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Recent perspectives of pediatric leukemia – an update

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Abstract. – Leukemia is defined as an aberrant hyper-proliferation of immature blood cells that do not form solid tumor masses (i.e., liquid cancer). Usually, leukemia could be either of the myeloid or lymphoid lineages, and is classified as acute or chronic in nature. Chronic leukemias tend to have more mature cells and are rare in pediatric patients. Acute leukemias, on the other hand, are typically less mature and commonly occur in patients of all ages and are potentially rapidly fatal if not readily treated. The acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. Similar to AML, and in some cases, on the same disease spectrum, are the myelodysplastic syndromes (MDS). The present review is focused on the recent perspectives of pediatric leukemia.

Key Words:
Leukemia, ALL, Pediatrics, MDS.

Introduction

The acute lymphoblastic leukemia (AML) is one of the more common childhood cancer[1]. The risk for developing AML in the majority of cases is biological rather than environmental, with the only established pediatric AML cause being *in utero* exposure to ionizing radiation[2]. Other exposures, e.g. maternal chemical exposure and parental age, have only limited evidence supporting their association with AML. In US, the overall incidence of pediatric AML is approximately 7.7 cases per million children. The race appears to play only a very minor role in AML risk among Americans, with Asian and Pacific Islanders having the highest incidence (8.4 per million) and African Americans having the lowest risk (6.6 per million)[3]. The risk of childhood AML peaks early in life at 18 per million in infants less than 1 year of age, reaches an incidence of approximately 4 per million in children aged 5-9. The factors that convey the direct risk are genetic in nature. Down syndrome (DS) is the most common genetic risk factor. However, less common diseases, especially those associated with DNA repair deficiencies like Fanconi anemia and ataxia telangiectasia are also associated with an elevated risk to develop pediatric AML.

Biology of Pediatric AML

Originally divided into few morphological subtypes, advancements in molecular medicine have allowed for the reclassification of AML subtypes based on the vast array of different morphological, cytogenetic, and genetic variations. One of the first major attempts at classification came with the development of the French-American-British (FAB) system in 1976[4]. The FAB system divided AML into 8 different subtypes, M0-M7, which correspond to: acute myeloblastic leukemia, minimally differentiated (M0); acute myeloblastic leukemia, without maturation (M1); acute myeloblastic leukemia, with granulocytic maturation (M2); acute promyelocytic leukemia (APL) (M3); acute myelomonocytic leukemia (M4); acute monoblastic/monocytic leukemia (M5a/b); acute erythrocytic leukemia (M6); acute megakaryocytic leukemia (M7, AMKL). Though less commonly used for prognostic indications, the FAB classification is well entrenched in modern hematology and is commonly referred to (WHO) classification.

The WHO classification of myeloid neoplasms represents an attempt of offering a comprehensive classification scheme based on all available clinical, morphologic, cytochemical, immunophenotypic, and genetic data. While substantially more complicated than the older FAB systems, the WHO classification (now in its 4th edition) allows for a much finer differentiation. Therefore, it offers a more accurate prognostic correlations. The major categories are: AML with recurrent genetic abnormalities; therapy-related myeloid neoplasms; myeloid proliferations related to Down

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syndrome; and AML A full discussion of this classification system is presented by Vardiman et al[5] providing a good starting point for those with further interest.

Core Binding Factor Leukemia

Core binding factor (CBF) AML describes a subset of leukemias that possess genetic alterations in one of the two proteins that make up the family of protein complexes known as CBFs. The CBF transcription factor plays an important role in the normal hematopoiesis, and small perturbations of its expression or function can have deleterious consequences (6). Together, two common cytogenetic abnormalities make up the largest group of CBF leukemias: t(8;21)(q22;q22) and inv(16)(p13.1q22), representing approximately 15% and 6% of pediatric AML cases, respectively.

