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Triphenotypic acute leukaemia: the contribution of multimodality laboratory diagnostic techniques

Sir,

Mixed phenotype acute leukaemia (MPAL) represents approximately 4% of all acute leukaemias, and most commonly manifests with myeloid and either B- or T-lymphoid defining markers.^{1–3} Mixed phenotype acute

leukaemias co-expressing B- and T-lymphoid markers are much less common, and those expressing a combination of B-, T- and myeloid markers ('triphenotypic acute leukaemia') are even rarer, accounting for approximately 2% MPAL, and <0.1% of acute leukaemia cases.^{1,2} Here we describe how the use of multimodality laboratory diagnostic techniques was required to confirm the diagnosis of trilineage mixed phenotype acute leukaemia, and its subsequent clinical course.

A 47-year-old man presented with dyspnoea and was found to have large pleural and pericardial effusions on chest X-ray and echocardiogram. Full blood examination revealed Hb 129 g/L, WCC $14.6 \times 10^9/L$, ANC $5.1 \times 10^9/L$, Plt $338 \times 10^9/L$ and blood film demonstrated 57% blasts with agranular cytoplasm and no Auer rods.

Bone marrow aspirate demonstrated 88% blasts (Fig. 1A) which were pleomorphic, intermediate to large in size, with high N:C ratio, scant basophilic cytoplasm, fine chromatin with occasional prominent nucleoli.

An initial acute leukaemia orientation flow cytometry panel (CD45, CD34, MPO, cytCD3, sCD3, CD7, CD19, cytCD79a) was performed to assign lineage, which identified an expanded population of cells within the progenitor gate, accounting for 80% of total white blood cells (WBCs) and comprising two distinct subpopulations which differed by their myeloperoxidase (MPO) expression. To further characterise the blast populations, our standard B-acute lymphocytic leukaemia (B-ALL), T-ALL and acute myeloid leukaemia (AML) panels were performed, in addition to a hybrid single tube 10 colour panel (CD45, CD19, CD38, CD7, CD22, CD24, CD117, CD66b, CD34, CD20) to confirm cross-lineage antigen expression. The dominant blast population (representing 90% of blasts) co-expressed B- and T-lymphoid markers (CD34+, cytCD3+, smCD3+, CD7+, CD19+dim, CD22+dim, CD79a+dim, CD24+dim, CD10–, MPO–, CD13+, CD33+), whereas the smaller population (10% of blasts) demonstrated co-expression of B- and T-lymphoid and myeloid markers (CD34+, cytCD3+, smCD3+, CD7+partial, CD19+dim, CD22+dim, CD24+dim, CD79a–, CD10–, MPO+, CD117+, CD13+br, CD33+br) (Fig. 1B).

Given the rarity of true triphenotypic leukaemia, cytochemistry was performed to confirm MPO expression which was positive in 15% of blasts (Fig. 1D). Immunohistochemistry on trephine confirmed that CD19, CD3, CD79a were positive in the majority of blasts. Immunohistochemistry for MPO and lysozyme was positive in 10% (Fig. 1C).

Conventional karyotyping demonstrated an abnormal karyotype (46,Y,add(X)(p11),del(3)(p21),add(10)(p11),-13,add(16)(q21),-18,add(22)(q13),+2mar[cp14]/46,XY[15]), with no *BCR-ABL1* fusion or *KMT2A*-rearrangement detected by fluorescence *in situ* hybridisation (FISH) and no *BCR-ABL1* transcripts detected by reverse transcription polymerase chain reaction (RT-PCR).

A next generation sequencing panel identified an *EZH2* mutation [NM_004456.4:c.1717A>G; p.(Thr573Ala)] at 74% variant allele frequency as the only mutation of the 76 genes assessed, including no evidence of *TP53* mutations. RNA sequencing was performed to further investigate gene fusions and identified a *DDX3X-MLLT10* in-frame fusion involving *DDX3X* exon 3 (NM_001356.3, located at Xp11) and *MLLT10* exon 17 (NM_004641.3, located at 10p12).

