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A motivational narrative

From basics to dynamics: The laboratory story

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Introduction

From ancient times, blood has been seen as the essence of life. The Holy Bible declares, "The life of the flesh is in the blood." Throughout history, blood was not only revered as vital for survival but also used in rituals – from bathing in it, to drinking it, to offering it in sacrifices.

Early physicians experimented with ways of transferring blood from humans and animals in the hope of curing disease. By the mid-19th century, the fundamental theory of blood clotting was known, but effective treatment for haemophilia only became available in the 1960s. Remarkably, the Babylonian Talmud records that Rabbi Judah exempted families with a history of excessive bleeding after circumcision from undergoing the ritual, noting that mothers could transmit the condition to their sons – identifying the carrier state of haemophilia centuries before Mendel's genetic discoveries.

This article recounts how, within just 13 years, the haematology laboratory at the National Hospital of Sri Lanka (NHSL) evolved from a modest unit into a state-of-the-art facility – thanks to the collective efforts of medical officers, postgraduate trainees, medical laboratory technologists (MLTT), orderlies and support staff working toward a common vision.

Modernising the laboratory

When I assumed duties as the Head of Haematology at the Department of Pathology, NHSL in the late 1990s, the hospital was the country's premier teaching centre for haematology trainees. It had 65 ICU beds across nine units, a major accident service, 19 operating theatres, several outpatient clinics (OPD) and emergency treatment units.

At that time, laboratory practices were basic: Blood samples arrived in glass penicillin bottles sterilised and prepared with anticoagulants by orderlies. Coagulation samples were processed in glass tubes.

Haemoglobin was measured by the cyanmethemoglobin method using a colorimeter, PCV with a microhaematocrit centrifuge, WBC counts with a Neubauer chamber and differential counts on Leishman-stained smears. Platelets were counted manually using chambers and slides.

The introduction of a 3-part analyser in 1998 was a turning point. It could measure red cell parameters, total WBC with a 3-part differential and platelets, though slides still had to be examined manually. With no printer attached, results were handwritten onto request forms.

Haemoglobin controls were prepared in-house using near-expiry blood packs from the blood bank, adjusted to high, normal and low values by adding saline or removing plasma. The tri level values were 20 g/dL, 12 g/dL and 6 g/dL respectively. Thus, prepared samples were kept in the refrigerator and used as daily controls with the results plotted on Levy-Jennings charts.

At the time, immunophenotyping with CD markers were unheard of. Instead, stains such as Sudan Black B and PAS were used to identify blasts.

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Coagulation testing remained manual and done in the water bath using a stop watch to record time. Thrombokinase reagents for Prothrombin Time (PT), often unaffordable, were prepared from cadaver brain tissue. It was a tedious job to remove all the capillaries from the surface of the brain by using forceps and none of us wore masks. In that era, wearing masks in a laboratory set up was unheard of. Masks were issued only to the operating theatres.

The brain matter was blended in the laboratory, reagents were prepared and the concentration of the reagent was checked by doing the test on several normal individuals. In that era, we couldn't assign the ISI value to this prepared PT reagent. We assigned an arbitrary ISI value as 1.

Bleeding and clotting times were performed using the old ear-lobe lancet method. The lancet was sterilized in the laboratory.

Blood collection for outpatients was done by medical officers on roster. Syringes and needles were sterilised in the laboratory and brought to the bleeding rooms, most of the times the needles were blunt and one could imagine how the patients reacted when the doctor had to prick several times to get into a vein.

The laboratory operated 24 hours and the night lab had only a skeletal staff. Any abnormal counts were referred in the morning to the medical doctor in charge. The night request forms were of a different colour to identify them from day reports. There was no night roster for doctors and any abnormal counts were informed to the on-call ward registrar. Any urgent opinion was obtained from the laboratory consultant over the phone.

By 2000, the first 5-part analyser arrived – complete with a printer. Results could now be issued in printed form and an air-conditioned room was prepared for the machine. A coagulometer soon followed, speeding up PT/ INR testing and reducing patient waiting times. Disposable plastic tubes gradually replaced penicillin bottles, marking a shift towards safer, more efficient practices.

Introducing advanced diagnostics

With the installation of a semi-automated coagulometer in 2002, we were able to introduce tests for thrombophilia – previously unavailable in the government sector: Screening for lupus anticoagulant, Antithrombin III levels, Protein C and S levels and Activated protein C resistance (APCR) (Table 1).

Before starting these tests, we wanted to establish the prevalence of Factor V Leiden (APCR) in our population. For that we collected blood from patients with unexplained thrombotic events, their family members and laboratory staff. As these reagents were expensive, we limited the testing to arbitrary 35 people. This study revealed a prevalence of <2%, a rate far lower than in Western populations and routine testing for APCR was not pursued.

Around the same time, extensive bleeding disorder diagnostics were introduced: factor assays, inhibitor screening, platelet aggregation studies and factor XIII levels. Patients came from across the country, even during the civil war, often waiting all day for results while technicians worked through lunch.

We collaborated with Christian Medical College (CMC), Vellore, for external quality assurance (EQA) for coagulation. The consultant in charge of the laboratories Dr Sukesh Nair agreed to send control plasma to 4 centres in Sri Lanka. These were couriered and immediately distributed to National Hospital, Medical Research Institute (MRI), Peradeniya Teaching Hospital and Lady Ridgeway Hospital. The test results were sent over to Dr Nair. From time to time the Vellore university sent their representatives to do workshops here.