APL with PML-RARα Fusion Gene

Somewhat unique among the early FAB classifications was the M3 subgroup, which represents APL. It is associated with an extremely high rate of fatal hemorrhage. Notably, almost every case (approximately 95%) of APL has a characteristic t(15;17)(q22;q21) translocation which results in the fusion of the *PML* and *RARA* genes. The *PML* gene product is important for nuclear body formation and plays a role in transcriptional regulation and tumor suppression. The *RARA* gene product, retinoic acid receptor alpha (RARα) is a nuclear receptor that normally plays a role in a host of differentiation processes. When these two genes fuse, the resultant protein prevents both of the physiologic functions. Conversely, the PML-RARα fusion protein binds to DNA and acts in a dominant negative fashion to repress gene expression, as well as nuclear body formation. This prevents further differentiation of the APL cell, and while not sufficient, is considered necessary for the development of APL. Though APL is considered to be universally fatal, this unique biology allows the use of targeted therapies that makes APL one of AML subtypes with a more favorable prognosis. In approximately 5% of cases, the RARA gene is fused with another partner protein, resulting in variable changes to chemotherapy sensitivity and prognosis[7].

Therapy-Related Myeloid Neoplasms

In a departure from most WHO classifications, myeloid neoplasms (both AML and MDS) that are believed to be sequelae of a previous therapy are grouped together into the same category (tAML). Many types of cancer treatments act by inducing structural damage to DNA. In the process of repairing this damage, it is possible that new genetic abnormalities might arise, some of which may aid in the progression to AML. As this process takes time, tAML is less common in children, mostly because by the time tAML develops the patient has reached adulthood. However, tAML is a potential consequence of many types of therapy, especially topoisomerase II poisons and radiotherapy, both of which are commonly used to treat childhood malignancy.

Though tAML is not defined by any specific set of genetic abnormalities, there are trends that are arising. The use of certain chemotherapy drugs, especially anthracyclines and etoposide, are highly correlated with the development of tAML with chromosomal abnormalities involving 11q23. At this locus, *MLL* gene (officially referred to as *KMT2A*) encodes a histone methyltransferase. Rearrangements at this site are common in infant AML, with different fusion partners having different prognostic significance. However, in the context of tAML, the presence of these common genetic abnormalities help to differentiate tAML from *de novo* primary AML.

FLT3-ITD AML FLT3 is a receptor tyrosine kinase encoded by the *FLT3* gene on chromosome 13. As mentioned above, FLT3 plays an important role in the normal hematopoiesis. Further, tyrosine kinases like FLT3 exist primarily as a monomer that upon ligand binding dimerizes and autophosphorylates. This activates an intracellular signaling cascade that has consequences for maturation and proliferation. In approximately 10-20% of pediatric AML cases, an internal tandem duplication of variable length (FLT3-ITD) in exons 14 and 15 promotes ligand-independent activation of FLT3. The unfortunate consequence of this activation is a decreased maturation and an increased proliferation of myeloid progenitor cells. Similar to FLT3-ITD are point mutations in FLT3 that have similar effects, though these mutations are less common[8].

AML with CEPBA Mutation

The CCAAT enhancer binding protein family (C/EBP) is made up of several related transcription factors, the α-isoform of which (C/EBPα) is commonly mutated in pediatric AML. These proteins play an important role in the differentiation of many tissue types. Specifically, C/EBPα is heavily involved in the maturation of the granulocyte lineage, binding to the promoters and supporting

transcription of a variety of genes necessary for this process. Mutations in the CEBPA gene in the context of AML resulted in the expression of a 30 kDa form of the C/EBPα protein (full length is approximately 42 kDa) so as to prevent appropriate promoter binding and gene transactivation[9].

