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Cancer stem cells and the side population

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Review Article
Cancer stem cells and the side population.

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Original Article
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Cancer stem cells and the side population

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Abstract

The cancer stem cell (CSC) theory states that only a small percentage of cancer cells have the characteristics to self renew, proliferate, differentiate and most importantly, initiate tumours. This small sub-population is able to maintain and replenish the bulk of tumours and has therefore emerged as an important therapeutic target. Up to date, putative CSCs can be identified by surface markers such as CD133, CD44+CD24– or the side population (SP) phenotype resulting from the efflux of the fluorescent dye Hoechst 33342 (Ho342) and detected by flow cytometric analysis. In this review, evidence and some characteristics of this distinct entity of SP cells which can initiate and maintain tumour cells in various cancers will be introduced and discussed in detail. Overall, our understanding of the essential properties of putative CSCs offers a gateway to understand the basic cell biology of these cells, which may culminate and translate clinically into the development of novel and more efficient cancer treatments that target the root problem of the neoplastic clone.

Keywords: Cancer, cancer stem cell (CSC), side population (SP)

Introduction

The occurrence of cancer in Australia is soaring, just as it is in the United States and other developing countries. An overview of cancer in Australia in 2008 reported that the risk of being diagnosed with cancer before age 75 was one in three and before age 85 was one in two (based on 2005 data). The number of new cases of cancer diagnosed in Australia in 2005 was 100514 and this alarmingly high figure is projected (2006–2010) to increase by 3090 annually, mainly because of the increasingly ageing population in Australia (AIHW and AACR 2008). Despite the improving technologies and introduction of new drugs, the average annual change in mortality rates for all cancers from 1999 to 2007 decreased by only 1.2%. This minor improvement does not reflect the large amount of time and money committed to the search for an efficient cancer treatment (AIHW).

The existing therapeutic approaches have been based largely on the model which predicts that every cell within the tumour is potentially tumour-initiating but that entry into the cell cycle is governed by low probability stochastic events (Reya et al 2001). However, the failure of these therapies to cure most solid cancers suggests that there may be an alternative theory: the hierarchical theory which proposes that the tumour is functionally heterogeneous and only a limited number of cells are capable of initiating the tumour, and are termed as CSC. There is growing data which support the theory that all cancers are clonal and represent the progeny of a single cell. Therefore the problem underlying efficient cancer treatment is to identify and target the cell type responsible for sustaining the growth of the neoplastic clone.

This review is presented four sections. It begins with a brief discussion of various types of normal stem cells. It will then go on to describe the evidence of CSC in various cancers. Finally it focuses on the aspect of SP cells as putative cancer stem cells. The review finishes with a discussion of the possible mechanism of transporter activities in relation to SP and some possible limitations of current CSC identification methodologies.

(A) Normal stem cells

Stem cells are defined as unspecialised cells that have the capability to self-renew and proliferate, and to differentiate to generate mature cells of a particular tissue (Bjerkvig et al 2005). In other words, stem cells have the notable potential to develop into many different cell types in the body. They serve as the repair system...
Stem cells, will be introduced briefly as below.

Categories of normal stem cells, the embryonic and adult endothelial cells. However, these cells can self-renew (e.g. haemangioblast stem cells can produce blood cells as red blood cells, and platelets) or to closely related cells (e.g. haematopoietic stem cells give rise to white blood cells, brain cells, or muscle cells). Depending on the stem cells’ level of differentiation, stem cells can be divided into totipotent stem cells, pluripotent stem cells, multipotent stem cells, or progenitor (or unipotent) cells (in order of increasing specialisation) (Wobus and Boheler 2005). Totipotent stem cells refer to a fertilised egg or the limited divided cells at the early stage of the blastocyst (up to eight-cell stage of the morula). Totipotent stem cells have the capability to grow into any type of cell in the body without exception. Pluripotent stem cells are the progeny of totipotent stem cells. These cells can also grow into all cell types from the embryonic germ cell layer (endoderm, mesoderm, and ectoderm) except the totipotent stem cells. Multipotent stem cells are able to give rise to a certain group of cells within a family (e.g. haematopoietic stem cells give rise to white blood cells, red blood cells, and platelets) or to closely related cells (e.g. haemangioblast stem cells can produce blood cells as well as endothelial cells). Progenitor (or unipotent) cells are more senescent cells and can produce only one cell type (e.g. endothelial progenitor cell can only produce endothelial cells). However, these cells can self-renew and are thus different from the mature cells. Two main categories of normal stem cells, the embryonic and adult stem cells, will be introduced briefly as below.

Embryonic stem cells are stem cells derived from a blastocyst, an early stage of embryo consisting of 50–150 cells. A blastocyst is principally formed by three structures: (i) the trophoblast, which is a monolayer of cells that form the outer coating that surrounds the blastocyst; (ii) the blastocoel, which is the hollow cavity inside the blastocyst; and (iii) the inner cell mass, a group of cells which consists of approximately 30 cells, at one end of the blastocoele. Basically embryonic stem cells are derived from the inner mass cells that are harvested from a blastocyst, and cloned in a specific culture medium and condition (Cowan et al 2004; Odorico et al 2001; Sjogren et al 2004; Talbot et al 1995). Blastocysts may be sourced from a discarded in vitro fertilised egg or can be cloned by fusing a denucleated egg cell with a patient’s cell. The isolation and maintenance of the embryonic stem cells (ES) in their pluripotency is extremely difficult. Despite the potential of the human embryonic stem (hES) cells, the ethical controversies associated with the destruction of human embryos and the clinical potential of ES cells in regenerative and reparative therapies remains a significant issue from technical, legal and ethical standpoints.

Adult stem cells can be divided into three categories (Young and Black 2004): (i) epiblast-like stem cells, (ii) germ layer lineage stem cells, and (iii) progenitor cells. Epiblast-like stem cells (ELSCs) were regarded as pluripotent stem cells (Young et al 2004) based on the inherent differentiation potential of the stem cells and the developmental nomenclature of Young and Black. An ELSC consists of a single stem cell that will form cells from all three embryonic germ layer lineages. Unlike the hES cells, ELSCs were demonstrated to remain quiescent unless stimulated by specific proliferative and/or inductive agents (Shamblott et al 1998; Thomson et al 1998). Germ layer lineage stem cells consist of the ectodermal stem cells, mesodermal stem cells and endodermal stem cells. As the names suggest, each of the stem cell groups is able to form cells that are limited to the embryonic ectodermal, mesodermal, or endodermal germ layer lineage. Progenitor cells are composed of a multipotent, tripotent, bipotent, and unipotent progenitor cells. In solid tissues these cells are located near their respective differentiated cell types. Progenitor cells are pre-programmed to commit to particular cell types and are uni-directional in their ability to form differentiated cell types (Young et al 2005).

(B) Cancer stem cells (CSCs)

Increasing evidence has revealed that many cancers, like normal organs, seem to be maintained by a hierarchical organisation that includes (i) quiescent or slow-dividing stem cells with unlimited self-renewal capability, (ii) rapidly proliferating cells (precursor cells) with limited self renewal, and (iii) differentiated or end-stage resting cells (Fig. 1). Stem cells have been reported to exist in certain kinds of tumours such as acute myeloid leukaemia (Bonnet and Dick 1997; Lapidot et al 1994), breast cancer (Al-Hajj et al 2003), pancreatic cancer (Li et al 2007), brain cancer (Singh et al 2004a; Singh et al 2003; Singh et al 2004b), colon tumours (O’Brien et al 2007; Ricci-Vitiani et al 2007), lung cancer, nasopharyngeal carcinoma etc. Some of the evidence will be discussed in the following sections.

![Cancer stem cells theory](image.png)

*Cancer Stem Cells Theory*

Cancer cells are heterogeneous and seem to be maintained by a hierarchical organisation. Only the cancer stem cell subset (CSC; yellow) has the ability to self-renew, proliferate extensively and form new tumours.
Human acute myeloid leukaemia initiating cells

Evidence of cancer stem cells was first documented in haematological malignancies. Lapidot et al (1994) demonstrated that only a minute population of human acute myeloid leukaemia (AML) cells was capable of forming new tumours in severe combined immunodeficient (SCID) mice. These cells were known as SCID leukaemia-initiating cells (SL-IC) and were exclusively CD34+CD38-. In contrast, the CD34+CD38 and CD34- fractions failed to form such tumours. Further limiting dilution analysis showed that the SL-IC in the peripheral blood of AML patients engrafted at a rate of one in 250 000 cells. A subsequent study reported three years later, using non-obese diabetic mice with severe combined immunodeficiency disease (NOD/SCID), showed that the SL-ICs could differentiate in vivo to reacquire the same leukaemic phenotype as seen in the patient while retaining extensive self-renewal capability. This provides the formal proof that the SL-IC assay detects AML stem cells (Bonnet and Dick 1997).

In the above model, the SL-ICs were always found in the CD34+CD38- subfraction. The injection of 105 CD34+CD38- cells into NOD/SCID mice resulted in 10% CD45+ human cells that mostly co-expressed CD34, CD38, and CD33, indicating that the SL-ICs were able to acquire lineage markers and/or differentiate in vivo. In addition, the engrafted human cells also retained the CD34+CD38- phenotype, demonstrating SL-ICs' capability to replenish itself, thus suggesting its capacity to self-renew. To determine whether SL-ICs have self-renewal potential, leukaemic cells were transplanted serially into secondary recipients, and results showed that all samples (n = 4) could be successfully transplanted into secondary recipients with equivalent levels of human cell engraftment. The CD34+CD38- SL-ICs were able to generate large numbers of AML colony-forming units and leukaemic blasts that expressed the same aberrant combinations of surface antigens typical of the patient sample. Taken altogether, these data provided functional evidence that the AML leukaemic clone is organised as a hierarchy in which the SL-ICs represent the most primitive leukaemic stem cell.

Human breast cancer-initiating cells

Evidence of CSCs existence in solid tumours was first reported in breast cancer. Tumourigenic breast cancer cells have been identified as lineage negative CD44+CD24-/low (lineage markers: CD2, CD3, CD10, CD16, CD18, CD31, CD64, and CD140b) (Al-Hajj et al 2003). As few as 100 cells with this phenotype were able to form tumours in NOD/SCID mice, whereas tens of thousands of cells with other phenotypes failed to form tumours. Lineage negative CD44+CD24-/low cells or lineage negative CD44+CD24- cells from primary breast cancer patients were injected into the mammary fat pads of NOD/SCID mice. Six months after injection, the injection sites were examined by histology. The lineage negative CD44+CD24-/low cells injection sites contained tumours approximately 1 cm in diameter and contained malignant cells as judged by haematoxylin and eosin-stained sections, whereas the lineage negative CD44+CD24- cells injection site contained no detectable tumours and only normal mouse mammary tissue was seen. The tumourigenic subpopulation could also be serially passaged: each time cells within this population generated new tumours they contained additional lineage negative CD44+CD24-/low tumourigenic cells as well as the phenotypically diverse mixed populations of non-tumourigenic cells as present in the initial tumour. The phenotype of the secondary tumour arising from lineage negative CD44+CD24-/low tumourigenic cells expressed heterogeneous patterns of ESA, CD44 and CD24, resembling the phenotypic complexity of the tumours from which they were derived. Within these secondary tumours, the lineage negative CD44+CD24-/low cells remained tumourigenic (even after four rounds of serial passage in mice), whereas other populations of lineage-negative cells remained non-tumourigenic. In conclusion, lineage negative CD44+CD24-/low tumourigenic breast cancer cells undergo processes analogous to self-renewal and differentiation of normal stem cells in vivo. This has proven that distinct tumourigenic breast cancer cell subpopulations do exist, and again, increases the evidence of cancer stem cells.