Female patients with a history of frequent abortions were sent from De Zoysa Maternity and Castle Street Hospital to get their thrombophilia profiles. Most had lupus antibodies. Some received subcutaneous heparin throughout the pregnancy. Most had successful pregnancies, and they always came to the laboratory with their newborn babies to show us the miracle bundle.

Table 1. Statistics of the tests done for inherited thrombophilia

Year	Protein C (Clotting method)			Protein S (Clotting method)			Antithrombin (Clotting method)			Activated Protein C Resistance - APCR		
	Total	Positive Male	Positive Female	Total	Positive Male	Positive Female	Total	Positive Male	Positive Female	Total	Positive Male	Positive Female
2002	82	10/82	06/82	82	05/82	10/82	82	03/82	04/82	30	01/30	01/30
2003	37	06/37	06/37	37	02/37	03/37	37	01/37	0/37	37	01/37	00/37
2004	113	21/113	06/113	131	14/113	07/113	113	05/113	13/113	113	01/113	01/113
2005	62	12/62	09/62	62	07/62	08/62	62	02/62	01/62	62	01/62	00/62
2006	59	08/59	06/59	59	08/59	07/59	59	03/59	02/59			
2008	30	02/30	03/30				30	01/30	01/30			

(Normal ranges: Protein C - 66-129%, Protein S - 61.9-145.3%, AT III - 75.6-122.4%, APCR Ratio - 2.18-3.38% (Ref: Dacie and Lewis 10th edition)

When bone marrow biopsies were requested from wards, some days up to 5-6, the medical officer had to go to these wards carrying the tackle box with all the paraphernalia and the MLT would follow.

This was rectified when we divided the bleeding room with a screen and obtained a theatre bed and started to perform the procedure in the laboratory. Times were allocated; therefore, the procedures were streamlined. In the event of a bleed, we obtained permission from the hospital director to wheel the patient to the adjoining surgical ward. We were not allocated any nurses. We had 3 female minor staff, and they assisted the doctors.

Towards a centre of excellence

One of the challenges for local trainees was limited exposure to clinical haematology, unlike colleagues abroad. Determined to bridge this gap, we began patient-centred services within the laboratory setting.

We first started with the coagulation, so called INR clinics. We had to take permission from the hospital director to start patient clinics which was a new chapter for laboratory haematology. Then submitted our specimen signatures to the OPD pharmacy for them to issue medicine. Once established, within one day the patients could give their blood sample, get their PT/INR report and the required medicine.

Thrombophilia clinics were established to investigate causes of recurrent thrombosis and provide targeted treatment. Peri-operative monitoring protocols were developed for patients with thrombotic history undergoing surgery, adjusting heparin dosing across perioperative phases.

Patients with habitual abortions were monitored in a special clinic and prophylactic heparin dose was worked out, after discussing with the obstetric team.

The next development was the “FR clinics”. When the Bed Head Tickets (BHTs) were referred to us requesting a comprehensive blood work evaluation, we performed all the required tests, sometimes culminating in a bone marrow or trephine examination, and the medication was issued accordingly. After some time the physicians/

surgeons were happy to register them in our clinics.

The greatest success, however, came with the Haemophilia Clinic. Partnering with the Haemophilia Association and World Federation of Haemophilia (WFH), we created a comprehensive care programme. Patients were transitioned from paediatric care at Lady Ridgeway Hospital to adult care at NHSL. The patient referral to NHSL increased after 2006 as patients from other hospitals were also referred after we started the comprehensive Haemophilia care programme (Table 2). The referred patients had their full profile registered which included the deficient factor and the factor level, the inhibitor screening and the inhibitor level. This data was collected monthly.

Table 2. Newly registered patients with haemophilia

Year	Haemophilia A	Haemophilia B
2002	2	0
2003	1	0
2004	1	2
2005	6	0
2006	9	2
2007	17	5
2008	10	4
2009	10	0
2010	8	1

Home therapy was introduced and parents were trained to administer factor VIII or IX at home when the bleeding signs or aura (sense that a bleed is about to occur) appear at home. The factors were allocated name patient basis to the indoor pharmacy. To obtain the next quota of factors the patient's party had to provide us with the empty vials. We had to be double cautious as the factors are quite expensive. By this method the admission to the wards were reduced considerably. As the factor levels got stable due to this home therapy and patients visited us less frequently, we had to look for other means to collect factor deficient plasma with factor level <1% for APTT mixing studies, a screening test used for the diagnosis of haemophilia. With time we moved to using commercially prepared deficient plasma for this test.

With the availability of the factors, minor and major operations could be performed in haemophilia patients.

One of our patients with severe haemophilia A became a national and international medals-winning table tennis player thanks to prophylactic therapy. He secured a bronze medal in the 19th Commonwealth games held in India.

The unit gained recognition from the WFH, securing fellowships, training opportunities in India for technologists, and support for EQUAS (external quality assurance service) programmes. By 2008, tests for red cell membrane disorders, thalassaemia, haemoglobinopathies, paroxysmal nocturnal haemoglobinuria (PNH), chronic myeloid leukaemia (CML) and platelet function defects were fully established.

Notable firsts

The first Sri Lankan patient with aplastic anaemia successfully treated with anti-thymocyte globulin (ATG) at NHSL.

The first administration of recombinant Factor VIIa for postpartum haemorrhage at De Zoysa Maternity Hospital, in collaboration with Prof. Chandrika Wijeratne.

The unit also became the first clinical haematology training centre for postgraduate trainees in Sri Lanka.

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