956)”.

Arthur Bearup was a great Australian parasitologist and needs to be recognised as the founding editor of this journal 75 years ago.

**References**


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Figure 2. AJ Bearup with a group of boys from Moge area, Mt Hagen, New Guinea in 1934. Courtesy of National Library of Australia.

Figure 3. AJ Bearup with a group near Mt Hagen collecting Anopheles mosquito larvae. Courtesy of National Library of Australia.

Figure 4. AJ Bearup removing his dentures to the amazement of the indigenous population. Courtesy of National Library of Australia.
Cytopathology of the Glandular Lesions of the Female Genital Tract
Jiménez-Ayala, M.
Karger
Hard Cover XVI + 110 Pages
ISBN: 978-3-8055-9464-6
RRP: US$168.00

Cytopathology of the Glandular Lesions of the Female Genital Tract is the 20th volume in the continuing Karger series of Monographs in Clinical Cytology (series editor Svante R. Orell). The lead authors are Matias Jiménez-Ayala and Beatriz Jiménez-Ayala Portillo of the Instituto Jiménez-Ayala, Madrid with contributions from other internationally recognised cytopathologists.

Glandular lesions have been of increasing prominence in recent years and the authors’ aim is to provide a suitable reference work for cytopathologists and cytotechnologists that covers the cytopathology of benign and malignant glandular lesions of the female genital tract.

This reference book comprises twelve chapters and begins with a comprehensive contents section, with each chapter listing divided into headings and subheadings. There is a reference list provided at the end of each chapter. The text is well-illustrated with numerous papanicolaou stained images together with haematoxylin and eosin histopathologic images where relevant. The images are generally of good quality. There could be more consistency in the descriptions of images, many do not state the type of stain, magnification or origin of the sample, whether it be a liquid based preparation or conventional smear, which would be of value when reviewing morphologic criteria. There are also many summary tables that aid in the understanding of the text and provide summaries of the descriptive criteria for the various glandular lesions.

The text is comprehensive beginning with chapters on cytopathologic techniques and the Bethesda Classification System of glandular lesions. Subsequent chapters follow covering both benign and malignant glandular lesions of the cervix and vagina. Endometrial hyperplasias are then discussed, together with the current status, prevention and early diagnosis of endometrial adenocarcinoma. The intraoperative approach to the cytology of ovarian lesions is well covered. The final chapters move through glandular lesions of the vulva, fallopian tube and then metastatic lesions to the female genital tract. The text concludes with a brief chapter discussing the value of ancillary techniques in the diagnosis of the previously described glandular lesions.

The comprehensive reference list provided at the end of each chapter is valuable for further reading, if required. The authors have succeeded in providing a useful and detailed text on the cytopathology of glandular lesions of the female genital tract that would be particularly useful as a reference in gynaecological cytology laboratories particularly for cytotechnologists and cytopathology registrars.

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A Balanced Omega-6/Omega-3 Fatty Acid Ratio, Cholesterol and Coronary Heart Disease
World Review of Nutrition and Dietetics
Volume 100
Simopoulos AP, DeMeester F.
Karger
Hard Cover XVI + 126 p
ISBN: 978-3-8055-9224-6
RRP: US$156.00

Are you confused about which type of fat is best or how low your cholesterol level should be in order to maximize health and minimize the risk of heart disease? If you answered ‘no’ to this question, you are probably lying.

There is no doubt that the main message we are hearing these days from all directions is that lowering cholesterol is good, and will protect you from an early death from ‘high cholesterol induced’ heart disease. The message frequently comes from prime time television - where various ‘natural’ cholesterol lowering agents including vegetable based spreads, oats and most recently cheese are marketed to all and sundry. The message also comes from our GPs, who may prescribe various cholesterol lowering agents (viz, statins) in order to reduce our cholesterol to below a certain ‘healthy range’.

According to the popular Wikipedia, the ‘desirable level corresponding to lower risk for heart disease’ is
<5.0 mmol/L, whereas a level between 5.2–6.2 represents a borderline high risk, and a level > 6.2 poses a high risk for heart disease. These values probably derive from the American Heart Association guidelines. A simple internet search of the term ‘cholesterol level’ produced nearly 10 million hits, while that for ‘cholesterol lowering’ produced over 3 million, and that for ‘cholesterol lowering medication’ over 1 million.

