Reports of Practical
Oncology and Radiotherapy

Megakaryocytes morphology in idiopathic (primary) myelofibrosis

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Rep Pract Oncol Radiother 2004;9:223-8, original paper

Received March 29th, 2004; received in a revised form August 26th, 2004; accepted September 3rd, 2004

Summary

Background: The cause of idiopathic myelofibrosis (IM) originating from a haemopoietic stem cells with development of secondary marrow fibrosis is unknown. Increased production of platelet-derived growth factor by megakaryocytes causes amplified activity of fibroblasts. Thus, an analysis of changes in megakaryocyte population affecting fibrous expansion of the haemopoietic base is relevant.
Methods: Analysis and calculation of megakaryocytogram were carried out using bone marrow smears during routine examination of 12 patients with IM and 12 individuals without haematological disorders.
Results: Hyperthrombocytemia prevailed in the peripheral blood of patients with IM. A significant increase was revealed in the content of megakaryoblasts ($p<0.05$), naked nuclei ($p<0.01$) both with diminished count of polychromatophilic megakaryocytes ($p<0.05$).
Cells with picnotic nuclei surrounded with cytoplasm completely decomposed into platelets were distinguished as a separate population with a mean number of 21 $\pm$ 3.0%, out of which basophilic cells made up 9 $\pm$ 1.5% and polycromatophilic cells - 12 $\pm$ 1.9% (vs. 1 $\pm$ 0.4% in control) of the group investigated.
Decrease in the megakaryocyte population with 4-8 nuclei lobules ($p<0.001$) at the expense of an increase in the megakaryocytes number with tight picnotic nuclei, naked nuclei and an increase in the number of 1-2 lobuled forms ($p<0.05$) were found.
Contents of atypical micromegakaryocytes, giant hyperlobuled and dysplastic forms of megakaryocytes was increased.
Conclusion: The changes revealed characterise differentiation disorder in the process of megakaryocytopoiesis. Megakaryocytes are subject to accelerated ageing and enhanced destruction with simultaneous prominent platelet production.
Key words: idiopathic myelofibrosis, megakaryocytes, platelets.

Morfologia megakariocytów w samoistnym (pierwotnym) zwłóknieniu szpiku

Streszczenie

Wstęp: Przyczyna powstawania samoistnego zwłóknienia szpiku, pochodzącego z macierzystych komórek krwiotwórczych, któremu towarzyszy wtórne zwłóknienie, jest nieznana. Zwiększona wartość czynnika wzrostowego pochodzenia płytkowego dzięki megakariocytom powoduje większą aktywność fibroblastów. Dlatego też ważną rzeczą jest przeprowadzenie analizy zmian populacji megakariocytów mającej wpływ na rozwój włóknisty podłoża krwiotwórczego.
Metoda: Przeprowadzono analizę i wyznaczono megakariocytogram używając rozmazów szpiku kostnego podczas rutynowego badania 12 pacjentów z samoistnym zwłóknieniem szpiku oraz 12 innych osób bez zaburzeń hematologicznych.
Wyniki: W krwi obwodowej pacjentów przeważała hypertrombozytoza. Wykryto znaczny wzrost megakarioblastów ($p<0.05$) i jąder pozbawionych cytoplazmy ($p<0.01$) przy zmniejszonej liczbie barwnikochłonnych megakariocytów (0.05).
W badanej grupie chorych procent populacji komórek z jądrami piknotycznymi otoczonymi cytoplazmą rozpadającą się całkowicie na płytki wynosił 21$\pm$3.0%, z których jądra pozbawione cytoplazmy ($p<0.1$) stanowiły 9$\pm$1.5%, zaś komórki wielobarwnikowe 12$\pm$1.9% (w porównaniu do 1$\pm$0.4% w grupie kontrolnej).
Wykryto spadek populacji megakariocytów z 4-8 jąder płatowatych ($p<0.001$) kosztem wzrostu liczby megakariocytów posiadających zwarte jądra piknotyczne oraz jądra pozbawione cytoplazmy oraz wzrost liczby 1-2 postaci płatowatych ($p<0.05$).
Zwiększyła się zawartość nietypowych mikromegakariocytów, olbrzymich hyperpłatowych i dysplastycznych postaci megakariocytów.
Wnioski: Wykryte zmiany charakteryzują zaburzenia różnicowania w procesie tworzenia się megakariocytów, które podlegają przyśpieszonemu starzeniu się i wzmożonej destrukcji, której towarzyszy równoczesna produkcja wyraźnych płytek.
Słowa kluczowe: samoistne zwłóknienie szpiku, megakariocyty, płytki.

