Hematology Case Studies using Digital Cell Image Analysis

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Goals for Today

- Obtain a working knowledge of digital cell image analysis.
- Learn briefly about how automated digital systems work.
- Obtain an understanding of some of the advantages of using automated cell image analysis in the clinical laboratory.
- Demonstrate how automated cell image analysis can provide better and more rapid diagnoses through the use of examples.
Laboratory Trends

- Reduction of Qualified Medical Technologists
  - More Generalists
  - Less Specialists
- Increased Workloads
  - Decreased Staffing

Laboratory Trends

- Demand for Accurate Results
- Demand for Shorter TAT
- Increased Capital Equipment cycles
Options To Manage “The Slide”

- Review Remains Significantly a Manual Process

Concerns with Manual Microscopy

- Labor intensive
- Not standardized
- Challenging to train
- No historical images
- Limited consultation
- No traceability
Why Automate the Manual Differential

- The need for a greater level of standardization and consistency for the manual differential
- Increasing demand for connectivity between healthcare providers

Location and Pre-Classification of WBCs

1. Whole blood sample in lavender top tube
2. ~20% tests flagged in cell counter
3. Peripheral blood smear
4. Barcode slides loaded on analyzer
5. Automatic location and pre-classification of cells performed by analyzer
How it Works

- Finding the WBC Monolayer
  - Centre line
  - Start scanning
  - Collecting Locations of WBCs

Pre-Classification with help from the Neural Network

- Feature Extraction
  - Categories: shape, size, colour, texture, etc.

- ANN-based Classifier
The RBC analysis

The RBC monolayer can be:
- Within the WBC monolayer
- Partly within the WBC monolayer
- Completely outside the WBC monolayer

RBC Monolayer

• RBC monolayer is defined at 50x magnification
• Separate RBC monofinder process
• The RBC monolayer is typically in a slightly thinner area of the smear
• ~35 images are acquired for the RBC pre-characterization
Pre-Classified WBC’s Presented on Screen

RBC Characterization Presented on Screen
Platelet Estimates Presented on Screen

Consultation

Cell Image Analyzer
Clinical Hematology

RRS
Conference Room

RRS
Pathology

RRS
Oncology
Key Points

• Automated Digital Imaging systems are *not* just about pre-classification .......they *are* about providing powerful tools that improve the speed and accuracy of the Technologist and the Lab.
• The Med Tech remains an integral and important part of the differential.
Cell Image Analysis Portfolio

Introducing: DI-60 Cell Image Analyzer: Complete integration to the XN line or in configuration with an SP-10 called the Integrated Slide Processor (ISP). Targeted for automation customers. Expected Release: Q4 2013

CellaVision® DM1200 Cell Image Analyzer: Designed for mid-size labs. Analyzes both blood and body fluid samples.

CellaVision® DM96 Cell Image Analyzer:

Medica EasyCell® assistant, Cell Image Analyzer Provides automated assistance and standardized results for blood samples for smaller laboratories.

Portfolio Comparison

<table>
<thead>
<tr>
<th></th>
<th>DI-60</th>
<th>DM1200</th>
<th>EasyCell</th>
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<tr>
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<td>Sample Loading Access</td>
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<td>On Board Slide Capacity (per run)</td>
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Case Studies

Case #1

- A 44-year old woman presents to her GP with the following symptoms:
  - Bloated Feeling
  - Heartburn
  - Chronic Diarrhea
  - Constipation
  - Two months prior the patient had itchy, flat red rashes with scalloped edges for which she tried topical anti-bacterial and anti-fungal creams with only limited success.
  - Lab results were mostly normal with a slightly elevated WBC count.
Systemic Candida Disease

- The Candida yeast is normally harmless and resides in the mouth and the digestive track. When your immune system is lowered or favorable conditions like warm and humid atmosphere are available, Candida grows uncontrollably and becomes an infection.

- Systemic Candida is most often caused by a simple infection that goes untreated or insufficiently treated.
Case #2

- A 35 year old man went to visit relatives for vacation. Two weeks after his return, he presented to the ER and told them he wasn’t feeling well. He told them he had just returned from visiting family. Nothing out of the ordinary.
- He reported high fevers, shaking chills, and flu-like symptoms.
- Upon examination he was found to have an enlarged liver and spleen.
- Lab Results: see below along with a slightly elevated TBili.