The 120-page book, “A Balanced Omega-6/Omega-3 Fatty Acid Ratio, Cholesterol and Coronary Heart Disease” largely challenges the popular view that high cholesterol levels per se represent a true risk factor for heart disease and associated mortality. The book primarily consists of presentations from the satellite workshop of the Second Congress of the International Society of Nutrigenetics/Nutrigenomics that took place in Geneva in 2008. Although now two years old, the book is still well worth the read. Although the book challenges the role of high cholesterol in developing coronary heart disease (CHD), and questions the role of statins in reducing heart disease via reducing cholesterol levels, the main focus of the book is really that a balance of the omega 6 to omega 3 fatty acid (FA) ratio is most essential to health and that cholesterol levels are in themselves a poor marker of CHD.

The importance of the Omega 6 to Omega 3 ratio has its basis in several pieces of evidence. First is the long held recognition that people in European countries have relatively high fat diets but low CHD rates. For example, people in Crete have a long survival and low CHD but their total fat intake is similar to people in the US. Notably, however, their Omega 6 to Omega 3 ratio is substantially lower (comparatively about 2:1, vs some 17:1 for the US), and largely due to their use of olive oil (vs vegetable oil in the US). In some countries, a lowered Omega 6 to Omega 3 ratio is facilitated by greater consumption of Omega 3 containing foods such as fish. In summary, there is a wealth of data supporting the lower atherogenic potential of a diet relatively high in Omega 3 fatty acids.

Several pieces of evidence are also presented in this book to support the claims made by the group of authors in relation to cholesterol. For example, an analysis of the 30 year Framingham Heart study follow-up data resulted in several striking findings – (i) only 50% of individuals who developed CHD were identified by using total cholesterol alone, (ii) high cholesterol was not a risk factor for CHD after age 47, (iii) both coronary and total mortality was higher in those whose cholesterol had decreased than in those whose cholesterol had increased. Strikingly, for ‘each 1% md/dL drop of cholesterol there was an 11% increase in coronary and total mortality.’ The question of whether statins were used to facilitate the cholesterol lowering associated with the elevated mortality risk was also raised by authors in this book. In one chapter, the ‘disappointing recent cholesterol-lowering drug trials’ are reviewed and the question ‘is it not time for a full reappraisal of the cholesterol theory’ raised. In essence, most of the drug trials have not shown positive outcomes for lowered CHD risk from lowered cholesterol. There was a suggestion raised that publication of such negative trials may be delayed by the drug manufacturers. This is certainly understandable, given that such news could not be used to promote sales of such drugs. The Jupiter Trial did report positive findings for drug intervention, but results were questioned within the book as being inconsistent and possibly biased.

There are many other notable facts presented, and interesting thoughts proposed, in the book, including: (i) despite the five-fold increase in statin prescription in the UK during 1996 to 2002, admission rates for myocardial infarction have shown little reduction; (ii) according to the Framingham Heart study, 80% of individuals suffering a myocardial infarction (MI) have similar total cholesterol levels to those who did not have an MI; (iii) elevated cholesterol intake increases the size rather than the number of low density lipoprotein (LDL) particles, resulting in less atherogenic large buoyant LDL particles rather than the highly atherogenic small dense LDL particles; (iv) current medical interventions are aimed at treating unproven surrogates of CHD rather than true causes of CHD.

The view that atherogenesis is not just a passive process of lipid deposition but rather a specific type of systemic chronic inflammation is also explored and explained in an easy to read scientific style.

The first three chapters from the book summarise the scientific evidence for the importance of the Omega 6/Omega 3 ratio and consider the need to measure blood fatty acids (FA) in order to better define the risk of CHD and other chronic disease.

The physiological functions of cholesterol in muscle is explored in another chapter that suggests cholesterol may in fact positively affect skeletal muscle through its role in steroid hormone production and that the long term effects of aggressive cholesterol lowering is not fully understood.
The relationship between dietary cholesterol intake and serum levels is reviewed in the context of evolutionary aspects of diet relative to cholesterol intake and the effect of eggs on body weight and cholesterol metabolism and low density lipoprotein (LDL) levels. It appears that our ancestors had high cholesterol intakes but probably low serum cholesterol levels, giving food for thought and maybe further implied evidence that with such easy access to food that generally people within developed countries eat too much.

Another chapter challenges the accepted dogma that dietary saturated fat raises cholesterol and that high cholesterol leads to atherosclerosis and CHD. Instead, the author considers high cholesterol levels to be potentially protective primarily via an immunoprotective role. The concept that low cholesterol is in fact a risk factor for cancer and early mortality is also explored in this chapter and another in this book. One author concludes with a theory that the inflammatory process is possibly secondary to aggregated complexes formed by lipoproteins and microorganisms or their toxins, which in turn may occlude major arteries and form plaque. The healthy role of eggs, recently criticized in terms of high cholesterol potential, is also explored, and concludes with the view that ‘the clear benefits of these nutrient-rich animal products in the diet greatly exceed the undocumented risk people associate with dietary cholesterol’.