In a separate study, xenotransplanted human breast cancer cells bearing lineage negative CD44+CD24+/low with increased aldehyde dehydrogenase 1 activity (ALDEFLUOR®) was demonstrated to have higher tumourigenic capacity. The lineage negative CD44+CD24+/lowALDEFLUOR® cells generated tumour in vivo from as few as 20 cells but not the lineage negative CD44+CD24+/lowALDEFLUOR® cells, even when implanted in numbers of 50 000 cells per fat pad (Ginestier et al 2007). This study further supports the CSC hypothesis, but importantly, it also indicates great cellular heterogeneity within the lineage negative CD44+CD24+/low population. As this population contributes to only some but not all CSCs in breast tumour, other markers related to breast CSCs should be investigated.

Human brain tumour initiating cells

Further evidence of CSCs existence in solid tumours is in human brain tumours. CD133+ human brain tumour fraction has been demonstrated to have potent in vivo self-renewal and proliferative capacities (Singh et al 2004b). In a xenograft assay, as few as 100 CD133+ cells were sufficient to produce a tumour in NOD/SCID mice that were analysed at 12–24 weeks post injection whereas injection of 105 CD133+ cells engrafted but did not cause...
a tumour. The self renewal ability of CD133⁺ cells was demonstrated in a serial transplantation experiments. One thousand CD133⁺ cells from a primary tumour were reinjected into secondary mice. After five weeks, five out of five secondarily xenografted mice had brain tumours that recapitulated the phenotype of the original patient tumour and primary xenograft. However, none of the mice injected with CD133⁺ tumour cells developed brain tumours when analysed at 12 weeks post-injection. Interphase FISH analysis of murine brain sections using species-specific centromeric probes was carried out and human cells were detected in small clusters near the original injection site. This indicated that CD133⁻ cells were engrafted but were not able to form tumours in mouse brain owing to their inability to be adequately supported in the brain environment after transplantation. The CD133⁺ fraction ranged from 19 to 29% (n = 6) and 16–21% (n = 3) in uncultured glioblastomas (including two corresponding tumour xenografts before serial retransplantation) and medulloblastomas respectively as quantified by flow cytometry.

**Clonogenic multiple myeloma cells**

Matsui et al (2004) have identified CD138⁻CD34⁻ subset as the clonogenic multiple myeloma (MM) cells. Further characterisation revealed that these cells expressed the B cell antigens, CD19 and CD20. CD138⁺CD34⁻ cells from all 24 MM patients tested were unable to form colonies in vitro in a colony formation assay, while CD138⁻CD34⁻ cells generated colonies that consisted of morphologically mature plasma cells by two to three weeks. When CD138⁻CD34⁻ or CD138⁺CD34⁻ BM cells from four MM patients were injected intravenously into NOD/SCID mice, engraftment of human cells was not detected in any of the mice injected with CD138⁻ cells. However, CD138⁻ cells from a single patient engrafted in three of three animals. Engraftment was demonstrated by the presence of human CD138⁺ cells with light-chain restriction matching the original patient sample within the murine bone marrow. Once again, this shows the existence of a subpopulation of cells within myeloma cancer cells which have the capability to proliferate, and differentiate in vivo.

In recent years, increasing reports have shown that a subset within the bulk of cancer cells can be distinguished via Hoechst 33342 (Ho342) staining. This method appears to offer a promising marker to isolate stem cells from various sources. The subset is now identified as “side population (SP)”. Cancerous SP is highly enriched for tumour-initiating cells that show similar properties owned by the CSCs. Matsui et al (2008) have demonstrated that the SP cells in myeloma were almost exclusively CD138⁻ (97%) compared with the bulk of the population that was CD138⁺. In line with this report, our laboratory reported that SP cells were identified in 85.7% (n = 27) of bone marrow samples of patients with myeloma and that the majority of these bone marrow SP cells were CD138⁺ (Loh et al 2008). This suggests that SP identification method is able to enrich for the CD138⁺ clonogenic MM cells. SP cells also have an extensive capacity to proliferate, differentiate, and self-renew. This is discussed further below.

**(C) Side population: alternate CSC identification method**

![Figure 2](image)

**Figure 2**

**SP phenotype in bone marrow samples from patients with myeloma**

SP cells were identified in 18 out of 21 BM samples from patients with myeloma (A) Patient with highest SP percentage (4.9%). (B) Patient with median SP percentage (0.7%). (C) Patient with lowest SP percentage (0.06%). Left panel: Non-treated cells. Right panel: Cells were treated with 200 μM of R(+)-verapamil (Sigma) prior to Ho342 staining (Loh et al 2008).

**Origin of SP**

SP cells were originally identified in murine bone marrow and have been defined by Goodell et al as a population of cells detected by flow cytometry that has the capability to efflux the fluorescent dye, Ho342 (Goodell et al 1996). Figure 2 depicts the SP phenotype detected in bone marrow samples from patients with myeloma.
(Loh et al 2008). The SP scattergram portrays a distinct staining pattern based on fluorescence-activated flow cytometric analysis due to the dye efflux activity that may be mediated by ATP-binding cassette (ABC) transporters, such as p-glycoprotein or the breast cancer resistant protein (BCRP1). The fact that SP is highly enriched for normal stem cells has also been verified with reconstitution studies in lethally irradiated mice. Bone marrow-derived SP cells, excluding the non-SP (NSP) compartment, not only demonstrated potent haematopoietic engraftment in mice but also had the potential for plasticity (Gussoni et al 1999; Jackson et al 2001; Uchida et al 2001). Evidence of cancerous SP cells to initiate tumour in vivo has also been shown in many human cancers such as AML (Wulf et al 2001), breast cancer (Patrawala et al 2005; Zhou et al 2007), glioblastoma, prostate cancer (Patrawala et al 2005), nasopharyngeal carcinoma (Wang et al 2007), and lung cancer (Ho et al 2007). A few of these examples are given in more detail in the following sections.

**Evidence that SP is enriched with tumour-initiating cells**

**Human AML**

In 2001 the leukaemic stem cell in AML was revisited by Wulf et al (2001). It was reported that more than 80% of 61 bone marrow samples from patients with AML contain a distinct SP. Four out of 10 leukaemic blood samples also contain SP. In an in vivo study Wulf et al transplanted an average of 340 CD34+ SP cells (range: 3.8 x 10^4 to 1 x 10^5) per NOD/SCID mouse and successfully resulted in engraftment of AML-like disease in three of 28 the recipient NOD/SCID mice, each resulting in growth of high number of AML cells (1 x 10^4 to 1 x 10^5). Low but persistent numbers of leukaemic SP cells were detected by immunocytology and polymerase chain reaction (PCR) assay in half of the remaining mice. Interestingly, in contrast to previous report (Bonnet and Dick 1997), these cells were predominantly CD34low/−.

**Human breast cancer**

SP cells obtained from MCF7 human breast cancer cell line has been shown to be significantly more tumourigenic than the NSP cells (Zhou et al 2007). A total of 2000, 20 000, and 200 000 SP and NSP cells were inoculated into NOD/SCID mice with oestrogen supplement. SP cells gave rise to tumours in seven of 10, 11 of 12, and seven of eight mice respectively, whereas the NSP cells gave rise to smaller tumours only in two of 10, three of 12, and three of eight mice. Histological analysis indicates that the SP cell-derived tumours showed typical pathological features of cancer with many poorly differentiated round cells, whereas NSP cell-derived tumours had a different pathology profile with more differentiated cells and fewer poorly differentiated cells.

**Human glioblastoma carcinoma**

Patrawala et al (2005) reported that one thousand cells from U373 human glioblastoma cell line when injected into NOD/SCID mice, gave rise to a prominent tumour within about one month. The tumour histological analysis revealed palisade-like structures and was highly vascularised, resembling clinical samples. By contrast, inoculation of 50 000 NSP cells yielded no tumour even after seven months of observation. This was also the first report that documented SP cells’ capability to serially transplant tumour in recipient mice. One hundred SP cells obtained from an SP-derived tumour in the same study generated tumours with ~ 60% (seven of 11) efficiency, whereas 200 000 of the NSP counterpart was unable to form any tumour. This suggests that SP cells possess self-renewal capacity in vivo and are probably > 200 times more tumourigenic than the NSP cells.

**Human lung cancer**

Ho et al demonstrated that SP exists in six of six human lung cancer cell lines ranging from 1.5% to 6.1% of total viable cell; and in 16 of 16 clinical samples obtained from surgical resections from lung cancer patients, ranging from 0.03% to 1.12% of total viable cells (2007). SP cells from H460, A549, and H441 human lung cancer cell lines when subcutaneously injected into NOD/SCID mice generated tumours. The NSP cells of these cell lines also generated tumours but at a delayed time point and only when a higher number of cells (versus SP) were inoculated into the mice. For example, 1000, 5000, and 50 000 A549-SP cells generated tumours two of four, three of four, and four of four in recipient mice, whereas only zero of four and three of four mice gave rise to tumours when 5000 and 50 000 A549-NSP cells were inoculated. A similar trend of tumour engraftment was seen using the H460 and H441 cell lines.

**Important features of SP**

Overall, studies of SP cells have collectively demonstrated the maintenance and tumourigenic potential in a few cancer cell lines. Although SP phenotype should possibly not be considered as a sole marker to identify putative stem cell populations, the assessment of SP is indisputably important and useful. Identification and characterisation of SP in various cancers may provide us with more information regarding the etiology and the tumourigenicity of cancer stem cells. A few important features of SP cells are discussed in detail below.
Higher level of ABC transporter expression

The ability of SP to efficiently expel Ho342 dye has been attributed to higher expression of ATP-binding cassette (ABC) transporter expression in SP compared to the NSP cells, and suggests a mechanism by which such cells could escape the lethal effects of anti-cancer drugs. The family of ATP-binding cassette (ABC) transporters includes a number of members that are located in the plasma membrane and mediate the ATP-dependent efflux of endogenous and xenobiotic substances (Dean et al 2005). Based on the amino acid sequence similarity and phylogeny, the 48 known human ABC transporters are grouped into seven subfamilies designated A through G (Dean and Annilo 2005). Members of the ABC transporter superfamily participate in diverse cellular processes, including drug resistance, drug metabolism, transport of lipids and organic anions, and iron metabolism. The ABC transporter superfamily is also associated with multidrug resistance and exhibits overlapping substrate specificities.