Proceedings from the Conference "Current Achievements in Oncology" Poznań, 6-8 November 2003
Praca prezentowana na konferencji "Współczesne Osiągnięcia w Onkologii" Poznań, 6-8 listopada 2003

Introduction

Idiopathic myelofibrosis (IM) is a haemopoietic stem cell disorder with hypercellular bone marrow (with ineffective erythropoiesis and an increased immature granulocytes/total granulocytes ratio), increase in abnormal megakaryocytes, a variable degree of marrow fibrosis and extramedullary haematopoiesis [1].

Megakaryocytes and monocytes have been implicated as sources of the nosogenic cytokines that may augment fibroblast proliferation (platelet-derived growth factor and calmodulin), collagen synthesis (transforming growth factor-β), angiogenesis (vascular endothelial growth and basic fibroblast growth factors), and osteogenesis (the transforming growth factor-β and that of the basic fibroblast growth). The above factors cause development of bone marrow fibrosis [2].

In the case of idiopathic-primary myelofibrosis there appears a cluster of abnormally differentiated, often bizarre forms of megakaryocytes [3,4].

However, the degree of differentiation and functional activities of megakatyocytes and their prognostic role in the pathogenesis of idiopathic-primary myelofibrosis is not well understood. The aim of our study was to assess the degree of maturation, dependence of functional activity on the degree of maturation, number of lobules, cytological features of megakaryocyte, and platelet number in IM.

Methods

In our study, the morphology of megakaryocytes was ascertained in smears of bone marrow from 12 patients with idiopathic myelofibrosis (8 males and 4 females) at a chronic stage and 12 patients having no haematological disorders (a control group). Average age of patients with IM and in the control group was 54 ± 1.9 and 50 ± 5.8 respectively. Bone marrow smears were prepared using a routine method and stained by Pappenhaim.

The definition of idiopathic myelofibrosis score was used according to the criteria adopted by the Italian Consensus Conference on Diagnostic Criteria for myelofibrosis with myeloid metaplasia [5].

A staging system to classify megakaryocyte maturation has been proposed based on light microscopic criteria employing the nuclear/cytoplasmic ratio, nuclear shape, basophilia, and granularity (megakaryoblast with lobed nucleus, basophilic, granular, and mature megakaryocytes) [6].

Taking into account prominent morphological features in the megakaryocyte population we employed broader criteria to classify megakaryocytic cells into subclasses with their quantitative analysis. Megakaryocytic cells were divided into megakaryoblasts, promegakaryocytes, basophilic, polychromatophilic, oxyphilic, and naked/bare-nuclei megakaryocytes according to the stage of maturation. Megakaryoblasts were identified as cells with nonlobuled nuclei, basophilic cytoplasm with characteristic cytoplasm extension without azurophilic granule, their size of up to 15 μm. Promegakaryocyte was a cell with lobuled nuclei, basophilic cytoplasm with sometimes scant amount of azurophilic granules of 15-25 μm in size. Basophilic, polychromatophilic and oxyphilic megakaryocytes had lobuled nuclei, a different degree of cytoplasm maturation with great amount of azurophilic granules of up to 25-120 μm in size. Naked (bare) nuclei were free nuclei of megakaryocytes without cytoplasm. The calculation was made as percent of 100 megakaryocyte cells.

Megakaryocytes that formed platelets were considered functionally active. A specific group of highly functional active cells included megakaryocytes with tight picnotic nuclei, some lobules being not visualized and with cytoplasm completely represented by platelets formed (referred to below as megakaryocytes with tight picnotic nuclei under active platelet formation). These cells were either in basophilic or polychromatophilic degree of maturation. The percentage of functional cells was calculated from the total number of promegakaryocytes, basophilic, polychromatophilic and oxyphilic megakaryocytes except for megakaryoblasts and naked/bare-nuclei megakaryocytes. The percentage of megakaryocytes with a different number of lobules except for megakaryocytes with tight picnotic nuclei with an active platelet formation and naked nuclei was also calculated.

