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Earlybird registration closes 13 August 2010
Presenter registration deadline 13 August 2010
Some of the speakers include:

**Professor Dennis Lo**, who will be known to some with his work in the Molecular Technology field. He discovered the presence of cell-free foetal DNA in maternal plasma in 1997 and has continued his work in prenatal diagnosis as well as cancer testing. He received the IFCC-Abbott Award for Outstanding Contribution to Molecular Diagnostics. Professor Lo will be giving the David Curnow Plenary Lecture.

**Professor Stefan Grebe** has varied research interests, including thyroid disease, endocrine malignancies, application of mass-spectrometry, in particular high throughput applications and the use of mass-spectrometry for analysis of steroids and proteins.
An association between normal D-dimer levels at diagnosis and improved survival in a group of acute myeloid leukaemia patients.

Averil Drummond¹, Luke Coyle²

¹ PaLMS Pathology, Royal North Shore Hospital, Sydney, NSW.
² Haematology Dept. Royal North Shore Hospital, Sydney, NSW.

Abstract

It has been accepted for many years that fibrin remodelling plays an important role in the ability of many solid tumours to increase in size and to metastasise. Normal levels of both D-dimer and other components of the fibrinolytic system, measured at diagnosis, have been shown to relate to a favourable clinical outcome in many of these tumours. In some cases normal D-dimer levels have been shown to have equal or greater prognostic significance than more commonly used tumour markers such as carcinoembryonic antigen (CEA) and the tumour associated antigen CA 125. This study postulates that, in spite of apparent differences in tumour biology, normal D-dimer levels at diagnosis may have an equally favourable prognostic significance in patients with acute myeloid leukaemia (AML). Sixty-one patients who presented with *de novo* AML, excluding acute promyelocytic leukaemia (APML), from January 2000 until October 2008 were divided into two groups: those with normal (n=9) and those with elevated (n=52) D-dimer levels at diagnosis. Normal levels of D-dimer correlated with a lower peripheral blood white cell count and serum lactate dehydrogenase (LDH). Most importantly patients in the normal D-dimer group showed a significantly reduced rate of relapse and improved overall survival. If this effect is confirmed by a larger study it is proposed that D-dimer may be incorporated into a prognostic model for patients with AML.

Keywords: Acute Myeloid Leukaemia, D-dimer, fibrin, fibrinogen, uPAR

Introduction

D-dimer is one of several small peptide products, known as fibrin degradation products (FDPs) which are formed when the bonds of stable, cross-linked fibrin which are the end product of the coagulation pathway, are hydrolysed by the protease plasmin. This process of fibrinolysis is an essential part of the regulation of haemostasis, ideally preventing deposition of fibrin in areas other than those immediately adjacent to a site of blood vessel damage. There are, however, many pathological conditions in which the inappropriate formation and breakdown of fibrin occurs, and D-dimer measurement has become a useful diagnostic aid in these conditions. Plasmin itself is the activated product of the breakdown of plasminogen by plasminogen activators the most important of which are tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA). Plasminogen activation is negatively regulated by inhibitors, principally plasminogen activator inhibitor 1 (PAI-1).

In many solid tumours an elevated D-dimer at diagnosis has been shown to be a poor prognostic indicator for survival. This association has been observed most often in breast cancer (*Blackwell et al. 2000; Dirix et al. 2002; Nijziel et al. 2003*), but also in colorectal (*Blackwell et al. 2004*), ovarian (*Mirshahi et al. 1992; Gadducci et al. 1996; Henic et al. 2008*) and lung cancers (*Taguchi et al. 1997; Pavey et al. 2001; Buccheri et al. 2003*). An association between elevated D-dimer levels and survival in AML patients has not been extensively investigated, although elevated levels of other components of the fibrinolytic system, such as plasminogen activation inhibitor (PAI) (*Yilmaz et al. 2006*) and urokinase plasminogen activation receptor (uPAR) (*Graf et al. 2005*) do appear to be associated with poor prognosis. The objective of this study was to establish whether a normal D-dimer at diagnosis would also prove to be a favourable prognostic
marker in AML, in spite of apparent differences in the disease process between AML and solid tumours.

The malignant cells in both AML and solid tumours have similar requirements such as the need to adhere to tissue components, to migrate and to enter the circulation. In the bone marrow haematopoietic cells are maintained in an environment consisting of stromal cells such as myofibroblasts and adipocytes and stromal proteins such as fibrous proteins, glycoproteins and proteoglycans. Mature haematopoietic cells usually exit from the marrow by passing through the endothelial cells of the capillaries and sinuses. Stromal factors including plasminogen activators (PAs) facilitate the escape of normal cells and also immature myeloid cells from the marrow into the circulation and homing back from the circulation into the marrow (Greer et al 2004). The blasts in AML are also capable of invading other tissues and crossing the blood/brain barrier in a manner analogous to the metastatic behaviour of solid tumours. Both plasminogen activation on the surface of malignant blasts and the actions of PAs in adhesion, migration and extravasation of blasts appears to play a role in the pathology of AML (Tapiovaara et al 1996) and this may provide an explanation for a correlation between elevated D-dimer levels (as a marker of plasminogen activation) and less favourable prognosis in AML patients. Data on this limited number of patients shows that both relapse rate and overall survival were significantly improved in those patients with normal D-dimer levels at diagnosis: normal vs elevated. The two groups were examined for differences in treatment (intensity of induction chemotherapy and the use of bone marrow transplant as initial therapy) and for outcome (response to induction chemotherapy and rate of relapse). Bone marrow blast percentages could not be obtained for two patients as one patient had a necrotic marrow and the other a dry tap.

**Patient treatment**

Induction chemotherapy consisted of Anthracycline and Cytosine +/- Etoposide. Cytosine dose was either high (3000mg/m2) bd, day 1,3,5,7 or standard (100mg/m2) day 1-7 as a continuous infusion. Remission status was defined on bone marrow aspirate morphology (<5% bone marrow blasts). Cytogenetic risk assessment was according to the MRC AML 10 (Grimwade et al 1998) and AML 11 (Grimwade et al 2001) trials. Patients were supported with red cell and platelet transfusions and treated with antimicrobials in line with unit policy. Granulocyte colony stimulating factor (G-CSF) was administered after induction chemotherapy until neutrophil recovery.

**Haematological assays**

For the D-dimer assay, venous blood was collected into 0.106M (3.2%) sodium citrate, in a ratio of 1 volume citrate to 9 volumes blood. Plasma was separated within 4h of collection. Platelet poor plasma was obtained by centrifuging for 7 min at 4000 rpm at 16-18°C. D-dimer was measured using the immuno-turbidometric method (Liatest D-dimer), on STA analysers (Diagnostica Stago, Asnières-sur-Seine, France). A cut-off value of <0.5ug/ml FEU has been validated as negative for the presence of any D-dimer (Ghanima et al 2006). This value was confirmed in the laboratory setting by the testing of 60 normal individuals. Complete blood counts were performed on a Coulter LH750 automated haematology analyser (Beckman-Coulter, Fullerton, USA). Bone marrow aspirates were collected into EDTA and were processed within 2h of collection. A diagnosis of AML on morphological assessment was made when greater than 20% myeloblasts were detected using a 400-cell differential (Jaffe et al 2001). Diagnostic material was further assessed using cytochemical, cytogenetic, molecular genetic and flow cytometric techniques.

**Biochemical assays**

Lactate dehydrogenase (LDH) was measured by UV assay (IFCC recommended method) L-lactate to pyruvate, using a Roche Modular analyser (Roche Diagnostics, Indianapolis, USA).

**Materials and methods**

**Patients**

Adult patients (>18yr) presenting to the hospital with de novo AML between January 2000 and December 2006 and who had a D-dimer level performed on admission were selected retrospectively. Patients with APLM, those developing AML from known antecedent myelodysplastic syndrome (MDS) or myeloproliferative disorders (MPD) and patients not treated with curative intent were excluded leaving a final cohort of 44. New patients admitted from January 2007 to the beginning of November 2008 fulfilling the above criteria (n=17) have been added to the study and the data combined. The final patient cohort consists of 61 patients. No patient was known to have any other condition which causes elevated D-dimer levels at presentation compared to those in which D-dimer levels were elevated.
Statistical analysis

Statistical analyses were performed using SPSS Statistics (Version 16, IBM, Chicago, USA). Two-sided p values of <0.05 were considered statistically significant. Relationships between categorical variables were compared using the chi-squared test. Relationships between continuous variables were compared using the Mann-Whitney U test. Kaplan-Meier survival curves were constructed for the total patient cohort with the cohort subdivided according to cytogenetic risk group and into those with normal and those with elevated D-dimer at diagnosis. Differences in survival were tested by Log Rank calculation (Mantel-Cox). Multivariate analysis of the data was not performed. D-dimer and favourable cytogenetic risk groups both consisted of less than 10 patients. It has been demonstrated that significant errors may occur using proportional hazard regression in cases where there are less than 10 events/variable (Peduzzi et al 1995). It was therefore decided to defer this analysis until more data has been collected.

Ethics approval

The study has been approved as a Database Research Request by the Human Research Ethics Committee of the Northern Sydney Central Coast Area Health Service. Protocol no: 0712-238M.