The greater efflux activity in SP was initially reported due to higher activity of multidrug resistance protein 1 (MDR1), also known as P-gp or ABCB1, the first member of the ABC subfamily B (Goodell et al 1996). It was verified that SP was specifically eliminated when bone marrow cells were stained with Ho342 in the presence of the verapamil, a drug and an inhibitor of MDR1 (Asakura et al 2002; Benchaouir et al 2004; Gussoni et al 1999; Uchida et al 2001). However the breast cancer-resistant protein, BCRP1, also known as ABCG2, was subsequently reported to be more closely related to the SP phenotype (Zhou et al 2001). ABCG2, like the MDR1, is also a member of the ABC transporter family (Allikmets et al 1998; Miyake et al 1999) and is likewise expressed on the cell surface (Rocchi et al 2000; Scheffer et al 2000).

In 2001, an MDR1-knockout mice (Mdr1a/1b−/−) model was reported to have normal numbers of SP cells as the wild type, suggesting that the SP phenotype is clearly distinct from that of the MDR efflux system (Zhou et al 2001). ABCG2 gene was detected to be highly expressed in the normal and Mdr1a/1b−/− mice. The expression of ABCG2 was also detected in SP cells obtained from murine skeletal muscle, murine embryonic stem cells and rhesus monkey bone marrow. This suggested that the ABCG2 expression is conserved in different types of SP cells. Using a monoclonal antibody that recognises an external epitope of ABCG2 on living cells, ABCG2 expression was shown to be strongly correlated to the SP phenotype. In the NSP subset, 17% of cells showed relatively low levels of ABCG2 expression. In contrast, 48% and 91% of cells were positive in middle and distal portions of the SP region, respectively. Enforced expression of the ABCG2 cDNA directly conferred the SP phenotype in bone marrow cells and caused a reduction in maturing progeny both in vitro and in transplantation-based assays.

In spite of that, in Mdr1a/1b−/− Bcrp1 triple knockout mice, some SP cells in the bone marrow were still retained. This suggests that ABCG2 is not the sole drug efflux pump that is responsible for the SP phenotype (Jonker et al 2005). Taken altogether, the Ho342 efflux mechanism of SP cells has yet to be fully elucidated. It was suggested that either redundancy in transporter function and/or the mechanism by which the SP phenotype is determined, is not solely conferred through the expression of ABC transporter proteins (Wu and Alman 2008).

SP cells are radiation and drug resistant

SP cells have been shown to be chemoresistant. Mitoxantrone and daunorubicin are cytotoxic drugs that are commonly used as a treatment for some types of cancer. The reactivity of SP towards these cytotoxic drugs has been studied. Mononuclear bone marrow cells from AML patients were simultaneously incubated with Ho342 and either daunorubicin or mitoxantrone. The efflux of drug from the gated SP and NSP cells was measured by its specific emission fluorescence. Comparison of the emission profiles of SP versus the main population MP cells demonstrated greater efficiency of cytostatic drug efflux from SP cells (Wulf et al 2001). In a separate study, in a clonal growth assay, both sorted SP and sorted NSP cells from JF human neuroblastoma line formed colonies by day 14 in the absence of mitoxantrone. However, no colonies from the NSP cells were formed when cells were cultured in the presence of 1 ng/ml mitoxantrone, while the number of colonies formed by the SP cells remained unchanged. These studies illustrate that, in the presence of mitoxantrone, the drug efflux capacity of SP cells contributes to their survival advantage over NSP cells (Hirschmann-Jax et al 2004). In clinical samples Loh et al (2008) showed that there was no statistically significant difference in the SP % in bone marrow samples of treated versus non-treated patients with myeloma indicating that the SP cells are resistance to conventional drug treatment for myeloma.

SP cells have also been demonstrated to be resistant to radiology treatments. SP and NSP cells from CNE-2 human nasopharyngeal were cultured for two weeks after exposure to X-ray at 2 Gy. There were significant differences in the numbers of clones generated between these two subsets. The colony forming efficiency of SP cells was not significantly different before and after radiation (P > 0.05); however, there were significant differences between NSP cells before and after radiation (P < 0.05) (Wang et al 2007). Together this demonstrates the chemoresistant and radio-resistant capability of SP cells compared the NSP counterpart.
SP cells are more tumourigenic than NSP cells

In vitro

Growth characteristics of SP and NSP cells from cancer cell lines have been compared. Equal numbers of sorted SP and sorted NSP cells were cultured in vitro. The expansion rate of each subset, measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, or [H3]thymidine incorporation assay revealed that SP cells have a higher proliferative rate than the NSP cells (Hirschmann-Jax et al 2004; Wang et al 2007). SP cells have also been reported to have higher colony-formation ability. Inoculation of equal numbers of SP and NSP cells in soft agar demonstrated that SP cells consistently formed a higher number of colonies than the NSP cells. SP cells also have the capability to divide asymmetrically generating both SP and NSP population more efficiently than the NSP cells (Hirschmann-Jax et al 2004; Ho et al 2007; Wang et al 2007) suggesting the differentiation ability of SP cells. Taken together these findings demonstrated by SP cells are more tumourigenic than the NSP cells.

(D) Limitations of current CSC identification methodologies

Limitations of using surface markers to identify

Although there has been increasing evidence of the presence of CSC, the problems associated with identification, quantitation, and stable or transient state of CSC remains. Cell surface markers are commonly used to identify the subpopulation cells of interest such as the examples given above. The surface markers are characterised by their molecular ‘cluster of designation’ (CD), which represents a number given to a specific cluster of molecules. The expression of these CD markers represents the activation of specific set of genes for such molecules on the cell surface to carry out the biological functions required for the survival and function of this specific cell.

The problem is that for any evolving and differentiating stem cells, in response to various signals from their micro-environment, the gene expressions of such cells change and cell surface markers evolve. Simply culturing and plating to expand the cell population had been shown to alter their RNA and protein markers in embryonic stem cells (Clark et al 2004). Therefore the evolving CD surface markers results in confusion and may be playing a significant role for the controversies in stem cell biology. One example of this controversy is the CD138– phenotype in clonogenic myeloma cells reported by Matsui et al was challenged by another report that successfully xenografted CD138– myeloma plasma cells into SCID mice implanted with rabbit bone fragments (Yata and Yaccoby 2004). This demonstrated that the selection of putative myeloma cancer stem cells based on CD138–CD34– surface marker is questionable.

Limitations of using SP to identify CSC

Despite the increasing popularity of using SP to identify putative tumour initiating cells, there remains many problems associated with this method. One major problem is the poor reproducibility of SP quantitation that differs greatly due to the variations in experimental parameters. Because efflux of a dye is a dynamic process, slight variations in cell counting, Hoechst concentration, staining time, temperature, and stringency in selection of SP cells by FACS gating can dramatically affect the viability, homogeneity and apparent yield of SP cells (Montanaro et al 2004). In addition, although SP fraction could greatly enrich for cells with ‘stem cell’ activity, the purified cell population are still heterogeneous. It remains difficult to separate the most primitive cell compartment from closely related progenitors. Finally, the phenomenon where not all cell lines contain SP (Mitsutake et al 2007) indicates that tumourigenicity is therefore unlikely to be solely dependent on their presence. For that reason a purification method using various cell surface markers in combination with optimised SP identification suggested by Sales-Pardo (Sales-Pardo et al 2006) is proposed to achieve a higher enrichment in the future.

CSC controversies

In cancer research, although the CSC concept is highly plausible, there remain many unresolved issues surrounding CSC that should be acknowledged and will be briefly discussed here. Firstly, the question of whether there is a stem cell at all is one of the many ongoing controversies in translational research. Current evidence of CSC has been based on tumour formation after injecting human tumour cells into an experimental animal. However, animals could not provide a complete model of human disease therefore the true clinical relevance of the CSC in human is yet to be revealed. Besides, there is a possibility that the rare CSCs may be inadvertently selected during xenotransplantation, whereas the majority of non-CSCs are incapable of surviving in their foreign environment owing to the lack of appropriate supporting factors. Secondly, the fact that certain cells do not initiate tumours in mice may only reflect the limited robustness of the mouse model used for assessing tumourigenic potential. A good example is a recent study comparing the growth of melanoma cells from patients in NOD/SCID and more immunocompromised NOD/SCID interleukin-2 receptor gamma chain null (NOG) mice. Although one in 100 000 unselected melanoma cells initiated tumours in NOD/SCID mice, as few as one in four melanoma cells were tumourigenic when transplanted into NOG mice (Quintana et al 2008). Thirdly, disparate findings surrounding the exact phenotype and biology of CSC with the capacity to initiate tumour remains. For
example in multiple myeloma, when CD138–CD34+ cells from the BM of MM patients were injected intravenously into NOD/SCID mice, engraftment of human cells was only detected in mice injected with CD138– cells but not in mice injected with CD138+ cells. (Matsui et al 2004). In contrast to this report, CD138+ MM plasma cells have been reported previously to successfully xenografted into SCID mice implanted with rabbit bone fragments (Yata et al 2004).

Conclusion

The definition and identification of stem cells is constantly evolving and there is no current consensus or a gold standard assay to isolate or identify stem cells. Despite the many controversies discussed above, it is still highly plausible that CSCs exist and are enriched in a distinct sub-population of cancer cells, based on their ability to reconstitute the original tumour after transplantation in immunodeficient mice. Therefore, finding the source of cancer cells and chemotherapy that targets the residual chemo-resistant CSC will be crucial for successful cancer treatments. In addition, understanding the basic scientific aspects of the cell biology of CSC may culminate into the development of novel efficient cancer treatment that targets the root problem of the neoplastic clone caused by CSC.

Acknowledgements

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References


Evaluation of the Sysmex pocH-100i analyser for use in remote laboratory settings

Craig Williams
Haematology Department, Sullivan Nicolaides Pathology, Brisbane

Abstract

The pocH-100i (Sysmex Corporation, Kobe, Japan) is a fully automated haematology analyser with the ability to perform a full blood count with nineteen parameters, including a three part differential which includes neutrophils. It has a compact design with a view to point-of-care testing. The pocH-100i was compared to the XE2100 analyser (Sysmex Corporation, Kobe, Japan). The evaluation included basic operational characteristics of the analyser to ensure non-scientific staff could process specimens in the absence of scientific staff without difficulty. This would cover those situations in remote locations where scientific staff are absent at the time of receipt of a stat specimen. Non-scientific staff would be able to process specimens allowing scientific staff in a core laboratory to review and release preliminary results using defined criteria. The pocH-100i showed good correlation for all parameters, including for haemoglobin ($r^2 = 0.9934$), neutrophils ($r^2 = 0.9648$) and platelets ($r^2 = 0.9860$), making it an ideal analyser for a small volume laboratory, or as a backup analyser for a small to medium sized laboratory. The pocH-100i also proved to be easy to use, confirming its suitability for use by non-scientific staff in a remote laboratory.