The final chapter considers the conflict between pharmaceutical industries, food industries and consumers and supports a push for the ‘Columbus Concept’, which promotes a balance between Omega 6 and Omega 3 in plasma total lipids, which in turn may protect against many chronic degenerative diseases.

In conclusion, this book provides an easy, informative and valuable narrative that represents an opposing view to the ideology that low cholesterol levels equate to a lowered risk of CHD as well as valid reasons to question the no doubt over prescribed use of statins. For members of AIMS, perhaps most noteworthy is the chapter reporting on the recent and disappointingly unsupportive cholesterol lowering drug trials, as detailed previously. However, additional chapters related to nutrition and the balance of Omega 6 to Omega 3 fatty acids are also worth considering. Perhaps it is indeed time for a full reappraisal of the cholesterol theory, and to replace the largely unvalidated approach to cholesterol lowering as a strategy to reduce CHD by alternate concepts based on improved nutrition and lowered Omega 6 to Omega 3 ratios.

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Illness in Colonial Australia
F. B. Smith
Australian Scholarly Publishing Pty Ltd
371 Pages
ISBN: 978 1 921509 19 3
RRP: AUD $49.95

Illness in Colonial Australia is written by Professor F. B. Smith who has been one of Australia’s leading historians for longer than I have even been alive. His latest work highlights the common diseases facing indigenous and colonial Australians from the arrival of convicts until the early 20th century.

Professor Smith provides an extensively researched insight not only into the diseases facing Australians but also into the changing social, financial and geographical landscape that shaped society’s responses and attitudes to health. Illness in Colonial Australia attempts to arrange its rich body into 12 chapters in order to draw attention to topics such as mothers, children, crowd diseases, hygiene, hospitals and irregular practices.

Smith traces the evolution of healthcare from the acceptance of less than ideal living conditions in early Colonial Australia to the advent of almost modern hospitals employing almost modern methods. It is interesting to learn of Australia’s earliest public health interventions and the logic behind them finding a place in the healthcare system.

Over 1200 works are referenced in this book and Smith describes situations vividly and expertly. The now redundant language used in much of the cited material reminds us that we have indeed come a long way. Smith describes an investigation into a Typhoid outbreak in Brisbane in 1878 where the sewage system (or lack thereof) was justified by Deuteronomy 23:13; “And thou shalt have a paddle upon thy weapon; and it shall be, when thou wilt ease thyself abroad, thou shalt dig therewith, and shalt turn back and cover that which cometh from thee.” The author’s portrayal of both the people’s and governments’ attitudes to health and life, with reference to specific incidents will leave the reader informed, amused and likely, flabbergasted.

Illness in Colonial Australia would be most suited for use as a text to accompany public health or social science units at either undergraduate or graduate level in tertiary institutions, or for medical or medico-legal professionals wishing to broaden their knowledge.

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Medical Scientist,
Dili TIMOR-LESTE

Australian Journal of Medical Science August 2011 Vol. 32 No. 3
Pathophysiology for the Health Professions
(4th Edition)
Barbara E Gould and Ruthanna M Dyer
Saunders Elsevier 2011
Hard cover   736 Pages
ISBN: 9781437709650
RRP: AUD$88.20

This is the fourth edition of this quite popular book with a new edition being published approximately every 4 - 5 years since 1997. This frequent updating ensures that the material is current, with the following significant changes since the third edition:

- New content on the causes and trends related to disease, new drugs, technology, and treatment;
- Coverage of obesity and its complications, including metabolic syndrome;
- Multiple disorder syndromes in the aged patient;
- DNA, genetics and the Human Genome Project with current research on protein pathways in health (proteomics) and the implications for drug treatment and disease causation;
- Coverage of autism;
- Updated content on the H1N1 virus and communicable diseases;
- HIV, cancer causation, and immunology;
- Substance abuse including both illicit drugs; and prescription medications.

Case studies revised to emphasize chronic diseases, prevention, and acute care, and to apply to a wider range of health professions; as well as a reorganisation of the Appendices. There is also a companion website with additional resources and updates. This new content reflects the contemporary health care issues of an ageing population and the genetics revolution.

This is a very readable and well laid out reference with clearly defined learning objectives supported by concise text, well designed diagrams and illustrations, case studies, study questions and addition text and online resources.

