CCTC Clinical Chemistry Trainee Council | AACC Better health through laboratory medicine.

PEARLS OF LABORATORY MEDICINE

www.traineecouncil.org

TITLE: Acute Myeloid Leukemia
PRESENTER: Kamran M. Mirza, MD PhD

Slide 1:
Hello! My name is Dr. Kamran Mirza and together with my resident Dr. Aadil Ahmed I would like to welcome you to this Pearl of Laboratory Medicine on “Acute Myeloid Leukemia”.

Slide 2:
The accepted paradigm for hematopoiesis begins with a multipotent progenitor cell. This cell has the ability to differentiate into any hematopoietic cell. It also has the ability for self-renewal. In general it is thought that this cell leads to the common lymphoid and myeloid precursors, which can then differentiate down paths to make all the cells of the hematopoietic system Any cell in this system can undergo malignant transformation that leads to uncontrolled growth.

An Acute leukemia is defined as the uncontrolled, clonal expansion of hematopoietic progenitors, or blasts. Acute leukemias typically present with symptoms of impaired hematopoiesis and bone marrow failure, due to the crowding out of normal marrow by malignant cells. Patients may present with recurrent infections due to neutropenia, bleeding or bruising due to thrombocytopenia, and weakness or lethargy due to anemia. Since these progenitor cells can become either lymphoid or myeloid cells, two main types of leukemia exist.

Expansion of blasts in the myeloid lineage leads to Acute MYELOID leukemia. Acute lymphoblastic leukemia, the lymphoid counterpart of AML is discussed separately. AML is the most common type of acute leukemia in adults, accounting for 3-5 cases per 100’000 people in the US. AML can arise de novo from blast cells, be associated with an underlying hematological disorder, be a result of prior chemotherapy, or be associated with congenital disorders such as Down syndrome

It is thought that continued accumulation of chromosomal abnormalities or genetic mutations leads to the formation of chimeric proteins that alter the maturation process of myeloid blasts, leading to uncontrolled or unchecked growth of these cells without any maturation.

Slide 3:
The WHO classification of tumors of hematopoietic and lymphoid tissues requires 20% blasts either the blood, or bone marrow to diagnose AML. Some considerations or exceptions to this rule include. Certain cytogenetic abnormalities such as translocation 8, 21 and inversion 16 do not require a count of 20% since just their presence is diagnostic of AML.

Pure erythroid leukemia requires >80% of the bone marrow cells to be erythroid with > 30% pro-erythroblasts

© 2016 *Clinical Chemistry*

In the case of acute promyelocytic leukemia, or APL, abnormal promyelocytes are also counted blasts equivalents.

Lastly, extramedullary presentation of AML, or myeloid sarcoma, may not have 20% blasts in blood or bone marrow.

To establish a diagnosis of AML we need to confirm the cells being counted are truly myeloblasts, since morphologically myeloid blasts and lymphoid blasts could have similar features. For this, ancillary tests such as immunophenotyping by flow cytometry and/or immunohistochemistry and cytochemical stains can be performed

The second question is of enumeration of blasts. This can be done by differential counts of the blood or bone marrow. Sometimes CD34 immunostaining or assessment of cellular events in the blast gate of flow plots are thought to help in this enumeration – however, not all blasts are CD34 positive and the flow specimen may not be representative of the marrow. Therefore flow and IHC should not be used to establish the 20% cut-off.

Morphologically, myeloid blasts are large cells, with a high nucleus to cytoplasmic ratio. Typically there is a fine chromatin pattern with one or more nucleoli. The cytoplasm can be variably granular. The presence of Auer rods, as indicated by the arrow, is a defining feature of myeloid lineage. The presence of myeloperoxidase can be confirmed by a cytochemical reaction. In this blast, the myeloperoxidase is present in blue.

Immunohistochemistry for stains such as CD34 helps estimate the overall numbers of blast cells in core biopsy or clot specimens. Expression of MPO by either cytochemical reaction, immunohistochemistry or flow cytometry is considered evidence of myeloid differentiation. Monocytic differentiation demonstrated by non-specific esterase, lysozyme, CD14, CD64 or CD11c is also used to help define myeloid origin. While CD117, CD13, CD33, CD65, CD14, CD15 and CD65 are commonly expressed by myeloid cells, they are not definitive markers of myeloid origin.