Results

Sixty-one patients who presented with de novo AML between January 2000 and October 2008 and who were treated with standard or high dose induction chemotherapy were included in this study. Approximately half the patients received a bone marrow transplant and half did not. The mean age of patients at diagnosis was 48.7 years with 72.1% less than 60 years. This represents a somewhat younger cohort than is normally found in AML (Grimwade et al 2001; Milligan et al 2006). This is explained by selection for inclusion only of patients considered fit to receive treatment with curative intent. Patients placed on palliative regimes were excluded.

At the time of admission and prior to treatment the following parameters were measured for each patient: plasma D-dimer, haemoglobin, total WCC, platelets and LDH. The patients were then divided into two groups: those with normal D-dimer at presentation (n=9) and those with elevated D-dimers (n=52). The frequencies of the following characteristics in the two groups were compared: sex (male/female), mean values and range for age for the WCC, LDH, platelet count, bone marrow blast percentage, and cytogenetic risk group (good/intermediate/poor) (Table 1). The groups were then compared for treatment protocols: induction chemotherapy (standard/intensive), and bone marrow transplant (allogeneic/autologous//none) (Table 2). They were also compared for outcome: complete remission after induction, relapse proportion (Table 3) and for median survival (Fig. 1).

A significant difference (p<0.05) was found between the two groups for WCC at diagnosis (Fig. 2), LDH at diagnosis (Fig. 3), occurrence of relapse after induction (Table 3) and survival (Fig. 1). No significant difference was found between the two D-dimer groups for age at diagnosis, sex, platelet count, bone marrow blast percentage and cytogenetic risk group. Treatment (cytosine intensity at induction, use of bone marrow transplant) did not differ significantly between the two groups. A number of variables are measured at diagnosis in patients with AML in an attempt to predict outcome. The most powerful positive predictor for survival is still favourable cytogenetic risk group. Hyperleucocytosis, defined as a WCC >100 x 10^9/L, and age >60 yr are considered to be predictors of inferior survival (Milligan et al 2006). Eight of the nine patients in the normal D-dimer group (88.9%) were aged <60 yr compared to 36 of 52 (69.2%) in the elevated D-dimer group. All nine patients in the normal D-dimer group had a WCC on presentation of <100 x 10^9/L compared with 45 of 52 patients (86.5%) in the elevated D-dimer group. Table 4 compares the significance of normal D-dimer at diagnosis as a prognostic indicator to the above predictive variables. A significant difference in overall survival was found by univariate analysis for normal D-dimer group (n=9) and good cytogenetic risk group (n=8). No significant difference was found between those patients with WCC <100 x 10^9/L (n= 54) at diagnosis vs those with a count of >100 x 10^9/L (n=7) or age group <60yr (n=44) vs >60yr (n=17) in this group of patients.

Kaplan-Meier survival curves have been constructed for the cohort overall (Fig. 4) and the cohort divided by criteria shown to be predictive for survival by univariate analysis: D-dimer group (Fig. 1) and cytogenetic risk group (Fig. 5).

Most AML patients fall into an intermediate risk group for survival using conventional cytogenetic techniques. In an attempt to further stratify prognosis in this group of patients additional cytogenetic and molecular techniques are now applied. A simple test such as D-dimer would be of value if it proves to help in the stratification of these patients. Therefore the patients with normal D-dimer were subdivided according to cytogenetic risk group and their survival compared to the mean survival for the whole cohort. Results are shown in Table 5 and illustrated in Fig. 6. One patient in the poor cytogenetic risk group died shortly after diagnosis. With this exception, patients in intermediate and poor cytogenetic risk groups with normal D-dimer levels appear to show survival times in excess of the average for their risk group. Numbers were too small, however, for
Table 1.
Frequency of characteristics at diagnosis for the total patient cohort and the cohort divided into those with normal and elevated D-dimer at presentation. The p values of <0.05 are significant and in bold.

<table>
<thead>
<tr>
<th>Presenting characteristics</th>
<th>Group</th>
<th>Chi squared</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Normal DD group</td>
<td>Elevated DD group</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>35/61 (57.4%)</td>
<td>4/9 (44.4%)</td>
<td>31/52 (59.6%)</td>
</tr>
<tr>
<td>Female</td>
<td>26/61 (42.6%)</td>
<td>5/9 (55.6%)</td>
<td>21/52 (40.4%)</td>
</tr>
<tr>
<td>Age</td>
<td>49 (19-75)</td>
<td>43.3 (22-73)</td>
<td>49.6 (19-75)</td>
</tr>
<tr>
<td>WCC x 10^9/L</td>
<td>45.7 (1.6-322.9)</td>
<td>10.1 (1.9-27.7)</td>
<td>51.9 (1.6-322.9)</td>
</tr>
<tr>
<td>LDH IU/L</td>
<td>727 (154-3964)</td>
<td>427 (154-1442)</td>
<td>779 (181-3960)</td>
</tr>
<tr>
<td>Platelet count x 10^9/L</td>
<td>91 (3-286)</td>
<td>109 (12-286)</td>
<td>87 (3-282)</td>
</tr>
<tr>
<td>Bone marrow blast count %</td>
<td>62 (8.94)</td>
<td>60 (32-76)</td>
<td>62 (8.94)</td>
</tr>
<tr>
<td>Cytogenetic Risk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>8/61 (13.0%)</td>
<td>2/9 (22.3%)</td>
<td>6/52 (11.5%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>40/61 (65.6%)</td>
<td>4/9 (44.4%)</td>
<td>36/52 (69.2%)</td>
</tr>
<tr>
<td>Poor</td>
<td>13/61 (21.4%)</td>
<td>3/9 (33.3%)</td>
<td>10/52 (19.3%)</td>
</tr>
</tbody>
</table>

Table 2.
Comparison of treatment protocols for the whole patient cohort and the cohort divided into those with normal and elevated D-dimer at presentation.

<table>
<thead>
<tr>
<th>Treatment protocol</th>
<th>Group</th>
<th>Chi squared</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy induction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensive</td>
<td>31/61 (50.8%)</td>
<td>6/9 (66.7%)</td>
<td>25/52 (48.1%)</td>
</tr>
<tr>
<td>BM transplantation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allogeneic</td>
<td>18/61 (29.5%)</td>
<td>3/9 (33.3%)</td>
<td>15/52 (28.8%)</td>
</tr>
<tr>
<td>Autologous</td>
<td>11/61 (18.0%)</td>
<td>0</td>
<td>11/52 (21.2%)</td>
</tr>
<tr>
<td>No transplant</td>
<td>32/61 (52.5%)</td>
<td>6/9 (66.7%)</td>
<td>26/52 (50.0%)</td>
</tr>
</tbody>
</table>

Table 3.
Comparison of outcomes for the whole patient cohort and the cohort divided into normal and elevated D-dimer levels at presentation. The p values of <0.05 are significant and in bold.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Group</th>
<th>Chi squared</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Normal DD group</td>
<td>Elevated DD group</td>
</tr>
<tr>
<td>Induction Response</td>
<td>42/61 (68.8%)</td>
<td>6/9 (66.6%)</td>
<td>36/52 (60.2%)</td>
</tr>
<tr>
<td>Relapse</td>
<td>17/61 (27.9%)</td>
<td>0/9</td>
<td>17/52 (32.7%)</td>
</tr>
</tbody>
</table>

Figure 1.
Kaplan-Meier survival curve for the cohort divided into normal and elevated D-dimer groups.
Figure 2. Comparison of normal and elevated D-dimer groups with WCC at diagnosis. Box-whisker plots showing medians, quartiles, extreme values and outliers.

Figure 3. Comparison of normal and elevated D-dimer groups with LDH at diagnosis. Box-whisker plots showing medians, quartiles, extreme values and outliers.

Figure 4. Kaplan-Meier survival curve for whole patient cohort.

Figure 5. Kaplan-Meier survival curve for the cohort divided into good, intermediate and poor cytogenetic risk groups (p=0.028).

Figure 6. Survival times for normal and elevated D-dimers divided by cytogenetic risk group.
any definitive conclusions about the prognostic value of D-dimer in intermediate and poor cytogenetic risk groups at the close of the study.

Table 4.
Comparison of overall survival of the whole patient cohort divided according to risk groupings. The p values of <0.05 are significant and in bold.

