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CORRESPONDENCE

Acute myeloid leukaemia with *NPM1* mutation: no longer having an absolute blast (count)

To the Editor,

Increasing recognition of diagnostic and prognostic implications of molecular abnormalities in acute myeloid leukaemia (AML) have led to the incorporation of entities with disease-defining mutations in recent revisions of the World Health Organization (WHO) classification of haematolymphoid tumours.[1,2] While nucleophosmin (*NPM1*) mutation is considered a founding mutation of AML, its presence alone has not hitherto defined AML in absence of blast percentage >20%,[1] although this blast threshold is set to be abolished in the fifth revision of the WHO classification.[2] We present a case which demonstrates the diagnostic challenges haematopathologists and clinicians face in AML with *NPM1* mutation and supports the proposed elimination of blast percentage requirements for this entity.

A 44-year-old otherwise well female presented with sepsis requiring inotropic support, secondary to a large vulval abscess. She had reported the onset of new skin lesions on the face (Fig. 1) and vulval region 2 months prior to her presentation. The vulval lesion, in particular, had rapidly progressed, requiring debridement by the gynaecology-oncology team. Histopathological examination of both vulval and facial lesions were consistent with neutrophilic dermatosis (Sweet syndrome). The patient was treated with a course of pulse methylprednisone 500 mg IV for 5 days.

Whilst at time of admission the full blood count (FBC) demonstrated only normocytic anaemia and mild neutrophilia [haemoglobin (Hb) 116 g/L, mean corpuscular volume (MCV) 91fL, total white cell count (WCC) 17.6×10^9/L, neutrophils 15.6×10^9/L, platelets 176×10^9/L], over the course of several days this evolved to a leukoerythroblastic blood picture with 4−6% circulating blasts (Hb 89 g/L, WCC 28.7×10^9/L, neutrophils 22.9×10^9/L, myelocytes 0.5×10^9/L, blasts 1.1×10^9/L, platelets 206×10^9/L). A bone marrow biopsy was performed to exclude a haematological driver underlying Sweet syndrome. This demonstrated a markedly hypercellular marrow and marked granulocytic dysplasia (Fig. 2) with accompanying dysmegakaryopoiesis to a lesser degree. Eleven percent blasts were enumerated by morphology on the aspirate sample, though <1% CD34+ cells were identified on flow cytometry and trephine immunohistochemistry. Canonical myeloproliferative neoplasm (MPN) mutations (*JAK2, BCR-ABL, MPL* and *Calreticulin*) were negative. The karyotype was normal. The provisional conclusion was that this was suggestive of an evolving myelodysplastic syndrome (MDS) or a MPN, with its interpretation impacted by the significant inflammatory response at the time of sampling. Given this diagnostic dilemma and to confirm the presence of a clonal process, next generation sequencing (NGS) for myeloid-associated mutations was forwarded off-site for further processing.

The patient was discharged on a tapering dose of oral prednisone initially at 60 mg daily. Her Sweet syndrome improved on moderate steroid dosing, however, re-occurred each time when weaned below 10 mg daily. The haematological parameters gradually normalised 12 weeks following initial presentation (Hb 120 g/L, WCC 5.2×10^9/L, neutrophils 4.1×10^9/L, platelets 244×10^9/L) although occasional dysplastic neutrophils were still noted on film review. Despite normalisation of the FBC parameters, a progress bone marrow biopsy enumerated 7% blasts with improvement in the degree of dysplasia seen in granulopoiesis and megakaryopoiesis.

Of note, at the time of conducting this progress bone marrow biopsy, the myeloid NGS results returned from the initial aspirate. This demonstrated a type A frameshift mutation in the *NPM1* gene NM_002520.6: c.860_863dup; p.(Trp288Cysfs*12) at variant allelic frequency (VAF) of 40−45%, as well as a missense mutation in DNA methyltransferase 3 alpha (*DNMT3A*) gene NM_022552.4: c.2644C>T; p.(Arg882Cys) with VAF 30−35%.

