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MORPHOLOGICAL FEATURES FOLLOWING G-CSF TREATMENT

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RhG-CSF is widely used in hematology and oncology for the amelioration of chemotherapy induced neutropenia, for the restoration of neutrophil production after bone marrow transplantation, and for other conditions such as myelodysplastic syndromes, aplastic anemia and severe chronic neutropenia.[1] Moreover, RhG-CSF is able to bring about a lot of functional and phenotype modifications in the induced neutrophils,[2] but little has been reported about the morphological modifications induced by this growth factor. In particular, toxic granules, hypogranularity, Dohle bodies, abnormal nuclear lobulation (hypersegmentation or hyposegmentation), pseudo-Pelger-Huet nuclei, corkscrew-shaped nuclei, circular nuclei and dyschronism between nuclear and cytoplasmic maturation have all been reported;[3] however, it must be pointed out that these findings refer to high rhG-CSF dosages (40-60 µg/kg/day) given for a long period (about three weeks) immediately after high dose chemotherapy. We report some hitherto undescribed images of neutrophil abnormalities detected in peripheral blood smears of patients undergoing rhG-CSF at standard dosage(5 µg/kg/day) for a short period (5 days), far from direct cytostatic drug effects; in fact, the growth factor was administered in the last week of a 14-day interval between two chemotherapy courses. Moreover, to avoid any activation due to the circulating growth factor, the blood smears were prepared 24 hrs after the last rhG-CSF dose. Under these conditions, about 30% of neutrophils displayed several peculiar aspects along with the presence of abundant toxic granules: some cells presented membrane *blebs*, others showed two-horned pseudopodia, some were *hand mirror shaped* and others were *cigar-shaped* (Figure 1). These features might be the morphologic expression of hyperactivation, in agreement with the previously reported capacity of the drug to induce neutrophil polarization[4] (which is considered to be the initial reaction of chemotaxis). Alternatively, they might be the morphologic expression of structural defects

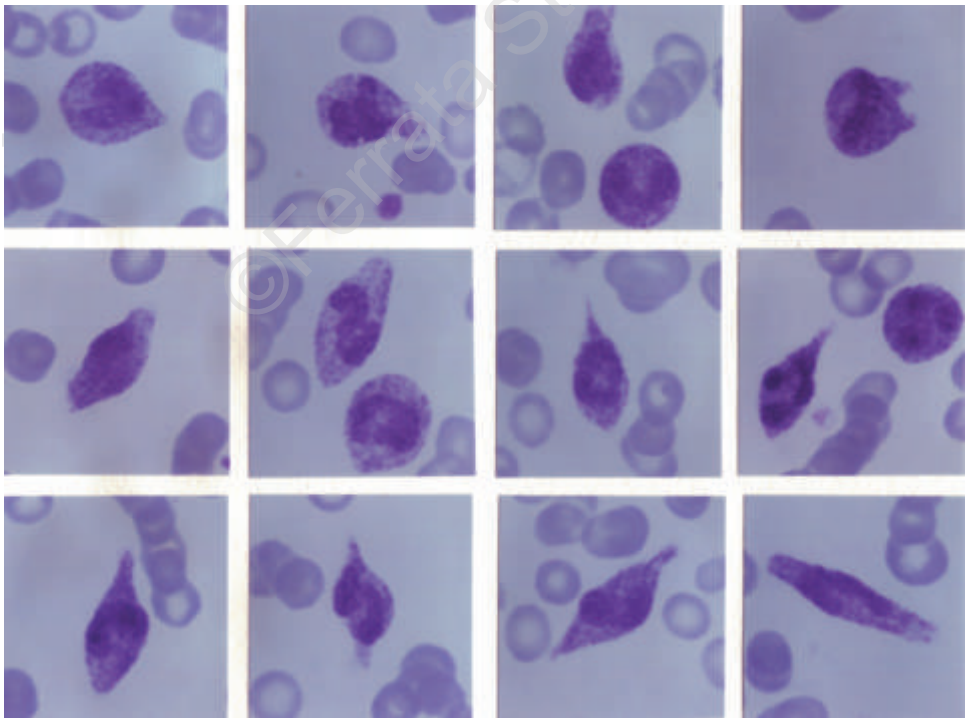

Figure 1. May-Grünwald-Giemsa staining of blood smears prepared 24 hrs after the final rhG-CSF dose (5 µg/kg/day for 5 days) in patients previously treated with chemotherapy, far from any cytostatic effects. The cells (which display the presence of abundant toxic granules) are strongly polarized, *hand mirror-shaped, cigar-shaped,* with membrane blebs, with two-horned pseudopodia.

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responsible for functional disorders. Recently, using a very sensitive computer-assisted image processing system[5] capable of identifying the kinetics of cell migration in micropore filters,[6] we reported a study into random and stimulated migration of rhG-CSF-induced neutrophils in these patients.[7] Random motility, though reduced, displayed its normal gaussian pattern before and after rhG-CSF; by contrast, stimulated motility, which was also strongly reduced, lost its typical migratory kinetic pattern so that only a gaussian pattern, just like that of the unstimulated condition, was detectable. Taking into account classical studies[8] demonstrating that leukocytes with disassembled microtubules lose their directional movement, whereas they still move at random, and that increased polymerization of microtubules is needed by neutrophils migrating through micropore filters, our results were consistent with imperfect cytoskeletal assembly. Moreover, membrane deformability strictly depends on a well-assembled cytoskeletal system, and it is known that membrane deformability increases during PMN maturation, while PMN metabolic functions are partially independent of membrane deformability in PMN from neutropenic subjects.[9] This may explain the simultaneous finding of an enhanced respiratory burst observed in rhG-CSF-induced PMN in these patients.[2] Our hypothesis was that, apart from the uninfluent number of band cells, rhG-CSF induced PMN are mature with regards to enzymatic contents and metabolic activities, but *structurally* altered because of the short maturation time and accelerated bone marrow transit. More recently, our data were confirmed by *in vivo* studies which demonstrated elevated dose-dependent counts of circulating neutrophils, with a simultaneous decrease in the accumulation of neutrophils in skin chambers, after rhG-CSF treatment.[10]

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