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PATHOLOGY MATTERS

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Flow Cytometry in Acute Leukaemia

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Acute leukaemia is a heterogeneous group of malignancies originating from bone marrow and have varying clinical, morphologic, immonophenotypic, genetic and molecular characteristics[1]. It can be divided into acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML) (Figure 1a and 1b).

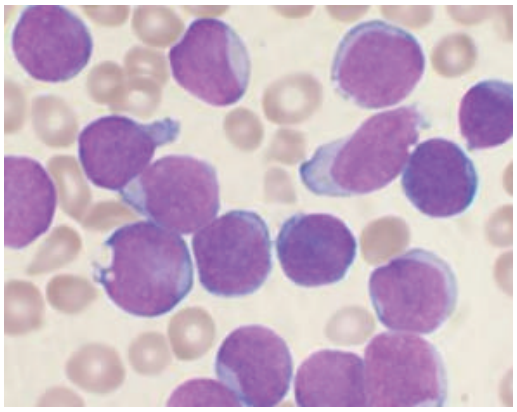

Figure 1a: Acute Lymphoblastic Leukaemia

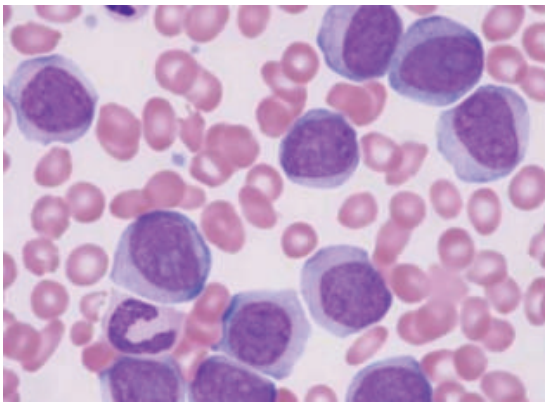

Figure 1b: Acute Myeloid Leukaemia

Flow cytometry is a technology that simultaneously measures and then analyses multiple physical characteristics of single particles, usually cells, as they flow in a fluid stream through a beam of light[2]. The cell populations are labelled with various Monoclonal Antibodies (Mab) that is tag with fluorochrome through antigen-antibody interactions prior to analysis. The presence or absence of antigens on or in the cell populations gives characteristic immunostaining defining the cell phenotypes[3].

Principles of flow cytometry

There are three main components of flow cytometry (Figure 2):

i) Fluidic system - transports the cells in a stream to the laser beam for interrogation.

ii) Optic system - consist of lasers to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors.

iii) Electronic system - converts the detected light signals into electronic signals that can be processed by the computer.

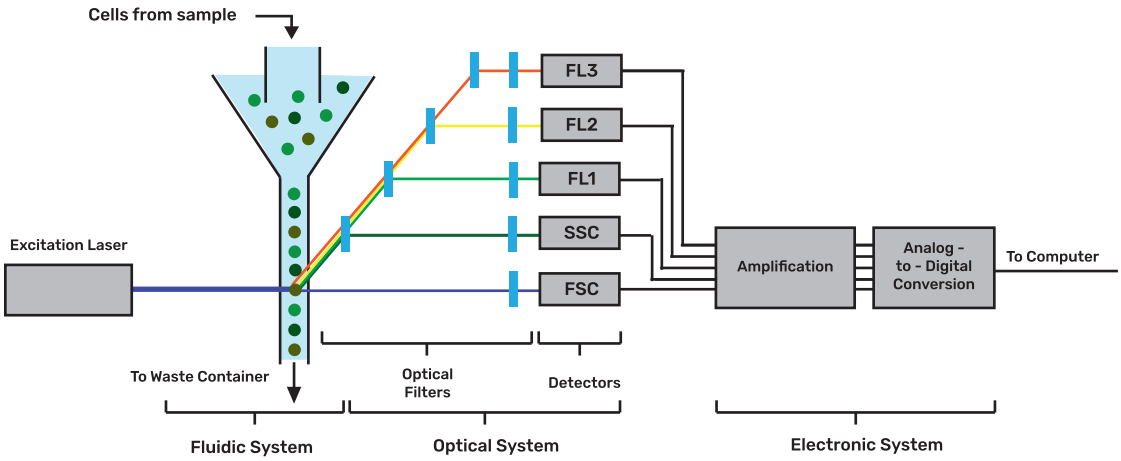

Figure 2: The three main components of flow cytometer

Data analysis requires selection of the cell population(s) or interest, followed by determination of the proportion of positive cells for each antigen studied in each population. To identify the cell populations of interest, forward vs. side scatter or CD45 vs. side scatter are commonly used (Figure 3).