Keywords:pocH-100i, automated haematology analyser, three part differential, neutrophils

Introduction

The Sysmex pocH-100i analyser (Sysmex Corporation, Kobe, Japan) is a compact, fully automated haematology analyser designed for easy operation. It consists of a single analytical unit housing all components. The analyser footprint is only 185 mm wide, 350 mm in height and 460 mm deep, and it weighs < 15kg. A liquid crystal touch screen displays results and troubleshooting information.

Analysis is available in whole blood mode on a number of different collection tube sizes and a pre-dilute mode (although it should be noted that the three part differential is not available in pre-dilute mode). The volume of sample required is 15 μL in whole blood mode (although a dead volume of 1mL is stated for a 13 mm diameter collection tube, and 500 μL for a paediatric collection tube), meaning the pocH-100i analyser is suitable for small sample volumes. Throughput is approximately 25 samples per hour.

The pocH-100i performs analysis of nineteen parameters, including haemoglobin (HB), red cell count (RBC), white cell count (WBC), platelets (PLT) and a three part differential comprising neutrophils (NEUT), lymphocytes (LYM) and mixed (MXD). The mixed count consists of monocytes, eosinophils and basophils.

WBC is obtained by the direct current (DC) detection method following red cell lysis. A white blood cell histogram is created and discriminators used to measure the WBC. To determine the three part differential, the histogram is used by dividing it into small, middle, and large sized cells. Two distinct troughs are identified on a normal histogram: T1 and T2. Lymphocytes are located between the lower WBC discriminator and T1, the mixed cell population is located between T1 and T2, and neutrophils are located beyond T2.

HB is measured photometrically on this same lysed sample as the WBC. A non-cyanide method is employed.

RBC and PLT are measured by a hydrodynamic focusing DC detection method. Red blood cells and platelets are plotted on histograms, and discriminators used to determine each parameter. The other red cell and platelet parameters are derived from the histogram or calculated.

A flagging system exists on the pocH-100i, based on both numerical limits (eg. linearity) and the identification...
of abnormal populations on the white cell, red cell or platelet histograms. Table 1 details those flags encountered during our evaluation.

Table 1
Instrument generated flags on the pocH-100i analyser encountered during evaluation*

<table>
<thead>
<tr>
<th>Flag</th>
<th>Possible Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>!</td>
<td>Value is outside the linearity limit</td>
</tr>
<tr>
<td>*</td>
<td>Result exceeds upper result limit</td>
</tr>
<tr>
<td>-</td>
<td>Result exceeds lower result limit</td>
</tr>
<tr>
<td>*</td>
<td>Result is unreliable</td>
</tr>
<tr>
<td>**<em>,</em></td>
<td>Result could not be calculated because of analysis error</td>
</tr>
<tr>
<td>---,*</td>
<td>Result could not be calculated because of data error</td>
</tr>
</tbody>
</table>

WL: Error at the lower discriminator for WBC
Incomplete lysing of red blood cells, presence of NRBCs, presence of large platelets, platelet aggregates, fibrin or lipids

RL: Error at the lower discriminator for RBC
Presence of fragmented red blood cells, large platelets, platelet aggregates, micro red blood cells

PU: Error at the upper discriminator for PLT
Presence of large platelets, fragmented red blood cells, platelet aggregates, micro red blood cells

T2: Trough discriminator T2 could not be determined
CML, immature granulocytes, aged specimens, incomplete lysing of red blood cells

*WBC: white blood cells; RBC: red blood cells; PLT: platelets; NRBC: nucleated red blood cells; CML: chronic myeloid leukaemia.

Results are displayed on the LCD screen and can also be printed or sent to the laboratory’s host computer. The pocH-100i also has the capacity to store results of up to 20 samples. Data is preserved after the analyser has been switched off and only lost on a first in, first out basis when there are already 20 samples stored.

The pocH-100i requires only two reagents for general use. The pocH-pack D is a diluent for DC detection and absorbance analysis of whole blood. The pocH-pack L lyzes red blood cells, allowing for white blood cell counting by the DC detection method and haemoglobin by a photometric measurement. A third reagent, Cellclean, is a strong alkaline detergent that is used as part of preventative maintenance procedures.

Quality control is achieved using a tri-level (low, normal and high) set of controls called EIGHTCHECK-3WP. All parameters are controlled, and up to 60 data points are stored.

The pocH-100i was evaluated for correlation in comparison to the Sysmex XE2100 analyser (Sysmex Corporation, Kobe, Japan). Also evaluated were carryover, within-run precision (reproducibility) and linearity. As the aim of our study was to evaluate the pocH-100i as an analyser that could be used in after-hours settings by non-scientific staff, the evaluation also included basic operational characteristics.

Materials and methods

Residual specimen from patients presenting for full blood count analysis on the day of the evaluation were used. All were collected into 4.0 mL BD Vacutainer tubes containing 7.2 mg of K₂EDTA (Beckton Dickinson, Franklin Lakes, NJ). Specimens were analysed within four hours of collection.

Correlation studies between the pocH-100i and the XE2100 analyser were performed by analysing a total of 70 samples on each analyser. Approximately 75% of the samples were obtained from healthy individuals. The remaining samples were a mix of hospital inpatients and oncology patients. Included were samples with high white cell count, cold agglutinins, nucleated red blood cells (NRBC), chronic lymphocytic leukaemia (CLL), immature granulocytes, marked pancytopenia and thalassaemia to assess performance of significantly abnormal specimens that could be expected to be seen in a clinical laboratory.

The WBC, RBC, HB, HCT, mean corpuscular volume (MCV), PLT, LYM%, MXD% and NEUT% parameters were determined on both analysers. As MXD% is not measured on the XE2100, for the purpose of this evaluation, the sum of the MONO%, EO% and BASO% was used.

Within-run precision was determined by running a normal specimen 10 times on the pocH-100i. The mean and coefficient of variation (CV) was established for WBC, RBC, HB, HCT, MCV, PLT, LYM%, MXD% and NEUT%.

Carryover for WBC and PLT was assessed by running a markedly abnormal specimen (WBC = 205 x 10⁹/L, PLT = 1040 x 10⁹/L) in duplicate followed by a markedly pancytopenic patient (WBC = 0.1 x 10⁹/L, PLT = 5 x 10⁹/L) in triplicate.

Linearity studies for WBC were performed using dilutions of a markedly abnormal specimen. Stability studies were not performed as they were not considered relevant within the scope of our evaluation.

Results

As shown in Table 2, the pocH-100i analyser showed excellent correlation to the XE2100 over a wide range of results, confirming the studies of Briggs et al (2003), Whisler and Dahlgren (2005) and Gopal et al (2005). The parameters WBC, RBC, HB, HCT, MCV, PLT, LYM%, MXD% and NEUT% all demonstrated an r² of greater than 0.95. MXD% did not achieve this level of correlation (r² = 0.5815), which was in line with the findings of Whisler.
This reduced correlation is to be expected, as the MXD% is a smaller population of cells compared to the NEUT% and LYM%. Poor correlation for monocytes, which would make up at least 50% of the MXD population in a healthy individual, has been well documented in automated haematology analysers in several studies, including those by Briggs et al. (1999), Kern (2001) and Johnson et al. (2002), which could also, in some part, account for the level of correlation seen in our evaluation.

Table 2
Results of blood count and automated differential comparability study, XE2100 versus pocH-100i analysers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>( r^2 )</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC x 10^9/L</td>
<td>0.1–153.8</td>
<td>0.9943</td>
<td>0.9923</td>
<td>0.0650</td>
</tr>
<tr>
<td>RBC x 10^12/L</td>
<td>3.02–7.91</td>
<td>0.9928</td>
<td>1.0275</td>
<td>-0.0328</td>
</tr>
<tr>
<td>HB g/L</td>
<td>42–235</td>
<td>0.9934</td>
<td>0.9655</td>
<td>2.7274</td>
</tr>
<tr>
<td>HCT %</td>
<td>0.205–0.697</td>
<td>0.9760</td>
<td>1.0100</td>
<td>0.0060</td>
</tr>
<tr>
<td>MCV fl</td>
<td>47.1–133.2</td>
<td>0.9934</td>
<td>0.9416</td>
<td>5.5125</td>
</tr>
<tr>
<td>PLT x 10^12/L</td>
<td>8–864</td>
<td>0.9860</td>
<td>1.0386</td>
<td>-8.6734</td>
</tr>
<tr>
<td>LYM %</td>
<td>6.2–59.8</td>
<td>0.9823</td>
<td>0.9824</td>
<td>-0.0024</td>
</tr>
<tr>
<td>MXD %</td>
<td>1.0–21.4</td>
<td>0.5815</td>
<td>0.6622</td>
<td>0.8773</td>
</tr>
<tr>
<td>NEUT %</td>
<td>0.5–88.9</td>
<td>0.9648</td>
<td>1.0580</td>
<td>-0.0541</td>
</tr>
</tbody>
</table>

*WBC: white blood cells; RBC: red blood cells; HB: haemoglobin; HCT: haematocrit; MCV: mean corpuscular volume; PLT: platelets; LYM: lymphocytes; MXD: mixed cells (monocytes, eosinophils, and basophils); NEUT: Neutrophils.

Johnson et al. (2002) and Kang et al. (2007) have shown good correlation between the XE2100 analyser and the analysers of other manufacturers, suggesting that the good correlation shown between the pocH-100i and the XE2100 would be transferable across these platforms.

Table 3
Within-Run Precision for pocH-100i

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average</th>
<th>Carryover %</th>
<th>Manufacturers Claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC x 10^9/L</td>
<td>7.6</td>
<td>7.1</td>
<td>&lt;3.5%</td>
</tr>
<tr>
<td>RBC x 10^12/L</td>
<td>4.68</td>
<td>24.0</td>
<td>&lt;3.5%</td>
</tr>
<tr>
<td>HB g/L</td>
<td>139</td>
<td>7.1</td>
<td>&lt;3.5%</td>
</tr>
<tr>
<td>HCT</td>
<td>0.42</td>
<td>68.86</td>
<td>&lt;3.5%</td>
</tr>
<tr>
<td>MCV fl</td>
<td>89.7</td>
<td>24.0</td>
<td>&lt;3.5%</td>
</tr>
<tr>
<td>PLT x 10^12/L</td>
<td>244</td>
<td>7.1</td>
<td>&lt;3.5%</td>
</tr>
<tr>
<td>LYM %</td>
<td>24.0</td>
<td>68.86</td>
<td>&lt;3.5%</td>
</tr>
<tr>
<td>MXD %</td>
<td>7.1</td>
<td>24.0</td>
<td>&lt;3.5%</td>
</tr>
<tr>
<td>NEUT %</td>
<td>68.86</td>
<td>7.1</td>
<td>&lt;3.5%</td>
</tr>
</tbody>
</table>

*WBC: white blood cells; RBC: red blood cells; HB: haemoglobin; HCT: haematocrit; MCV: mean corpuscular volume; PLT: platelets; LYM: lymphocytes; MXD: mixed cells (monocytes, eosinophils, and basophils); NEUT: Neutrophils.

