Hematopathology / Megakaryocyte Size and Age

Developmental Differences in Megakaryocyte Size in Infants and Children

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Key Words: Megakaryocyte; Bone marrow; CD61; Thrombocytopenia

DOI: 10.1309/AJCP4EMTJYA0VGYE

Abstract

Developmental differences in megakaryocytes between neonates and adults have been described. However, the age at which megakaryocytes make a transition to an adult phenotype is unknown. Small megakaryocytes are often described as “dysplastic” in the pathology literature. Thus, recognizing the normal features of megakaryocytes at different ages has diagnostic implications. We identified 72 samples from 61 patients, aged 3 days to 80 years, who had negative staging based on bone marrow examination. Megakaryocyte diameters, as highlighted with anti-CD61, were measured. A scatter plot of megakaryocyte size by age revealed a normal distribution of sizes at the youngest ages, with a shift to multiple peaks starting at 24 months indicating that neonates have megakaryocytes of uniform sizes, which diverge into separate clusters of smaller and larger cells beginning at 2 years; this is followed by an overall shift toward larger megakaryocytes at age 4 years. These observations have direct implications for the evaluation of bone marrow megakaryocytes in young children.

Bone marrow morphologic characteristics, including the size and frequency of megakaryocytes, are assessed in cases of unexplained cytopenias (including thrombocytopenia), suspected lymphoid or myeloid neoplasms, and other clinical scenarios. In young children, bone marrow samples are most often examined for possible acute leukemia or staging for solid tumors. In these situations, the focus is on finding leukemic blasts or foci of metastatic disease. Age-related differences in peripheral blood and bone marrow findings are well known in each lineage, including higher leukocyte counts in children than in adults and change from neutrophilia in the neonatal period (defined as birth to 30 days of life) to a relative lymphocytosis that persists through childhood. At the same time that lymphocytes are increasing in number, serum immunoglobulin levels also increase. In the erythroid lineage, precursors remain stable in number and percent in the bone marrow. However, production shifts from fetal hemoglobin to hemoglobin A in the first year of life. In the megakaryocytic lineage, although platelet counts are similar in healthy full-term newborn infants, children, and adults, a growing body of literature describes developmental differences between neonatal and adult megakaryocytes. We previously published our observations that megakaryocyte phenotype (size and ploidy) is different in neonatal (<1 month) bone marrow specimens from that of adult specimens, with nonthrombocytopenic neonates having smaller megakaryocytes than adults. This finding is in agreement with those of previous studies of fetal bone marrow.1,5 In adult patients who have thrombocytopenia associated with increased platelet consumption, the bone marrow compensates by increasing megakaryocyte number,
and neonatal megakaryocytes may be mediated by increases in thrombopoietin.\(^8,9\) Challenging common paradigms, however, we recently demonstrated that despite their small size, neonatal megakaryocytes are fully mature as evidenced by bright expression of CD42b; presence of alpha granules containing p-selectin and von Willebrand factor; and a well-developed, open, demarcated membrane system.\(^10\) This difference in maturational pattern between adult and neonatal megakaryocytes may be mediated by increases in mTOR signaling and GATA-1 levels.\(^10\) Developmental differences between neonatal and adult megakaryocytes may account for the frequent severe thrombocytopenia found in sick neonates in intensive care units, and has implications for several clinical situations including time to engraftment after transplantation of cord blood stem cells compared with peripheral blood stem cells.\(^11,12\)

The age at which megakaryocytes make a transition from a neonatal to an adult phenotype is currently unknown. Previous studies examining developmental differences have compared neonatal with adult megakaryocytes with little or no examination of megakaryocytes from infants and children. Thus, the age at which transition occurs has not been previously documented. Studies in this field have been hampered because bone marrow biopsies cannot be performed, for ethical reasons, on hematologically normal infants and children. However, recognizing the normal features of megakaryocytes at different ages has implications for the diagnosis of megakaryocyte disorders (such as congenital amegakaryocytic thrombocytopenia and Fanconi anemia) and other bone marrow disorders, including acute leukemia and myelodysplastic syndromes.\(^13\)

Based on data from previous studies, we hypothesized that the shift from a neonatal to an adult megakaryocyte phenotype would occur within the first 5 years of life. We therefore retrospectively collected bone marrow biopsy and clot sections from pediatric and adult patients ranging from 1 month to 80 years of age; the patients did not have thrombocytopenia and had undergone bone marrow biopsies, in most cases, for staging of solid tumors. Using CD61 immunohistochemical staining, we measured the megakaryocyte diameter for each patient and plotted the results against age. In this study, we report for the first time that neonates have more homogeneously sized megakaryocytes, which diverge into separate heterogeneous populations of larger and smaller megakaryocytes starting at approximately 24 months. We further demonstrate more frequent megakaryocytes of larger size beginning at 4 years of age, a finding that continues into adulthood. These observations have direct useful implications for the morphologic evaluation of bone marrow for various disorders in infants and children.