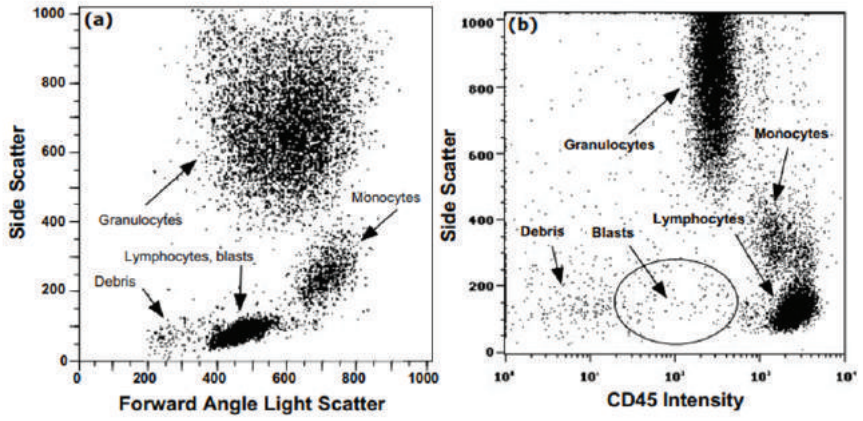

Figure 3: Left: "Dot plot" of FSC vs. SSC demonstrating the characteristic position of different cell populations b) Right: "Dot plot" of CD45 vs SSC demonstrating the CD45 intensity of different cell populations. Leukemic blast usually shows decreased CD45 expression

Utilities of flow cytometry in acute leukaemia

Flowcytometric immunophenotyping is a rapid and reliable method for the diagnosis of acute leukaemia. It relies on the expression of lineage specific CD markers on the blasts population to quantify, determine the lineage and maturation stage, and detect any aberrant features in the blast population. It is also useful in assessing prognosis and monitoring residual disease after chemotherapy. CD2, CD34, and CD56 expression in APML, CD20 expression in adult precursor B ALL, and bright CD45 expression in B and T cell ALL are all associated with a poor prognosi[4-6].

Flow cytometry also may predict few genetic aberrations in acute leukaemia, such as AML with high side scatter, negative for both CD34 and HLA-DR, bright CD33, and heterogeneous CD13 raises the suspicion of APML with t(15;17). BCR::ABL1 positive B-ALL usually express CD13 or CD33 positivity, and t(8,21) is associated with B lymphoid marker expression such as CD19 or CD79a.3

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Procalcitonin:
A Diagnostic Tool for Sepsis and Antibiotic Therapy

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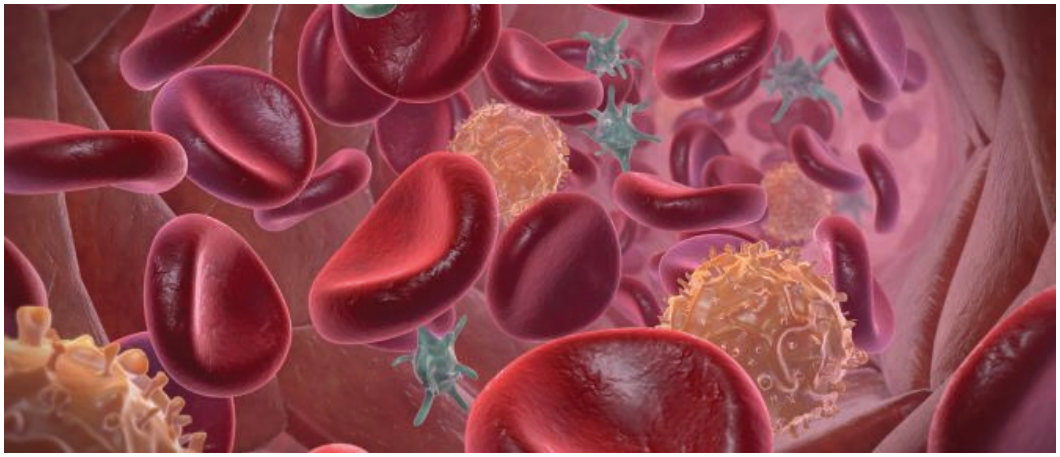