<table>
<thead>
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<tr>
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<tr>
<td>%Neut</td>
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<td>%LY</td>
<td>12</td>
</tr>
<tr>
<td>%MO</td>
<td>3</td>
</tr>
<tr>
<td>%EO</td>
<td>1</td>
</tr>
<tr>
<td>%BAS</td>
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</table>

Did anyone ask him where his family lived?
Case #2  Image B

Plasmsodium Vivax
**Plasmodium Vivax**

- Malarial parasites work by digesting red cell proteins and making the RBC membrane less deformable, causing hemolysis, increased splenic clearance, and anemia.
- Red cell lysis stimulates release of cytokines and TNF-α. The systemic manifestations of malaria such as headache, fever and rigors, nausea and vomiting, diarrhea, anorexia, tiredness, aching joints and muscles, thrombocytopenia, immunosuppression, coagulopathy, and central nervous system manifestations have been largely attributed to the various cytokines released in response to these parasite and red cell membrane products.
- P. Vivax makes up 16% of cases reported in US.
- Not found in West Africa as no Duffy Antigen, which is required for entry in to the RBC.
- Characterized by:
  - Low to Normal Platelet Count
  - Anemia
  - White blood cell (WBC) counts during malaria are generally characterized as being low to normal, a phenomenon that is widely thought to reflect localization of leukocytes away from the peripheral circulation and to the spleen and other organs, rather than actual depletion or stasis.
  - In P. Vivax it is common to see more than one stage in the life cycle at the same time in the Peripheral Blood.

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**Case # 3**

- A 38 year old; HIV positive man presented at the clinic with a chronic cough, bloody sputum, fever, night sweats and recent weight loss.
- The MD ordered chest films as well as bacterial, fungal, and AFB cultures on the sputum.
- CBC values were unremarkable accept for a WBC count of 17.2
Monocytosis

• Monocytosis itself is not a disease, but a symptom.

• Inflammatory conditions such as infection or autoimmune disturbances are primary causes of monocytosis. Some of the common types of infection that might lead to this condition include tuberculosis, syphilis and Rocky Mountain spotted fever. Autoimmune disorders such as lupus or rheumatoid arthritis might also lead to monocytosis.

• Some blood disorders might lead to a high number of monocytes as well.
  • In this particular case, Tuberculosis was the diagnosis
Case # 4

- 19 year old Male
  - WBC 41.5
  - HBG 8.7
  - PLT 61
  - MCV 90.0
  - PT 15.5 sec
  - aPTT 27 sec

- Ecchymosed (bruised) skin
- Bleeding Gums
- Fever
- Bleeding time greater than 30 minutes.

- Cytochemical Stains
  - Myeloperoxidase Strongly Positive
  - Chloroacetate Esterase (specific) Positive
  - α-Naphthyl Acetate Esterase (nonspecific) Negative

- Immunophenotyping
  - Abnormal cells mark with CD13 and CD33, but not with HLA-DR or CD34, consistent with myeloid lineage.

- Cytogenetics
  - 46, X, Y, t(15;17), (q22;q11.2) Abnormal male karyotype.

Acute Myeloid Leukemia (AML)
Acute Myeloid Leukemia (AML)

- **Auer rods** can be seen in the leukemic blasts.

- Auer rods are classically seen in myeloid blasts of M1, M2, M3, and M4 acute leukemias.
  - These cytoplasmic inclusions are named for John Auer, an American physiologist (1875-1948).

- The increased production of blasts creates a blockade of the production of normal marrow cells, leading to a deficiency of red cells causing anemia, platelets and white blood cells that are found in the peripheral blood.

- AML is the most common acute leukemia that affects primarily adults and accounts for over 1 percent of cancer deaths here in the United States.

- AML is initially treated with chemotherapy with the intent of inducing remission.

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Case # 5

- 6 year-old boy
- Repeated illnesses, colds, etc.
- Fatigue
- No weight gain
- Initial testing: CT scan, Spinal Fluid
  - CT Scan showed an Abdominal Mass
Burkitt’s Lymphoma

- **Burkitt’s Lymphoma** is a non-Hodgkin’s Lymphoma that has an especially high incidence among children 3 to 16 years of age. The disease is characterized by tumors of the jaw bones and abdomen and is named after Denis Burkett, who mapped its peculiar geographic distribution across Africa in the 1950s.

- The Epstein-Barr virus, which causes infectious mononucleosis, is present in almost 100% of persons afflicted with Burkitt’s lymphoma.

- Burkitt’s lymphoma occurs more readily in persons who have been weakened by malaria and in persons suffering from AIDS.

- Research suggests that Burkitt’s Lymphoma caused by a genetic mutation.

- Similar disease characteristics to diffuse large B cell lymphoma (DLBCL)

- Synonym(s): Mature B cell high-grade lymphoma; Mature B cell acute lymphoblastic leukemia, L3 type (FAB classification);

- Despite the fast-growing nature of this tumor, Burkitt’s lymphoma is also one of the most curable types of lymphoma, depending on the stage of the disease at the time it is diagnosed.

Read more: [http://www.livestrong.com/article/3759-need-burkitts-lymphoma/]
Case #6

- 60 Year Old male. Abdominal pain, Neck Pain, Armpit, and sore throat.
- Night sweats and fever.
- Unexplained weight loss (over 10%)
- Swelling of lymph nodes
- CBC results normal w/ suspect flag for variant lymphocytes on automated differential.
- Flow cytometry
  - CD19+
  - CD5+

Mantle Cell Lymphoma
Mantle Cell Lymphoma

- Mantle cell lymphoma is a non-Hodgkins lymphoma that occurs in the B cells found on the outer edge of the lymph node follicle. The uncontrolled growth of these B cells causes the lymph nodes to swell. Mantle cell lymphoma can also affect the bone marrow, liver and gastrointestinal tract.

