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LETTER TO THE EDITOR

Puzzling neutrophilic inclusions in a child with autoimmune hepatitis

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Dear Sir,

A 6-year-old girl who was on therapy for autoimmune hepatitis (first detected when the patient was 2 years of age) was referred to Haematology when she developed persistent leucopenia while on azathioprine. Her hemoglobin was 96 gm/l, total leukocyte count 2.6×10^9/l, and absolute neutrophil count 1.3×10^9/l with normal range platelet and reticulocyte counts. The leucopenia did not improve despite tapering of immunosuppression over 1 month, and therefore a bone marrow examination was performed. This revealed borderline normal cellularity with adequate and proportionate representation of erythropoiesis, granulopoiesis and megakaryocytes.

Remarkably, both the bone marrow and the accompanying blood smears showed one to two small, round, densely basophilic and hyperchromatic cytoplasmic inclusions in neutrophils (strikingly resembling red cell Howell Jolly bodies), a few of which were hypolobate (Fig. 1a–d). In the marrow, these inclusions were also present in the myelocytes and metamyelocytes. They were separate from the nucleus hence excluding Barr bodies. The patient was afebrile and otherwise well and the neutrophils did not display other toxic changes like hypergranulation, maturational shift to the left or cytoplasmic vacuoles, hence they were felt to be unlikely to be the usually larger and less well-defined, blue gray Döhle bodies or intra-cytoplasmic infective agents.

Hereditary/congenital conditions with neutrophilic inclusions like May–Hegglin anomaly (MHA) and Chediak–Higashi syndrome were considered next, but were excluded when a review of blood and bone marrow aspirate smears done at the time of diagnosis of autoimmune hepatitis (at age 2 years) did not reveal similar inclusions (Fig. 1e). Moreover, these punctuate, round inclusions were smaller and more densely staining than the giant gray-red secondary (specific) Chediak–Higashi granules or the Döhle body-like ribosome-related amorphous May–Hegglin inclusions. Periodic acid Schiff and myeloperoxidase stains were negative. Subsequent to a literature survey, the inclusions were suspected to be detached nuclear fragments, arising as a result of the child's immunosuppressive therapy [1–3]. This was confirmed when fluorescent staining with acridine orange resulted in bright staining equal in intensity to that of the neutrophil nucleus (Fig. 1f).

These detached nuclear fragments were previously described by Bain as rare dysplastic changes in neutrophils, interestingly also first in a patient on azathioprine [1]. They and other authors commented on their striking resemblance to Howell Jolly bodies (which are also nuclear remnants, albeit in erythroid cells), and their tendency to mimic inclusions present in congenital disorders [1–3]. Azathioprine, a prodrug of 6-mercaptopurine is a purine metabolism antagonist that inhibits RNA and DNA synthesis by getting incorporated into replicating DNA [4]. The neutrophilic inclusions are possibly morphological manifestations of this drug-induced DNA damage.

The disordered granulopoiesis giving rise to these inclusions may be seen with HIV infection, administration of drugs interfering with DNA synthesis, e.g., chlorambucil,