From the total number of megakaryocytes we calculated the percentage of micromegakaryocytes, dysplastic megakaryocytes (cells with asynchronous maturation of nuclei and cytoplasm), megakaryocytes with an emperipolesis, and cells with vacuolized cytoplasm.

The number of blood platelets was calculated using a hemanalyzer Sysmex F-800 (Japan). To detect statistically significant differences between group means we used the Student t-test.

Results

In the group of 12 patients with IM hyperthrombocytemia ($606 \pm 116.7 \times 10^9$/L) was revealed in 10 patients; the number of platelets on the average in the group being $554 \pm 105.3 \times 10^9$/L i.e. considerably higher vs. the control group ($278 \pm 18.2 \times 10^9$/L, $p < 0.05$). To ascertain changes in megakaryocyte cells (i.e. platelets precursors) accompanying an increased amount of blood platelets a detailed cytologic analysis was made of the bone marrow megakaryocytes under IM.

The analysis of bone marrow megakaryocytes distribution according to the differentiation stage is shown in *Table 1*. As compared with the control group, in the group of patients with idiopathic myelofibrosis there is a significant increase in megakaryoblasts ($p < 0.05$) and naked-nuclei megakaryocytes ($p < 0.01$) against a background of a decrease in polychromatophilic megakaryocytes ($p < 0.05$),

the latter being the largest population of the megakaryocyte cells in the controls. In the case of IM these changes indicate differentiation stimulation and increased destruction of megakaryocytes. The percentage of promegakaryocytes, basophilic and oxyphilic megakaryocytes did not differ from that of the controls.

The study of megakaryocyte functional activity, i.e. platelets formation depending on differentiation is shown in *Table 2*. A total number of functioning megakaryocytes being 49±4.0% in the control group. Polychromatophilic megakaryocytes were most functionally active. In idiopathic myelofibrosis the total number of functioning megakaryocytes does not differ from that of the control group. Functional activity of megakaryocytes does not depend on differentiation. It is worth admitting that the bone marrow analysis showed presence of megakaryocytes with picnotic nuclei surrounded by cytoplasm completely represented by formed platelets; the average number of such cells constituted 21±3.0% of functioning megakaryocytes, out of which basophilic cells 9±1.5% *(Figure 1a)* and polychromatophilic cells 12±1.9% *(Figure 1b).* In persons without blood disorders there were zones or some cytoplasmic strands

Table 1. Distribution of megakaryocytes according to the degree of differentiation (± standard error).

	megakaryoblasts	promegakaryocytes	basophilic megakaryocytes	polychromatophilic megakaryocytes	oxyphilic megakaryocytes	naked nuclei of megakaryocytes
control group (n=12)	2 ± 0.5%	7 ± 1.4%	24 ± 1.8%	41 ± 3.3%	14 ± 1.7%	11 ± 2.3%
idiopahtic myelofibrosis (n=12)	5 ± 1.0%*	6 ± 0.9%	22 ± 1.7%	32 ± 2.8%*	14 ± 1.3%	21 ± 2.5%**

Table 2. Functional activity of megakaryocytes at a different maturation stage (± standard error).

	total number of functional megakaryocytes	functional basophilic megakaryocytes	functional polychromatophilic megakariocytes	functional oxyphilic megakaryocytes	megakaryocytes with picnotic nuclei and active platelet formation
control group (n=12)	49 ± 4.0%	15 ± 2.5%	30 ± 3.0%	4 ± 0.9%	1 ± 0.4%
idiopahtic myelofibrosis (n=12)	49 ± 4.3%	18 ± 1.8%	26 ± 2.7%	5 ± 1.0%	21 ± 3.0%***

Table 3. Distribution of megakaryocytes with different number of lobules (± standard error).