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>p value (univariate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WCC at diagnostic: &lt;100 vs &gt;100 (x10^9/L)</td>
<td>0.184</td>
</tr>
<tr>
<td>Age group: &lt; 60 vs &gt;60 yr</td>
<td>0.072</td>
</tr>
<tr>
<td>D-dimer group: normal vs elevated</td>
<td>0.041</td>
</tr>
<tr>
<td>Cytogenetic risk group</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Table 5.
Survival in weeks of the nine patients with normal D-dimers divided according to cytogenetic risk group.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Cytogenetic risk group</th>
<th>Survival*</th>
<th>Mean for normal DD group*</th>
<th>Mean for whole patient group*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Good</td>
<td>174</td>
<td>281</td>
<td>321</td>
</tr>
<tr>
<td>2</td>
<td>Good</td>
<td>402</td>
<td>281</td>
<td>321</td>
</tr>
<tr>
<td>3</td>
<td>Intermediate</td>
<td>181</td>
<td>123</td>
<td>73</td>
</tr>
<tr>
<td>4</td>
<td>Intermediate</td>
<td>127</td>
<td>123</td>
<td>73</td>
</tr>
<tr>
<td>5</td>
<td>Intermediate</td>
<td>397</td>
<td>123</td>
<td>73</td>
</tr>
<tr>
<td>6</td>
<td>Intermediate</td>
<td>466</td>
<td>123</td>
<td>73</td>
</tr>
<tr>
<td>7</td>
<td>Poor</td>
<td>123</td>
<td>80</td>
<td>44</td>
</tr>
<tr>
<td>8</td>
<td>Poor</td>
<td>256</td>
<td>80</td>
<td>44</td>
</tr>
<tr>
<td>9</td>
<td>Poor</td>
<td>5</td>
<td>80</td>
<td>44</td>
</tr>
</tbody>
</table>

* Includes censored and uncensored data at end of study

**Discussion**

The object of this study was to establish if a normal level of D-dimer at diagnosis is associated with good prognosis for survival in AML patients. This positive association has been demonstrated on many occasions in solid tumours. There is already some evidence that low levels of some other components of the fibrinolytic system, PAI (Yilmaz et al 2006) and uPAR (Graf et al 2005) relate to improved survival in AML, but these are not so easily quantitated as D-dimer. This study is restricted to patients presenting with de novo AML with no known antecedent haematological disorder. Patients developing AML from myeloproliferative or myelodysplastic disease are often of an older age group with associated co-morbidities which may elevate D-dimer levels and this may confound the data. There are also potential difficulties in measuring D-dimer at the exact time of transformation in patients with progressive disease. Most patients in the cohort were found to have D-dimer levels elevated above the normal range of <0.5ug/ml FEU.

However a small subset of patients (nine out of 61), had D-dimer values in the normal range. Since patient numbers were limited it was decided to divide the patients into two groups only: those with normal D-dimer at diagnosis and those with elevated levels. The two groups were compared for sex, age, WCC, LDH, platelet count, percentage of bone marrow blasts, cytogenetic risk group, type of induction chemotherapy, type of bone marrow transplantation (if any), response after first induction regime and duration of remission. A normal D-dimer at diagnosis was found to correlate with lower numbers of circulating white cells and lower (though usually elevated) LDH levels, perhaps indicating a less aggressive disease process. The power of D-dimer as a prognostic indicator for survival in this cohort was compared to other known prognostic indicators. Although they were few in number, the patients with normal levels of D-dimer at diagnosis had significantly lower rate of relapse (p=0.050) and overall survival (p= 0.041) compared with those in whom levels were elevated. No patients with normal D-dimer had relapsed at the completion of the study compared with relapse in 32.7% of the elevated D-dimer group. There was no difference in response to first induction between the two groups. Interestingly, in the normal D-dimer group, two of three patients in the poor, and all four patients in the intermediate cytogenetic risk group are still surviving (Table 5 and Fig. 6) However, numbers are small and no statistical significance could be attached to this finding at the conclusion of the study. The favourable survival and reduced rate of relapse noted in this small group of patients awaits confirmation in a future larger study.

The potential biological explanation of these findings lies in the function of some components of the fibrinolytic system in malignancy. In solid tumours it has become increasingly evident over more than twenty years that many components of the coagulation/fibrinolytic system play an important role in tumourgenesis. Fibrin remodelling occurs in the formation of tumour stroma, in the migration of many types of normal and neoplastic cells and in neovascularization in a manner analogous to its role in wound repair (Palumbo et al 2000). Vascularization is essential in tumour growth for the provision of metabolites and oxygen, cellular growth factors and cytokines. Angiogenesis stimulators such as vascular endothelial growth factor (VEGF) and fibroblast growth factors (FGFs) are often up-regulated in the tumour environment, along with many components of the coagulation and fibrinolytic systems, which interact in a synergistic fashion (Wojtukiewicz et al 2001). As elevation of D-dimer is a marker of fibrin remodelling it is to be expected that raised D-dimer levels have been shown to correlate with elevations of Interleukin 6 (IL6), VEGF and bFGF in cancer patients (Dirix et al 2002; Salgado et al 2002). Perhaps more surprisingly in prognostic studies, normal D-dimer levels have been shown to be an equal
or better predictor for survival than the more commonly used tumour markers CEA in colorectal cancer (Blackwell et al 2004) and CA125 in ovarian cancer (Mirshahi et al 1992; Gadducci et al 1996). In advanced malignancies a fall in D-dimer levels after treatment with prophylactic low molecular weight heparin (LMWH) has been shown to be associated with improved survival (Di Nisio et al 2005).

In tissue, fibrinolysis is initiated when uPA binds to its receptor on the cell surface activating plasminogen with resulting generation of plasmin. The generated plasmin also directly proteolyses the extracellular matrix (ECM) and releases growth factors whose activity will induce further ECM degradation (Mazar 2008). The uPA/uPAR complex also appears to have a major role in the metastatic process independent of plasmin formation; in intercellular signalling, as shown by its accumulation at cell-cell contact sites, and in cell/ECM interactions, such as with the ECM protein vitronectin which aids cellular migration and tumour cell invasion (Andreasen et al 1997). Histochemically the complex has been located on the leading edge of migrating monocytes and invading tumour cells and anti-uPA antibodies have been shown to have the ability to block tumour metastases (Tapiovaara et al 1996). uPAR also has a role in intracellular signalling. High levels are required for β1 intergrin mediated signalling (Rigolin et al 2003; Wei et al 2006), and it has been shown to up-regulate MAPK, ERK, and p38MAPK (Aguirre-Ghiso et al 2001) and possibly many other intracellular signalling pathways either directly or indirectly (Mazar 2008). Like D-dimer, high levels of u-PA and uPAR have been associated with poor prognosis in many solid tumours; in fact the group of neoplasms is almost identical (Dass et al 2008). As yet it does not appear that a direct correlation between elevated levels of uPA, uPAR and D-dimer has been measured in any solid tumours or in AML.

While the activity of uPA/uPAR, other plasminogen activators and D-dimer is becoming increasingly understood in solid tumours, their roles are less easily explained in a malignancy such as AML. Tissue invasion, akin to metastasis, does occur, especially in monocytic leukaemias whose blasts exhibit bright uPAR expression (Lanza et al 1998), and malignant blasts occasionally cross the blood/brain barrier aided by the fibrinolytic proteases, but neither event occurs with such frequency as to cause substantial statistical variability in patient survival. There is increasing indirect evidence however that plasminogen activation on the surface of blast cells plays a role, yet to be fully explained in AML. The activation of plasminogen in AML was reviewed in 1996 (Tapiovaara et al 1996). Neither in this review nor in subsequent studies has there been a definitive explanation for the role of plasminogen activation on the surface of leukaemic blasts. The current general consensus is that the activity of PAs plays an essential role in adhesion, migration and extravasation of leukaemic cells from the bone marrow (Gräf et al 2005; Yılmaz et al 2006; Tapiovaara et al 1996). No doubt the uPA/uPAR complex also plays a role in cellular signalling in leukaemic blasts, in a manner analogous to solid tumour cells. Generated plasmin may be involved in the regulation of haemopoiesis as it is capable of releasing and activating cytokines (Tapiovaara et al 1996). In another haematological malignancy, multiple myeloma, high expression of uPAR is also correlated with poor survival, bone marrow involvement and the activation of cytokines causing bone matrix degeneration (Rigolin et al 2003).

Conclusion

In conclusion, it is likely that plasminogen activation on the surface of blast cells plays a role, yet to be fully defined, in the disease process in AML. Elevated plasma D-dimer levels are a marker of plasminogen activation and normal levels in a small subset of AML patients at diagnosis appears to confer favourable prognosis for survival. D-dimer is a simple and readily available assay. A larger study would be required to assess its value as a useful prognostic marker in AML and it is to be hoped that when more data has been collected these findings can be confirmed. D-dimer may prove to be a valuable addition to the panel of prognostic tests available in AML especially in those patients with intermediate and poor cytogenetic risk.

Acknowledgements

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References


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Harmonising quality to the lowest clinical diagnostic standard?
The case against regulation of in vitro diagnostics (IVDs) for use in clinical diagnostic laboratories.

Emmanuel J Favaloro
Department of Haematology, Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, NSW.

Abstract

A revised framework for the regulation of in vitro diagnostic devices (IVDs) will come into force on July 1, 2010 that aims to ‘ensure that public and personal health are adequately protected’, but which instead may lead to adverse outcomes in clinical diagnosis and management. The regulatory process aims to regulate all IVDs, including those used by clinical diagnostic laboratories, which are already subject to scrutiny as part of the current laboratory accreditation process. The intended aim of the IVDs regulatory process also appears to imply that the current procedure for regulation of clinical laboratories and their use of current IVDs is somehow inadequate and is placing the public at risk, although the evidence clearly indicates the opposing view, showing ongoing improvements over time in clinical diagnostic practice, driven by current accreditation requirements. In contrast, existing evidence related to regulation of IVDs in the USA, for example, highlights several failings in this process that leads to ongoing use of inappropriate test panels or methodologies in clinical diagnostics. This is largely due to direct and indirect costs associated with, and the effort needed to fulfil, regulatory requirements. The end result is that manufacturers of IVDs will conform to existing regulatory standards ahead of clinical best practice standards, and that future improvements to clinical best practice standards may not be translated into clinical practice because the regulatory burden may act as an impediment to manufacturers to undertake any future improvements in existing IVDs. It is therefore difficult to identify how the new framework will achieve its goal of ‘ensuring public and personal health’ with respect to the regulation of IVDs used in clinical diagnostic laboratories, since ‘public and personal health’ is best served by the timely introduction of appropriate diagnostics. Thus, the alternative outcome, that public and personal health will suffer following its implementation is the clear danger.