AML with NP1 Mutation

Nucleophosmin, or NPM1 protein encoded by the NPM1 gene, has pleiotropic functions, but is well known for its role as a nuclear chaperone that is involved in the import and export of a wide variety of substrates. When mutated, these functions typically cease, and many cellular processes go into disarray. Mutations in NPM1 are relatively uncommon in pediatric AML (up to approximately 10%, 20% in cytogenetically normal AML [CN-AML]); however, their prevalence increases with patient age. Importantly for AML, NPM1 is involved in hematopoiesis, DNA replication, and repair, as well as gene transcription. When mutated, these functions are affected to varying degrees, and *NPM1* mutations are considered to be primary events in the development of malignancy in some cases[10,11].

Treatment and Prognostic Considerations for Pediatric AML

When treating AML, there are many factors that need to be considered. The first is urgency; AML, especially with certain presentations, is a medical emergency that requires immediate intervention. The second is efficacy; not all interventions are equally effective against each disease subtype. Finally, complicating efficacy is toxicity. Though advances have been made in the treatment of pediatric AML, the most effective therapies are profoundly toxic even when administered properly. Therefore, it is imperative that measures be taken to maximize efficacy and minimize toxicity.

To rapidly decrease the leukemic burden, the patient is also treated with induction chemotherapy[12]. Typically given in multiple rounds, induction chemotherapy typically consists of moderately high-intensity dosing schedules. The goal of induction chemotherapy is to safely induce remission so as to allow restoration of normal hematopoiesis. A patient with newly diagnosed AML is typically very ill, and care must be taken to ensure patient safety.

Once remission is achieved, therapy transitions to a phase known as consolidation. Consolidation therapy is usually maximally intense, both with regard to dosing (high doses) and timing (short latency between doses)[13,14]. The goal of consolidation is to eliminate any remaining leukemic blasts, ideally resulting in a cure. Both the duration and types of treatments used for consolidation vary between AML subtypes. When patient's disease does not respond to treatment, a remission in refractory disease is often used and it is known as salvage therapy[15]. Highly variable salvage therapy includes treatments that are associated with unfavorable side effects. Similarly, if a patient's disease that was once in remission returns, it is considered to be relapsed. For many AML subtypes, relapse is not uncommon and is associated with varying prognoses. In the event of relapse, re-induction is attempted. If unsuccessful, the disease is considered secondary refractory. When chemotherapy alone is likely to be insufficient to achieve a cure, hematopoietic stem cell transplant (HSCT), or bone marrow transplant, might be pursued[16].

Prognosis

With the discovery of new drugs and improvements in supportive care, survival among pediatric AML patients has risen to approximately 70%. With greater treatment experience and understanding of the biology underlying AML, it is now possible to identify patients with higher- and lower-risk disease. This allowed a less aggressive treatment in patients with more favorable prognoses.

Favorable Prognostic Indicators

There are several disease characteristics that are associated with a favorable prognosis (survival >70%). Fortunately, CBF AML represents one of the largest AML subgroups and is associated with a favorable prognosis[17]. Similarly, APL with the standard cytogenetics is also associated with good outcomes[18]. Further, AML in the DS population is associated with one of the most favorable prognoses[19].

Adverse Prognostic Indicators

In contrast to those findings listed above, there are several cytogenetic or genetic abnormalities associated with poor outcomes (survival <50%). Certain translocations, including t(10;11)(p12;q23), t(6;9)(p23;q34), inv(3)(q21126.2), t(3;3)(q21;q26.2), t(7;12)(q36;p13), t(7;12)(q32;p13), and t(5;11)(q35;p15.5) are poor prognostic markers. Though not a specific finding, tAML is always considered to have an adverse prognosis. Finally, mutations in Flt3 or Flt3-ITD are less favorable.

Moreover, Flt3 abnormalities carry an adverse prognosis.

Intermediate Risk

For many subtypes of AML, the prognosis is considered to be intermediate (survival 50-70%). In some cases, this could be the result of underlying biology, but often the assignment of intermediate risk is due to inconclusive results. Many findings had variable outcomes between trials and, therefore, it is up to the clinician's judgment to assign risk and subsequent treatment strategy.