	singlelobuled megakaryocytes	twolobuled megakaryocytes	threelobuled megakaryocytes	megakaryocytes with 4-8 nucleus lobules	hyperlobuled megakaryocytes
control group (n=12)	3 ± 0.6%	3 ± 0.6%	13 ± 2.1%	59 ± 2.9%	11 ± 1.7%
idiopahtic myelofibrosis (n=12)	10 ± 2.4%*	6 ± 1.1%*	6 ± 1.4%*	27 ± 3.5%***	14 ± 2.2%

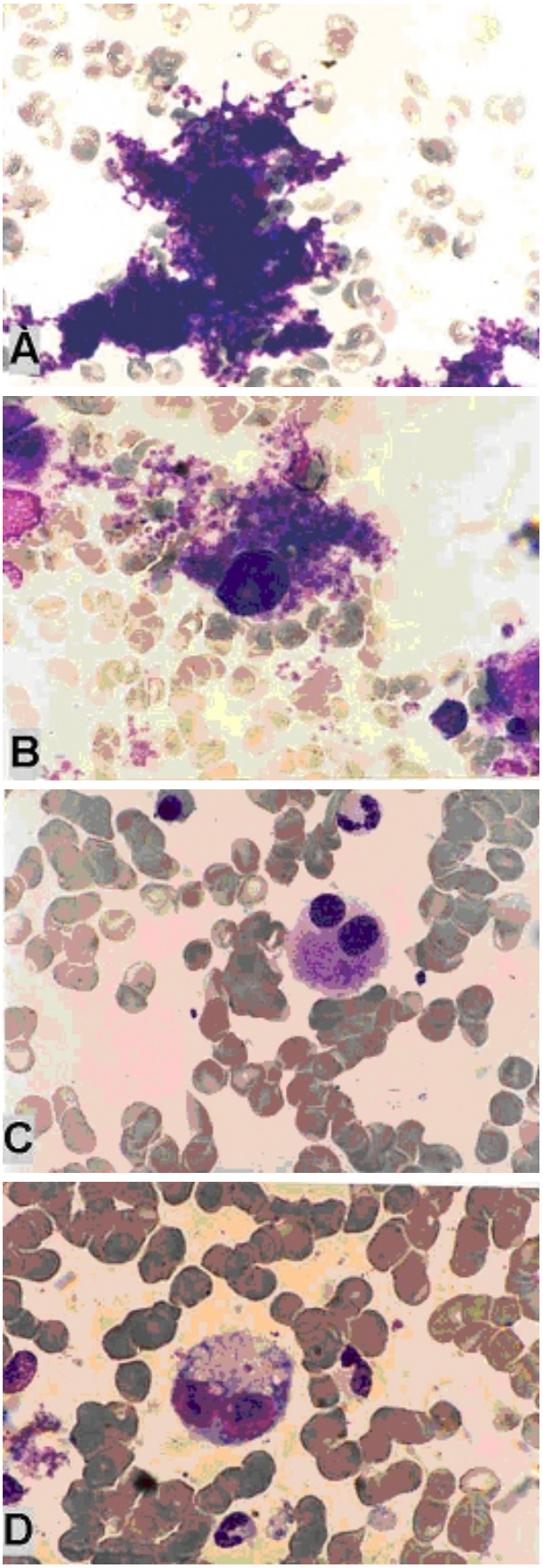
* $p<0.05$, ** $p<0.01$, *** $p<0.001$

in megakaryocytes that fragmented into platelets. The number of megakaryocytes with picnotic nuclei and those with cytoplasm composed of platelets was minimal (1±0.4%). A great number of such cells in the group with IM indicates stimulation of active megakaryocytes towards platelet production and accelerated cell ageing.

