



The Symbiotic Relationship between a Clinical Hematologist and Hematopathologist in the Management of Children with Cancer

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Introduction

Hematological malignancies, namely, leukemias and lymphomas comprise the major part of clinical practice for most centers that manage children with cancer.¹ Survival of children with common pediatric malignancies such as acute lymphoblastic leukemia (ALL), Hodgkin's lymphoma, and Burkitt's lymphoma has improved by leaps and bounds over the last few decades. This can be largely attributed to superior diagnostic techniques and risk-stratified approaches that tailor the therapy for different patient subgroups. The pathology laboratory, by performing indispensable tests ranging from cytology and histomorphology to immunohistochemistry (IHC), flow cytometry, and molecular methods, has veritably paved the way for this success story.

The evolution of hematology as a specialty can be attributed to doctors who played the dual role of managing patients at the bedside and examining their peripheral smears and bone marrow aspirates in the laboratory.² It is only in the last 20 years that clinical and laboratory hematologists are emerging as distinct specialists. The dichotomy is necessitated by the growing number of children being diagnosed with hematological disorders and the multitude of investigations that are now mandatory for their standard of care. Yet, it is noteworthy that contemporary courses that train clinical and laboratory hematologists ensure the rotation of the trainees in the laboratory and clinical areas, respectively. In this perspective, the authors illustrate the importance of a healthy relationship between the two groups of professionals with the help of some real-life scenarios.

Case 1: A Smear in Time Saves Nine

A 9-year-old boy presented with high-grade fever and vomiting for 1 week and altered sensorium for 1 day. Neuroimaging revealed intraparenchymal hemorrhage. A complete blood count (CBC) showed a hemoglobin (Hb) level of 5.8 g/dL, white blood cell (WBC) count of 93,200/mm³, and platelet count of 35,000/mm³. Prothrombin time and activated partial thromboplastin time were both prolonged. The clinician suspected the possibility of acute promyelocytic leukemia (APML) and called his hematopathology colleague to review the peripheral blood smear, and the smear was promptly reported to show blasts and abnormal promyelocytes. The child was immediately initiated on all-trans retinoic acid and administered platelet and plasma transfusions. By the next day, a bone marrow test was performed, which corroborated the diagnosis of APML on morphology and flow cytometry. After 3 days, a polymerase chain reaction (PCR) test for PML-RAR α decisively established the diagnosis. Timely initiation of therapy based on good clinical suspicion and reliable smear findings while waiting for the confirmatory diagnostic results enabled complete recovery of the child.

The importance of the peripheral blood smear cannot be undermined. A simple Romanowsky smear seen by experienced eyes can reliably demonstrate atypical cells in leukemia and enable the clinician to plan further investigations and treatment. In a typical case of leukemia with pallor, bleeding, lymphadenopathy, and hepatosplenomegaly, the smear can confirm the clinical suspicion. The smear findings can raise the possibility of leukemia in atypical situations too. A child with severe musculoskeletal pains due to

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leukemia may be misdiagnosed as juvenile rheumatoid arthritis, and an astute hematopathologist can call the clinician after seeing the blood smear to prompt a bone marrow test before starting steroid therapy. A smear can also rule out a malignant etiology and redirect the diagnostic pathway. For example, an infant with transfusion-dependent β -thalassemia can present with pallor and hepatosplenomegaly. WBC may be spuriously elevated due to nucleated red cells (NRBC) and the platelet count may be low due to untreated Vitamin B12/folic acid deficiency. An unnecessary and invasive bone marrow test can be prevented and the clinician can be directed to obtain a hemoglobin variant analysis to confirm the diagnosis. Even in today's age of the automated hematology analyzer giving CBC reports in most laboratories, abnormal cells are flagged and smears are prepared for review by the pathologist.³ No machine is a replacement for the trained eyes of a hematopathologist! As the adage goes, "The eye cannot see what the mind does not know." Therefore, it is also important that the clinical differential diagnoses are effectively communicated to the hematopathologist.