At the time, the entity of AML with *NPM1* mutation was recognised, however this still required an absolute blast percentage >20%.[1] The case was discussed at the local haematology multidisciplinary meeting and further opinion was sought internationally. A consensus was reached that the patient should be treated as AML with *NPM1* mutation, despite her normal blood count parameters and blast percentage <20% on the bone marrow sample. The patient was admitted for her first cycle of induction chemotherapy with daunorubicin-cytarabine (DA 3+8) 4 months following her original presentation with Sweet syndrome.

Following cycle 1, bone marrow biopsy demonstrated complete remission (CR). Bone marrow biopsy following cycle 2 of induction therapy continued to show CR with incomplete platelet recovery. *NPM1* minimal residual disease (MRD) analysis showed no detectable *NPM1* in peripheral blood and low level MRD (1.332 copies per 10^5 *ABL* copies) in the bone marrow in keeping with CR with molecular MRD detection at low level (CR-MRD-LL). She received three cycles of high dose cytarabine (HIDAC) consolidation therapy. Bone marrow *NPM1* following cycle 2 HIDAC continued to show CR-MRD-LL with 3.87 *NPM1* transcripts per 10^5 *ABL*, reaching CR with negative MRD (CR-MRD) at end of consolidation therapy. She continues to remain in a CR-MRD negative state, now 12 months following completion of therapy. Three-monthly bone marrow MRD is planned to continue for 2 years following completion of therapy in line with current European Leukaemia Net 2021 guidelines.[3]

The *NPM1* mutation has been considered specific to AML since it was originally reported close to two decades ago, seen in approximately 30% of all adult *de novo* AML.[1] Whilst multilineage dysplasia (MLD) can often be observed in AML with *NPM1* mutation, the morphological detection of MLD has not been shown to bear any clinicopathological or prognostic implications in AML patients found to have an *NPM1* mutation.[4] Therefore, presence of the *NPM1* mutation has been an accepted disease-defining feature of AML, with the presence of MLD being subsidiary to the detection of the *NPM1* mutation.[1]

In the case illustrated, the initial diagnostic challenge related to the presence of both MLD features and *NPM1*

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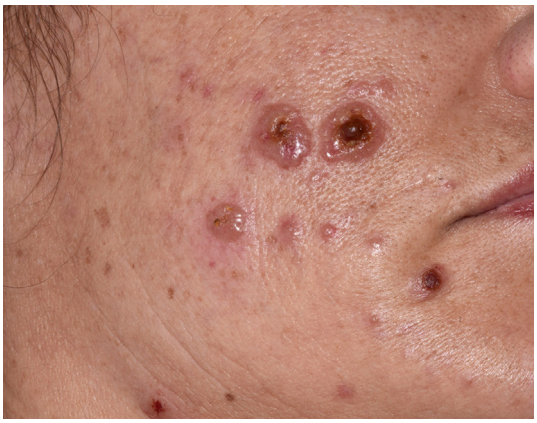

Fig. 1 Skin lesions on patient's face at presentation, confirmed histopathologically in keeping with neutrophilic dermatosis (Sweet syndrome).

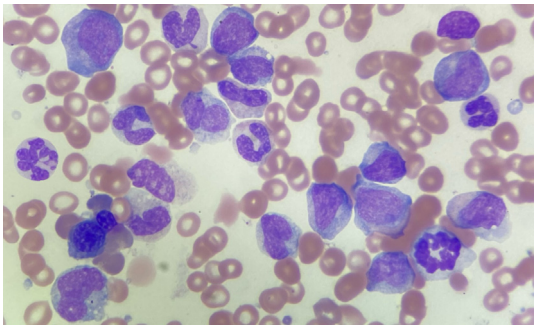

Fig. 2 Bone marrow aspirate demonstrating granulocytic dysplasia and myeloblasts.