Slide 4:
Once the diagnosis of acute myeloid leukemia is established, the next step is subclassification. Over the past few decades, numerous classification schemes addressed the various types of acute leukemias. The French-American-British or FAB classification from 1976 subtyping AML, M0 through M7 was based on morphology and cytochemistry. The more recent WHO classification continues to include this scheme under AML, not otherwise specified. From 1976 to 2001 the FAB classification was used.

In 2001 the World Health Organization brought forward it’s Classification of Tumors of Hematopoietic and Lymphoid tissues. This scheme has subsequently been revised in 2008 and 2016. The WHO classification takes into account morphology, cytochemistry,

immunophenotype, cytogenetics, molecular genetics, and clinical outcomes – making it a robust system

The 2016 WHO classification includes the following main subtypes of AML and related precursor neoplasms:
AML with recurrent genetic abnormalities
AML with myelodysplasia related changes
Therapy-related myeloid neoplasms
AML, not otherwise specified (NOS)
Myeloid sarcoma
and Myeloid proliferations associated with Down syndrome

In the recurrent genetic abnormality subgroup – the following abnormalities are recognized
AML with translocation 8-21
AML with inversion 16, or translocation 16-16
Acute Promyelocytic Leukemia, APL with PML-RARA fusion
AML with translocation 9-11
AML with translocation 6-9
AML with inversion 3, or translocation 3-3
Acute myeloid leukemia (megakaryoblastic) with translocation 1-22
AML with BCR-ABL1 fusion

The AMLs with recurrent gene mutations are also contained within this group and include
AML with mutated NPM1
AML with biallelic mutation of C-E-B-P-alpha
And AML with mutated RUNX1

Within this subgroup, AML with 8-21, inv(16) or t(16;16) and APL are regarded AML irrespective of blast count. This means that the finding of one of these three abnormalities on cytogenetics or molecular studies immediately imparts a diagnosis of AML, even when the blast count has not reached 20%.

Slide 5:
AML with translocation 8-21 typically presents with needle-shaped Auer rods. Salmon-colored granules that typically cloak part of the nucleus and some blasts show large, pseudo-Chediak-Higashi granules.

These cases usually show expression of CD34, with dim CD33 staining, frequent expression of CD19 and variable expression of CD56. AML with translocation 8-21 is associated with a high rate of complete remission and disease-free survival when treated with intensive consolidation therapy. Acute promyelocytic leukemia demonstrates two morphologic subtypes. The hypergranular variant, shown here

demonstrates abnormal promyelocytes and cells with numerous Auer rods as shown here. In contrast, the microgranular variant demonstrates bilobed nuclei, often with a coin-on-coin appearance and are devoid of obvious granules. Both variants demonstrate intense positivity for myeloperoxidase on cytochemical staining. This is one acute leukemia which is a true emergency as many patients present in DIC and an early morphologic diagnosis can help start ATRA therapy.

Slide 6:
Typically presenting as an acute myelomonocytic leukemia, AML with inversion 16 may also present as isolated myeloid sarcoma at initial diagnosis or at relapse. AML with inversion 16, or translocation 16-16 is best known for its association with eosinophils at all stages of maturation, with abnormalities in immature eosinophilic granules. As shown here, these abnormal eosinophils demonstrate purple-violet granules which sometimes are so dense that they obscure the nucleus. AML with inversion 16 is associated with a high rate of complete remission when treated with intensive consolidation therapy. With regard to the other AMLs in the recurrent genetics category some salient morphologic findings include AML with translocation 9-11 resulting in KMT2A-MLLT3A fusion demonstrates monocytic features and is associated with intermediate survival.

Slide 7:
AML with translocation 6-9 may demonstrate monocytic features and is usually associated with basophilia and multilineage dysplasia. In both children and adults, AML with translocation 6-9 generally carries a poor prognosis. AML with inversion 3, or translocation 3-3 frequently presents with increased platelet counts and demonstrates unilobed or bilobed, dysplastic megakaryocytes. This AML usually has an aggressive course with short survival. Acute megakaryoblastic leukemia with translocation 1-22 is very rare, and commonly occurs in females with Down syndrome. AML with 1-22 responds well to AML chemotherapy with long disease-free survival.

