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High abundance of circulating megakaryocytic cells in chronic myeloid leukemia in Indian patients. Revisiting George Minot to re-interpret megakaryocytic maturation

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ABSTRACT

Circulating megakaryocytic cells abound in chronic myeloid leukemia (CML) seen in India and uniquely provide a setting for observing megakaryocytic maturation in the peripheral blood, a milieu not native to megakaryocytes. Peripheral blood megakaryocytic cells were studied in 324 cases of CML (235 chronic, 65 accelerated and 24 blastic phases). Two maturation themes were evident. Megakaryocytic blasts, especially in some cases of blast crisis, precociously make a foray into platelet formation and end up producing huge agranular or poorly granular cytoplasmic lobulated masses, that break off and come to lie in the circulation. This evidence of unsuccessful effort may exist, in a considerably attenuated form in chronic phase, alongside of the second major theme of megakaryocytic maturation centered around the familiar micromegakaryocyte, characteristic of the chronic phase. This cell is regarded as dysplastic, but produces morphologically normal platelets. The possibility that this occurs via a hitherto unstudied alternative path of platelet maturation that plays out in the peripheral blood, and the contrasting disorderly premature attempt of blasts to form platelets, represent exciting maturation processes that need further study. Our observations fortuitously constitute a revisit of the insightful exposition on the subject by George Minot nearly a century ago.

1. Introduction

Knowledge about how megakaryocytes (Mk) mature and platelets form has come chiefly from, (a) direct observation on bone marrow (BM), the primary site of Mk development, and (b) studies on Mk cells grown in culture. Peripheral blood (PB) is less useful in this regard as Mk cells only uncommonly circulate and the occasional ones that normally do, are the well-described lobulated bare nuclei [1–8], that are little more than curiosities. Indeed, even in chronic myeloid leukemia (CML), cells of megakaryocytic series, including micromegakaryocytes, have been rarely mentioned as standard PB findings in textbooks and other accounts [2–29], reflecting a rare occurrence in CML as seen in the West. In contrast, even a casual assessment of the peripheral blood of our CML patients shows that such cells abound in all phases of the disease and thus represent an unstudied population. In this study, therefore, we evaluated the morphology of peripheral blood Mk cells in 324 untreated CML patients, to systematically document observations that clearly add up to hitherto unreported themes of megakaryocytic maturation, one that has gone awry right from the start, and the other, that produces morphologically normal looking platelets but needs, nevertheless, to be investigated.

2. Patients and methods

A total of 324 cases of unselected, untreated CML were studied. Of these, 235 were patients in chronic phase (CP) at presentation, 65 in accelerated phase (AP) and 24 in blast crisis (BC). Diagnosis was based on characteristic PB and BM picture and cytogenetics. Assignment to the three phases was done using standard criteria [30,31]. Peripheral blood smear and BM slides were stained by Jenner Giemsa. A 400-cell differential leukocyte count was performed on each PB smear that was submitted to the laboratory as part of the initial routine diagnostic work-up. Note was made of the morphologically identifiable Mk cells. Immunophenotyping using DAKO monoclonal antibodies for CD41 was done in selected cases using alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique, also part of the routine diagnostic procedure.

Abbreviations: Mk, megakaryocytes; BM, bone marrow; PB, peripheral blood; CP, chronic phase; AP, accelerated phase; BC, blastic phase; APAAP, alkaline phosphatase-anti-alkaline phosphatase.

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3. Results

I. **Frequency**
106/235 (45.1%), 41/65 (63.1%) and 8/24 (33.3%) patients in chronic (CP), accelerated (AP) and blastic (BC) phases had up to 18/400 (4.5%), 34/400 (8.5%) and 67/400 (16.75%) circulating Mk cells, respectively. Among those patients in whom Mk cells were present, the median figure was 0.75% for CP, 2.0% for AP and 6.0% for blast crisis. There were two cases of blast crisis where practically the only finding was blasts and large numbers of micromegakaryocytes, the latter in masses. These cases were excluded from this count.

II. **Megakaryocytic maturation in peripheral blood**

1. *Megakaryocytic maturation in blast crisis* (Figs. 1–3). Large lobulated cytoplasmic masses linked to each other by narrow bridges of cytoplasm were seen coming off from blasts. The lobulated masses were either agranular, at times irregularly vacuolated, or had granules recognizable as platelet/megakaryocytic, features that enabled such blasts to be identified as megakaryocytic. Cytoplasmic masses having one or more lobes that evidently had broken off from these cells, were seen in the blood smear, where they were recognizable as dysplastic megakaryocytic masses or platelets. Cells showing increasing maturity by way of deepening chromatin condensation and decreasing nuclear size, right down to the smallest, having small nuclei with condensed chromatin, showed the same kind of cytoplasmic excrescences.
2. *Megakaryocytic maturation in chronic phase* (Fig. 4). The chronic phase was generally characterized by micromegakaryocytes, morphologically mature, small, lymphocyte-like cells with non-lobulated, typically megakaryocytic nuclei, and small to negligible amount of cytoplasm having granules characteristic of platelets. Platelets could be seen budding from the cytoplasm or pinching off from thin cytoplasmic processes of these cells. Larger cells with characteristic platelet / megakaryocytic granules or vacuolations that were not considered blastic because of the nuclear features, were included in this category. Patients with increased platelets and large platelet aggregates in the peripheral blood smear, always had one or two entrapped micromegakaryocytes in the midst of the platelet masses. In a substantial minority of chronic phase and in several patients in accelerated phase, there were megakaryocytic cells showing changes similar to those present in blast crisis described above, though much attenuated in comparison. Large dysplastic cytoplasmic fragments identical to the cytoplasm of these cells could also be seen.
3. Circulating Mk nuclear fragments well described in the Western literature were only occasionally observed and were not included in the analysis.

