BRIEF REPORT

NEGATIVE INITIAL BONE MARROW ASPIRATE DOES NOT RULE OUT ACUTE LYMPHOBLASTIC LEUKEMIA

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Abbreviations Table

B-ALL	Precursor B-cell acute lymphoblastic leukemia
BMA	Bone marrow aspirate

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ABSTRACT

Patients with precursor-B-cell acute lymphoblastic leukemia (B-ALL) may initially present with a prodrome, cytopenia(s) with abnormal bone marrow cellularity, but without clonal abnormalities. Prior cases of "indolent ALL" report infections preceding B-ALL diagnosis. Here we describe our institutional experience, eight patients over a 15-year period with a prodrome (2% of B-ALL diagnoses) prior to definitive diagnosis. Patients ranged from 3-15 years of age (median 5 years), requiring a median 3.5 months from presentation to diagnosis, with a median 3 bone marrow aspirates (BMA) to reach definitive diagnosis. Practitioners must be aware that initial negative BMA does not rule out B-ALL.

Keywords: Bone marrow; leukemia, lymphoid; pancytopenia; preleukemia

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy with an average of 6,000 new diagnosis each year in the United States.[1] While much progress has been made in understanding prognostic factors associated with ALL, the pathogenesis of ALL remains elusive.[1] Two hypotheses have been proposed regarding the development of ALL. Greaves' "delayed infection hypothesis" proposes that individuals who have delayed or diminished exposure to microorganisms perinatally or during infancy may have an under-developed immune system and, when these individuals are exposed to common infections post infancy, an immune response dysfunction occurs, resulting in ALL.[2] Kinlen et al. describe a "population mixing hypothesis" suggesting an epidemiological association that childhood leukemia may be related to epidemics occurring amongst vulnerable populations.[3] Like Greaves, Kinlen suggests that childhood leukemia arises from an inappropriate immune response to infection. However, a meta-analysis of 36 studies performed by Hwee et al. concluded that the association between infection and ALL cannot be confirmed or refuted based on the current literature.[4]

While the connection between childhood infections and ALL is still under debate, several case reports have described an aleukemic pre-ALL phase preceding definitive ALL diagnosis associated with infection. Heegaard et al. and Yetgin et al. detailed several cases where parvovirus B19 infections and cytopenia(s) preceded ALL diagnosis.[5,6] Other reports similarly describe infection with transient cytopenia(s) prior to ALL diagnosis.[7-9] In all cases, initial bone marrow aspirate (BMA) was negative at presentation of cytopenia(s), and time to ALL diagnosis ranged from one month to eighteen months. However, an infectious event precipitating ALL development was not confirmed. An aleukemic prodromal phase has been reported in

approximately 2% of ALL diagnoses but clear data regarding the pathogenesis and clinical presentation are lacking.[10,11] Therefore, we sought to describe the clinical presentation, laboratory evaluations, and clinical evaluations of children who presented with initial cytopenia(s) but negative BMA prior to the eventual diagnosis of B-ALL in our patient population.

DESIGN AND METHODS

We conducted a retrospective chart review of patients diagnosed with B-ALL over a fifteen-year period (2002-2017) at UCSF Benioff Children's Hospital Oakland who initially presented with cytopenia(s) but negative BMA. We collected baseline demographic data including age, sex, and self-reported race and ethnicity in addition to clinical data including laboratory and pathological findings, clinical symptoms at presentation, number of BMAs until diagnosis, and time from initial BMA to diagnostic BMA.