Case 2: A Good Bone Marrow is a Bull's Eye Hit by an Arrow

A 14-month-old boy presented with fever and easy bruisability for 6 months. He had been treated for immune thrombocytopenia (ITP) elsewhere and had undergone bone marrow test twice with inconclusive reports. The trephine was reported as myelofibrosis. At the time of presentation to our center, the CBC showed Hb level of 5.1 g/dL, WBC of 7,600/mm³, and platelet count of 10,000/mm³. The disproportionate anemia and presence of hepatosplenomegaly alerted the clinical hematologist that this was not ITP. The hematopathologist reported the smear to have NRBC, leukoerythroblastic blood picture with 11% blasts in the differential, which morphologically resembled megakaryoblasts. The bone marrow aspirate was dilute yet again. The trephine biopsy was reported as fibrotic with some atypical megakaryocytes. The flow cytometry of the peripheral blood was able to immunophenotype the circulating blasts as megakaryoblasts. The colleague in the cytogenetics laboratory was alerted to look for t(1;22), which was promptly identified in a week, confirming the diagnosis of acute megakaryoblastic leukemia. The child was treated with intensive chemotherapy for acute myeloid leukemia (AML) and is now in remission 6 months post therapy.

A bone marrow investigation done by an experienced person is indispensable to the diagnosis of hematological malignancies. Extra samples of the aspirate must be sent for genetic analysis and to the flow cytometry laboratory in all cases with suspicion of malignancies. In the above case, it was a combination of morphology, flow cytometry, and genetic techniques that led to the establishment of a diagnosis. In the absence of the cytogenetic aberration identified, it would have been arduous to label the child as AML based on peripheral blood flow cytometry alone. A cytogenetics report that normally takes a minimum of 2 weeks was expedited by the laboratory consultant at the request of

the clinician, leading to the initiation of chemotherapy within a week of presentation to our center.

Case 3: Laboratory Milestones on the Clinical Road to Remission

A 4-year-old boy was admitted with fever, pallor, hepatosplenomegaly, and pancytopenia. A bone marrow test was performed with aspirate samples sent to the flow cytometry, molecular hematology, and cytogenetics laboratories. Six hours later, the flow cytometry laboratory confirmed the diagnosis of pre-B ALL to the clinician. He was assigned to the "standard risk" category based on his age and a presenting white cell count of 5,600/mm³ (<50,000/mm³). After 1 week of the oral prednisolone prephase, an absolute blast count in the blood of 200/mm³ suggesting a good prednisolone response (<1,000/mm³ on day 8 after the steroid prephase) and the absence of blasts in the cytospin preparation of the cerebrospinal fluid (CSF) corresponded to a CNS1 stage. By 2 weeks of therapy, the molecular hematology laboratory reported a PCR positivity for ETV6-RUNX1, which carries a good prognosis in ALL. The cytogenetics laboratory reported a normal karyotype. At the end of induction chemotherapy, a repeat bone marrow showed no residual leukemic blasts, and a flow cytometry-based minimal residual disease (MRD) was reported by the hematopathologist to be 0.15% (positive MRD taken as $\geq 0.01\%$). The chemotherapy protocol was then escalated to "high risk" based on the positive MRD. The child completed his intensive and maintenance chemotherapy successfully and remains in remission 5 years after treatment.

ALL is the poster child for how a clinician and pathologist work closely together to establish the diagnosis, prognosis, and final risk group for treatment. In the past 50 years, ALL has transformed from an almost incurable cancer to cancer that is cured in almost 90% of children in the developed world.⁴ Flow cytometry has revolutionized the diagnosis of leukemia and establishes the immunophenotype for deciding treatment. From the first report of Philadelphia chromosome (Ph) positivity in ALL 60 years back to the recent discovery of novel genetic entities such as Ph-like ALL with advanced molecular techniques, the concept of a blanket diagnosis of "ALL" or "AML" has changed to a genetically heterogeneous wide spectrum of leukemias.^{5,6} The latest classifications of hematological malignancies incorporate both immunophenotype and characteristic genetic aberrations in defining the categories.⁵

Cytotoxic treatment-related mortality is a major hurdle to the successful treatment of cancers.⁷ Factors such as the presenting blood counts, absolute blast count after prephase, CSF cytology, genetics, bone marrow morphology, and MRD are now used for optimal risk stratification.⁸⁻¹⁰ MRD measurement by flow cytometry or genetic techniques has superseded morphological assessment of remission in ALL and emerged as an independent predictor of outcome.⁹ Risk stratification permits escalating intensities of cytotoxic therapy for different patients. This has significantly reduced toxic morbidity and mortality. AML is stratified completely based

on the genetic aberrations detected and the MRD response post-induction chemotherapy.¹¹ The decision of offering a hematopoietic stem cell transplant in the first remission is also based on the same factors.

