ISLH Webinar Q&A
Thursday, March 18, 2021
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Question: How can autofluorescence be differentiated?

Answer: *Answered live. In short, different populations will have a different autofluorescence. Assessing unstained cells can help to assess this. FMO controls are helpful as well.*

Question: How can we discriminate promonocytes from atypic monocytes by flow cytometry?

Answer: *Answered live. The following link from the ICCS ask an expert segment provides some tools for assessing monocytic differentiation and maturation.*

https://www.cytometry.org/web/q_view.php?id=201&filter=Interpretation%20and%20Clinical%20Application

Question: How to differentiate between mixed lineage or biphenotypic leukemia?

Answer: *Answered live. In short, the WHO provides strict criteria for when one should consider an MPAL.*

Question: As you mentioned flow cytometry has limitation on enumerating blasts due to hemodilution of partial lysis of NRBCs, then why we use flow for MRD assessment?

Answer: *In the diagnostic setting the threshold for defining the blast percentage from the WHO is 20% of the nucleated cells and is assessed by morphology. We can't generate the same denominator as is used by morphology using flow cytometry. For MRD assessment, flow cytometric assays have a denominator (often WBC, mononuclear cells, or nucleated cells after RBC lysis) defined by flow cytometry rather than morphology.*

Hemodilution impacts all blast enumeration regardless of method (morphology, flow, molecular). In general peripheral blood (and hence hemodilute marrow) specimens will provide a lower sensitivity (by at least a factor of 10 in most studies) than good quality marrow for BALL

or AML MRD assessment. Some studies suggest that TALL MRD assessment is closer between blood and marrow samples.

Question: For megakaryocytic marker CD61, do you use cytoplasmic or surface staining? Which one is better?

Answer: *Answered live. In our lab, we do several washing steps and the use surface staining. I don't have personal experience with cytoplasmic CD61 but I have heard of others who use it and like it.*

Question: What is your experience with NK lymphoblastic leukemias? Which antigens are the most prevalent? thank you!

Answer: *These are rare so I have limited experience with these. These are mentioned on page 213 of the 2016 WHO and I would refer you there for more information. I would caution anyone that before making this diagnosis, make sure you have excluded other processes that may express CD56: For instance, CD56 positive AML including monocytic leukemias and AML with a RAM immunophenotype, plasmacytoid dendritic cell neoplasms, T lymphoblastic leukemia with CD56 expression.*

Question: How often can we see aberrant CD14 expression in neoplastic promonocytes / monoblasts?

Answer: *It is seen sometimes and is clone dependent so I cannot give a single answer. You should interrogate the CD14 clone you use to determine how it performs. I can also refer you to the following article that addresses this subject:*

https://academic.oup.com/ajcp/article-pdf/124/6/930/24984027/ajcpath124-0930.pdf

Question: Can you comment on MRD flow analysis in terms of the ability to differentiate between normal regenerating hematogones from lymphoblastic leukemic cells since both lineage express similar phenotypic expression?

Answer: *Neoplastic B lymphoid blasts can be separated from hematogones using a panel of antigens expressed on both. The answer is broader than what I can answer in this QA but I have two resources for you to consider:*

YouTube video from ICCS: https://www.youtube.com/channel/UCoYQq-3CvuP7nGr-fsvmKtQ

Article from AJCP: *https://academic.oup.com/ajcp/article/155/1/38/6000688*

Question: How do you gate the blast equivalent in case of CD34- hidden in the monocyte gate?

Answer: *You have a few options. For immature monocytic populations, I recommend looking for a monocytic population that lacks CD14. Abnormal immature monocytic cells often also lack CD13 but express CD15, Bright HLA-DR, CD4, CD33 and CD64. CD56 may be positive. Neoplastic cells are abnormal though so they are rule breakers and you need to be careful about relying too heavily on any one antigen (normally expressed antigens by show increased or decreased intensity of expression). Comparing flow with morphology data is critical in my opinion. Looking for and characterizing all CD117 positive populations and populations falling in your CD45 versus SSC defined blast gate is helpful as well.*

Question: What is your experience with tdt?