RESULTS

Eight children presented with cytopenia(s) and normal BMA before eventually progressing to B-ALL (Table 1). All patients had fever and either proven or presumed infection at initial presentation. The laboratory confirmed infections included *Salmonella, Streptococcus, Moraxella, Epstein-Barr virus, Respiratory Syncytial Virus, and Human Metapneumoniae Virus*. Four patients had lymphadenopathy, fatigue and abdominal pain. Three had anorexia. Blood analyses showed median hemoglobin 8.3 g/dL, median white blood cell count 1.8 $x10^9$/L, median absolute neutrophil count 120.0 $x10^6$/L, and median platelet count of 224 $x10^9$/L. Sixty-three percent of patients presented with pancytopenia, 13% with bi-lineage cytopenia, and 25%

with single lineage cytopenia. Six patients had an initial hypocellular bone marrow aspirate, while two had a hypercellular marrow. None had evidence of clonal abnormalities. Of note, three patients (patients 3, 5, 6) had evidence of abnormal "lymphoblast-like" cells noted on flow cytometry at the time of initial BMA but did not meet diagnostic criteria required for ALL. Two of these three demonstrated resolution of these "lymphoblast-like" cells on subsequent BMA prior to diagnosis. All patients had initial normal cytogenetics, four of which progressed to clonal abnormalities at diagnosis, three of which were favorable. The median time from initial presentation to ALL diagnosis was 3.5 months, with a median of three BMAs prior to definitive diagnosis. Six patients were male, six were self-reported Hispanic, and one was African American. Six of eight were NCI standard-risk, while two were NCI high-risk based on age at diagnosis. All eight patients and alive and well post completion of therapy.

DISCUSSION

Our patient cohort represented 2% of all patients diagnosed with B-ALL at our institution during this time period, similar to previously reported incidence of "indolent ALL." All patients presented with proven or presumed infection at the time of initial presentation. This supports both Greaves' and Kinlen's hypotheses that common childhood infections may be associated with ALL development and may be involved in a prodromal phase of leukemia presenting as cytopenias with negative initial BMA. Zimmermannova described a clonal evolution within eight patients, who similarly presented with cytopenias but negative initial BMA, who went onto develop leukemia.[10] Zimmermannova demonstrated genetic aberrations in conjunction with the timing of first cytopenias (up to five different clones at said time point), with the prevalence of two of these five clones predominant at the time of diagnosis of B-ALL. She hypothesized

that acute infection allows for the clonal evolution of 1-2 subtypes of genetic aberrations, leading to the development of ALL. This was further discussed by Lyngarrd et al. and Li et al. who hypothesized that ongoing infection may indirectly be involved in the presentation of preleukemia by causing bone marrow suppression, with a small leukemic clone already present, and interval remission of the pancytopenia occurring due to increased endogenous glucocorticoid production acting as a temporary anti-leukemic agent.[12,13] Similarly, Savasan *et al.* demonstrated that infection with Parvovirus B19 may yield suppression of non-leukemic hematopoiesis, which could provide advantage for the malignant clone.[14] While a single infectious agent was not noted in our study or in prior studies, we demonstrate the need for continued clinical follow up for such patients after infection and cytopenia resolution to ensure no recurrence of symptoms or cytopenias prompting the need for repeat BMA evaluation.

In our population, the timing of definitive B-ALL occurrence post infection associated with cytopenias was quite variable and therefore causality cannot be determined. Other patients diagnosed with ALL may have had a prior infection with associated cytopenias that was not identified due to lack of significant symptoms at the time of presentation. As compared with prior case reports and case series, which noted a female and non-Hispanic white preponderance, the majority of our patients were male and self-reported Hispanic.[6] Given the small sample size of subjects in the prior reviewed literature as well as population differences, it is not possible to generalize specific demographic-based risk factors for this prodrome. Additionally, multiple cytogenetic findings were noted in these patients. The overall outcome for patients with an "indolent leukemia" seems favorable based on limited patient numbers.

Practitioners should be aware that a leukemic prodrome with cytopenias but a negative BMA may precede a final diagnosis of ALL by months. Based on the literature available to date, it is unclear if more sensitive minimal residual disease testing by multiparameter flow cytometry would help diagnose these cases earlier. Therefore, in a portion of patients, a normal BMA will not definitively disprove a diagnosis of ALL. We recommend close clinical follow up and laboratory monitoring in such patients, with repeat BMA in those with recurrent symptoms or cytopenias.

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