Sepsis is a systemic inflammatory response syndrome (SIRS) that affects all organs. Due to the fact that clinical signs for a definite or suspected sepsis can be heterogeneous and often ambiguous, its diagnosis and treatment remains challenging. To date, no gold standard exists for the detection of sepsis caused by bloodstream infections[1]. The use of conventional diagnostic approaches such as blood cultures, inflammatory biomarkers [i.e C-reactive protein (CRP), and white blood count (WBC)] among patients clinically suspected of infection or sepsis are limiting[2]. The use of blood cultures for the identification of pathogens, can provide information about the type of microorganism and susceptibility towards antibiotic therapy. However, only a small percentage of the analyzed cultures are positive and in around 40–90% of patients with an assumed systemic infection, the blood culture results are negative, with no evidence of pathogen growth[3]. In addition, the emergence of antibiotic resistance calls for a more stringent effort to reduce antibiotic overuse. This is especially true for acute respiratory tract infections where antibiotics are prescribed despite the majority of infections being caused by viruses rather than bacteria. In order to improve diagnostic work-up, there are biomarkers which have been established to address the deficiencies of conventional tests that have been shown to be reliable markers of infection.

Among these biomarkers are procalcitonin (PCT). PCT, the precursor of the hormone calcitonin, has been used as a biomarker to aid in diagnosis of bacterial infection or sepsis, as well as in differentiating bacterial pneumonia from viral pneumonia and chronic obstructive pulmonary disease (COPD). In systemic microbial infections, circulating PCT increases up to several thousand-fold. In healthy individuals, serum PCT is not detectable, since the protein is not released into the blood in absence of systemic inflammation[4]. In cases of sepsis caused by bacterial infections, PCT synthesis is induced in practically all tissues and therefore, detectable in the blood. PCT synthesis is triggered by bacterial toxins, such as endotoxin and cytokines[5]. Due to cytokines released during viral infections that inhibit the production of TNF-alpha, PCT synthesis is rarely induced in viral infections[4]. Moreover, PCT has a wide biological range, a short time to induction after bacterial stimulation and a long half-life[6]. Thus, it has good discriminatory properties for the differentiation between bacterial and viral inflammations with rapidly available results.

The level of PCT may be useful in estimating the probability of severe bacterial infection[7]. PCT level rises rapidly and peaks within a very short time; moreover, if the patient responds appropriately to the treatment, the level of

PCT returns to normal range faster which makes it a reliable biomarker for monitoring progression of sepsis[8]. In general, PCT alone or in combination with other biomarkers could serve as a promising tool for understanding the prediction, cause, diagnosis, progression, regression and outcome of the treatment regimes in sepsis.

PCT is a useful tool for antimicrobial stewardship and its utilization may lead to significant reduction of unnecessary administration of antimicrobial therapy. There is increasing evidence for the use of PCT in guiding antibiotic therapy, both for initiation and for discontinuation of antibiotics. Clinical algorithms with specific PCT cut-offs in various clinical settings and patient populations are used as part of the antibiotic stewardship program. Most compelling evidence for PCT use is in adults with respiratory tract infections and in the critically ill, where randomized controlled trials (RCT) have demonstrated the safety and efficacy of PCT guided antibiotic therapy[9].

Implementation of antibiotic stewardship helps to control unnecessary antibiotic prescribing as well as to ensure the efficiency of treatment[10]. Inappropriate usage of antibiotics may lead to the development of antibiotic resistance[11]. It is obligatory to reduce 'blind' prescription of drugs to avoid the evolution of secondary infection to antibiotics and obstruct the occurrence of drug resistance. An ideal marker should assist early diagnosis and capabilities to monitor the disease progression and facilitate therapeutic decisions and interventions. PCT is a better choice, compared to other markers which satisfy these features. An algorithm based on serial measurement of PCT can reduce antibiotic exposure in septic patients[11] . Other causes of increase and decrease of PCT[12]:

1. Factors which may cause a raised PCT apart from a bacterial infection include recent major surgery, severe trauma, severe burns and prolonged cardiogenic shock. However, in the absence of infection, these patients should have decreasing PCT levels on subsequent measurements.
2. Fungal and malaria infections could raise the PCT level.
3. Medications which stimulate cytokine release such as OKT3, antilymphocyte globulins, alemtuzumab, IL-2 and granulocyte transfusion will also have an elevated PCT level.
4. Dysregulated PCT production leading to a high PCT is seen in patients with paraneoplastic syndromes due to medullary thyroid and small cell lung carcinomas.
5. Higher than normal baseline PCT levels are seen in patients with chronic kidney disease (CKD).

An early diagnosis and the initiation of an appropriate antibiotic treatment are still the cornerstones of effective sepsis care. In this respect, PCT has shown promising results for the treatment of patients with sepsis. However, it should be noted that PCT values are not intended to replace good clinical practice, but should be used as a complementary tool combined with other available clinical and diagnostic parameters. Combinational biomarkers with PCT-guided antibiotic stewardship could be properly outlined to develop a safer and affordable strategy for diagnosis of sepsis and its prognosis.

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