The major topics dealt with are Inflammation and Healing; Immunity and Abnormal Responses; Infection; Neoplasms; Fluid, Electrolyte and Acid-Base Imbalance; Congenital and Genetic Disorders; Complications in Pregnancy; Aging; Stress; Immobility; Pain; Substance Abuse; Environmental Hazards; Cardiovascular Disease; Disorders of the Respiratory System, Digestive System and Urinary System; Acute and Chronic Neurologic Disorders; Endocrine and Reproductive System Disorders; Skin and; Musculoskeletal Disorders.

The target reader is someone with little background in medicine or science and the text aims to deliver a fairly detailed understanding of the wide range of topics to a level consistent with a final year undergraduate. Each Chapter contains all the common significant diseases for the system being studied plus the detailed causation, symptoms, diagnostic tests and treatment.

The authors provide sufficient detail in the Chapters for the text to be a useful companion to any undergraduate student of an Allied Health degree. The text also contains reflective questions, current research approaches and emergency procedures. There are also case studies with detailed questions and an extensive set of Appendices which contain a lot of useful information and an extensive Glossary.

I would recommend this text for any Allied Health discipline as an introductory text to Pathology.

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Frontiers of Hormone Research: Volume 33
Growth Hormone Deficiency in Adults
JOL Jorgensen & JS Christiansen
Karger
Hard cover   IX+227 pages
ISBN: 3-8055-7992-6
RRP: US$180.00

This book sets out to address an issue not usually discussed in standard text books. The area of growth hormone deficiency in adults has been largely ignored as many believe that growth hormone is redundant in adults. The book focuses on the reasons for, and the effects of growth hormone replacement in adults with hypopituitarism, consequent to pituitary tumours, surgery/radiotherapy or traumatic brain injury.

The specific aim of the book is to address the traditional end points of growth hormone replacement which includes reduced risks of cardiovascular disease, improved body composition and increased exercise capacity. In addition it also addresses the novel end
The contributors to this book, clinicians and scientists, put together an interesting array of chapters covering aspects of growth hormone deficiency. The book begins with a chapter describing the clinical aspects of growth hormone deficiency leading into the next chapter on the epidemiology of the condition. Critics may point out that the issue of adult hormone deficiency is contentious, let alone population based studies on the subject. However this chapter focuses on pituitary tumours, their treatment and the replacement of hormones other than growth hormone. They provide evidence to support that non-replacement of growth hormone in these adults lead to higher risks of cardiovascular disease, respiratory disease and cancer as well as mortality from any of these conditions. In addition to the various aspects of treatment, end points and monitoring treatment response, the chapters devoted to diagnosis are of particular interest to the medical scientist. Dynamic stimulation tests for growth hormone are well described. Some of these are commonly used to diagnose growth hormone deficiency in children and almost never used for adults in Australia. Of interest to the medical scientist, this chapter also discusses aspects of using immunoassays to detect growth hormone. The description of various circulating forms of growth hormone and other factors influencing immunoassays is in depth and comprehensive. The chapter on IGF-1 is also particularly useful to the medical scientist. It discusses biological variation, physiological variation and its usefulness in diagnosis and in monitoring treatment.

This book is intended for clinicians but the various diagnostic aspects discussed will be of particular use to medical scientists in endocrinology laboratories.

Overall, this book highlights the need for particular attention to growth hormone in hypopituitarism and in head injury patients. It also addresses the controversial issue of growth hormone replacement in adults, and its benefits in reducing the risks of metabolic syndrome and osteoporosis. The book meets its aim of drawing attention to a controversial issue and providing scientific data from reputable sources to strengthen the view of the authors.

The organisation of chapters follows a logical sequence. Each chapter is divided into concise subsections which allow quick access to specific facts.
immunohistochemical characteristics of both non-Hodgkin and Hodgkin lymphomas. The book begins in describing the technical aspects as well as the cytological features of lymph node disorders both inflammatory and neoplastic lesions, based on the recent WHO lymphoma classification. Thus, it is a valuable resource for Cytopathologists, Pathologists, Oncologists and Hematologists. Moreover, the technical methodology on immunostaining procedures and Flow Cytometry from the second and third chapter of this book proves it to be a very useful reference book for Medical Scientists working on Immunohistochemistry and Flow Cytometry.

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MALAYSIA

Manual of Surgical Pathology - 3rd Edition  
Susan C Lester  
Elsevier Inc  
Soft Cover  608 Pages  
ISBN: 978-0-323-06516-0  
RRP: AUD$174.60

Susan Lester MD, PhD is an Assistant of Pathology at Harvard Medical School and The Chief of Breast Pathology Services, Brigham and Women's Hospital Boston Massachusetts. This book is an interesting and an informative resource on pathology cut up and reporting.