Key words: Accreditation, regulation, external quality assurance, pathology, professional status.

Introduction

If it’s broken, fix it. If it’s not broken, leave it alone.

An imminent change is planned — a brave new world of costly, laborious and inadequately justified regulation of in vitro diagnostic devices (IVDs) for use in clinical diagnostic laboratories. The Australian Government Department of Health and Ageing Therapeutic Goods Administration (TGA 2010) has introduced a new framework for the regulation of IVDs. This has been enacted because the TGA believes that this is ‘international best practice’ and will ‘ensure that public and personal health are adequately protected’, as recently highlighted in this journal by Cohen (2010). The new legislation intends to cover all IVDs, including those for laboratory use as well as those for non-laboratory (e.g., home) use. The current report is not concerned with the regulation of IVDs for non-laboratory use, as this is currently largely unregulated. In contrast, IVDs used by clinical diagnostic laboratories are already subject to scrutiny as part of the current laboratory accreditation process. The current report is therefore focussed on the potential adverse impact that this new regulatory process may have on future laboratory diagnostic practice in Australia.

Address correspondence to:
Dr EJ Favaloro
Senior Hospital Scientist
Department of Haematology
Institute of Clinical Pathology and Medical Research (ICPMR)
Westmead Hospital, WSAHS
Westmead NSW 2145
As mentioned, the TGA believes that the new legislation reflects ‘international best practice’ and will ‘ensure that public and personal health are adequately protected.’ Of course, just because the TGA believes that this is so does not make it so, and the claim that this process will ensure that public and personal health are ‘adequately protected’ implies that the current process is inadequate and is placing the public at risk. The obvious questions here are: (i) where is the evidence that this change truly reflects ‘international best practice’ for clinical diagnostics, and (ii) where is the evidence that the current process of accreditation and laboratory scrutiny (inclusive of laboratory utilised IVDs) is inadequate and is placing the public at risk?

What does the change mean?

Cohen (2010) nicely summarised most of the intended changes and their general outcomes in his review, but the clinical practice changes actually reflect the potential for far wider adverse outcomes (as summarised in Tables 1 and 2).

(i) Cost and supply issues

As Cohen (2010) highlighted, the TGA operates as a full cost recovery agency, deriving its income from fees levied to sponsors and manufactures that by law are required to submit documentation related to safety, quality and efficacy, prior to inclusion on the Australian Register of Therapeutic Goods (ARTG). Full cost recovery will also apply to the regulation of IVDs. Does the fact that the TGA will receive income from a process it is promoting as necessary to protect the public warrant further independent assessment?

Although the fee structure has been defined and is currently available on the TGA website, the actual level of cost and the total burden of impact can only be speculated, and also too the effect on future supply of clinical diagnostic products. The overall cost is certainly likely to be substantial, and will not only include direct costs associated with levied fees, but also indirect costs associated with the requirements for manufacturers/suppliers/distributors to undertake additional clinical studies and to collate and provide documentation to the TGA in the format required for their assessment.

The ‘Cost Recovery Impact Statement’ (2006) from the TGA website notes that the “Australian market accounts for 1.35% of global sales of IVDs”, and further that although “the number of businesses engaged in supplying IVDs and associated equipment to pathology laboratories in Australia is estimated to be 160” that the “market is highly concentrated with the four main suppliers accounting for 70% of sales in Australia”, and also that the “majority of suppliers would be classified as small business”.

These manufacturers and suppliers/distributors will need to assess whether costs can be borne by the diagnostics market, with profits typically narrower than those of the therapeutics market, and without a doubt, any costs ensued will eventually be passed onto laboratories, which may then eventually pass them onto their customer base. Alternatively, manufacturers/suppliers/distributors may decide that the process is not viable for some IVDs, and therefore abandon supply of those IVDs to the Australian market. Where IVDs continue to be available but at a greatly increased cost, laboratories may decide to abandon such testing as economically unviable, thereby reducing services, or else changing to cheaper, potentially clinically inferior products or test panels, thereby compromising patient safety and simply countering the intended purpose of the new regulatory process that claims to ‘ensure that public and personal health are adequately protected’. For public and tertiary level pathology institutions, extra costs will need to be borne by additional public funding, since the actual costs of providing these levels of services are typically greater than that recouped by revenue. There will also likely be claims for additional Medicare and directed health funding. Thus, one or other health branch of the government will in effect be paying the costs of another health branch of the government.

In any case, this regulatory process is likely to shrink market supply, reduce competition and favour larger manufacturers and suppliers with larger economies of scale. As noted by Cohen (2010), suppliers of low volume tests and small companies may decide that this process is not commercially viable. These companies are often the cornerstones of progressive or niche-level diagnostic clinical testing. What this may also therefore mean is the reduction in overall diagnostic test quality to the lowest common denominator. Several examples related to my area of interest are provided later in this report.

(ii) Double regulation and more.

For clinical laboratories to operate in Australia, they are subject to strict laboratory accreditation requirements, as set out by the National Pathology Accreditation Advisory Council (NPAAC 2010) and monitored by the National Association of Testing Authorities (NATA; 2010). Although the regulation of IVDs for use in clinical diagnostic laboratories does not intend to regulate clinical laboratory practice per se, the regulation of laboratory IVDs will in effect have a similar outcome, and will thus reflect an additional ‘regulatory oversight’ of laboratory practice. There are additional implications in regards to ‘in-house’ assays as highlighted later in this report.

Clinical laboratory accreditation by NATA requires laboratories to adhere to specific ISO guidelines as well as to NPAAC guidelines. This process is supplemented by many other organisations including external quality
Table 1.
Summary of potential advantages of IVDs legislation to clinical diagnostic practice

<table>
<thead>
<tr>
<th>Theoretical advantages</th>
<th>Comments</th>
<th>Questions Raised</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Conformity in production and testing</td>
<td>• Conformity to a inferior scientific and/or clinical standard will not improve or protect public and personal health.</td>
<td>• Does the TGA have the scientific and clinical expertise to identify world best practice for the wide diversity of IVDs that currently exist or that may be introduced over time?</td>
</tr>
<tr>
<td>• Protects public and personal health</td>
<td>• Effective laboratory regulatory systems that oversee IVDs are already in place for clinical diagnostic testing</td>
<td>• Where is the evidence that the current regulatory process for clinical laboratory testing overseeing the use of IVDs is inadequate and is placing the public at risk?</td>
</tr>
<tr>
<td>• Involves assimilation of regulatory burden for laboratories, consumers and government</td>
<td>• Implication in the wording used in the legislation is that the current laboratory testing process is inadequate and is placing the public at risk.</td>
<td>• Where is the evidence that this new change reflects ‘international best practice’ for diagnostics and clinical practice?</td>
</tr>
<tr>
<td>• The new legislation creates an additional regulatory burden that is not sufficiently justified.</td>
<td>• The new legislation creates an additional regulatory burden that is not sufficiently justified.</td>
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Table 2.
Summary of potential disadvantages of IVDs legislation to clinical diagnostic practice