AML with BCR-ABL1 fusion is a provisional entity in the WHO classification of 2016 These cases should represent a de novo AML with no evidence, with or without therapy, of previously diagnosed chronic myeloid leukemia. The morphologic features are non-specific, but patients generally present with leukocytosis. It appears to be an aggressive disease with poor response to traditional chemotherapy

Slide 8:
The diagnosis of acute myeloid leukemia with myelodysplasia-related changes requires the presence of several criteria. The case should demonstrate more than or equal to 20% blasts.

The second criterion requires either a family history of MDS or MDS/MPN, a MDS-related cytogenetic abnormality, or multilineage dysplasia. Of note, multilineage dysplasia itself is not sufficient to diagnose AML with MRC in a de novo case of AML with mutated NPM1 or biallelic mutation of CEBPA.

This diagnosis requires the absence of prior cytotoxic or radiation therapy for an unrelated disease and lastly for this diagnosis cases cannot harbor any of the abnormalities described in AML with recurrent genetic abnormalities.

For an AML to be placed in this category based on morphology alone, greater than 50% of cells in at least two hematopoietic cell lines need to demonstrate dysplasia

Dysmegakaryopoiesis is characterized by micromegakaryocytes, non-lobated nuclei, or nuclei with widely separated nuclear lobes

Dyserythropoiesis is characterized by megaloblastoid changes, nuclear irregularity, nuclear budding, and ring sideroblasts.

Dysgranulopoiesis is characterized by hypogranular neutrophils, hyposegmented nuclei, pseudo-Pelger Huët anomaly, or bizarrely segmented nuclei.

AML with myelodysplasia-related changes generally carries a poor prognosis with a lower rate of complete remission than in other AML subtypes.

Slide 9:
Myeloid malignancies occurring as a late complication of cytotoxic chemotherapy and or radiation therapy for a prior neoplastic or non-neoplastic process qualify as therapy-related myeloid neoplasms, or t-MNs.

Cytoxic agents implicated in therapy-related myeloid neoplasms including alkylating agents, ionizing radiation to large fields of marrow, topoisomerase II inhibitors, certain antimetabolites and antitubulin agents.

The presentation of these cases could be 20% or more blasts, which would be therapy-related acute myeloid leukemia or t-AML, less than 20% blasts and dysplasia which would qualify as therapy-related myelodysplastic syndrome, or t-MDS, or lastly a therapy-related overlap myelodysplastic syndrome and myeloproliferative neoplasm

Irrespective of which entity a case may fall based on the blast count in the blood or marrow, these cases are best regarded therapy-related myeloid neoplasms – a unique category distinguished by prior iatrogenic exposure to the therapies listed above.

TWO main subsets of therapy-related myeloid neoplasms are seen with increased frequency.

The more common are therapy-related myeloid neoplasms arising after alkylating agents and/or ionizing radiation. These tend to present 5-10 years after exposure and typically have an t-MDS phase before t-AML. This subset is commonly associated with

© 2016 *Clinical Chemistry*

unbalanced loss of genetic material, often chromosome 5 and or chromosome 7. The second group has a shorter latency of 1-5years and occurs after exposure to topoisomerase II inhibitors. These cases typically present with over 20% blasts and often have balanced chromosomal translocations. Although strongly influenced by associated chromosomal abnormality, the prognosis is generally poor with overall 5 year survival rates of less than 10%.

Slide 10:
Cases of acute myeloid leukemia that do not fit any category within AML with recurrent genetic abnormalities, or therapy-related disease, are lumped together in the category of acute myeloid leukemia not otherwise specified.