The analysis distribution of megakaryocytes according to number of nuclear lobules is shown in *Table 3*. In the control group the largest population of megakaryocytes was that of cells with 4-8 nuclear lobules. The persentage of single-lobuled megakaryocytes was 3±0.6%, the majority of them being represented by megakaryoblasts. Hypolobuled megakaryocytes (two-, three-lobuled) were mainly represented by promegakaryocytes and basophilic megakaryocytes. Hyperlobuled megakaryocytes amounted to 11±1.7%. The latter type of cells were naked-nuclei megakaryocytes (11±2.3%) not involved in our estimation. In the case of IM the number of the largest megakaryocytes population with 4-8 nuclear lobes was lower ($p<0.001$) at the expense of the increase in the number of megakaryocytes with picnotic nuclei and free-nuclei megakaryocytes. Moreover, there was an increase in single-lobuled ($p<0.05$) and two-lobuled ($p<0.05$) megakaryocytes not only at the expense of immature hypolobuled forms (i.e. megakaryoblasts and promegakaryoblasts), but also due to the appearance of mature (basophilic, polychromatophilic and oxyphilic) hypolobuled megakaryocytes *(Figure 1c)*. On the contrary, the number of three-lobuled megakaryocytes was decreased ($p<0.05$). These changes indicate some disorder in megakaryocyte differentiation, which is manifested by a decrease in the number of megakaryocytes with 4-8 nuclei lobules (the major cell population of the control group) with an increase in the number of hypolobuled megakaryocytes.

It should also be mentioned that in 10 out of 12 patients with IM hyperthrombocytemia was accompanied by an increased number of giant hyperlobuled megakaryocytes in the bone marrow (17±2.1%, $p<0.05$) *(Figure 1e, 1f)*. Such megakaryocytes are supposed to be highly poliploidic cells destined at forming a great number of platelets.

A study of cytologic features of megakaryocytes in the case of IM showed an increase in the number of atypical micromegakaryocytes (5±1.6%) and dysplastic megakaryocytes (3±0.9%) as compared with the control group (1±0.4%, 1±0.4%, respectively, $p<0.05$). The number of megakaryocytes with isolated whole or part of nuclear lobules *(Figure 1f)* was increased (2.5±0.9% vs. 0.3±0.2%, $p<0.05$ in the control group) though the number of megakaryocytes with partial fragmentation of nuclear lobules was not different (5±1.0%, 5±1.6%). The number of megakaryocytes with emperipolesis (2±0.6% vs. 1±0.5% in the control group) and vacuolized megakaryocytes *(Figure 1d)* (2±1.1% vs. 1±0.3% in the control group) in both groups did not differ though there was a tendency to an increase in patients with IM.



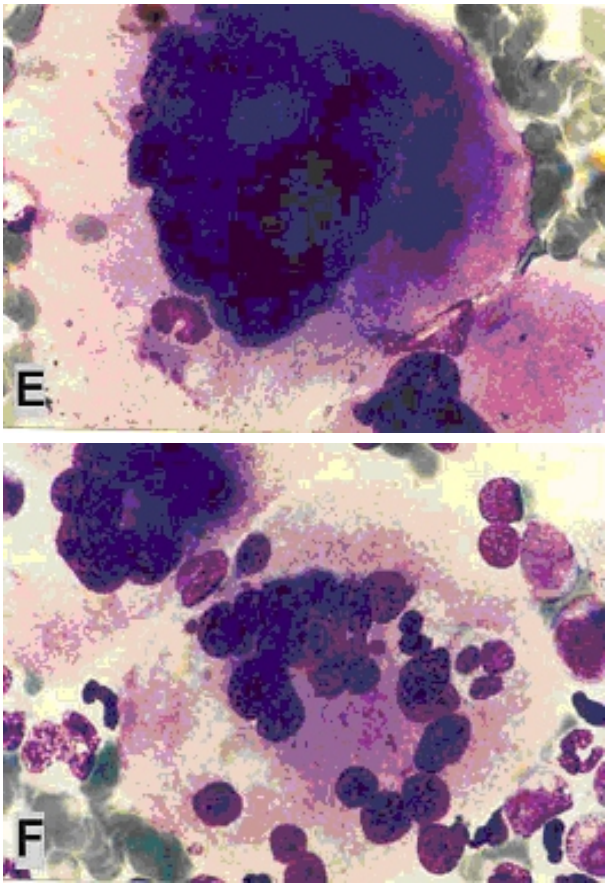


Figure 1. Bone marrow smears from patients with idiopathic myelofibrosis. (**a**) basophilic, (**b**) polychromatophilic megakaryocytes with tight picnotic nuclei and active platelet formation; (**c**) polychromatophilic two-lobed micromegakaryocytes with isolated lobules of nuclei, cells of 25 μm in diameter; (**d**) two-lobed promegakaryocytes with vacuolized cytoplasm; (**e**) Giant hyperlobulated polychromatophilic megakaryocytes, cells of 120 μm in diameter; (**f**) Giant hyperlobulated oxyphilic megakaryocytes with an isolated part of nuclear lobules and emperipolesis, cells of 100 μm in diameter. Stained by Pappenhaim. Magnification x1000.