Table 4
Carryover for pocH-100i

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Average</th>
<th>Carryover %</th>
<th>Manufacturers Claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC x 10^9/L</td>
<td>205</td>
<td>0.1</td>
<td>&lt;3.5%</td>
</tr>
<tr>
<td>PLT x 10^12/L</td>
<td>1040</td>
<td>0.8</td>
<td>&lt;5.0%</td>
</tr>
</tbody>
</table>

*WBC: white blood cells; PLT: platelets.

Within run precision (Table 3) and carryover (Table 4) were both within the manufacturers specifications. Linearity for the parameter studied, WBC, confirmed the manufacturers stated specifications (Figure 1).

The minimum volume required to process a sample was compared to the manufacturers stated volume. Using 13mm EDTA tube, we were able to process samples with as little as 700uL of blood. The paediatric tube insert was unavailable at the time of the evaluation to assess the stated minimum volume on a paediatric tube.

Results of samples from several commonly encountered conditions were scrutinised. In general, the pocH-100i handled these specimens satisfactorily. A thalassaemia specimen with a significantly reduced MCV (47 fl) correlated well for MCV between the pocH-100i and XE2100. Although the analyser flagged RL and PU (Table 1), the RBC and PLT also correlated well, showing no obvious interference at this low MCV. Cold agglutinin specimens were easily identified by an increased MCHC, and showed correction upon warming in line with results obtained on the XE2100. Results for several patients on chemotherapy with markedly reduced white cell count (< 0.5 x 10^9/L) and platelets (< 20 x 10^9/L) showed good correlation at this lower result range. Samples with WBC > 50 x 10^9/L showed good correlation to the XE2100, and the correlation for the HB on those samples with WBC > 100 x 10^9/L was also good, indicating no significant interference for HB at this significant WBC. Chronic lymphocytic leukaemia (CLL) samples showed good correlation, however a complete differential was not always obtained on both analysers. Samples with significant numbers of immature granulocytes also showed good correlation. The WBC obtained on the pocH-100i for samples with a significant number (> 15%) of nucleated red blood cells (NRBC) was
consistently lower (approximately 10%) than the WBC obtained from the XE2100.

![WBC Linearity](image)

**Figure 1**

Linearity for WBC for pocH-100i

*WBC: white blood cells.*

Of the 50 normal samples, the pocH-100i gave an incomplete differential on four occasions (all generated a T2 flag). No cause was identified, and specimens were not rerun to confirm. The XE2100 was able to perform a differential on all of these samples. Of the 25 abnormal samples included in the evaluation, the pocH-100i was unable to perform a complete differential on ten samples, of which seven were chemotherapy patients with a WBC <0.5 x10^9/L. The remaining three samples contained either a high number of NRBC (two – both flagged WL) or significant lymphocytosis in CLL (one – flagged T2). One sample with significant numbers of immature granulocytes did not produce any flagging. The XE2100 was unable to perform a complete differential on four samples from this abnormal group. Overall, the pocH-100i did not perform as well as the XE2100 in relation to the frequency that a complete differential was obtained and in flag generation, but this is to be expected as the technology of this analyser is not equivalent to the technology of large haematology analysers like the XE2100.

The basic operational characteristics of the pocH-100i were also assessed by two staff with differing levels of experience. The simplicity of the analyser meant both were able to process specimens with no formal training, relying solely on the operational manual and on screen instructions. In fact, the on screen instructions were informative such that the operational manual was rarely required during the evaluation. Analysis was a simple process of inputting the sample’s laboratory ID, either via the touch screen keypad or barcode reader, selecting the correct tube insert for the tube type (all conveniently colour coded), mixing the sample, inserting into the analyser and closing the door. The process of analysing quality control samples was equally simple, and failed QC runs were sufficiently highlighted at the end of the analysis process. Errors encountered during analysis were identified by an audible alarm, and the on screen troubleshooting that accompanied each error was sufficient to assist us through the remedial action required without the need to consult the operational manual. It should be noted that only minor troubleshooting issues such as reagent replacement were encountered during our evaluation. Preventative maintenance is minimal. A daily shutdown process is required to clean chambers and sample lines. This process is activated from the touch screen and requires nothing more than selecting the shutdown sequence. Other scheduled maintenance is basic and recommended at two weekly intervals (or every 150 samples) and three monthly intervals (or every 1500 samples). A number of as needed troubleshooting procedures are also detailed in the manual.

**Conclusion**

Our evaluation of the Sysmex pocH-100i analyser demonstrates the potential of this analyser to perform an accurate full blood count over a wide range of results, confirming the findings of previous evaluations. It met or exceeded instrument specifications for correlation, carryover, reproducibility, and linearity for all parameters evaluated, including the three part differential. This would allow the pocH-100i to function as a reliable analyser for a low volume laboratory, or backup analyser in a small to medium sized laboratory.

No technical difficulties were encountered during the evaluation. Staff were able to process specimens with no formal training on the analyser due to its ease of operation, excellent operational manual and informative on screen help.

While not a handheld point-of-care device, the compact design, with minimal reagents, makes the analyser transportable, either on a trolley within a laboratory or into a hospital ward in an after hours setting, or with minimal packaging between laboratories.

One major advantage of the pocH-100i over the majority of other compact haematology analysers currently available in Australia is its ability to accurately determine the parameters that most clinicians would require in a critical afterhours scenario: haemoglobin, neutrophils and platelets. A number of other compact analysers which produce a differential report a granulocyte count, consisting of neutrophils, eosinophils and basophils rather than reporting an individual neutrophil count.

All of these features made the pocH-100i analyser ideal for non-scientific staff in a remote laboratory processing specimens. In some remote regional settings, where there are limited scientific staff, this could become a very important process to reduce the fatigue that scientific staff in these areas may encounter due to the demands of after-hours testing. Conceivably, collection staff or laboratory assistants could collect and then process full blood count specimens after hours with little difficulty on the pocH-100i, without the need for scientific staff to
be present. Results, including any flags generated, could upload into the laboratory information system, allowing staff in a core laboratory to review and either release a report, or suggest further action as appropriate, including the requirement for an on-call scientist to be called in to review a blood film.

Beyond these routine roles for the pocH-100i, Briggs et al (2007) have even demonstrated extended possibilities for the analyser by evaluating it in the measurement of CD4+ T cells.

Acknowledgements

Thank you to Anneke Vanderham who assisted in processing of specimens on the pocH-100i and XE2100 analysers.

References


Microscopic Haematology 3e

a practical guide for the laboratory

Gillian Rozenberg, Senior Medical Scientist, Department of Haematology, SEALS Randwick, Prince of Wales Hospital, Sydney, NSW

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The platelet section follows on and adheres to the same format.

There is a section on haematology relating to paediatrics which describes red cell, white cell and platelet disorders occurring in cord blood, the neonate and childhood.

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Applicants would be expected to have some basic knowledge and the scholarship is particularly suitable for members who either do not have resources for continuing education available to them or have a need for retraining due to rationalisation or multiskilling in their workplace.

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☐ My resume detailing my place of work, qualifications and employment history is enclosed.

In 50 words or less, please explain why you believe you should receive the scholarship:
_________________________________________________________
_________________________________________________________
_________________________________________________________
_________________________________________________________
_________________________________________________________
A six months old child with Down syndrome presented to the Paediatric Casualty Department with severe pallor. She had a past history of having had an atrioventricular septal defect (AVSD) repair at two months of age. A full blood count and reticulocyte count were performed with the following results:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/L)</td>
<td>43</td>
</tr>
<tr>
<td>Reticulocyte count</td>
<td>9.9% (161.7 x 10^9/L)</td>
</tr>
<tr>
<td>Mean Cell Volume (fl)</td>
<td>77.3</td>
</tr>
<tr>
<td>White Blood Cells (x 10^9/L)</td>
<td>6.0</td>
</tr>
<tr>
<td>Platelets (x 10^9/L)</td>
<td>488</td>
</tr>
</tbody>
</table>

The blood film revealed a severe microangiopathic haemolytic anaemia with a marked number of schistocytes and moderate polychromasia. The white blood cell count and differential were normal as was the platelet count. The microangiopathic haemolytic anaemia was believed to be directly related to the AVSD repair.

Children born with Down syndrome, or trisomy 21, often have a related congenital heart disease. One of the most frequently occurring defects is an AVSD. When this defect occurs, the valve at the junction between the right and left atriums and the right and left ventricles is shared so that there is effectively one valve rather than the normal two valves. This allows blood to flow between the atriums and ventricles which in turn leads to an increase in pressure in the lung circulation. Early surgical intervention is necessary. This involves the use of two patches, one closing off or repairing the atrial septal defect (ASD) while the other repairs the ventricular septal defect (VSD). The atrioventricular valve is separated into two valves which are in turn attached to two patches. This results in each side of the heart having its own valve thus allowing the two sides of the heart to function independently as in a normal heart.

In the case presented, one of the patches had pulled away from the repaired valve allowing blood to gradually leak back through the valve. The small orifice through which the red cells were passing was smaller than the size of the red cells so the cells were being 'ripped' as they passed across the valve. This led to the presence of a marked number of schistocytes whilst the platelets, being smaller, passed straight through the small orifice, resulting in a normal platelet count which in a six-month-old child ranges between 205 and 553 x 10^9/L.
In view of the low haemoglobin, 43 g/L, some additional tests were performed. These tests included haemoglobin electrophoresis (EPG). The results were as follows:

Hb EPG Cellulose Acetate (pH 8.6): abnormal band detected in HbD/S position.
Hb EPG Agar Gel (pH6.0): abnormal band with HbA.
Abnormal band: HbD 36.8%
HbA2: 2.1% NR (2.0–3.5) %
HbF: 5.3%

Considering the age of this child, the result was reported as a probable heterozygous HbD. A repeat Hb EPG was suggested when the child reaches 12 months of age.

A Hb EPG was also performed on the parents. The mother’s results were normal whilst the father’s results were as follows:

Hb EPG Cellulose Acetate (pH 8.6): abnormal band detected in HbD/S position.
Hb EPG Agar Gel (pH6.0): abnormal band with HbA.
Abnormal band: HbD 38.1%
HbA2: 2.0% NR (2.0–3.5) %
HbF: 1.0%

Consistent with HbD trait.

HbD, commonly referred to as HbD-Los Angeles or HbD-Punjab is inherited as an autosomal recessive variation of HbA. HbD-Los Angeles results from a substitution of glutamine for glutamic acid at position 121 on the β chain. HbD-Los Angeles is found in the Punjab region of India and Pakistan where the prevalence of HbD trait is 3%. It is known as HbD-Los Angeles after the city where it was first discovered.