This paperback book comprises of 608 pages and covers 34 chapters. The first part of the book covers general histopathology procedures while the second part covers tissue specimen types. There is also an expert consult online facility with this book. Searchable on line text is available. It is activated by registering and activating with an activation code that is scratched off inside the front cover.

The first chapter covers requesting pathology and the fixatives required for certain tissue types. It also covers terminology for tissue orientation. Chapter two describes cutting up tissue for placement into cassettes. There are very helpful diagrams on cutting to determine margins. It also covers how to describe tissue. The next chapters describe the number of levels to be cut, special stains, fixation and reporting. The book is American so the guidelines for consultations are based on American Associations. There is a chapter on common operating room consultations which includes frozen sections and skin scrapings.

The chapter of special studies goes into a lot of detail for special stains and immunohistochemical studies. There are tables of tumour type and IHC results. There are also extensive tables with histochemical stains and immunoperoxidase studies. Electron microscopy is briefly touched upon. Cytogenetics is extensively covered set out clearly in table form. A chapter is dedicated to safety precautions of infectious tissue.

The optical properties of non cellular material are well covered as are basic microscopy techniques. The second part of the book has chapters devoted to specific organ groups. The covered topics are adrenal glands, amputations and large resections, small biopsies, bones and joints, breast, cardiovascular specimens and so on. There are 23 of these chapters in all. Lymph nodes, gastrointestinal and gynaecological specimens were particularly well covered.

The relevant clinical history, gross features, what to look for and how to cut these specimens including the number of slides suggested is covered. There are tables of tumour types and a number of different classifications. The diagrams and tables enable easy consultation. There are no actual photographs of organs or tissues.

All the diagrams are very well hand-drawn diagrams with clear labels.

A number of orientations are often shown for placement of tissue onto slides. This would be very good for scientists performing the orientation into blocks and then cutting the slides.

Overall the book is an easy reference that could easily be used as a bench resource within the Laboratory. It would be ideal for Anatomical Pathology Registrars and Scientists with an interest in Histopathology. More experienced pathologists may also consult the immunohistochemistry and cytogenetics tables.

Lesley Affleck MAIMS  
Supervising Scientist  
Queensland Pathology Caboolture
Australian Professional Acknowledgement of Continuing Education (APACE)

5 APACE Group 1 credits per set of questions will be awarded if at least 8 out of 10 questions are answered correctly. 40 credits maximum per year can be claimed.

### Journal-based CPD No.29

**Page 1 of 2**

Questions relating to *The fifth human malaria species – Plasmodium knowlesi*, page 82 of this issue.

1. The *Anopheles hackeri* mosquito is attracted mainly to humans.  
   True/False

2. The drug Artesunate is successful for treating severe malaria.  
   True/False

3. There is a high incidence of *P. knowlesi* infections in Vietnam.  
   True/False

4. The long-tailed and pig-tailed Macaque monkeys die from *P. knowlesi* infections.  
   True/False

5. The fatalities from *P. knowlesi* infections are due to acute renal failure, acute respiratory distress and shock.  
   True/False

6. The incidence in Malaysia and Malaysia Borneo is between 28% and 100%.  
   True/False

7. Thrombocytopenia is present in 88% of patients.  
   True/False

8. It is usually mistaken morphologically for *P. malariae*.  
   True/False

9. Rhesus monkeys only get chronic *P. knowlesi* infections.  
   True/False

10. *P. knowlesi* infections were used to treat neurosyphilis in the 1930s.  
    True/False

Name: ___________________________________ Membership No: _______________________

Email: __________________________________________________________________________

Please photocopy this page or print it from the AJMS on the AIMS Member Library in the AIMS Member Lounge at www.aims.org.au, circle your answers and post or fax by 31 January 2012 to:

AJMS APACE Questions, AIMS National Office, PO Box 2426, Toowong DC Qld 4066. Facsimile: 61 7 3876 2999
Questions relating to *The use of the automated parameter RDW-SD as a discriminator of both alpha and beta thalassaemia trait from iron deficiency*, page 88 of this issue.

