CD14/16 Monocyte Profiling in Juvenile Myelomonocytic Leukemia

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May 6, 2020

Abstract

Monocyte subset analysis by flow cytometry has been shown to be a useful diagnostic tool in chronic myelomonocytic leukemia in adults. An increase in the classical monocyte fraction (CD14++/CD16-) greater than 94.0% of total monocytes is considered highly sensitive and specific in distinguishing CMML from other myeloproliferative disorders. In a pilot study of juvenile myelomonocytic leukemia cases, we noted that CD14++/CD16- monocyte fraction was >95% in de novo JMML with somatic PTPN11 mutations but normal in those with monosomy 7 or Noonan syndrome. Monocyte subgroup profiling by itself is not diagnostic of JMML but may distinguish molecular subgroups within JMML.

Introduction

Abnormal monocyte proliferation is a central feature of both juvenile myelomonocytic leukemia (JMML) in children and chronic myelomonocytic leukemia (CMML) in adults. Flow cytometry based monocyte profiling has been found to be a useful aid in the diagnosis of CMML.[1-5] Monocytes can be classified into three functionally and immunologically distinct cell populations based on expression of CD14 and CD16 surface markers: classical monocytes (CD14++/CD16-), intermediate monocytes (CD14+/CD16++), and non-classical monocytes (CD14-/CD16++).[6-9] Classical monocytes comprise about 90% of the circulating monocytes with only 10% intermediate and non-classical monocytes. [7, 10]Absolute count of monocyte subsets by flow cytometry can vary in various disease conditions and provide clinically useful information in management of diseases.[7, 11, 12] An increase in the classical monocyte fraction (CD14++/CD16-) greater than 94.0% of total monocytes is considered highly sensitive and specific in distinguishing CMML from other myeloproliferative disorders. [2, 3, 9]A specificity of 97% in peripheral blood (PB) and 100% in bone marrow (BM) samples was observed in one study.[4]

While the distribution and expression of CD14/CD16 surface markers is very well characterized in adult CMML, no such categorization has been reported in JMML. In this pilot study, we evaluated the monocyte partitioning in 14 JMML samples (PB and BM).

Methods

Samples

Between the years 1979-2019, total of 14 patients were clinically diagnosed with JMML at Children's Hospital of Michigan, Detroit MI. Frozen archived mononuclear cells isolated from PB or BM samples were available for 12 patients and fresh samples were available for the 2 patients presented in 2019. The study was approved by the Institutional Review Board at Wayne State University.

Monocyte profiling

CD14/CD16 monocyte profiling was carried out by flow cytometry. Briefly, cryopreserved mononuclear cells were rapidly thawed under warm water, washed with complete medium and resuspended in staining buffer. For fresh samples, mononuclear cells from heparinized peripheral blood or bone marrow samples were obtained by density gradient separation using Fico/Lite-LymphoH (Atlanta Biologicals; Flowery Branch, GA), washed in complete medium (RPMI1640 + 10% fetal bovine serum + gentamycin) and re-suspended in phosphate-buffered saline (PBS) + 30% adult bovine serum as a blocking agent for non-specific staining of immunoglobulins. Cell viability was assessed by trypan blue staining. Surface markers were assessed for 3-color immunophenotyping by incubating mononuclear cells for 20 minutes in the dark with fluorescence-conjugated monoclonal antibodies against CD45 (PC5), CD16 (FITC) and CD14 (PE) (Beckman Coulter; Brea, CA), followed by washing in PBS and re-suspension in PBS + 0.4% formaldehyde as a fixative prior to acquisition using a Coulter XL-MCL flow cytometer (Beckman Coulter; Brea, CA) equipped with an Argon laser. Monocyte populations were gated based on CD45/SS and CD14/SS analysis. Thereafter, CD14/CD16 double parameter plots were used to further define the three subsets of mature monocytes, CD14++/CD16- classical (MO1), CD14+/CD16++ intermediate (MO2), and CD14-/CD16++ non-classical (MO3) monocytes.[6-9] The distribution of monocytes was reported as percentages within the total monocyte gate.

Results and Discussion:

Samples from 14 cases diagnosed to have JMML between 1979 and December 2019 were available for testing; all patients were under age 13; 3 of these cases were included in previous publications.[13, 14] Four of the 12 archived samples showed increased necrosis on flow cytometry and therefore were excluded from the analysis. Figure 1 shows CD14/CD16 monocyte profiling on the remaining 10 JMML samples (8 archival samples; 2 fresh samples) along with the associated JMML mutation.

In four patients (Panel A), MO1 monocytes (CD14++/CD16) were >95% of total monocytes (range 95.1 to 98.8%). Three of these had somatic *PTPN11* mutations (*PTPN11* p.A72V,*PTPN11* p.E76A, *PTPN11* p.A72T), while one case had a novel*CBL* p.L62F mutation. No such increase in MO1 population was observed in any of the four Monosomy-7 patients (despite having concurrent *PTPN11* or *KRAS* mutations) or the two Noonan syndrome cases with *PTPN11* p.E73I mutation (Panel B). This distinct clustering of JMML cases based on monocyte subsets was unexpected. Molecular studies have identified clinically significant clustering of JMML cases with somatic *PTPN11* mutations associated with poorer prognosis than those with germline mutations as seen in Noonan syndrome. Epigenetic studies have also reported increased global 5-methylcytosine in JMML associated with somatic *PTPN11* mutations as compared to the Noonan syndrome cases.[15, 16]

In summary, this pilot study is the first to evaluate monocyte subsets in JMML and correlate with underlying JMML genetics. Flow-based monocyte profiling of JMML patients is not diagnostic of JMML per se but the presence of >95% classical monocytes identifies a subset of cases with underlying deleterious somatic *PTPN11* mutations. In adult CMML, the monocyte subset repartitioning showed normalization in patients who responded to hypomethylating agents (HMA).[3] Thus, monocyte profiling may be a useful tool in characterizing the abnormal monocyte proliferation in JMML and for therapeutic monitoring.

Conflict of interest: The authors have no conflicts of interest

Acknowledgments:

This study was funded by a grant from The Children's Hospital of Michigan Foundation and funds from the Georgie Ginopolis Chair in Pediatric Cancer and Hematology award to Dr. Y Ravindranath.

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Figure Legend:

Figure 1: CD14/16 monocyte profiling in JMML (A) 4 cases of JMML with increased classical monocytes, >95%, (B) JMML cases with normal monocyte profile. * indicates BM sample

Figure 1