In the heterozygous state, HbD-Los Angeles constitutes between 35 and 50% of the total haemoglobin. HbD-Los Angeles is an asymptomatic condition. Patients have a normal haemoglobin, normal red cell parameters and no evidence of haemolysis. However the heterozygous state should be distinguished from HbD in combination with β°-thalassaemia, in which case the patient will have a mild microcytic hypochromic anaemia. HbD-Los Angeles also copolymerizes with HbS to produce severe sickling. This is due to enhancement of polymerization due to the substitution of glutamine for glutamic acid at position 121 on the β chain, an important contact point for polymerisation of HbS.

The child was transfused and subsequently returned to the operating theatre to have the AVSD repaired.

**Figure 2**
Schematic representation of the atrioventricular septal defect and its repair.
Reproduced by kind permission of Dr James Wilkinson, Royal Children's Hospital, Melbourne.
YEAR 2011

MARCH 13–16: 11th National Rural Health Conference, Perth Convention Centre, Perth, Western Australia

MARCH 18–20: Laboratory Diagnosis of Infectious Diseases: From Basics to Molecular Methods Centre for Infectious Diseases and Microbiology (CIDM) Workshop
Enquiries: Ms Lou Orszulak
Email: lou_orszulak@wsahs.nsw.gov.au


APRIL 8–10: AIMS NSW South West Division 2011 Conference, Batemans Bay Soldiers Club, 2 Beach Road, Bateman’s Bay http://www.aims.org.au/


JULY 4–8: ASM Annual Scientific Meeting & Exhibition


AUGUST 2-5: 28th Annual NRL Workshop on Infectious Diseases (formerly NRL Workshop on Serology) Hotel Realm, Canberra.


AUGUST 8–12: SPC 2011 AIMS/NZIMLS combined scientific meeting Gold Coast Queensland http://www.allocasionsgroup.com/aimsnzimls11/


OCTOBER 30 - NOVEMBER 2: The Combined Annual Scientific Meeting of HSANZ, ANZSBT and ASTH. Sydney, Australia http://www.hsanz.org.au/news/events.cfm

NOVEMBER 4–6: AIMS NSW North Coast annual conference. Darlington beach Resort, Arrawarra (35 km north of Coffs harbour, NSW). 0267769840 Email: neil.horton@hnehealth.nsw.gov.au

AIMS SCHOLARSHIPS
AIMS/NZIMLS South Pacific Congress 2011
8-12 August 2011

Six scholarships will be offered to financial members of AIMS in 2011 to support attendance at the AIMS/NZIMLS South Pacific Congress 2011.

- Three Remote Attendee scholarships of $1250
- One Young Scientist Scholarship of $1250
- Two scholarships of $1000 each will be offered for first time presenters at the South Pacific Congress – one for an oral presentation and one for a poster.

Applicants must be current members of AIMS and must have held membership for at least six months at the time of the application. Affiliate, retained or student members are not eligible to apply. For full details and application forms see the AIMS website www.aims.org.au or contact National Office.

Closing date for applications: COB 1 May 2011
CALL FOR ABSTRACTS

AIMS NZIMLS South Pacific Congress
8-12 August 2011
Gold Coast Convention Centre, Queensland, Australia

The Australian Institute of Medical Scientists and the New Zealand Institute of Medical Laboratory Science is proud to host and invite you to the South Pacific Congress, 8-12 August 2011. The Congress will bring to the Gold Coast Convention Centre a top level forum of leading national and international speakers to address topical issues in medical science.

SUBMISSION CATEGORIES

The Congress theme ‘Lights! Camera! Action!’ has been chosen as a call to action for delegates to spend a focussed 4 ½ days in the vibrant Gold Coast at a Congress filled with topical and relevant presentations. Daily sub-themes will logically group presentations and have a little fun based on the Congress theme!

* ‘Waterworld’ – Water trauma/diseases
* ‘Basic Instinct’ – Back to basics
* ‘Aliens’ – The immune system
* ‘Back to the future’ – New technology
* ‘That’s all folks!’ – Closing day

SUBMITTING ABSTRACT

This information is to be submitted electronically through the Congress website: www.alloccasionsgroup.com/AIMSNZIMLS11.

All submissions are to include the following:
* A 100 word biography
* 250 word abstract providing the essence of the presentation and how it links with the Congress theme(s)
* Full contact details (name, organisation, position, phone, fax, address and email).

Please refer to the Congress website for information regarding formatting requirements and submission guidelines.

SELECTION CRITERIA

* Originality and interest
* Structure and clarity
* Scientific validity
* Potential significance

POSTER PRESENTATION

You are welcome to send an abstract that will describe your poster submission. Please refer to the Congress website for information regarding formatting requirements and submission guidelines.

Posters will be displayed for the duration of the Congress and the presenting author should be by their poster as often as possible, ideally during the breaks in the Congress.

KEY TIME FRAMES

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For further information:

Shanna Sheldrick, Manager - Conventions & Events
Tracy Fisher, Coordinator - Conventions & Events

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2011
RCPA QAP / AIMS
MORPHOLOGY WORKSHOP

20-21 May & 5-6 August 2011

Applications available online on 22/02/2011

Venue:
The Australian Technology Park,
Redfern, Sydney Australia
The Anatomy of the Human Embryo
A Scanning Electron-Microscopic Atlas
Karger 2008
By G Steding
Hard cover, xiv+516 pages
ISBN: 978-3-8055-9705-0
Price: out of print, soft cover version soon to be released.

This is an extraordinary book. It follows a pictorial journey along human development visualised through high resolution scanning electron microscopy (SEM) images of the human embryo.

The book is separated into six chapters: external aspects, head and neck, thorax, abdomen urogenital and finally, brain and sensory organs. Each chapter is further subdivided into subsections of which there are 27 in all. These highlight specific components relevant to the main section, for example the chapter on abdomen comprises development of the small intestine, liver, stomach, spleen, great vessels and pancreas while that on thorax examines development of the heart and lungs. Across the full text, all the major organ systems are covered.

The technique used to demonstrate the specific structure involved sputter coating the most external surface first which was then examined by SEM and images recorded. The surface of the specimen was then dissected to reveal deeper structures which were in turn sputter coated and examined in the SEM. The process was repeated until the entire structure is visualised level by level. This creates a remarkable series of images moving ever deeper through a sample in a stepwise fashion revealing the developmental process of individual structures and their interrelationships. In some cases micrographs have been taken from different angles to further emphasise tissue arrangements.

Micrographs are rarely labelled so as not to obscure the content but matching annotated line drawings do accompany some images to clarify the arrangements of various organs. The amount of text is also minimal and only provides a very brief coverage of the developmental changes illustrated. As the author states, this is not intended to be an instructive text but is rather designed to be illustrative providing a different perspective on human embryonic anatomy from that normally available by routine histological techniques.

It is stated that embryos were all obtained legally and therefore there is no sample earlier than four weeks gestational age. Embryos were only used for imaging for the atlas hence none shows any unrelated sampling or damage. Specimens were collected over a 25 year period and were prepared according to standard SEM protocols. There are some 818 images in total. All are of extremely high quality with no artefact or flaws evident reflecting a consistently outstanding level of technical skill. This is indeed an exceptional production which is the culmination of a substantial and sustained body of work. The author is to be highly commended for this utterly unique contribution.

The book will be of major interest to embryologists and developmental biologists both as a resource and as a powerful teaching tool. However I would strongly encourage anyone who has even a broad interest in human biology to look over this work as it is unlikely to be bettered in the foreseeable future.

AE Woods
Associate Professor
School of Pharmacy and Medical Sciences
University of South Australia

Case Files: Neuroscience
By EC Toy, R Jandial, EY Snyder and MT Paukert
McGraw Hill 2009
Soft cover, 408 pages
ISBN: 978-0-07-148921-8
AUD$48.00

This book is part of a series, Case Files, published by McGraw Hill Medical and deals with a variety of cases in neurology and neurosurgery. These cases include both acute and chronic presentations, covering emergency cases as well as outpatient clinic visits.

The intended audiences of this book are medical students preparing for examinations in neurology, advanced trainees in neurology and neurosurgery and for those who are preparing for the USMLE (US Medical Licensing Examination). It addresses the critical and essential needs of its audience by presenting the cases in a unique format. Each case is presented succinctly, in a manner that a consultant would expect to hear his or her trainee present a case. Following each case are a few questions pertaining to the pathophysiology or biochemistry of that case. The answers are provided and are again succinct and to the point.

There are unique features of this book that set it apart from other case series books. One is the detailed Clinical Correlation that ties in the specific disorder pertaining to the case with its epidemiology, aetiology and natural history. Armed with a good background knowledge of the disorder of that case, the reader is then lead to an approach to understanding of the specific cell type or structure involved in that disorder. This section includes Learning Objectives that immediately alerts the reader to the salient points that should be understood by the end of that case, followed by Definition of Terms. The Discussion that follows addresses the learning objectives.

Australian Journal of Medical Science    February 2011    Vol. 32    No. 1
After the discussion, there are a few Comprehension Questions, in the multiple choice question style. These questions may include further cases on that disorder or cell type/structure. The Answers to these questions then follow. At the end of each case is a set of Neuroscience Pearls which I feel are extremely helpful if one is revising just before an exam.

The cases are chosen from all areas of neuroscience and are commonly encountered situations in any teaching hospital. This allows the reader to related these cases to patients he or she has seen. This helps consolidate the knowledge. I find the cases extremely fascinating. The cases are likely to hold the readers’ attention so that they will attempt to answer the questions and will be encouraged to read the answers as well as the following pages of discussion on that case. The comprehension questions will test the readers’ knowledge so that they can be certain that they have achieved the learning objectives. For those who want more on that particular disorder or structure, there is a list of references at the very end of each case. Those quoted are text books, likely to be those set by exam boards or medical schools rather than the latest journal articles. However the intended audience may have these books at hand.

Contributors to each section are mostly neurosurgery residents and medical students, and therefore the cases chosen are what junior doctors and medical students are likely to encounter on their clinical attachments. The style of writing is easy to read and would appeal to junior doctors.

This book is clearly a gem when preparing for exams because it has been set out to achieve a high yield, by providing all necessary key points with each case. This saves time the reader otherwise would have spent looking up definitions etc.

Being a soft bound book of only 408 pages it is easy to carry around and use as a guide or reference on the wards or in the clinic. Therefore even after the exams, this book will continue to provide neuroscience pearls and help solve mysterious cases in neuroscience.

What I find most helpful, and what many students of neuroscience overlook is what is set on the first 2 pages: The Approach to Learning Neuroscience. Relating the symptoms to the anatomical pathways is the only way to learn and understand neuroscience, which unfortunately many medical schools fail to impress upon their students. If I had my time over in medical school I definitely would consider buying this book.

Although the intended audience is clearly junior doctors and medical students, this book is a fascinating and enlightening read for anyone with an interest in neuroscience and how the nervous system functions.