1. Both thalassaemia trait and iron deficiency are characterised by red cell microcytosis.
   - True / False

2. Alpha thalassaemia trait is characterised by a diagnostic change in the levels of the common haemoglobins.
   - True / False

3. RDW-SD is measured from a lower position on the red cell histogram than the RDW-CV on the Sysmex XE2100.
   - True / False

4. Long term stability studies showed significant variation in RDW-SD results over a six hour period at both room temperature and 4°C.
   - True / False

5. As RDW-SD is an automated parameter; its day to day performance can be monitored with the commercial control material provided by the manufacturer of the Sysmex XE2100.
   - True / False

6. Discriminative formulas outperformed the RDW-SD as a discriminator of thalassaemia from iron deficiency.
   - True / False

7. Individual laboratories should evaluate the performance of any discriminators of thalassaemia from iron deficiency that they are considering using.
   - True / False

8. Discriminative formulas that incorporate RDW-CV could be improved by replacing the RDW-CV in their formulas with RDW-SD.
   - True / False

9. Patients with beta thalassaemia and co-existing iron deficiency can show a reduction in HbA₂ levels.
   - True / False

10. Heterozygotes for hereditary persistence of fetal haemoglobin (HPFH) are generally anaemic.
    - True / False

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YEAR 2011

SEPTEMBER 30 – OCTOBER 1
ASM Tri-State Scientific Meeting
Indigenous, Rural & Regional Health issues – a Microbiological Perspective
ALICE SPRINGS NT AUSTRALIA
Register at: www.theasm.org.au
Email: tristate2011@icms.com.au

OCTOBER 2 – 6
12th International Congress of Therapeutic Drug Monitoring and Clinical Toxicology
STUTTGART GERMANY

OCTOBER 10 – 13
AACB 49th Annual Scientific Conference & Golden Jubilee
Sydney Convention & Exhibition Centre
SYDNEY NSW AUSTRALIA
http://aacb.asn.au/

OCTOBER 19 – 23
XXVI World Congress of the World Association of Societies of Pathology and Laboratory Medicine (WASPaLM)
Hosted by the American Society for Clinical Pathology
LAS VEGAS NEVADA USA
http://www.waspalm.org

OCTOBER 29
ASTH 2011 Scientific Workshop
Sydney Convention Centre
SYDNEY NSW AUSTRALIA

OCTOBER 30 – NOVEMBER 2
HAA Meeting
SYDNEY NSW AUSTRALIA

NOVEMBER 4 – 6
Fifth National Histology Meeting
Rosehill Gardens
SYDNEY NSW AUSTRALIA or NSW Website: http://www.histnsw.org.au
Kathy Drummond kdrummond@dhm.com.au

NOVEMBER 4 – 6
AIMS NSW North Coast annual conference.
Darlington Beach Resort
(35 km north of Coffs Harbour)
ARRAWARRA NSW AUSTRALIA
(02) 6776 9840
Email: neil.horton@hnehealth.nsw.gov.au

DECEMBER 10 – 13
American Society of Hematology Meeting
SAN DIEGO USA
http://www.hematology.org/Meetings/

YEAR 2012

JULY 22 – 25
Human Genetics Society of Australasia
36th Annual Scientific Meeting
National Convention Centre
CANBERRA ACT AUSTRALIA
www.hgsa.org.au
Email: hgsa@wsm.com.au

SEPTEMBER 24 – 27
NSM 2012 AIMS/NSM Meeting
Darwin Convention Centre
DARWIN NT AUSTRALIA
www.aims.org.au

OCTOBER 28 – 31
HAA Meeting
MELBOURNE VIC AUSTRALIA
BOOKS FOR REVIEW

Following is a list of books available for review by resource consultants and members of the Institute with particular expertise in the field.

The reviewer is invited to retain the complimentary copy of the book once the review is received.

As per our agreement with the book publishing companies, complimentary books are submitted to the Institute provided that all reviews are published in the Australian Journal of Medical Science. These reviews must be of a high quality as buying decisions and the reputation of the book and author are important considerations.

Books not requested will be allocated at discretion of the Editors for the Australian Journal of Medical Science. Reviews should be 300 to 700 words depending on the volume of the book. Time limit for return of review is six weeks.

Requests to: Australian Institute of Medical Scientists  PO Box 2426  Toowong  Qld  4066.
Tel: (07) 3876 2988  Fax: (07) 3876 2999    Email: aimsnat@aims.org.au

Latest Additions:

- Bile Acids as Metabolic Integrators and Therapeutics
- Environment and Lifestyle - Effects on Disorders of the Digestive Tract
- Emerging Trends in Antimicrobial Discovery: Answering the Call to Arms
- From Kurmond Kid to Cancer Crusader: Pioneering Integrated Cancer Treatment
- Sepsis - Pro-Inflammatory and Anti-Inflammatory Responses: Good, Bad or Ugly?
- Walzing with Jack Dancer: A Slow Dance with Cancer


15. Digestive Diseases The Keys to IBD 2010: Treatment, Diagnosis and Pathophysiology. Edited by G Rogler, W Sandborn. Karger. 188 pages


29. Ichthyoses: Clinical, Biochemical, Pathogenic and Diagnostic Assessment edited by PM Elias, ML Williams, D Crumrine and M Schmuth. Karger. x+144 pages.