Akin to flow cytometric immunophenotype, IHC is used for the diagnosis of lymphomas. The simple classification of Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL) is now replaced by a multitude of types of lymphomas that have been defined by contemporary IHC and genetic tests.¹² The nodular lymphocyte-predominant HL, for example, is a distinct entity that has a unique morphology and IHC, which is treated with different protocols from classical HL.¹³ The term NHL is expanding to include more and more entities with molecular advances. Despite the mind-boggling array of lymphoid neoplasms that are now described, the basic diagnostic approach stands firm and unshaken. For instance, in an older child who is operated on for intussusception, the clinical hematologist would suspect a Burkitt lymphoma and obtain a metabolic profile to demonstrate tumor lysis syndrome with hyperuricemia. The hematopathologist would then review the histopathology for the classically described "starry sky appearance." The rapidly proliferating lymphoma requires rapid initiation of chemotherapy and supportive care while awaiting further immunohistochemical confirmation of a mature B-cell phenotype and a *C-MYC* translocation by a fluorescence in situ hybridization. The concept of MRD in leukemias has led to the prospective evaluation of minimal disseminated disease in lymphomas as a tool for prognostication and risk stratification.¹⁴

Case 4: Putting Pieces Together with NGS

A 4-year-old girl was brought by her family to the oncology clinic. Her 27-year-old mother had recently succumbed to a disseminated gastric adenocarcinoma, and the family was anxious about the risk of cancer occurring in her. On examination, she was observed to have premature graying of hair. Her CBC and liver and renal function tests were all within normal limits and she had no other clinical concerns. A focused next-generation sequencing (NGS) for inherited marrow failure syndromes was performed on her peripheral blood, which detected a heterozygous mutation in the RTEL1 gene identified in dyskeratosis congenita. The family was counseled on the need for surveillance for the development of mucocutaneous features, bone marrow failure, and malignancies.

NGS has revolutionized the diagnostic machinery in the field of hematology. It has a wide range of uses including the identification of inherited disorders that predispose to malignancy and fine molecular characterization of malignancies such as ALL and AML. In pediatric ALL, NGS is now being used at diagnosis for identifying mutations that are prognostic and potentially targetable (e.g., Ph-like ALL), accurately quantifying MRD, identifying resistance to therapy (e.g., *CREBBP* mutations that induce glucocorticoid resistance), and pharmacogenomics (e.g., *TPMT* and *NUDT15* mutations that indicate a need to reduce the doses of 6-mercaptopurine).¹⁵⁻¹⁷

Conclusion

In this era of rapid advancement in medicine, it is vital for clinical and laboratory health professionals to work in constant liaison. In the absence of a complete clinical database and a list of differential diagnoses in the request form, the pathologist cannot be expected to give an accurate diagnosis on any test. Similarly, the clinical hematologist is heavily dependent on the details provided in each report from the laboratory for his or her practice. Any discrepancy between the clinical expectation and the pathology report must prompt a conversation to discuss the case in detail. Multidisciplinary discussions must be held regularly to discuss challenging cases. Further, research is the way forward to achieving the ambitious dream of curing all children with cancer in the future. Quality research in hematology mandates an active corroboration between clinical and laboratory-based researchers. Last but not least, in low- and middle-income countries, where the availability of advances lags behind high-income countries, the clinician and pathologist must make use of all modalities available to provide optimal care to children with hematological malignancies.

Author Contributions

The manuscript has been read and approved by all the authors that the requirements for authorship have been met and that each author believes that the manuscript represents honest work.

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Conflict of Interest

None declared.

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