**Materials and Methods**

**Patient Selection**

Hospital records of the University of Arizona Medical Center, Tucson, were searched for patients aged 0 to 80 years who had undergone bone marrow biopsies for staging of solid tumors (in most cases) between 1991 and 2008, were free of disease on the core biopsy and/or clot section, and had normal bone marrow cellularity. This search identified 74 cases with clot sections and/or core biopsy specimens from 61 patients. The study protocol was approved by the University of Arizona institutional review board in accordance with the Declaration of Helsinki.

**Megakaryocyte Size Measurement**

Specimens meeting the aforementioned criteria were stained with H&E for general evaluation of cellularity and suitableness for study. For immunohistochemical analysis, bone marrow clot and core slides were cut at 4 \(\mu\)m and deparaffinized on the Ventana Benchmark XT (Ventana, Tucson, AZ). The primary antibody was a mouse anti-CD61 (VMS 760–4249, clone 2f2, Ventana), which was incubated for 32 minutes at 37°C. Antigen retrieval was performed using a standard cell conditioning solution (CC1, Ventana) for 60 minutes. The iVIEW DAB v3 detection kit (Ventana) was used. Hematoxylin was added for 4 minutes to counterstain the slides. Each sample was evaluated by 1 of 2 investigators (S.G.M. or C.L.C.), who measured the largest diameter of each megakaryocyte using the calibrated measurement tool (at \(\times400\) of the Arcturus XT Laser Capture Microdissection System (Bucher Biotec, Basel, Switzerland). All megakaryocytes in the sections were measured if they represented identifiable cells with nuclei included in the plane of section. Nonnucleated cytoplasmic megakaryocyte fragments were not measured.

**Photomicrographs**

Photomicrographs of Wright-Giemsa–stained aspirate smears, H&E-stained sections, and slides stained for CD61 were captured using a Nikon digital Sight-FI1 camera (Nikon, Melville, NY) using a \(\times10\) objective and \(\times40\) lens for a total magnification of \(\times400\). Because of the thick background of clumped platelets and RBCs, the brightness and contrast of the aspirate smear photomicrographs were increased 10% to 30% using PowerPoint 2007 (Microsoft, Redmond, WA).
Statistical Methods

Statistical analysis was performed using JMP version 8.0.2 (SAS, Cary, NC). Analysis of variance was used for population comparisons, which, in the case of 2 populations, involves a simple t test. Megakaryocyte sizes were compared by age using standard logistic regression analysis. Statistical significance was defined as a P value less than .05. Bivariate nonparametric contour plots were used to summarize size versus age distributions with a contour line at each 0.1 quantile of the population. A standard cumulative distribution function plot was used to show the proportion of megakaryocyte population at or below any given size.

Results

A total of 72 bone marrow samples from 61 patients (37 male, 24 female) including 49 core biopsies and 23 clot sections were evaluated for megakaryocyte dimension. The patients’ ages at the time of the biopsy spanned from 3 days to 80 years, with most being examined within the first 4 decades of life (Table 1). Most biopsy specimens were evaluated for involvement by solid tumor (17 neuroblastomas, 8 Ewing sarcomas, 3 Wilms tumors, 2 synovial sarcomas, 3 breast carcinomas, 2 germ cell tumors, 3 rhabdomyosarcomas, and 3 peripheral neuroectodermal tumors). In 13 cases, either a biopsy was performed for a nonneoplastic disorder or no history was available (Table 2). All cases were free of disease on the core biopsy specimen and clot section, and demonstrated normal trilineage hematopoiesis with normal hematopoietic topobiological characteristics.

Size measurements obtained by both observers were compared and no significant differences were found (P > .3184). Comparison of megakaryocyte size between core and clot sections demonstrated a statistically significant difference (P < .0001). However, the size difference was small, with megakaryocytes averaging 1.2 μm larger on clot sections. The biological significance of this difference is likely negligible and likely artificially induced by slight differences in fixation length and decalcification of the core biopsy specimen.