Standard Induction and Consolidation

The standard induction therapy for pediatric AML has remained largely unchanged for over 20 years. It consists of a "3+7" or "3+10" strategy of administering cytarabine (araC) and an anthracycline (or substitute), typically daunorubicin (DNR)[20]. Both araC and DNR are genotoxic agents that non-specifically target replicating cells. The major toxicities associated with their use could be considered to be myelosuppression (araC) or late cardiac toxicity (DNR). Most protocols include a third drug for induction, often etoposide or thioguanine. The evidence supporting these inclusions are, for the most part, non-conclusive with regards to benefit to overall survival (OS). If complete remission (CR) is obtained, the consolidation therapy is appropriate. The consolidation therapy typically consists of high-intensity variants. For most cases, at least two rounds of high-dose araC (HiDAC) are given[21]. For most AML subtypes, post-consolidation maintenance therapy is inappropriate[22].

Role of HSCT

The use of HSCT in the treatment of pediatric AML, in many cases, is controversial[23]. Although HSCT is often considered to be a curative therapy, the profound toxicity associated with the procedure prevents its use for low-risk disease. The benefit of HSCT is that the combination of extreme-intensity condition regimens and graf versus tumor effects are highly effective[24]. However, the potential for graft *versus* host disease, the massive supportive care burden, and risks of mortality are of serious concern. One of the earliest hurdles that HSCT faces is the identification of a suitable donor.

Typically, the ideal donor is an HLA-matched sibling, although frequently unrelated donors are required. Before the transplant, the patient made a conditioning regimen of either total body irradiation or myeloablative chemotherapy. After conditioning, the cells are infused into the patient, but marrow reconstitution could take weeks, assuming successful engraftment. Current recommendations do not allow for HSCT in the first CR for low-risk patients, but for other patients a risk-benefit analysis is appropriate. Although HSCT has shown positive effects for high-risk patients but treatment-related mortality for HSCT in pediatric AML patients is as high as 41%[25].

Treatment of APL and Flt3-ITD AML

Both APL and Flt3-ITD AML present opportunities for treatment with agents that are not commonly used for other subtypes of AML. For the treatment of APL, the use of all-trans retinoic acid (ATRA) is the standard of care. When exposed to ATRA, the PML-RARα fusion protein relocalizes, and transcription of differentiation-inducing genes is increased. The use of ATRA has helped in changing APL from being one of the most fatal subtypes of AML to one of the most curable. For the case of Flt3-ITD AML, treatment with the multiple tyrosine kinase inhibitor sorafenib offers potential benefit. Early studies have shown favorable results, although the development of resistance and cross-resistance to other inhibitors remains problematic[26].

Role of MRD Monitoring

At specific stages of treatment, it is important to measure the response to treatment. Historically, this was performed by morphological analysis of bone marrow biopsies/aspirations. Unfortunately, this method is very subjective and offers poor sensitivity for detection of leukemic blasts. However, recent advances have allowed for more sensitive and objective methods for the detection of residual disease. If a specific fusion gene or other genetic abnormalities are identified, PCR detection could be used with a sensitivity of approximately 1/10,000 cells. Further flow cytometry is another option in hand. By identifying a patient-specific cell surface marker profile, it is possible to rapidly identify as few as 1/1000 cells as leukemic blasts for most patients. The utility of this approach was demonstrated to great effect in the St. Jude AML02 trial, as well as the recent COG-AAML0431 trial[27].

Conclusions

It is quite clear that a lot of approaches are being explored for the efficient management of

pediatric leukemia. However, the success rate is not good so far. So, it could be concluded that novel advanced approaches like HSCT are showing a good potential. However, more studies in the clinical setting are essential to confirm future use of HSCT as the gold standard therapy.

Conflict of interest

The authors declare no conflicts of interest.

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