<table>
<thead>
<tr>
<th>Theoretical disadvantages</th>
<th>Comments</th>
<th>Questions Raised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase in costs to manufacturers, suppliers, distributors, laboratories, consumers and government</td>
<td>Costs are substantive. Manufacturers/ suppliers/ distributors will either pass on costs or cease production or supply. Costs passed on to laboratories will then either be passed on to consumers or need to be paid by the government, thereby increasing health costs.</td>
<td>What evidence exists to justify this increased cost burden? Is the government truly aware that the increased costs of this legislation will be substantial and need to be largely covered by additional public funding? Does the fact that the TGA will receive income from a process it is promoting as necessary to protect the public warrant further independent assessment?</td>
</tr>
<tr>
<td>Shrinkage of market and competition</td>
<td>Small (local) manufacturers/ suppliers/ distributors unable to recoup costs will cease producing/ providing assay kits. Increased likelihood of importing overseas products, although alternatively possible that overseas manufacturers will also avoid the Australian market. Overall market will shrink and competition will fall.</td>
<td>What will be the effect on the balance of trade and Australian business and employment? What will be the adverse effects on patient diagnosis and management?</td>
</tr>
<tr>
<td>Some tests will not be supportable</td>
<td>Small volume or niche testing may become unsupportable. Tertiary level testing often represents small focussed diagnostic investigations, and this is most threatened. Test panels for complex disease will be reduced.</td>
<td>What will be the adverse effects on patient diagnosis and management?</td>
</tr>
<tr>
<td>Reduction of quality to lowest 'acceptable' standard or cheapest option</td>
<td>Cost rather than quality/world best practise will likely drive testing processes for some tests.</td>
<td>What will be the adverse effects on patient diagnosis and management?</td>
</tr>
<tr>
<td>Increased and unnecessary additional regulatory burden for laboratories</td>
<td>Laboratories are already regulated using a co-ordinated system that comprises several expert groups. There is no evidence that the system is not working to provide a safe diagnostic service for the public. There is no evidence that the new legislation will provide substantive improvements to clinical diagnostic practice, and the presiding evidence from overseas suggests that regulation of IVDs may hinder best practice and lead to delays in the introduction of some tests, reluctance to introduce diagnostically improved tests once a test is registered, and thus lowered quality in diagnostics and potentially compromised patient management.</td>
<td>What will be the adverse effects on patient diagnosis and management?</td>
</tr>
<tr>
<td>Compromised patient care placing the public at increased risk</td>
<td>Current evidence from the literature suggests that existing IVD regulation can create adverse outcomes in patient diagnosis due to laboratory incorporation of incomplete test panels and/or retaining old methodologies. Regulatory costs cause substantive delays (sometimes decades) in the introduction of some tests. Manufacturers preferentially produce and retain products that conform to an existing regulatory standard rather than create new products that reflect world best practice in diagnostics, if those changes then mean re-registration of said products.</td>
<td>What assurances can be given to negate these evidences and ensure that this will not occur in Australia due to the adoption of this legislation? What recourse do manufacturers, laboratories, scientists, clinical pathologists and expert groups have should they find evidence of these effects following the adoption of this legislation? Will there be an independent expert adjudicator?</td>
</tr>
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assurance (EQA) activities, as provided for example by the RCPA Quality Assurance Program (QAP) (2010). Adequate performance in an accredited EQA is mandatory for continued clinical laboratory accreditation in Australia (and for most other developed countries; Olson et al 2007). This process is overseen by clinical pathologists and laboratory scientists, typically members of professional organisations such as the Royal College of Pathologists of Australasia (RCPA; 2010) and the Australian Institute of Medical Scientists (AIMS; 2010). These organisations and professionals already assess laboratory practice, including the performance of IVDs for clinical diagnostic use, thereby ‘ensuring that public and personal health are adequately protected’. Indeed, this process, as undertaken by those experts most knowledgeable in each specific field, is best placed to ensure the adequacy of clinical diagnostic testing, and thus the application of, or drive towards, world best diagnostic laboratory practice.

There is substantial evidence in the literature that all of these co-ordinated processes already help drive quality improvements and standardisation. In regards to peer-driven changes influenced by EQA findings, and as an example in my field of expertise (haemostasis), test methods are constantly evolving and improving as a result of such activities. High variation methodologies such as electro-immunodiffusion (EID) were initially replaced by enzyme linked immunosorption assay (ELISA) procedures, and are currently being replaced by latex immuno assay (LIA) technology (Favaloro and Bonar, 2007). Laboratories will replace inferior methodologies as an ongoing natural process as techniques improve, in part because of automation, and in part driven by the EQA process itself – since older methodologies do not perform as well as newer or superior methodologies, as highlighted by comparative peer review EQA, and adequate EQA performance is a requirement of clinical laboratory accreditation. Thus, the EQA process itself will drive change towards improvement (or world best practice) in clinical diagnostics.

Importantly, EQA also provides evidence that method standardisation or regulatory conformity is not invariably favourable. For example, one commercial kit for one assay process used to identify the Antiphospholipid Syndrome (APS) was observed to generate occasional outlier findings to the major method groups, but the accompanying donor clinical information over the period of testing actually identified better diagnostic correlation with this outlier kit (Favaloro et al 2005; 2007a). Additional evidence for EQA driven improvements related to my specific area of interest is also provided below and elsewhere (Favaloro and Bonar 2007; Bonar et al 2010). It would be expected that other disciplines could provide similar evidence.

Indeed, within my own field of haemostasis, provision and assurance of clinical diagnostic testing quality to ‘ensure that public and personal health are adequately protected’ is additionally driven by many other expert organisations. At the risk of offending some organisations by omission, these include the World Health Organization (WHO 2010), the World Federation of Haemophilia (WFH 2010), the National Institute for Biological Standards and Control (NIBSC; 2010), and the Clinical Laboratory Standards Institute (formerly NCCLS – National Committee for Clinical Laboratory Standards; CLSI 2010). There are also a large range of expert committee groups, including the UK Haemophilia Centre Doctors’ Organisation (UKHCDO 2010), the Italian Association of Hemophilia Centers (Tagliaferri et al 2010), and perhaps most influential for general matters of standardisation in haemostasis and thrombosis, the many and various Scientific Subcommittees for Standardisation (SSC) of the International Society on Thrombosis and Haemostasis (ISTH 2010). For EQA, we can now include the External Quality Assurance in Thrombosis and Hemostasis (EQATH) group (Olson et al 2007). Health professional experts including scientists and clinicians have input into, or are members of, all these organisations.

It is unclear how adding another layer of bureaucracy via the current IVDs legislation will further assist to ensure public protection in clinical laboratory diagnostics, and as already stated, the perceived need to undertake this additional process implies that the current process is failing. Where is the evidence for this?

(iii) Improving quality, improving standardisation, or reducing quality to the lowest acceptable standard?

The application of TGA review of IVDs is likely to increase conformity, and may even improve standardisation, although this is not in itself necessarily a good outcome (Tables 1 and 2). The bigger question is whether the standardisation or conformity will result in a system that encourages adoption of world best practice for clinical diagnostic testing, or instead the adoption of the lowest common quality standard. The terms ‘quality’ and ‘standardisation’ have different meanings to different people, and also to different organisations. For scientists and clinicians involved in pathology testing, the emphasis is on using the best test process for the best diagnostic and patient management outcome. Medical scientists and pathologists will often apply differential testing on a case-by-case basis using detailed knowledge regarding the specific circumstances. In tertiary level pathology testing, many tests are in-house derived, and not just because they are cheaper to run than commercial methods; often, these tests are at the forefront of diagnostics, and commercial tests are often unavailable or diagnostically inferior.
That in-house assays may be superior to commercial methods, just like newer methodologies released by small niche companies may be superior to existing commercial methods that are mass produced, may not be well recognised by regulators and politicians.

The new legislation therefore potentially threatens world best practice in clinical diagnostic testing, not only because of increased (potentially unsustainable) cost burdens, but also because there is a need to continue to utilise these technologies and to integrate new technologies in a timely, sensible and cost-effective manner. It is difficult to see how the new legislation will promote these initiatives in laboratory diagnostics, and evidence from the literature suggests that the opposite is true (see below).

Regulation of IVDs – a world view

Although this topic is largely outside the scope of the intended report, it is useful to briefly overview current practice related to world-wide regulation of IVDs. In the USA, this process is undertaken by the Federal Food and Drugs Administration (FDA 2010), and overseen by the Clinical Laboratory Improvement Amendments (CLIA) of 1988 and the College of American Pathologists (CAP) (Lippi et al 2010). To bring an IVD to market in the USA requires either a new submission (a ‘Pre-market approval’, or PMA), or a submission that reflects on substantial equivalence to a predicate device (a so-called 510(k) submission). Since the latter reflects a substantially cheaper option (fee ~ $2000) than the PMA (fee ~ $50,000), most manufacturers head down this path. In Europe, manufacturers are required to register IVDs with a ‘Competent Authority’ before they can be sold in the European Union. The European Community mark is applied to the product by the manufacturer before it is placed on the European market. This mark may only be applied to an IVD for which the manufacturer has signed a declaration of conformity, indicating that it conforms to the requirements of the directive. However, these processes are subject to local laws that see the use of IVDs differentially applied in different geographies (Lippi et al 2010).

The process as intended to apply in Australia is different to both the US and European processes, and perhaps most closely aligns to that applied in Canada. What this means in practice is that an IVD will need to be registered by different processes and rules in each international geography. Prior registration in Europe or in the USA will not alter the need to register the same IVD in Australia under the rules of the new legislation and it will not even necessarily reduce the costs or labour burden associated with this process. The Australian legislation is also likely to reflect the greatest direct and indirect costs for registering an IVD in any international market. For a market that reflects <2% of the global market, the concern is that an international manufacturer is unlikely to focus too heavily on the Australian IVD market. Similarly, an Australian manufacturer may be better placed to export their IVDs to less heavily regulated markets.

Adverse effects of regulation of IVDs in clinical diagnostic practice - recent lessons from haemostasis

It is useful here to provide some examples from the field of haemostasis to highlight some additional issues related to clinical diagnostics, and how the new legislation may lead to a reduction in the quality of this testing.

(i) Investigation of von Willebrand disease

Von Willebrand disease (VWD) is a common bleeding disorder that arises from defects and/or deficiencies in a plasma protein called von Willebrand factor (VWF). VWD is diagnosed by clinical features (personal and sometimes family history of bleeding and/or bruising) and is confirmed by laboratory testing, which would ideally require the utility of a large panel of different tests to assess both levels and activity of VWF (Favaloro, 2006). Evidence has consistently shown that this test process is best achieved using a panel of tests that incorporate testing of VWF protein (VWF:Ag), VWF collagen binding (VWF:CB), VWF ristocetin cofactor (VWF:RCo), factor VIII coagulant (FVIII:C), and several additional tests as required on a case by case basis (i.e., multimer analysis, ristocetin induced platelet agglutination, VWF FVIII binding, and so on). This is since VWD is a complex and multifactorial disorder, and because a complete and appropriate diagnosis will best facilitate appropriate patient management. That is, inappropriate or incomplete diagnosis can lead to inappropriate management compromising patient care and health.