Dr Devika Thomas BSc(Hons), MBBS, M Surg, MAACB

Trainee Chemical Pathologist
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SA Pathology
Frome Road, Adelaide, SA

Clinical Cases in Infectious Diseases
A Public Health Approach
McGraw Hill 2007
By S Senanayake
Series editor: John R. Goldblum
Soft cover, 398 pages
Price: AUD$52.00

Clinical Cases in Infectious Diseases uses 22 case studies of mostly common (and some less common) infectious diseases to highlight the important clinical, diagnostic and public health aspects of each. The author, Sanjaya Senanayake is an infectious disease physician but has also worked as an epidemiology registrar in a previous life. He creates scenarios that begin in the emergency department or in the GP’s office and which are played out until well after the patients have recovered and after the public health response has been elicited. Each chapter begins with an ‘At a Glance’ box which lists the main characteristics of each disease. Senanayake then introduces a patient with typical symptoms, who is treated by hospital staff and specialists. They order the appropriate tests and importantly, take the reader through the differential diagnoses to highlight other diseases with similar symptoms. After the diagnosis has been made, the CDC or public health department is inevitably notified and the reader follows their response to the clinical case(s). These various characters all combine to present an illness to the reader like an episode of All Saints.

If one can manage not to get too caught up in all the melodrama, Clinical Cases in Infectious Diseases also presents the bare and relevant information about each disease by interjecting after each piece of character dialogue. Each chapter presents key information throughout under headings such as “What is the Disease?”, “Differential Diagnoses”, “Investigations”, “Treatment”, “Prognosis”, “Public Health Notification and Response” and then provides an extensive list of references at the end of each chapter for further, more detailed reading.

The theatrical pretence of each case is strengthened by the quality of the questions asked by each of the characters. The characters mostly ask quite predictable questions which allow Senanayake to answer with the predictable yet essential facts. However he also poses questions which perhaps some readers would not have ostensibly asked themselves and this is particularly effective when his target audience is wide and varied.

This book is not a comprehensive textbook of infectious disease. It is a light and easy read and is ideally
suited for use as a text for students in entry level public health subjects or for students of other disciplines who may touch upon public health but not in depth. Likewise, it may give non-clinical public health professionals or students an increased understanding of the processes that precede the commencement of their work. It can be strategically placed in the staff room of any hospital, laboratory, nursing ward or public health facility as the reader can easily ‘chip away’ at this book.

Clinical Cases in Infectious Diseases effectively achieves its aim to connect the worlds of the clinician, the laboratory scientist and the public health professional and show the reader how vital it is for workers in these areas to understand and appreciate each others’ role in combating infectious disease.

Anthony Draper MAIMS
Medical Scientist
Dili, Timor-Leste

Endocrine Development Volume 17
Pediatric Neuroendocrinology
Editors: S Loche, M Cappa, L Ghizzoni, M Maghnie, MO Savage
Karger 2010
Hard cover, vii+220 pages
ISBN: 978-3-8055-9302-1
RRP: EUR€98.50

This book attempts to cater for both the scientific community and the clinicians. However, in doing so, it unintentionally has created an appropriate text book on developmental endocrinology for diagnostic laboratorians. There are a few scientific papers that, at a glance may be disregarded by many clinicians as ‘basic science’ and those chapters on diagnosis and management of specific conditions may be ignored by basic scientists. However, to diagnostic and clinical laboratorians, as well as medical students, this mix will provide a valuable resource and a reference text.

There are several chapters in this book that satisfactorily address rarely discussed issues in standard text books. Examples are the chapter on the role of sleep or the deprivation of sleep on glucose metabolism and the metabolic syndrome as well as other endocrine organs, the neuroanatomical and endocrinology of sexual orientation and identity, the effects of commonly prescribed neuropsychiatric drugs on the hypothalamic-pituitary-adrenal axis and the chapter on the neuroendocrine consequences of anorexia nervosa. When interpreting abnormal endocrine tests, the effects of medication are rarely considered. This book alerts us to the importance of a good drug history.

Each chapter begins with an abstract, followed by a general introduction to that topic, and various aspects discussed under subheadings. At the end of each chapter is a list of references. The literature sited is from peer reviewed journals and are current and up to date. The authors of each chapter write with authority and have treated the given topic taking into consideration the mixed audience of the book.

I particularly enjoyed the clinically oriented chapters. The chapters on the diagnosis of central adrenal insufficiency and Cushing’s disease in children were of particular interest. These include guidelines and specific tests for screening and confirmation. Although prolactinomas are the most common pituitary tumours in children and adolescents, this topic is rarely covered in this detail in many pediatric text books.

The organisation of chapters could have been better. The book would better serve a browser or one who would use it for reference if the chapters were divided into sections. For example, several chapters would fit under the section headings ‘Metabolic Syndrome/Obesity’, ‘Growth/Development/Puberty/Sex Hormones’, ‘Hypothalamic-pituitary-adrenal axis’ etc. Unless one reads the book cover to cover or reads thoroughly the table of contents for all chapter headings, he or she is likely to miss an important chapter on a specific topic.

However, as mentioned above, as a chemical pathologist in a diagnostic laboratory, I find this book a useful resource to have on the shelf. I would encourage medical scientists in diagnostic laboratories to use this text when confronted with any queries regarding endocrine testing on children. General practitioners often contact medical scientists seeking advice on what tests to order and certain chapters in this book may help. Being a hard cover book it is more a reference book that sits on the shelf than a guide that is easily carried in a bag.

As mentioned above, I found the contents both fascinating and useful. It was a pleasure to review this book.

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Trainee Chemical Pathologist
Chemical Pathology
SA Pathology
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Hematopathology, 1st edition
Edited by ES Jaffe, NL Harris, JW Vardiman, E Campo, DA Arber
Elsevier Saunders 2011
Hard cover, 1058 pages
ISBN: 978-0-7216-0040-6
RRP: AUD$427.00

This is the first edition and besides the editors, there are nearly a hundred contributors, among them such familiar names as Barbara Bain, Nancy Rosenthal and Peter Isaacson.
The editors state in the preface that “Hematopathology is a discipline in which the traditional methods of clinical and morphologic analysis are interwoven with newer biologically based studies to achieve an accurate diagnosis”. The authors and editors have embraced the new technology available and have included in the discussion of each disease the morphologic description and the relevant immunophenotypic, genetic and clinical features. Chapters dealing with neoplastic disorders also incorporate information on prognostic factors and relapse.

The book is divided into seven parts as follows:

Part I (seven chapters): Technical Aspects

Part II (five chapters): Normal and Reactive Conditions of Hematopoietic Tissues

Part III (30 chapters) Prefaced with a chapter on the Principles of Classification of Lymphoid Neoplasms followed by Section 1: Mature B-cell B Neoplasms, Section 2: Mature T-cell and NK-cell Neoplasms, Section 3: Precursor B- and T-cell Neoplasms

Part IV (eight chapters): Myeloid Neoplasms

Part V (three chapters): Histiocytic Proliferations

Part VI (three chapters): Immunodeficiency Disorders

Part VII (six chapters): Site-Specific Issues in the Diagnosis of Lymphoma and Leukemia. There is also an appendix on staining techniques.

As you can see the parts group together specific disease entities or a group of related disorders, benign or malignant. The first part details the collection and processing of the samples needed for accurate diagnosis and is well set out and illustrated with instructions of how to collect and process lymph nodes, fine needle aspirates and biopsies and bone marrow aspirates and trephines. Part I also includes chapters on immunohistochemistry principles and tests, flow cytometry, molecular diagnosis and cytogenetic analysis and related techniques. There are many tables, figures and illustrations, most in colour and are clear and with adequate explanations and tabulations.

Part II is also beautifully illustrated with many histological sections and blood films and encompasses such topics as normal lymphoid tissues and bone marrow, reactive lymphadenopathies and bone marrow findings in inflammatory, infectious and metabolic disorders.

Part III is a huge section but again is well set out and illustrated. The different lineage lymphoid neoplasms are described with clinical presentation, diagnostic tests and results, treatment options and prognostic factors outlined in easy to follow text and in logical order. As most of the editors are also involved in the WHO classification, it is not surprising that this text follows and expands on this reference system.

Part IV Myeloid Neoplasms, is less in volume but not in importance, and the disorders are probably more familiar to the laboratory scientist. It begins with a chapter on the principles of classification of myeloid neoplasms and then continues with MDS, AML, myeloproliferative neoplasms and the other diseases as classified in the WHO structure. The sections on each of the disorders are necessarily brief, but include the relevant information in a concise manner and again are well illustrated with blood film and bone marrow images and trephine sections. There are also karyotype figures and diagrammatic representations of the molecular fusions and their effect on certain regulatory pathways.

Part V discusses the histiocytic proliferation disorders, both non-neoplastic and malignant. It covers such disorders as hemophagocytic syndromes, Langerhans Cell disease and sarcoma and storage disorders. Dendritic cell neoplasms are included and this relatively newly classified category is a welcome addition.

Part VI on the immunodeficiency disorders is a small but important section as it encompasses the primary immunodeficiencies, associated lymphoproliferative disorders and the hematopathology of HIV infection.

The final part is a section dealing with the specific issues relating to the biopsy material in the diagnosis of lymphoma and leukemia. It covers bone marrow, lymph nodes, the spleen and other extranodal sites and the challenges faced in diagnosing these malignancies after chemotherapy, co-existing inflammatory conditions or regeneration.

Each part of this book has a colour coded band at the top of the page, which makes it easy to navigate through the sections. The index is well cross-referenced and logical and I found no difficulty in finding specific topics easily.

References have been provided on the Expert Consult website only – this was decided due to the extensive referencing. To activate this website, direction and the code are supplied in the inside front cover of the book. This may be an inconvenience at times, but is outweighed by the reduction in pages (and weight) and the full access to those references through the PubMed links.

The stated goal “to provide concise, up-to-date, and practical information that is easy for the reader to access” is certainly met in this impressive and extremely well illustrated text. I think it will become a “must” for haematology registrars and contains a wealth of knowledge for the haematologists and scientists and I would recommend a copy for all larger haematology laboratories.

Robyn Wells
Senior Scientist, Core Haematology
Pathology Queensland Central Laboratory
Royal Brisbane and Women’s Hospital

**Fine Needle Aspiration of Bone Tumours**
The Clinical, Radiological, Cytological Approach
Monographs in Clinical Cytology Vol. 19
Karger 2010
By M Akerman, HA Domanski and K Jonsson
Hard cover, xii + 92 pages
ISBN: 978-3-8055-9214-7
Price: USD$132.00

Fine-needle aspiration cytology (FNAC) is a widely accepted procedure in a broad variety of diseases including diseases of the bone. With recent advances in immunohistochemistry and molecular genetics procedures, the role of FNAC in the investigation and diagnosis of primary bone lesions is greatly improved. The purpose of this book is to help facilitate the cytological evaluation of FNA smears from bone lesions and to provide diagnostic criteria based on the combined evaluation of clinical data, radiographic findings and the cytological features. The key objective of the authors is to thoroughly describe and illustrate the most common entities of bone tumours and tumour-like lesions, as well as the diagnostic use of ancillary techniques. A number of rare primary bone lesions are also illustrated.