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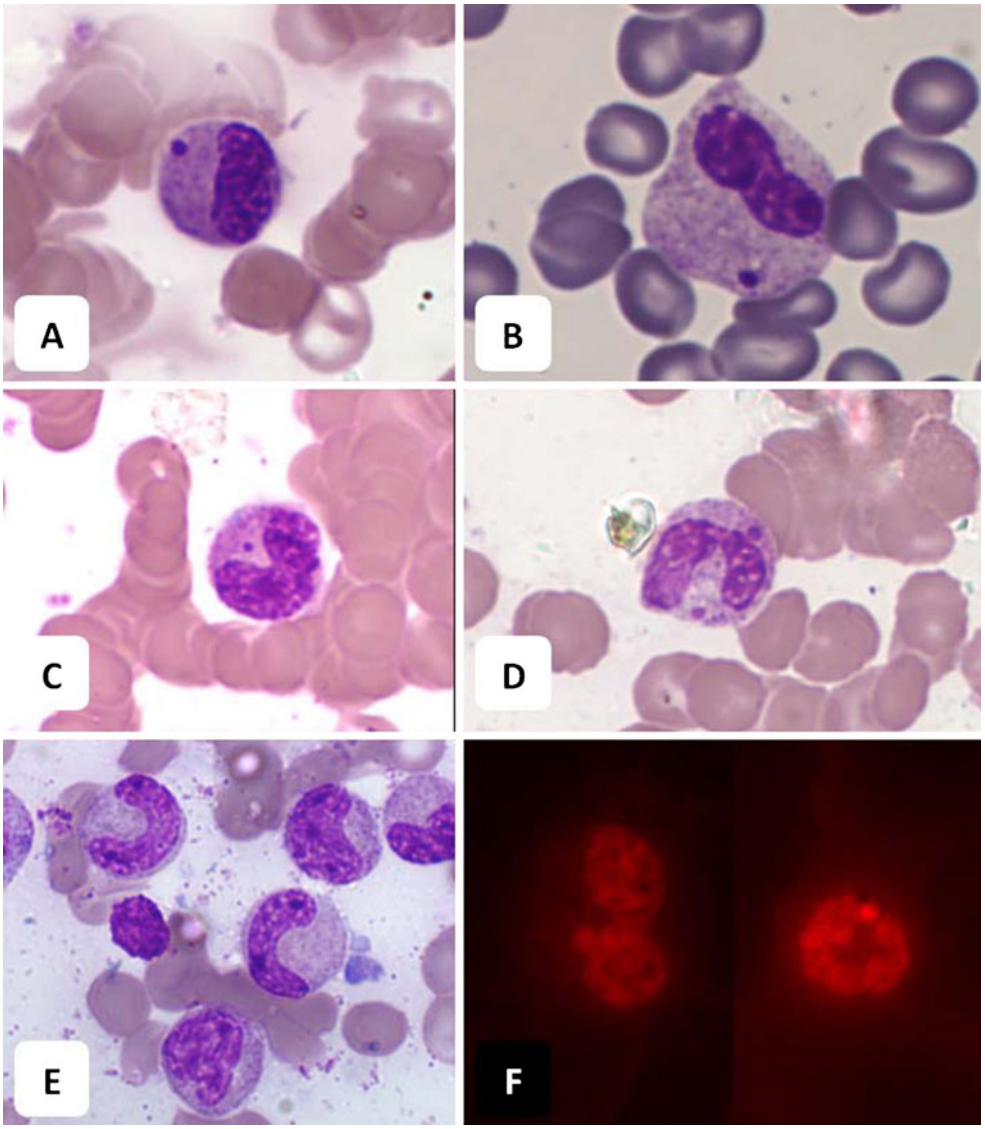

Fig. 1 Punctate, purple, hyperchromatic, cytoplasmic inclusions in neutrophils and their precursors that were mostly single (**a**–**c**), but occasionally two, per cell (**d**). The inclusions were absent in a previous bone marrow aspirate taken 4 years ago (**e**). They fluoresced with acridine orange at an intensity similar to that of the nucleus, thus corroborating their nuclear origin (**f**). Images **a**–**e**: MGG-Giemsa, ×1,000. Image **f**: Acridine orange, ×1000

mycophenolate mofetil and tacrolimus, as well as myriad anticancer chemotherapeutic agents. Follow-up blood counts and repeated smear examinations in our patient showed complete disappearance of the inclusions as well as recovery of the leukocyte count after approximately 3 months of tapering and then cessation of azathioprine therapy.

We report this intriguing finding to highlight the presence of these unusual neutrophilic inclusions. Although not of particular clinical significance beyond suggesting a drug-induced leukopenia, knowledge of their biogenesis and typical morphology is nevertheless relevant for the reporting hematopathologist to prevent erroneous over-interpretation. The case also re-emphasizes the importance of awareness of the complete clinical background, including all prior laboratory reports, while assessing pathological material.

Conflict of interest All authors declare that they have no conflict of interest.

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