The above diagnostic test process represents a complex and time consuming process when appropriately applied, and it is difficult to see how the new legislation will improve clinical laboratory diagnostic testing for this purpose. Indeed, each of these many test components may require independent assessment under the legislation. This will likely be prohibitively expensive for manufacturers, suppliers and laboratories. The legislation is therefore likely to restrict testing in VWD to fewer test components, or may cause laboratories to incorporate fewer tests into their test panels, and this will ultimately compromise patient safety, therebycountering the legislation’s intended purpose ‘to ensure that public and personal health are adequately protected’.

As a specific example, data from EQA studies has consistently shown that use of the VWF:CB can reduce...
diagnostic errors in VWD diagnosis by around 50% (Favaloro et al 2006). That is, laboratories that perform fewer tests within a test panel, or that do not perform the VWF:CB, have twice the diagnostic error rate in VWD than those that perform this assay or that perform more test components within a diagnostic panel. Indeed, the diagnostic error rate identified by EQA studies in laboratories that do not incorporate the VWF:CB is similar to that identified in overseas studies in a true diagnostic setting (Favaloro 2008). The VWF:CB assay has been adopted for use by some 50% of Australasian VWD testing laboratories for over 15 years, and these laboratories consistently perform better in VWD diagnostics as assessed via the EQA (Favaloro et al 2006). However, the VWF:CB is still hardly used within the USA (Favaloro 2002; 2007), perhaps being incorporated into <10% of VWD testing laboratories. The reason for non-use for clinical purposes in the USA is the burden of the requirement for manufacturers to register this assay with the US FDA (2010). The first commercial company to do so will encounter the greatest costs (both direct and indirect), which will then reduce, as future suppliers will subsequently only need to show equivalence to an existing approved product. Nevertheless, we continue to await the first registration for this assay in the USA, some 21 years following the original publication of the assay’s utility (Brown and Bosak 1986). In the interim, patients with VWD in the USA continue to be potentially clinically misdiagnosed. A similar issue related to non-registration of this assay in the USA most likely also prevents the better characterisation of VWF concentrates used as therapy for these patients (Favaloro et al 2007b). In Europe, where the registration burden is less than that of the USA, about 30% of laboratories incorporate the VWF:CB into their test panel (Meijer and Haverkate 2006).

(ii) Investigation of antiphospholipid antibodies

The Antiphospholipid Syndrome (APS) is a clinically serious condition, represented by a range of adverse effects including recurrent thrombosis and recurrent spontaneous miscarriage and foetal growth restriction (Wong and Favaloro 2008a). APS is diagnosed by clinical features and is confirmed by laboratory testing, which requires assessment of the presence of antiphospholipid antibodies (aPL), which may be identified by a range of test processes including solid phase (e.g., ELISA) as well as clot-based assays for the so-called lupus anticoagulant (LA) (Favaloro and Wong 2008). The solid phase assays can comprise a battery of different tests, including anticardiolipin antibody (aCL), anti-beta2 glycoprotein 1 (aB2GPI), anti-prothrombin (aPT), anti-phosphatidylerine (aPS), or complexes thereof (e.g., anti-prothrombin/ phosphatidylerine), as well as multiple isotypes of these (IgG, IGM, and potentially IgA). Clot based assays may also comprise a separate battery of tests, including dilute Russell Viper Venom Time (dRVVT), activated partial thromboplastin time (APTT), kaolin clotting time (KCT), dilute prothrombin time (dPT) and silica clotting time (SCT).

Like the case for VWD testing, the above represents a complex and time consuming process if appropriately applied, and it is difficult to see how the new legislation will improve clinical diagnostic testing for this purpose. Indeed, each of these test components may again require independent assessment under the legislation. This will likely be prohibitively expensive for manufacturers, suppliers and laboratories. The legislation is therefore likely to restrict testing in APS to fewer test components and may thereby compromise patient safety and again counter the legislation’s intended purpose ‘to ensure that public and personal health are adequately protected’.

Again, current evidence is that standardisation of test processes to world best practice in APS has actually been hampered by the regulation of IVDs. Various expert groups continue to be frustrated in this field (de Groot et al 2008; Reber et al 2008), as manufacturers reflect that they are required to standardise their assays towards regulatory requirements (Reber et al 2008; Favaloro and Wong, 2008; Wong and Favaloro 2008b), and that this therefore drives their production process ahead of expert or evidence based recommendations and guidelines that attempt to improve diagnostic practice. Thus, regulation of IVDs as applied to clinical diagnosis of APS is standardised to a regulatory conformity that does not represent current world best practice in diagnostics. Manufacturers are hesitant to improve these assays to reflect world best practice in diagnostics because this would require re-registration of the ‘new’ (improved) IVD. It is again therefore difficult to see how the new Australian IVDs legislation will improve current diagnostic practice for testing of APS in Australia.

Additional examples to the above can also be cited, and it is likely that other diagnostic disciplines could come up with similar examples for their own fields of expertise.

Consultation ignored?

To some extent, the above considerations are not new. There was a period of ‘consultation’ undertaken with stake-holder groups in 2003. Interestingly, several important stake-holders, including AIMS, were not identified by the original ‘consultancy’ process. Nevertheless, these expert groups, including AIMS (2003), and individual professionals, have since provided feedback on the new legislation. It appears that many of the concerns highlighted in these ‘consultations’ have been ignored. As noted by Cohen (2010), the TGA appears keen to push ahead with this process. As also
likely to undergo greater scrutiny by NATA.

In-house assays

The new legislation framework will include regulation of in-house IVDs. Laboratory scientists should recognise that what they consider to be an in-house IVD may not reflect what the TGA considers to be an in-house IVD. According to the legislation, an in-house IVD is: (i) developed de novo; or (ii) developed or modified from a published source; or (iii) developed or modified from any other source, or from its intended purpose, within the confines or scope of a laboratory or a laboratory network, and is not supplied for use outside the laboratory or laboratory network. Importantly, included in the definition of an in-house IVD are (i) commercial IVDs used for a purpose other than that intended by the manufacturer, or (ii) developed or modified from “any source” (this would therefore include a commercial IVD). What this means in practice is that if a laboratory uses a commercial IVD, but ‘modifies’ it for use in their laboratory, that constitutes the development of an in-house assay. That laboratory will then be required to list that ‘in-house assay’ (i.e., their modified commercial IVD method) with the TGA. These ‘in-house assays’ are also likely to undergo greater scrutiny by NATA.

It is unlikely that laboratory scientists, the TGA and NATA will all agree on what constitutes a ‘modification’ to an existing commercial IVD that would warrant listing as an in-house assay at the TGA.

Differential effects of the legislation on laboratory testing according to clinical importance?

The new legislation is likely to have a different effect on different manufacturers/suppliers/distributors as well as on different tests. For the former, larger companies are more likely to weather the impending storm. Since they have larger economies of scale, the associated costs (direct and indirect) and the laborious documentary requirements are likely to reflect a smaller proportion of their available resources. Conversely, smaller companies will be overwhelmed by the requirements, and will rationalise their future product lines. Similarly, routine tests that reflect low-end high-throughput tests will be less affected by the change than will high-end low-throughput tests. It is therefore these high-end tests that are at greatest threat from the new legislation - for a number of reasons. First, the market is smaller, and thus less likely to be developed, including any commercial drive to obtain regulatory approval for these tests. Second, these tests tend to be more complex and costly to perform, and generally less profitable – both to manufacturers/suppliers/distributors and laboratories alike; accordingly, any increased costs associated with their production or performance is likely to have a greater impact on their viability within Australian diagnostic practice. However, these are the tests that are most prized among tertiary level diagnostic centres, since they ultimately have the greatest diagnostic value for individual or complex disease. This can be viewed within a pyramid or iceberg analogy, as per Figure 1. This also once again highlights the potential for the new legislation to reduce Australian laboratory practice to the lowest clinical diagnostic standard.

Are all the stakeholders (including scientists, clinicians and government) truly aware of this potential and does the TGA have sufficient expertise in the complete (very broad) range of tests that currently make up the clinical diagnostics arena to understand the impact of the change? Current accreditation practice accomplishes this process by recruiting experts within each discipline to act as volunteer assessors during the accreditation procedure. For the TGA to understand, in-house, the breadth of clinical diagnostics it would need to replicate the staffing of a tertiary level pathology service.

Figure 1.
The new legislation represents a greater potential for adverse impact on complex, specialised, low throughput, diagnostic tests than on simple, routine, high throughput, screening tests. Thus, the potential for adverse impact is likely to be greatest on tertiary level diagnostic practice or complex disease.

Conclusion

There is a wealth of evidence to prove that the existing regulatory process for clinical laboratories, involving NATA accreditation and expert peer review, with evidence drawn from EQA activities, continues to improve diagnostic testing, with several examples from the field of haemostasis given in this report. In contrast, current evidence points to several failings in relation to existing regulation of IVDs in the USA, and essentially delaying the entry of new tests into diagnostics or leading manufacturers to retain existing methodologies
once already approved to a regulatory standard ahead of developing and releasing improved methodologies that can be evidenced as having superior clinical utility; i.e., they continue to market products that reflect existing regulatory requirements ahead of improved standards of excellence in clinical diagnostics. Accordingly, there is a great danger that Australian laboratories and clinical testing will become over-regulated, both indirectly from the effects of the legislation on commercial IVDs, and directly in relation to ‘in-house’ testing, without appropriate evidence that the current regulatory processes are failing or inadequate. There is also an increased likelihood of delay to the introduction of appropriate tests, the potential for non-introduction of appropriate tests, the potential for introduction or preference of cheaper potentially inappropriate or diagnostically inferior tests or test panels and the loss of world best practice in clinical diagnostic testing. The question that needs to be asked: is this the direction in which Australian clinical diagnostic testing should be heading?