Logistic regression analysis showed a significant decrease in the percentage of intermediate-sized megakaryocytes over time, from 72% at 1 month to 54% at 53 months (P < .0001). A bivariate plot of megakaryocyte size showed a divergence of distinct size populations over time. After approximately 24 months, we noted the emergence of clearly distinct megakaryocyte populations in the plot (arrow in Figure 1A). The pattern shown in Figure 1A continued in the older patients (>5 years of age), demonstrating that megakaryocytes continued to diverge into multiple subpopulations and increased in overall size (Figure 1B) as was also shown in photomicrographs of CD61 stains (Image 1). Finally, patients were divided into 6 age groups (see legend). As shown in Figure 2, the curves for ages 0 to 1 year, 1 to 2 years, 2 to 3 years, and 3 to 4 years overlapped. For patients aged 4 to 5 years there was a visible right shift in the upper portion of the curve, indicating more frequent megakaryocytes of diameters greater than 25 μm. For patients older than 5 years there was a clear shift to the right of the entire curve with increased size across the entire population.

Discussion

In the current study, we extended our previous observations of megakaryocyte size in neonates to include young children and adults to characterize the timing of the
transition of megakaryocytes from neonatal to adult phenotype. To our knowledge, this is the first study to examine this issue. Differences in neonatal and infant megakaryocytes compared with adults, while fairly well known in the neonatology literature, have not been recognized in the hematology or pathology literature. Based on our previous work, we hypothesized that this shift would occur in the first 5 years. In support of this hypothesis, our data demonstrate that neonates have megakaryocytes of more uniform sizes, which diverge into distinct clusters of larger and smaller megakaryocytes during childhood. This process begins at approximately 24 months and is maintained through adulthood. We further demonstrate more frequent megakaryocytes of larger size beginning at approximately 4 years of age.

Finding “normal” controls for pediatric bone marrow studies is particularly challenging because, for ethical reasons, bone marrow samples of healthy nonpatient infants or children cannot be obtained. Therefore, for this study, we used bone marrow specimens obtained for staging from patients with solid tumors. We recognize that paraneoplastic dysplasia could possibly affect our results. However, to do so, it would have to selectively affect only the youngest children. In our previous autopsy study, we also demonstrated the small size of neonatal megakaryocytes.5 Those patients, however, had multiple nonneoplastic medical problems leading to their hospitalization and subsequent death. In this study, we chose to use living patients with solid tumors to minimize possible postmortem artifact or effects of terminal illness on the bone marrow composition. Patients were further selected for having normal bone marrow cellularity and nonmetastatic disease in the marrow.

These observations have direct implications for the morphologic evaluation of bone marrow megakaryocytes in pediatric patients. Small megakaryocytes are often described as abnormal or dysplastic in pathologic descriptions of bone marrow.14-16 Small, hypolobated or monolobated megakaryocytes are typically seen in adult patients with chronic myeloid

Figure 1 Logistic regression analysis of megakaryocyte size vs age. All data are represented by black dots. The colored contour lines represent density of measurements for that size. Red lines indicate the highest density of measurements, followed by yellow, green, and blue. A, Data for children aged 0 to 53 months. In the younger infants aged 1 to 10 months, a single peak is identified, as illustrated by the red line concentrically surrounded by the other lines. At 24 months, separate peaks begin to be identified above and below the main peak, as indicated by the blue arrow with additional closed circles of blue peaks above and below the main central green peak. B, Data for patients aged 78 to 1,000 months. Distinct peaks surrounding the main central red peaks are clearly identified as evidenced by standalone circles separate from the main peaks.

Figure 2 Cumulative probability plot vs size divided by age of patients. There is a clear shift to the right of the curves for patients aged 4 to 5 years and older than 5 years, indicating an overall increase in size of megakaryocytes starting at 4 years of age.
leukemia and myelodysplastic disorders, particularly the 5q-
syndrome. Small dysplastic megakaryocytes are also described in leukemias that occur in infants and children.

However, because these leukemias occur most commonly in infants younger than 3 years, this appearance may actually represent typical megakaryocyte morphologic characteristics for this age group. Our current study demonstrates that small megakaryocytes may be a normal finding in this age group, which should not necessarily be construed as evidence of a pathologic state. We previously demonstrated that neonatal-type megakaryocytes are, in fact, fully mature cells but with limited capacity to increase their cell size in response to thrombocytopenia. Additional studies will be helpful to further define the normal morphologic and functional characteristics of megakaryocytes in infants and young children.

References