4. Discussion

Other than case reports of megakaryocytic blast crisis showing a large number of micromegakaryocytes in the peripheral blood, circulating Mk cells have received little attention in published work in the West [32–37]. Most textbooks and other reports [2–29] mention small, rounded, unilobate megakaryocytes in the bone marrow, but do not mention Mk cells even as findings in the peripheral blood in CML; the very few that do, make little more than a passing reference to Mk nuclear fragments or to micromegakaryocytes. Stray circulating Mk nuclear fragments appear to be the most commonly reported [1–8]. The usual absence or near absence of cytoplasm in these cells with characteristic mature megakaryocytic nuclear chromatin, their appearance as incidental findings in normal subjects and a wide variety of non-malignant conditions, shows them as inconsequential findings and quite justifiably, these cells evoke no further interest. In contrast, in the occasional texts that record their presence [38], circulating micromegakaryocytes have been accorded a more respectable mention, stating that they may be seen in the chronic phase [11], be associated with, or increase, in the course of acceleration [3,11], and when present in abundance in blast crisis, provide evidence of megakaryocytic lineage of the blasts [12,14].

Overall, accounts from the West are not representative of what is quite apparent from even a casual examination of the peripheral blood of our CML patients – that circulating Mk cells are far from rare or inconsequential findings. Our results show that as many as over 40% of our patients who are in chronic phase and 2/3 of those who are in accelerated phase have, respectively, up to 4.5% and 8.5% circulating micromegakaryocytes in the peripheral blood smear. Circulating Mk cells, up to approx. 15%, are present also in 1/3 of our blast crisis patients, higher percentages merging imperceptibly with pure megakaryocytic

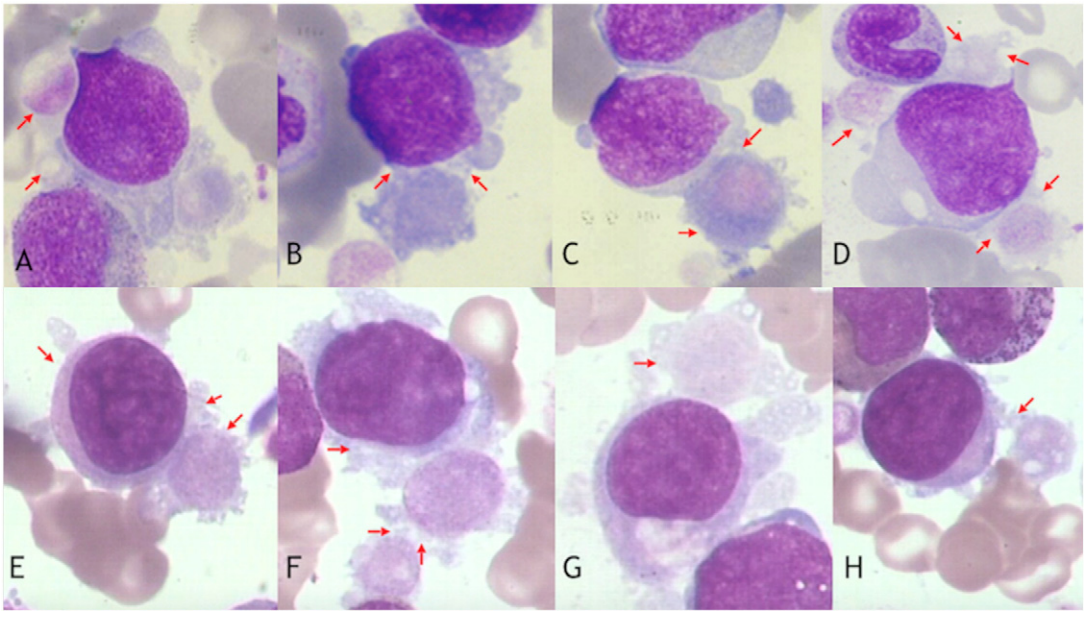

Fig. 1. A–H. Blasts, otherwise undifferentiated, from CML blast crisis, showing large cytoplasmic masses coming off from the cytoplasm. These are either agranular or have a central platelet-type granularity. (Magnification 1000×; oil immersion; Arrows point to cytoplasmic outlines.)