31. Laboratory Diagnosis in Neurology edited by B Wildemann, P Oschmann and H Reiber. Thieme (available through Elsevier Australia) 296 pages.


43. Neuromuscular Disorders authors Anthony A Amato and James A Russell. McGraw-Hill Medical. 775 pages.


47. The Regulator Genome: Gene Regulatory Networks in Development and Evolution author EH Davidson. Elsevier Australia. 289 pages.


The following instructions are based on the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” also known as the Declaration of Vancouver and on the Australian Government Style manual: for authors, editors and printers, 6th edition, 2002. URLs were correct on January 25th, 2008.

Manuscripts that do not fully comply with the following ‘Instructions to Authors’ may be returned for revision before they are considered for publication.

The Australian Journal of Medical Science (AJMS) will consider for publication any paper relevant to the field of Medical Science. Disciplines include Blood Banking, Clinical Biochemistry, Haematology, Histopathology, Immunology, Microbiology and Molecular Biology. Areas of general interest to medical laboratory scientists, including toxicology, epidemiology, public and community health, and professional and management issues will also be considered.

Papers published in the AJMS are in the form of:
- Review Articles
- Original Articles
- Brief Communications
- Technical Notes
- Case Studies
- Letters to the Editor
- Book Reviews

When the manuscript is submitted the authors must disclose any potential conflict of interest and/or commercial support.

Requirements and preparation of manuscripts

General

Articles should be submitted in electronic format to ajms@aims.org.au. If an article is too large to be submitted by email, it should be submitted on a CD.

Number pages consecutively commencing with the title page.

Arrange the article in the following sequence:
- Title page
- Abstract and key words
- Main Text
- Acknowledgements
- References
- Tables - each table, complete with title and footnotes, on a separate page
- Legends for illustrations.

Authors should ensure that their manuscript communicates their ideas and concepts simply and clearly so that the article is easily read and understood. Authors are strongly recommended to refer to the recommendations on reporting standards as outlined in the statements and checklists of the CONSORT group (see: http://www.consort-statement.org/) and similar groups such as STARD (see: http://www.stard-statement.org/website%20stard/). The principles outlined in these standards may be used as general guidelines and not just as applied to clinical trials and diagnostic studies.

Title Page

The title of the article should not exceed three lines (40 characters per line), including punctuation and spacing. All authors must be identified on the title page (e.g. William Smith, Susan Yeo, …”). Where applicable, the title page should also include the name of the institution with which each author is affiliated and to which the work should be attributed. In the case of multiple authors, the name, postal address, email address, telephone and facsimile number of the author responsible for correspondence relating to the manuscript should be indicated.
Abstract and Keywords

The abstract should be approximately 150 words and should make sense when read alone or in conjunction with the article. The abstract should be a concise overview that describes the important details of the article including the purpose of the study/investigation, basic procedures (study subjects/experimental animals/observational and analytic methods) and the results and principal conclusions. New and important aspects of the work and its implications may also be included. References should not be included.

Three to ten keywords may be listed. Authors are advised to comply with the terms from the Medical Subject Headings (MeSH) list from Index Medicus. (see: http://www.nlm.nih.gov/mesh/meshhome.html). Keywords should be given below the Abstract.

Text

The style of writing should conform to acceptable English usage. Do not use slang, medical jargon or unnecessary abbreviations. Accepted spelling is the first choice given in the latest edition of the Macquarie Dictionary.

Wherever possible observational or experimental articles should be divided into sections headed:
- Introduction
- Materials and methods
- Results
- Discussion
- References

For other types of articles such as commentaries, reports and reviews, use an appropriate format or consult the Editors for guidance.

Introduction

Clearly state the purpose of the article; lead the reader from the known to the unknown. Summarise the rationale for the study and state the question to be answered as appropriate. Give only strictly pertinent references, and do not review the subject extensively.

Materials and methods

Present the materials and methods in a logical sequence. Describe the selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Notification of ethics approval must be given where relevant. Identify the methods, apparatus and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Adequately describe new or substantially modified methods. Identify precisely all drugs and chemicals used, including generic name(s), dosage(s), and route(s) of administration. Do not identify patients or hospitals without consent.

Results

Present the results in the same sequence as given in the Materials and methods; use tables and illustrations where these will help the reader understand the work being presented. Do not repeat in the text all the data in the tables or illustrations.