Discussion

Idiopathic myelofibrosis is a chronic myeloproliferative disorder with three-lineage proliferation and bone marrow fibrosis. Proliferation of megakaryocytes producing fibroblasts growth factors is one of the causes of bone marrow fibrosis [6]. Hyperthrombocytemia is another myelofibrosis manifestation at the initial stage of the disorder, which is followed by reduction in the number of platelets at the late stages of the disorder [3]. These data indicate a change in cellular functioning of the haemopoiesis megakaryocytes lineage. Earlier studies showed that in the case of IM the bone marrow megakaryocytes are arranged by clusters and characterized by dysplastic changes [4,7]. In our studies of IM in the chronic stage with hyperthrombocytemia megakaryocytes dysplastic changes were shown as manifesting by appearance of micromegakaryocytes, hypolobular megakaryocytes, and those with asynchronous maturation of nucleus and cytoplasm (dysplastic megakaryocytes). The appearance of such cells is specific not only for IM. They are revealed in chronic myeloid leukaemia, myelodysplastic syndrome, etc. [8]. Thrombopoietin regulates production of multipotent haematopoietic progenitor cells, proliferation and differentiation of megakaryocytes manifesting by an increase in the number of megakaryocytes, gigant hyperlobuled forms and platelet production [9]. The level of thrombopoietin in the blood of persons with IM is increased while the expression of the receptors to the given growth and differentiation factor on megakaryocytes and platelets is impaired [10]. An increase in the number of megakaryoblasts, hyperlobuled megakaryocytes and megakaryocytes with tight picnotic nuclei and active platelet formation revealed in our studies of idiopathic myelofibrosis may be caused by a high level of thrombopoietin. Decrease in the number of thrombopoietin receptors on platelets may be due to the megakaryocytes differentiation disorder in the case of IM involving availability in the producing population of a great number of megakaryocytes with tight picnotic nuclei and active platelet formation, and immature basophilic megakaryocytes. In the control group the polychromatophilic megakaryocytes with 4-8 nucleus lobules were most functionally active. Decrease in the number of receptors on megakaryocytes may lead to an impaired response of the latter ones to the action of the differentiating factor (i.e. thrombopoietin) and formation of microforms and hypolobuled megakaryocytes, i.e. abnormal megakaryocytes.

While assessing cytological preparations from patients with idiopathic myelofibrosis we observed a significant increase in the megakaryocytes picnotic nuclei, free-nuclei megakaryocytes and those with isolated nuclear lobules. An increased number of megakaryocytes with free nuclei was also revealed in the histological preparations with idiopathic myelofibrosis by J. Thile et al. [11]. The authors consider “free” megakaryocytes nuclei as a manifestation of megakaryocyte involvement in the so-called para-apoptosis, in which chromatin intranucleosomal loop is not necessarily fragmented. There is, therefore, no positive staining in situ using end-labeling method which makes it possible to reveal DNA fragments characteristic of the apoptotic method.

Therefore megakaryocytes with picnotic nuclei, isolated nuclear lobules and free- nuclenuclei megakaryocytes may be considered as cytologic signs of accelerated ageing involving cells into the apoptotic process.

Conclusion

In I M, a number of structural changes in the bone marrow megakaryocytes as well as changes in the megakaryocytic functional activity were revealed to play a significant role in the pathogenesis of a given disorder, viz. hyperthrombocytemia, and development of bone marrow fibrosis. An increase in the number of megakaryocytes with picnotic

nuclei, isolated nuclear lobules, and megakaryocytes with free nuclei manifest accelerated ageing and destruction of these cells in the bone marrow. Appearance of atypical megakaryocytes is an important sign while diagnosing this disorder and it indicates a relation of megakaryocytes to the pathologic clone of cells incapable of profound differentiation.

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