Answer: *Good! I love TdT for helping to determine if an abnormal lymphoid blast is truly immature. Beware though rare abnormal lymphoid blasts (B or T) are negative for TdT and myeloid blasts can be positive.*

Question: Do you recommend interpreting and signing out flow cytometry data independently or morphology, relying on the hematopathologist reviewing morphology to integrate ancillary data (flow, FISH, karyotype, molecular)?

Answer: *This is a big question. I will provide a short answer to a big question ;) Integrating all data is always best. If you are providing only a part of the diagnostic data, be specific about potential limitations of what you are providing and recommend correlation with additional data for a complete evaluation and WHO classification.*

Question: How to report the MPO? Positive vs negative or in percentage in the context of the threshold of 3% published by WHO?

Answer: *The 3% threshold refers to MPO cytochemistry I believe. I don't think a lower limit is listed in the WHO for flow cytometry data. I use qualifiers, positive, negative, dim , bright, etc to describe MPO rather than a percentage. For cytoplasmic stains, we also use isotype controls to help determine where to draw the line for negative.*

Question: Don't you include LSC markers in your panels?

Answer: *Leukemic stem cells are CD34 bright and CD38 dim. We use both these markers in all myeloid tubes to help identify stem cells. With persistent or relapsed disease, it is often a subset with a stem cell like immunophenotype that persists and/or is treatment resistant. Including in your panel markers to identify stem cells is critical.*

Question: How do you rule out junk versus cells that have very low CD45 and SSC?

Answer: *We use a singlet gate to exclude doublets and use a FSC vs SSC gate to focus on viable/non debris events. Remember in the marrow you will also have erythroid precursors so we have CD71 included to specifically identify these.*

Question: How can reactive monocytes be distinguished from neoplastic ones?

Answer: *This is hard. I don't know of great markers. Proportions help but normal marrow will have some immature monos—especially with regeneration and sometimes these are especially increased post some AML therapy making things hard. If there is a discrete immature mono population with aberrant expression of an antigen (CD64 bright, CD45 dim, etc) that can help. CD56 helps if it is bright but it can also be expressed on regenerating monos. This is a challenging area. I will point you to the following article that uses a strategy of enumerating classical monocytes in the peripheral blood as a helpful feature in diagnosing CMML:*

https://ashpublications.org/blood/article/125/23/3618/34118/Characteristic-repartition-of-monocyte-subsets-as

Question: What is your preference for sample to exam with flowcytometry? Blood or bone marrow?

Answer: *Depends on the question one is asking. For acute leukemia maybe bone marrow though if there are 80% circulating blasts, blood should work. For MDS or acute leukemia MRD, marrow. For PNH, always blood.*

Question: Is isotypic control needed to define positive and negative markers, or we can rely only on internal controls (lymphocytes)?

Answer: *For most of our assays we rely on internal controls. Isotypes we use for cytoplasmic assays. Lymphocytes are not a good internal control for every population though, so be careful. Lymphocytes will have lower autofluoresence than monocytes, granulocytes, and many blast populations so they do not always serve as a good internal control.*

Question: Thanks for the great talk! Would clinician start treatment solely based on flow data? Eg APL where treatment is better to be as quick as possible. What is the TAT of other lab tests (genetic data, morphology) as compared to Flow Cytometry?

Answer: *Thank you! Sometimes our clinicians do start treatment based on flow data. It depends on how much information we can give them. If we can say with confidence that it is an acute leukemia and tell them lineage they will often start with flow alone. We do have a slide made in the lab to go along with every diagnostic flow study so I should note we have access to the slide though we don't typically report it as part of our flow report. For APL they will usually start ATRA based on flow and morphology and clinical concern but wait for FISH (TAT <24hrs here if ordered STAT) to add Arsenic. Flow TAT is 3-5 hours if a study is requested as a STAT.*

Aspirate smear stain and review can be <3 hours (core biopsy longer ~ 24-36 hours). Cytogenetics ~5 days.

Question: Do you recommend any reference for the changes that can occur in the normal marrow cells after chemotherapy, to help when assessing for residual disease. Thank you.