The subclassification within AML, NOS is based on morphologic, cytochemical, and immunophenotypic characteristics and extent of maturation in the blasts, often still referred to as M-types of FAB classification. Excluding AML with NPM1 and CEBPA mutations, all subgroups of AML, NOS are not prognostically different, except pure erythroid leukemia

AML with minimal differentiation demonstrates no morphologic or cytochemical evidence of myeloid differentiation. Myeloid nature of the blasts is confirmed by immunohistochemistry and/or flow cytometry

The myeloid nature of AML without maturation is demonstrated by MPO positivity
This acute leukemia has a high percentage of marrow blasts without significant evidence of maturation to neutrophils. Maturing cells account for less than 10% of all nucleated marrow cells.

AML with maturation reveals MPO positive blasts. In these cases the marrow demonstrates greater than 10% maturing cells of the granulocytic lineage. Of note, to be classified as M2, cells of monocytic derivation should be less than 20% of marrow cells. M3 is not listed here since it is APL.

Acute myelomonocytic leukemia is defined when blasts and promonocytes together account for greater than 20% of cells in the blood or bone marrow. Additionally, neutrophils and their precursors, and monocytes and their precursors should each account for greater than 20% of marrow cells.

Cases of leukemia where over 80% of leukemic cells have monocytic derivation – including monoblasts, promonocytes and monocytes are subclassified as Acute monoblastic and monocytic leukemia. In these cases, the neutrophil component is less than 20% of marrow cells.

Acute basophilic leukemia is very rare.

© 2016 *Clinical Chemistry*

Acute panmyelosis with myelofibrosis presents with an abrupt onset of fever and bone pain.

Slide 11:
Pure erythroid leukemia is a neoplastic proliferation of immature cells committed exclusively to the erythroid lineage. Diagnosing pure erythroid leukemia requires greater than 80% of bone marrow cells to be erythroid cells and additionally. Greater than or equal to 30% of cells should be proerythroblasts. There cannot be a significant myeloblastic component.

This is a very rare entity that can occur at any age, including childhood. There are no unique clinical features. Pure erythroid leukemia has a very aggressive clinical course with a median survival is 3 months.

In the previous edition of the WHO classification published in 2008 an erythroid/myeloid category of erythroleukemia existed. This diagnosis was made when erythroid precursors contributed greater than 50% of all marrow cells and myeloblasts were counted as a percentage of non-erythroid cells. In the 2017 revision, such cases are classified as MDS if there are less than 20% blasts and AML if greater than 20% blasts in the marrow or blood.

Slide 12:
Myeloid sarcoma, also known as chloroma or granulocytic sarcoma, is an extramedullary presentation of AML as a tumor mass. Infiltration of any site of the body by blasts is not classified as myeloid sarcoma unless it presents with tumor masses in which the tissue architecture is effaced. Most cases arise de novo and have similar etiologies as conventional AML. Any site can be involved, but the most common organ affected is skin. In one quarter of cases, myeloid sarcoma occurs in the absence of an underlying conventional AML. Clinical behavior and response to therapy do not seem to be influenced by age, gender or anatomic site. Allogeneic or autologous bone marrow transplantation leads to a higher probability of prolonged survival and cure.

Slide 13:
There is increasing recognition that some individuals or families with a germline predisposition face increased risk for myeloid neoplasms. The 2017 WHO classification acknowledges that constitutional symptoms and signs and family history are important clues to alert the Pathologist to this group of myeloid neoplasms which can have far-reaching consequences for entire families. There are three main categories within this group

First is myeloid neoplasms with germline predisposition without a pre-existing disorder or organ dysfunction. These include AML with germline CEBPA mutation or myeloid neoplasms with germline DDX41 mutation.

Second is myeloid neoplasms with germline predisposition and pre-existing platelet disorders. These include myeloid neoplasms with germline RUNX1, ANKRD26 and ETV6 mutations.

And third is myeloid neoplasms with germline predisposition and other organ dysfunction which include a mix of marrow failure syndromes, mutations, and importantly, Myeloid Neoplasms associated with Down syndrome (which will be discussed in a separate Pearl)

Slide 14: References
This marks the end of this pearl of laboratory medicine on acute myeloid leukemia. Thank you for participating. References for this presentation are listed here.

Slide 15: Disclosures
The authors have no disclosures or potential conflicts of interest.

Slide 16: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Acute Myeloid Leukemia”.