References


"Failure to thrive"

Gillian Rozenberg

South Eastern Sydney and Illawarra Area Health Services, Prince of Wales Hospital, Sydney, NSW

A seven weeks old infant was admitted to hospital with a clinical history of ‘failure to thrive’. A full blood count was performed with the following results:

Hb 123 g/L  WBC 10.2 x 10⁹/L  Platelets 586 x 10⁹/L

Examination of the blood film revealed a marked number of acanthocytes.

A provisional diagnosis of abetalipoproteinaemia was made.

The following chemistry tests were performed:

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>RR</th>
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<tr>
<td>Cholesterol</td>
<td>1.4 mmol/L</td>
<td>3.0-5.5</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>&lt;0.1 mmol/L</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>0.78 g/L</td>
<td>1.10-2.00</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>0.07 g/L</td>
<td>0.60-1.25</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0.4 umol/L</td>
<td>0.5-1.8</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>&lt;1 umol/L</td>
<td>5-14</td>
</tr>
<tr>
<td>Faecal fat</td>
<td>38 mmol/day</td>
<td>0-3.4</td>
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</table>

Abetalipoproteinaemia is a rare autosomal recessive disorder characterised by the presence of acanthocytic red cells in the peripheral blood. The primary defect is due to a mutation and lack of activity in the microsomal triglyceride transfer protein needed to bind lipids to the β-apolipoprotein in plasma. Intestinal absorption of lipids is defective. The plasma levels of cholesterol and triglyceride are extremely low and β-apolipoprotein is undetectable. There is an increase in sphingomyelin in the outer half of the red cell membrane bilayer increasing the surface layer of the cell. This β-apolipoprotein defect leads to the production of acanthocytic red cells, about 50-90% of the red cells being acanthocytes. The sphingomyelin accumulates with cell ageing thus the nucleated precursor red cells and reticulocytes are not affected while the older red cells are affected.

As a result of fat malabsorption and the absence of low density lipoproteins, which transport vitamin E, the red cells of patients with abetalipoproteinaemia are markedly deficient in vitamin E. The level of vitamin A is also reduced.

Peripheral blood film showing a marked number of acanthocytes
Abetalipoproteinaemia usually presents in early childhood with diarrhoea and failure to thrive and is characterised clinically by fat malabsorption, spinocerebellar degeneration, pigmented retinopathy and acanthocytosis. The initial neurologic manifestations are loss of deep-tendon reflexes, ataxia and development of a spastic gait. They also develop retinitis pigmentosa which ultimately progresses to near-blindness. The neurological abnormalities present between five and ten years of age and continue until death in the second or third decade. Despite the marked acanthocytosis and vitamin E deficiency seen in these patients, anaemia and haemolysis are mild. The haemoglobin levels are invariably normal.

Treatment consists of a low-fat, high caloric, vitamin-enriched diet accompanied by large supplemental doses of vitamin E. It is imperative that treatment be initiated as soon as possible to delay the onset of the neurological symptoms. New therapies for this debilitating disease are needed.

Address correspondence to:
Gillian Rozenberg FAIMS
Senior Scientist
Department of Haematology
Prince of Wales Hospital
Barker Street
Randwick NSW 2031
Email: gillian_rozenberg@yahoo.com

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<table>
<thead>
<tr>
<th>Day 1:</th>
<th>Day 2:</th>
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<tbody>
<tr>
<td>Red cell nomenclature</td>
<td>Malaria</td>
</tr>
<tr>
<td>WHO Classification of myeloproliferative neoplasms</td>
<td>Lymphocytes: reactive / neoplastic</td>
</tr>
<tr>
<td>WHO Classification of myelodysplastic syndromes</td>
<td>Paediatric Haematology</td>
</tr>
<tr>
<td>WHO Classification of acute myeloid leukaemia and related precursor neoplasms</td>
<td>Case Studies</td>
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JUNE 26-JULY 1: International Congress of the International Society of Blood Transfusion (ISBT) together with the German Society for Transfusion Medicine and Immunohematology (DGTI), Berlin. www.isbt-web.org/berlin/


JULY 4: Laboratory Leadership and Management Workshop, Sydney. AIMS President A/Prof Tony Woods will speak on the topic ‘What does it take to be a successful scientist?’ www.asm2010.org/workshops_leadership.htm

JULY 4-8: ASM 2010 Sydney - Bridging Diverse Cultures. Sydney Convention Exhibition Centre Darling Harbour - Sydney NSW. www.asm2010.org

JULY 23-24: AIMS/RCPA Morphology Workshops. Australian Technology Park in Redfern, Sydney. Email: haemqap@rcpaqap.com.au


AUGUST 14-15: ASM/ACTM Parasitology Masterclass RMIT City Campus www.parasitologymasterclass.org


AUGUST 22-27: 14th International Congress of Immunology (Immunology in the 21st Century: Defeating infection, Autoimmunity, Allergy and Cancer) Kobe, Japan. www.ici2010.org

AUGUST 24-27: NZIMLS Conference, Bay of Islands, NZ. Contact: rossh@adhb.govt.nz www.eenz.com.nzimls10/

AUGUST 29-SEPTEMBER 1: Endocrine Society of Australia and Society of Reproductive Biology Annual Scientific Meeting. Sydney, New South Wales www.esa-srb.org.au

SEPTEMBER 6-8: Australian Association For Quality In Health Care 8th Australasian Conference. www.aaqhc2010.org.au


OCTOBER: A National Forum on Safety and Quality in Health Care will be held in Canberra in October. www.achs.org.au

OCTOBER 16: ASTH Scientific Workshop, Auckland.

OCTOBER 17-20: HAA 2010 - The Combined Annual Scientific Meeting of HSANZ, ANZSBT and ASTH. SkyCity Convention Centre, Auckland, New Zealand. Contact: The Conference Company Ltd. Phone: +64 9 360 1240 Fax: +64 9 360 1242. Email: haa@tcc.co.nz www.haa2010.org
OCTOBER 25-29: AIMS AACB Combined National Scientific Meeting. 
Perth Convention Exhibition Centre 

NOVEMBER 5-7: NSW North Coast Division Meeting, Arrawarra. Contact Neil Horton at: neil.horton@hnehealth.nsw.gov.au or visit the AIMS website.

DECEMBER 4-7: 52nd American Society of Hematology Annual Meeting and Exposition. Orange County Convention Center, Orlando, FL, USA. 
www.hematology.org

YEAR 2011

AUGUST 8-12: AIMS/NZIMLS South Pacific Congress, Gold Coast Convention and Exhibition Centre, Gold Coast, Queensland.

OCTOBER 19-23: XXVI World Congress of the World Association of Societies of Pathology and Laboratory Medicine (WASPaLM), Las Vegas. Hosted by the American Society for Clinical Pathology. 
www.waspalm.org

OCTOBER 30 - NOVEMBER 2: The Combined Annual Scientific Meeting of HSANZ, ANZSBT and ASTH. Sydney, Australia 

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Questions relating to An association between normal D-dimer levels at diagnosis and improved survival in a group of acute myeloid leukaemia patients. Page 44 of this issue.

1. Data on this limited number of patients shows that both relapse rate and overall survival were significantly improved in those patients with normal D-dimer levels at presentation compared to those in which D-dimer levels were elevated. True/False

2. The malignant cells in both AML and solid tumours do not have similar requirements such as the need to adhere to tissue components, to migrate and to enter the circulation. True/False

3. No significant difference (p<0.05) was found between the two groups for WCC and LDH at diagnosis. True/False

4. It is unlikely that plasminogen activation on the surface of blast cells plays a role, yet to be fully defined, in the disease process in AML. True/False

5. Figure 1 shows the comparison of normal and elevated D-dimer groups with LDH at diagnosis. True/False

6. The potential biological explanation of these findings lies in the function of some components of the fibrinolytic system in malignancy. True/False

7. The patients were divided into two groups according to D-dimer levels at diagnosis: normal versus elevated. True/False

8. In many solid tumours an elevated D-dimer at diagnosis has been shown to be a poor prognostic indicator for survival. True/False

9. In tissue, fibrinolysis is initiated when uPA binds to its receptor on the cell surface activating plasminogen with resulting generation of plasmin. True/False

10. Figure 4 shows the Kaplan–Meier survival curve for the whole patient cohort and the overall survival after 400 weeks is 80%. True/False
Questions relating to *Harmonising quality to the lowest clinical diagnostic standard?* Page 56 of this issue.