- Though it looks like a slow growing, low-grade tumor under the microscope, it grows fast and behaves like a high-grade lymphoma. Mantle cell lymphoma accounts for approximately six percent of all non-Hodgkin's lymphoma related diseases, according to The Leukemia and Lymphoma Society.

- The overall 5 year survival rate for MCL is generally 50% (advanced stage MCL) to 70% (for limited-stage MCL).

Case #7

- 61 year old man
- Previous diagnosis of myelofibrosis
- Complaining of an increase in bone pain and fatigue with shortness of breath
- No Auer Rods seen in blasts

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<tr>
<td>NRBC</td>
<td>1</td>
<td>Blast</td>
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Worsening Progression of Myelofibrosis

Metamyelocytes
Myelocytes

Promyelocytes
Myelofibrosis

- Myelofibrosis is currently classified as a myeloproliferative disease where the replacement of the marrow with collagenous connective tissue fibers occurs.
- This replacement impairs the patient's ability to generate new blood cells.
- This is a fairly typical progression which will most likely continue with thrombocytopenia, gout, and often an acute leukemia.
- Only known cure is a bone marrow transplant. Other treatments are merely supportive.

Case #8

- 53 year old woman from Connecticut, no recent travel
- Fatigue, malaise, loss of appetite
- Occasional fever

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- Elevated ESR
- Liver function tests: Elevated TBili, LDH, and liver transaminases
Case #8  Image A

Babesia
**Babesiosis**

- **Where do most of the cases of babesiosis occur in the United States?**
  - Tickborne transmission of *B. microti* primarily occurs in the Northeast and upper Midwest, particularly in parts of New England, New York State, New Jersey, Wisconsin, and Minnesota. In the Northeast, babesiosis occurs in both inland and coastal areas, including off-shore islands such as Nantucket and Martha’s Vineyard (Massachusetts); Block Island (Rhode Island); and Shelter Island, Fire Island, and eastern Long Island (New York State).

- **Other possible ways of becoming infected with Babesia include:**
  - Receipt of a contaminated blood transfusion (no tests have been licensed yet for donor screening); or
  - Transmission from an infected mother to her baby during pregnancy or delivery.

  - The Centers for Disease Control and Prevention have issued a warning about babesiosis. According to the CDC the illness is transmitted through blood transfusions and has infected at least 122 people since 2000. This was released on Sept. 7, 2011.

**Case #9**

- **A 47 year old woman who is an avid spelunker came to her doctor with complaints of:**
  - malaise (a general ill feeling)
  - fever
  - dry or nonproductive cough
  - headache
  - shortness of breath
  - joint and muscle pains
  - chills

- She also noted this sore on her forehead.
- The physician ordered a CSF due to her severe headache.
Overview of CSF

Histoplasmosis
Histoplasmosis

- **Histoplasmosis** is a disease caused by the fungus *Histoplasma capsulatum*. Symptoms of this infection vary greatly, but the disease primarily affects the lungs. Histoplasmosis is common among AIDS patients because of their suppressed immune system.

- *H. capsulatum* grows in soil and material contaminated with bird or bat droppings (guano). The fungus has been found in poultry house litter, caves, areas harboring bats, and in bird roosts (particularly those of starlings).

- Histoplasmosis can be diagnosed by samples containing the fungus taken from sputum, blood, or infected organs. It can also be diagnosed by detection of antigens in blood or urine samples by ELISA or PCR. It can also be diagnosed by a test for antibodies against *Histoplasma* in the blood.

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Case #10

- 17 year old African-American girl
- Presents in the ER with Fever, joint pain, nausea and severe chest pain with shortness of breath

- CBC results upon presentation:

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
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<tbody>
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Sickle Cell Crisis

Sickle Cell Disease

- **Sickle-cell disease (SCD)**, or **sickle-cell anaemia**, is a recessive genetic blood disorder characterized by RBCs that assume an abnormal, rigid, sickle shape. Sickling decreases the cells' flexibility and results in a risk of various complications. The sickling occurs because of a mutation in the hemoglobin gene.

- There are different types of crisis events, including vaso-occlusive, aplastic, and hemolytic. Crisis events can be caused by physical stress, changes in temperature or seasons, emotional stress, etc. Different people have different triggers. The length of a crisis can very considerably.

- Hemolytic crises characterized by acute, accelerated drops in hemoglobin level. The red blood cells break down at a faster rate. Management is supportive and often involves blood transfusions.
Summary

• Automated Cell Image Analysis in the Clinical Lab can be useful for many reasons including:
  – Operational Capabilities
    • Ease of viewing and manipulating images
    • Saving time, faster TAT
    • More consistent training and learning for technologists
    • Long term storage of these images
    • Attractive Ergonomics for Staff
  – Clinical Capabilities
    • Ease of Sharing images for Real-Time Consultation
    • Faster and more reliable diagnoses
    • Improved Patient Care

Questions

Thank You