mutation despite a blast count <20%, so should this be diagnosed and treated as MDS or AML? Myeloid neoplasms with *NPM1* mutation presenting with blast percentage <20% are uncommonly reported, with *NPM1* mutations detected in <2−3% of MDS or CMML cases.[5] Our case shared pathological features of MDS bearing *NPM1* mutation as reported in the literature to date, including normal karyotype and CD34 negativity.[6] MDS harbouring *NPM1* mutations have been reported to bear an aggressive clinical course, rapidly evolving to AML within 0.5−16 months.[6] Indeed, MDS with *NPM1* mutation may simply be AML with *NPM1* mutation caught early in its natural history. Whilst acquisition of the *NPM1* mutation occurs during leukaemic transformation, most patients already harbour *NPM1* mutation during their 'MDS phase'.[7] In the case presented, evolution to AML (defined on the basis of blast percentage alone) was not apparent in the 4 months from initial presentation to induction, though the clinical course was modified by the multidisciplinary decision to commence induction chemotherapy despite normalisation of FBC parameters and marrow findings not meeting original 2017 WHO criteria for AML with *NPM1* mutation. We believe this illustrative case supports the move to allow diagnosis of AML with *NPM1* mutation irrespective of blast count, whilst exercising 'judicious clinicopathological correlation'.[2]

Furthermore, this case also illustrates pitfalls in morphological blast enumeration with significant interobserver variability amongst expert haematopathologists.[8] This, combined with the finding that two-thirds of AML with *NPM1* mutation are CD34-negative and another third negative for HLA-DR,[1] potentially contribute to underappreciation of blast percentage AML with *NPM1* mutation and misdiagnosis of MDS rather than AML based on the blast percentage requirement prior to the latest proposed iteration of the WHO classification.[2] Indeed, our case was subsequently forwarded to an external laboratory for a second opinion, which yielded a significantly higher blast count on the initial diagnostic marrow that would have met the >20% threshold for AML diagnosis from the outset. Notwithstanding potential bias arising from knowledge of the *NPM1* mutation that the external reviewers were privy to (which the original reporting haematopathologists were not), this case highlights the implications of interobserver discrepancies in blast enumeration. Immunohistochemical detection of cytoplasmic *NPM1* on bone marrow trephines has been shown to be predictive of *NPM1* mutation[9] and could be considered as a surrogate measure to determine blast percentage or even identify rare non-exon 12 *NPM1* mutations.[10]

Further supporting the proposed WHO changes,[2] the rapid evolution of previously recognised MDS with *NPM1* mutation to frank AML underscores a clear clinical argument to treat these patients as per AML regardless of blast percentage. In younger/fit patients diagnosed with MDS bearing *NPM1* mutation, treatment with intensive chemotherapy led to better outcomes than use of hypomethylating agents (HMA) alone.[6] Furthermore, in the age of venetoclax/HMA combinations and novel agents in older patients, it can be argued that the diagnosis of AML rather than MDS would afford this group of patients greater access to effective therapy for their disease.

Whether or not a downward revision in the blast percentage should apply more broadly to patients in the absence of recurrent genetic abnormalities remains contentious. Historical outcomes of patients with MDS with excess blasts appear dependent on the decision to employ intensive therapy rather than the absolute blast percentage.[11] There are calls for the establishment of a distinct MDS/AML category with blasts of 10−30% to allow patients better access to appropriate treatment and clinical trials. Preliminary presentations on the International Consensus Classification of AML suggest the use of an MDS/AML category with 10−19% blasts in presence of certain mutations such as *TP53*, whilst the use of >10% blasts is required for AML with mutated *NPM1*.[12]

This case represents a timely reminder regarding the challenges for haematopathologists and clinicians alike in the face of rapidly evolving developments in our understanding of the diagnostic and prognostic implications of molecular changes in the AML landscape.

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