1. The revised framework for the regulation of in vitro diagnostics devices (IVDs) will come into force on 20 July 2010. True/False

2. With regard to the new legislation AIMS was identified in the original consultation groups as a key stake holder in 2003. True/False

3. Current evidence points to several failings in relation to existing regulation of IVDs in the USA, which is delaying the entry of new tests into diagnostics. True/False

4. Von Willebrand Disease testing represents a complex and time consuming process. It is difficult to see how the new legislation will improve clinical diagnostic testing for this disease. True/False

5. TGA operates as a full cost recovery agency, deriving its income from fees levied to its sponsors and manufacturers that by law are required to submit documentation related to safety, quality and efficacy. True/False

6. It is clear that adding another layer of bureaucracy via the current IVDs legislation will further assist to ensure public protection in clinical laboratory diagnostics. True/False

7. Smaller companies will be overwhelmed by the new legislation and requirements, and will rationalise their future product lines. True/False

8. The new regulation will not include regulation of in-house IVDs. True/False

9. Figure 1 shows an increased potential for adverse impact on complex, specialised low throughput diagnostic tests than on simple routine high throughput screening tests. True/False

10. Clinical laboratory accreditation by NATA requires laboratories to adhere to specific ISO guidelines as well as to NPAAC guidelines. True/False

Name: ____________________________ Membership No: ____________________________

Email: ____________________________

*Please photocopy this page or print it from the AJMS on the AIMS Member Library in the AIMS Member Lounge at www.aims.org.au, circle your answers and post or fax by 30 November 2010 to:*

AJMS APACE Questions, AIMS National Office, PO Box 1911, Milton Qld 4064. Facsimile: 61 7 3876 2999
Frontiers of Neurology and Neuroscience Volume 22: 
Neurological Disorders in Famous Artists Part 2

Edited by Julien Bogousslavsky & Michael G Hennerici
Karger 2007
Hard cover, VIII+240 pages
ISBN: 978-3-8055-8265-0
Price: EUR88.00

This text is the second part in a series discussing neurological disorders in famous artists and the 22nd volume in the ongoing series “Frontiers of Neurology and Neuroscience”. The book is a small, easy to handle, 240 page hard-cover text divided into 14 chapters. The 60 figures (23 in color) and 6 tables are distributed sporadically throughout the book. In the preface, the editors outline the unknown relationship between normal brain function, neurological dysfunction and neuropathology to the fields of artistic, literary and musical creativity. Each chapter consists of an independent, free-standing article discussing the neurological conditions suffered by a specific artist of note. Each article is written by separate author/s with a small number of authors contributing to multiple articles.

The articles included in this text outline a wide range of neurological disorders affecting a number of artists from different genres. The first chapter discusses the effect of right-hemisphere stroke using case studies of 13 different professional visual artists. Although the artists studied in this first chapter all lived during different periods of the 20th century, there is great variation between the level of detail provided on each artist. For example, Anton Rädersheidt and Otto Dix each have detailed biographies discussing their artistic works and neurological findings. In contrast, comments about Guglielmo Lusignoli and “71-Year-Old Artist with Mild Left Neglect” are limited to four and two sentences respectively. Despite the lack of information supporting some artists suffering from right-hemisphere stroke, the first author is able to draw together some general conclusions discussing the impact of this neurological event on the artists’ professional capability.

Subsequent chapters in the text are written in a more standardized format as they generally discuss one artist in each article. This ensures that comprehensive case studies can be presented. Each chapter discusses the background (both professional and personal) of the artist of note. A thorough description of the neurological disorder is also included. On a pleasing note, many authors have also compared the medical knowledge available during the time of the artist’s illness and contrasted that with current accepted and published knowledge. These articles are by far more appealing as they allow the reader to develop their understanding of the artist, their disorder and potential impact on their artistic ability. In particular, I found most fascinating the chapters discussing the potential influence of Tourette’s Syndrome on Austrian composer Wolfgang Amadeus Mozart and the effect of syphilis on German poet/author Heinrich Hein. Examples of other subjects for articles include:

- Prussian painter Lovis Corinth
- Italian film directors Luchino Visconti and Federico Fellini
- French novelist Marcel Proust
- French poet Charles Baudelaire
- Swedish painter and sculptor Carl Fredrik Reuterswärd
- German pianist and conductor Hans von Bülow

The authors of each article have stated their affiliated organisations and they originate from a range of predominantly European academic institutes, including numerous departments of neurology and neuropsychiatry. Each chapter is comprehensively referenced so that the reader can further research any topic of interest. One major setback of this book is that the language of the text is variable between each author. In fact, a number of articles have credited a third party for revising the English text. Despite this, the quality of information is generally of a high standard with all information readily available. The use of diagrams thoroughly complements the discussion of the text.

Although this book is being reviewed for AIMS, the intended audience is definitely not medical scientists. Rather, it is recommended to scientific readers (including medical scientists) with an interest in both the arts and neurology.

Jarrod Phillips MAppSc BAppSc (RMIT), DipMgt (WCIT) MAIMS
Medical Scientist Second-in-Charge
Histopathology Department
St John of God Pathology WA
Perth, Western Australia
ABC Transporters in Microorganisms: Research, Innovation and Value as Targets against Drug Resistance

Edited by Alicia Ponte-Sucre
Caister Academic Press
Hard cover, xii+260 pages
Price: US $310

ATP Binding Cassette (ABC) Transporter family of proteins consist of well known membrane proteins which use the energy of ATP hydrolysis to transport a wide range of molecules across the cell membrane. Most ABC proteins are active transporters that move substrates against their concentration gradients. The study of these proteins has always received great attention mainly because they are considered major contributors to chemoresistance and efflux of drugs from microorganisms and cancer cells. However, in recent years, besides the role as drug transporters, the physiological roles of ABC transporters especially in the development or stem cell biology are also gaining increasing interest.

ABC Transporters in Microorganisms, Research, Innovation and Value as Targets against Drug Resistance is a good text book for basic science researchers in the area of multiple drugs resistant (MDR) proteins or microbiology. This book comprehensively discusses various types of ABC transporters and their implications for microorganisms such as bacterial, yeast, fungi, and parasites. Although the roles of ABC transporters are briefly reviewed from the human clinical and pharmacology aspects, it is relatively scarce. There is a total of 10 chapters in this book and it is divided into 3 main parts. The first part (Chapter 1-4) discusses the structure, evolution, and molecular function of ABC transporters, the second part (Chapter 5-8) explains the role of ABC transporters in the physiology of microorganism, and finally the third part (Chapter 9-10) discusses the classical and new approaches designed to fight against MDR.

This book provides extensive information regarding biological structure, molecular machineries of ABC transporters and their cellular functions in various microorganisms. The antibiotic resistance element (ARE) type ABC proteins, which do not have membrane spanning regions are also introduced. Importantly, the authors also covered 2 areas of experimental therapeutic strategies for overcoming MDR in microorganisms in the last sections of the book – 1) development of ABC transporter blockers, and 2) the potential application of RNA interference technology to reverse MDR activity.

Taken altogether, the book contents are not directly clinically relevant. However, because ABC transporter genes are highly conserved across species and are revolutionary ancient genes, this book offers valuable resources and may provide insights into the unknown functions and physiological role of ABC transporters in humans.

Dr YS Loh
Postdoctoral Research Associate
Faculty of Pharmacy
The University of Sydney
NSW 2006
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As per our agreement with the book publishing companies, complimentary books are submitted to the Institute provided that all reviews are published in the Australian Journal of Medical Science. These reviews must be of a high quality as buying decisions and the reputation of the book and author are important considerations.

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

Requests to: Australian Institute of Medical Scientists, PO Box 1911, Milton, Qld 4064.
Tel: (07) 3876 2988. Fax: (07) 3876 2999. Email: aimsnat@aims.org.au


7. **Clinical Cases in Infectious Diseases: A Public Health Approach** author Sanjaya Senanayake 480 pages


17. **Endocrine Development Volume 17: Pediatric Neuroendocrinology** edited by S Loche, M
Cappa, L Ghizzoni, M Maghnie, MO Savage. Karger 228 pages.


30. Lentiviruses and Macrophages: Molecular and Cellular Interactions by Moira Desport. Caister Academic Press. xii + 346 pages


39. Mycobacterium: Genomics and Molecular Biology


41. Neisseria: Molecular Mechanisms of Pathogenesis.

42. Neurological Disorders in Famous Artists - Part 3.

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


49. The Regulatory Genome: Gene Regulatory Networks in Development and Evolution


Instructions to authors

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

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

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

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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.

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

Requirements and preparation of manuscripts

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

Number pages consecutively commencing with the title page.

Arrange the article in the following sequence:

- Title page
- Abstract and key words
- Main Text
- Acknowledgements
- References
- Tables - each table, complete with title and footnotes, on a separate page
- Legends for illustrations.

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

Title Page

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

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

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

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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:

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- Materials and methods
- Results
- Discussion
- References

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

Introduction

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

Materials and methods

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

Results

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

Discussion

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

Acknowledgements

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

References

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

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

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

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

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

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

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

Examples of the correct form for references are given below:

i) Journal Reference:


ii) Personal Author(s) of a Book:


iii) Editor, Compiler, Chairman as Author:


iv) Chapter in Book:


v) Online documents:


Tables

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

For footnotes, use the following symbols in this sequence:

* † ‡ § ¶ ** ††

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and the magnification must be stated. Symbols, arrows, or
letters used in the photomicrographs should contrast with
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Cite each figure in the text in consecutive
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When symbols, arrows, numbers, or letters are used
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(b) substantive title.

Abbreviations

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

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

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

Commonly used abbreviations

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

Additional Information

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