The selection of entities illustrated in this comprehensive manual is based mainly on the authors’ own experience from data collected between 1966 and 2006 from patients referred to the Musculoskeletal Centre in Lund, Sweden. The 10 clinical case reports with brief commentary notes arranged according to the related chapter is an outstanding feature of this book. The book includes 88 figures: 61 colorful microscopic figures plus 21 radiological and CT scan pictures, and 10 tables, making it user-friendly and easily accessible.

The book is organised into 14 chapters beginning with brief information on epidemiology of bone tumours, an overview of radiological and morphological diagnosis of bone lesions as well as the use of adjuvant diagnostic techniques, followed by bone lesions using common classifications of bone lesions. All of the chapters incorporate the differential diagnosis and hence the book is very useful for the practicing clinicians. As it allows online web access and the publication is listed in bibliographic services, including current contents, researchers and scientists can easily perform quick reference searches.

This book is a valuable resource mainly for pathologists, cytopathologists, radiologists, oncologists and orthopaedic surgeons involved in the work-up and management of patients with bone pathology. Although technical methodology is not described in detail, additional information regarding immunohistochemistry means it is a useful reference book for medical scientists working on immunohistochemistry and cytogenetic and molecular markers.

Lavender Than Than Htwe
MBBS, D.Path, M.Sc (Medicine-immunology), MAIMS, FCAP
Associate Professor, Pathology Discipline,
University Kuala Lumpur – Royal College of Medicine
Perak, MALAYSIA

**How to Publish in Biomedicine**
500 tips for success - 2nd Edition
Radcliffe, distributed by Elsevier 2008
By J Fraser
Soft cover, xiv+516 pages
ISBN: 9781857751932
Price: USD$49.00

In his foreword to this book Richard Horton comments, “Writing is hard it is true.” Yet many scientists expect to write as though it were second nature with no training or experience needed – and they will be wrong! Communication is a critical element in science; it is the process that allows the distribution of empirical data and hypotheses for criticism and review; thus, the publication of experimental findings is an absolute requirement for scientific knowledge to progress. Allied to this there is a need for scientists to have high-level communication and writing skills and a good understanding of the structure and content of scientific articles and the publication process. *How to publish in biomedicine* is one of a series of texts produced by Radcliffe Publishers that aims to support all these aspects of scientific writing and communication (a review of one of these, Creating effective conference abstracts and posters in biomedicine: 500 tips for success, appears in *Aust J Med Sci* 30: 105).

How does this specific book help with the writing process? Jane Fraser, the author of *How to Publish in Biomedicine*, started her working life as a research scientist then moved into writing, editing and publishing. With this background she understands both the scientific and publishing processes, approaching her topic with an insider’s knowledge. The ‘500 tips’ format means that the reader is taken through the content in simple steps using a concise ‘dot-point’ style that makes each section easy to read and understand.

The first chapter focuses your attention on the task at hand by asking, “What do you want to write?” In other words, what type of article most suits your data: a research article, a short communication etc. – this leads into the publication process and selecting the right journal. Following this is a section on planning your article and how to organise your writing, wise advice that is echoed by eminent authors such as Stephen King, America’s most famous horror writer! The core of the book, some fifty pages, is devoted to developing an understanding of
what goes where – the detailed structure and content of a research paper with a brief section on conference abstracts and advice on tables, illustrations and referencing. The 'core' material ends with advice on checking and submitting your manuscript and dealing with Editor's and reviewer's comments. Following this there are brief chapters on writing reviews, books, book chapters, theses, dissertations and informal articles; the final chapters give tips on clear writing and the writing process.

Do I have any negative comments? Considering the focus on novice writers, I am not sure that the brief sections on reviews, books and book chapters (normally tasks only undertaken by senior staff) are particularly relevant – but this is hardly a major fault! More importantly, I would have liked more information about peer review criteria: these are critical because they are essentially the 'marking criteria' used by reviewers to determine whether a manuscript is accepted or rejected. Understanding these obviously helps the writing process.

In summary, I have no reservations about recommending *How to Publish in Biomedicine*: it covers the essentials of writing scientific research papers, is well-written and organised and easy to follow and digest – ideal for the novice writer. Providing your study was well constructed and has produced quality data, *How to Publish in Biomedicine* should help you write a better article and improve your chances of publication. Finally, and as always, I strongly encourage you to submit the fruits of your labour to the *Australian Journal of Medical Science*.

John W Stirling BSc(Hons), MLett, AFRCPA, MAIMS, FRMS
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Robbins and Cotran: Atlas of Pathology, 2nd Edition
By Edward Klatt
Saunders Elsevier 2009
Soft cover, 577 pages
ISBN: 978-1-4377-0170-8
AUD$79.00

The *Atlas of Pathology* is another instalment in the stable of pathology sources carrying the Robbins (and Cotran) title. This, the second edition, presents around a hundred new images taking the total set to well over one and a half thousand! All body organs and systems are included giving rise to some twenty chapters. Thus by any measure this is an extraordinary and impressive publication which any student and indeed practitioner of anatomical pathology should have in their collection.

The majority of images are photomicrographs of standard histological sections but many of these are complemented by a vast mix of supportive clinical material. This includes macro-photographs of anatomical specimens, X-rays, electron micrographs, scans from various modalities (CT, MRI scintigraphic) and the occasional immunofluorescence or immunohistochemically-stained section. In some cases diagrams are also added to assist with orientation and explanations. Recording sharp, colour-balanced images of standard haematoxylin and eosin specimens is notoriously difficult even with contemporary digitised imaging systems however the quality and consistency of images in the Atlas is outstanding. Similarly, the clarity and colour of the gross specimens is remarkable given the sheer range of specimens on display. The excellence of the images is another clear indication of the attention to detail one has come to expect from the Robbins (and Cotran) series of works.

There are only three figures on each page which is an effective compromise between maximising image size while holding the shear bulk of the atlas to a manageable level. Each image is accompanied by a brief section of explanatory text which rarely exceeds a hundred words to maintain emphasis on the images. Only key points or characteristic identifying features are described. In many instances clinical data is included to illustrate better the link between the pathophysiology and clinical observations. This is extremely useful for students and an expansion of this aspect in future editions would even further strengthen this work. It should be noted that while the descriptive content is brief for each image, collectively, the text is quite comprehensive given the extensive range and various aspects of the specimens covered. Moreover if the Atlas is used in conjunction with the major Robbins and Cotran reference, the *Pathologic Basis of Disease*, it would be difficult to imagine a wider and more detailed coverage of the discipline of anatomical pathology.

AE Woods
Associate Professor
School of Pharmacy and Medical Sciences
University of South Australia
Australasian Professional Acknowledgement of Continuing Education

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The healthcare industry is undergoing rapid change. We are expected to keep our knowledge and skills up to date to enable us to perform to the highest professional standard. The APACE scheme provides a method in which your professional activities may be recognised.

Credit where credit is due!

To learn more about how to become APACE accredited, tips and APACE news, visit the APACE pages on the AIMS website.

www.aims.org.au
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.

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2. Anaphylaxis edited by J Ring Karger (Series title: Chemical Immunology and Allergy) 2010 xxvi+228 pages.


18. Fighting Cancer with Knowledge & Hope: A Guide for Patients, Families and Health Care


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


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


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


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Questions relating to *Cancer stem cells and the side population*, page 2 of this issue.

1. Efficient cancer treatment includes targeting the cell type responsible for sustaining the growth of the neoplastic clone. **True/False**

2. Stem cells have the capability to self-renew and proliferate, and to differentiate to generate mature cells of a particular tissue. **True/False**

3. Totipotent stem cells are the progeny of pluripotent stem cells. **True/False**

4. Embryonic stem cells are derived from the inner mass cells that are harvested from a blastocyst. **True/False**

5. Evidence of cancer stem cells was first documented in gynaecological malignancies. **True/False**

6. CD45+ human brain tumour fraction has been demonstrated to have potent *in vivo* self-renewal and proliferative capacities. **True/False**

7. A subset within the bulk of cancer cells can be distinguished via Hoechst 33342 (Ho342) staining. **True/False**

8. SP cells were originally identified in murine bone marrow and have been defined by as a population of cells detected by flow cytometry that has the capability to efflux the fluorescent dye, Ho342. **True/False**

9. Wulf *et al* reported that less than 20% of 61 bone marrow samples from patients with AML contain a distinct SP. **True/False**

10. Identifying SP cells by flow cytometry is straight forward and readily adaptable in all routine flow labs. **True/False**

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Journal-based CPD No.27
Page 2 of 2

Questions relating to *Evaluation of the Sysmex pocH-100i Analyser for use in remote laboratory settings*, page 11 of this issue.

1. The red cell count and platelets are measured by a hydrodynamic focusing DC detection method.  
   True/False

2. The stated dead volume for a paediatric collection tube is 750 μl.  
   True/False

3. The correlation for a low MCV thalassaemia sample with the XE-2100 was poor.  
   True/False

4. The weight of the pocH-100i is less than 15 kg.  
   True/False

5. A differential was generated for all 50 normal samples that were tested.  
   True/False

6. Any errors that occurred during analysis were identified by a red flashing symbol.  
   True/False

7. The mixed count in the 3 part differential comprises the lymphocytes, eosinophils and basophils.  
   True/False

8. The pocH-100i can store up to 20 sample results.  
   True/False

9. The on screen instructions were informative enough that the manual was rarely required.  
   True/False

10. The correlation for the MXD% demonstrated an $r^2 > 0.95$.  
    True/False

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

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All individuals listed as authors must have made a substantial contribution to the conception and design of the study, the acquisition of data or the analysis and interpretation of data; the drafting of the article or revising it critically for important intellectual content; and final approval of the version to be published. The corresponding author must take responsibility for obtaining permission from all the authors for the submission of any version of the manuscript and for any changes in authorship.

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Number pages consecutively commencing with the title page.

Arrange the article in the following sequence:

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• Main Text
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• Tables - each table, complete with title and footnotes, on a separate page
• Legends for illustrations.

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**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.

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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.

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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**

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Throughout the body of the manuscript cite the author/s name and the publication year in parentheses as in the following examples:
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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.

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When plotting points, the following symbols are preferred:

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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
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Photomicrographs must have internal scale markers
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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
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Report measurements in the units in which
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Additional Information

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references.

Editors. Scientific style and format: the CBE manual
for authors, editors, and publishers. 6th ed. Cambridge

2. Style manual for authors, editors and printers. 6th ed.

3. O’Connor M, Woodford FP. Writing scientific papers
in English: an ELSE-Ciba Foundation guide for
authors. Amsterdam, Oxford, New York: Elsevier-
Excerpta Medica, 1975.

4. Day RA. How to write and publish a scientific paper.
Philadelphia, Institute for Scientific Information

5. Zeiger M. Essentials of writing biomedical research

6. Matthews JR, Matthews RW. Successful scientific
writing: a step-by-step guide for the biological and
medical sciences. 3rd ed. Cambridge, Cambridge
University Press, 2007 [Also available in eBook
format.]