Answer: *Here are a couple of suggestions:*

https://academic.oup.com/ajcp/article/155/1/38/6000688
https://onlinelibrary.wiley.com/doi/full/10.1002/cyto.b.21854

You can also suggest that ISLH does a webinar on the topic!

Question: Concerning leukemia blasts that express antigens from other lineages, has that any correlation with the patient epigenetics?

Answer: *Great question. I am not sure. I will say that in patients with B-LL receiving anti CD19 T cell engaging therapies, "reprogramming" has been reported as a mechanism of lineage switch. I refer you to the interesting manuscript below on this topic.*

Nat Commun . 2016 Jul 27;7:12320. doi: 10.1038/ncomms12320.
https://pubmed.ncbi.nlm.nih.gov/27460500/

Question: How do you diagnose Acute erythroid leukemia on Flow?

Answer: *This can be tough. Erythroid blasts often express CD71 with CD117 and lack myeloid markers. Without aberrant expression of a T cell marker or some definite abnormality it can be a challenge to separate a normal pronormoblast and abnormal erythroblast. I think correlating with morphology and genetic data is particularly helpful here.*

Question: How do you differentiate low levels of residual/recurrent B-ALL from hematogones in your lab?

Answer: *Neoplastic B lymphoid blasts can be separated from hematogones using a panel of antigens expressed on both. The answer is broader than what I can answer in this QA but I have two resources for you to consider:*

YouTube video from ICCS: https://www.youtube.com/channel/UCoYQq-3CvuP7nGr-fsvmKtQ

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Question: How do you classify an acute leukemia with two populations of blasts: B-lymphoid and myeloid, BCR/ABL+ without known history of CML? does it make a difference if it is a child or adult?

Answer: *Using the guidelines in the WHO I would call this MPAL (B/Myeloid) with t(9;22). This can be seen in kids or adults and I would use the same classification in either case.*

Question: Can we identify mixed lineage leukemias by Next Gen Sequencing and Gene expression profile

Answer: *MPAL are more likely to have rearrangements of BCR-ABL and KMT2A. I suspect we will learn more about what NGS has to contribute to this classification in the near future!*

Question: What do you mean more than 20% of blast express particular Ag is mean positive. Twenty percent from Nucleated differential cells or from what?

Answer: *I don't think I said that or if I did I did not intend to. Sorry for the confusion. The time I used 20% was to note that AML is defined in most settings by the presence of ≥20% blasts by morphology (denominator, nucleated cells).*

Question: Is there any minimal blast in marrow or peripheral blood that logic to performed flowcytometry examination?

Answer: *It depends on the question you are asking. Flow cytometry is more sensitive than morphology in picking up small abnormal blast populations. This has been demonstrated in acute leukemia residual disease testing and also in MDS with <5% blasts. If you are in a lab with a panel equipped to characterize blasts as normal or not, I would not place a lower limit on the blast percentage. You can find abnormal blasts when they are not increased by morphology. Sometimes, marrow might be more fruitful than blood. We don't have a lower limit for determining if a sample gets flow cytometry in our laboratory.*

Question: We have encountered a few leukemia cases that showed clear myeloid lineage and also showed moderate cytoplasmic CD3 (almost as high as background CD3 T cell intensity, but not quite), and we struggle with MPAL. What other features would you look at to see if you really have a T lineage expression in a potential MPAL?

Answer: *Seeing two separate lines of differentiation can help. It is hard to address this question further without having data to look at. I would refer you to the WHO. You can also send me some plots to look at!*

Question: Can you please elaborate more on your comment during lecture regarding blast enumeration using fresh blood sample specimen will be more accurate than in the marrow? If that so, may I know what is your take on cases like severe pancytopenia with low total WBC?

Answer: *The 2 main challenges to flow enumeration of blasts in the marrow is hemodilution and erythroid lysis. As blood does not suffer from blood contamination (it is blood after all) and as circulating NRBCs are usually limited, the numbers in the blood more often reflect the morphologic blast count. Granted, if the total WBC count is low in a leukopenic patient, morphologic blast counts may be unreliable. In this setting flow cytometry can be even more helpful given the ability to evaluate many more cells.*