Discussion

Indicate the new and important aspects of the study and emphasise the conclusions that follow. Do not repeat in detail data given in the Results section and do not add new data. Include in the Discussion the implications of the findings and their limitations and compare the observations to other relevant studies.Recommendations may be included if appropriate. Link the conclusions with the goals of the study and answer the experimental question stated in the Introduction. However avoid unqualified statements and conclusions not completely supported by your data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

Acknowledgements

Acknowledge individuals who have made substantial contributions to the study including technical work and financial support. Authors are responsible for obtaining consent from all the individuals acknowledged by name as inclusion may be interpreted as an endorsement of the article’s contents.

References

The AJMS uses a modified Harvard System (author-date system).

Throughout the body of the manuscript cite the author’s name and the publication year in parentheses as in the following examples:
(i) Research in this area (Jones 1999)...

(ii) It has been successfully demonstrated that ...(Smith and Brown 1981; Auteur 1995; Scienziato et al 2007).

(iii) Following further investigation, Wetenschapper (2002) highlighted the difficulties inherent in...

Where there are three or more authors, acknowledge only the first author, e.g. (Smith et al 2007). For two authors the following style should be used (Smith and Brown 2007).

The reference list should be in the format described below. Journal titles should be abbreviated in Index Medicus format (see: ftp://nlmpubs.nlm.nih.gov/online/journals/ljiweb.pdf) using standard abbreviations from the ISSN List of Title Word Abbreviations (see: http://www.issn.org/en/node/344). All authors should be given in the reference list.

Do not use abstracts as references. “Unpublished observations” and “personal communications” may not be used as references, although references to written, not verbal, Communications may be cited (in parentheses) in the text. Include in the references manuscripts accepted but not yet published, designate the journal followed by “in press” (in parentheses). Information from manuscripts submitted but not yet accepted should be cited in the text as “unpublished observations” (in parentheses).

Examples of the correct form for references are given below:

i) Journal Reference:


ii) Personal Author(s) of a book:


iii) Editor, Compiler, Chairman as Author:


iv) Chapter in Book:


v) Online documents:


Tables

Number tables consecutively with Arabic numerals and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in headings. Explain in footnotes all non-standard abbreviations used in each table.

For footnotes, use the following symbols in this sequence: * † ‡ § ¶ ** ††

In preparing tables, consideration should be given to the page width of the Australian Journal of Medical Science. All tables should be prepared for publication vertically. In the text, cite each table in consecutive order, and mark in the margin of the text its approximate location.

If data from another published or unpublished source is used, written permission must be obtained and a copy must accompany the manuscript.

Illustrations

Colour illustrations may be submitted on a CD. Images should be scanned at a minimum of 300 dpi.

When plotting points, the following symbols are preferred:

In most instances, figures will be reduced to one.
column in width. All letters and numbers should be drawn to be at least 1.5 mm high after reduction, symbols at least 1.0 mm. Titles for illustrations belong in the legends for illustrations and not on the illustrations themselves.

Photomicrographs must have internal scale markers and the magnification must be stated. Symbols, arrows, or letters used in the photomicrographs should contrast with the background.

Cite each figure in the text in consecutive order, e.g. “Figure 1 illustrates …” or “… as shown (fig. 2)”. If a figure has been published, acknowledge the original source and submit with the manuscript written permission from the copyright holder to reproduce the material. Permission is required, regardless of authorship or publisher, except for documents in the public domain.

Legends for Illustrations

When symbols, arrows, numbers, or letters are used to identify parts of illustrations, identify and explain each one in the legends. The figure legend must contain a boldface (a) name (“Figure” + arabic figure number) and (b) substantive title.

Abbreviations

Use only standard abbreviations (see list of commonly used abbreviations, above right).

Avoid abbreviations in the title. The full term for which an abbreviation stands must precede its first use in the text unless it is a standard abbreviation for a unit of measurement.

Report measurements in the units in which the measurements were made. In most countries the International System of Units (SI) is standard.

Commonly used abbreviations

<table>
<thead>
<tr>
<th>Abbreviation or Symbol</th>
<th>Standard Units of Measurement</th>
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Additional Information

The following are useful sources of information. The first two publications are used by the AJMS as standard references.


Conquer your space limitations

- Reliability
- Easy to use
- Quality results
- Comprehensive system
HAEMATOLOGY UPDATE
Severe anaemia in a 2-month-old child

REVIEW ARTICLE
The fifth human malaria species – Plasmodium knowlesi

ORIGINAL ARTICLE
The use of the automated parameter RDW-SD as a discriminator of both alpha and beta thalassaemia trait from iron deficiency