The patient commenced on Hyper-CVAD chemotherapy (cyclophosphamide, vincristine, doxorubicin, dexamethasone,

Fig. 1 (A) Bone marrow aspirate demonstrated 88% blasts (May-Grunwald-Giemsa stain). (B) Flow cytometry antigen expression on the blasts pictured in black. (C) Immunohistochemistry for MPO and lysozyme was positive in 10% (trephine). (D) Cytochemistry was performed to confirm MPO expression which was positive in 15% of blasts.

cytarabine, high dose methotrexate). He failed to achieve a complete remission (17% blasts on bone marrow aspirate by morphology) after cycle 1A, and subsequently had peg-asparaginase added in cycle 1B, resulting in morphologic remission but minimal residual disease (MRD) positive by flow cytometry at 1.2% of WBCs (CD2[−], cytoCD3⁺, smCD3⁺, CD4[−], CD5⁺, CD8[−], CD16/56⁺, CD7⁺, CD34^{+/−}). Immunophenotypic and cytogenetic remission was achieved following cycle 3A of therapy.

The patient then proceeded to a myeloablative allogeneic stem cell transplant from a haploidentical donor, and remains in a MRD negative complete remission 10 months post-transplant with full donor chimerism.

MPALs are rare, with the true incidence difficult to accurately determine given the changes in diagnostic criteria over the past 20 years. As a result, the prognosis and optimal therapy remain somewhat unclear, however they appear to represent a high risk category of disease with poor long term outcomes.^{3–6} The 2016 World Health Organization (WHO) criteria recognise three subclassifications within MPALs, namely those associated with *BCR-ABL1* or *KMT2A* rearrangements, and those without either of these genetic markers.^{1,2} MPALs can be further subclassified as bi-/tri-lineal cases which comprise two or more distinct blast

populations of different lineage, and bi-/tri-phenotypic cases with concurrent expression of B/T/myeloid lineage defining antigens on a single blast population.^{1,2,7,8}

Triphenotypic leukaemias account for <0.1% of acute leukaemias, though the true incidence is difficult to confirm given the change in diagnostic criteria in recent years.^{1,6} Given that expression of cross lineage markers is not uncommon in acute leukaemia, various criteria have been proposed for the definition of MPALs over the past 20 years, and have evolved with improvement in diagnostic techniques such as multiparametric flow cytometry and molecular testing. Current lineage-defining markers as per the 2016 WHO criteria are MPO or monocytic markers for myeloid lineage, cytoplasmic CD3 for T lineage, and CD19 in combination with cytoplasmic CD79a or CD22 for B lineage.² The EGIL criteria use a scoring system and incorporate a larger array of lineage defining antigens; however, these have been criticised as being overly inclusive and therefore potentially over-diagnosing MPAL cases.^{6,7} Regardless of the classification system used, current recommendations suggest MPO positivity by flow cytometry in lymphoblastic leukaemias requires confirmation by cytochemistry. This reflects the potential for false positive results using cytoplasmic MPO by flow cytometry due to the permeabilisation process,

as well as recognition of relatively frequent detection of MPO mRNA in ALL blasts, which does not correlate consistently with immunophenotyping and cytochemical findings.^{1,6}

Advances in molecular techniques have resulted in better subclassification of acute leukaemias and improved diagnostic certainty.^{2,6} Several sequencing studies have demonstrated a large degree of genomic heterogeneity in MPAL as well as differences between MPAL subtypes (B/myeloid and T/myeloid), although notably only a small number of triphenotypic leukaemia were included in these studies.^{9,10} *MLLT10* (also known as *AF10*) fusions involving multiple partner genes, including *DDX3X*, have been reported in both paediatric and adult T-acute lymphoblastic leukaemia and MPAL.^{9,11–13} Similar to previous reports the fusion detected in our patient retains the octapeptide motif-leucine-zipper (OM-LZ) domain of *MLLT10* at its C-terminus, a critical region required for leukaemogenesis.¹⁴ Although the role of the *DDX3X* RNA helicase in *DDX3X-MLLT10* fusions is less well understood, these fusions have only been observed in males, suggesting that the absence of wild type *DDX3X* may also contribute to leukaemia development.

Our case illustrates the diagnostic, and therefore therapeutic, implications of correctly identifying and subsequently confirming the triphenotypic lineage using multiple modalities. It highlights that in spite of the diagnostic advances in recent years, namely molecular testing and improvements in multiparametric flow cytometry, there remains a need for integration of multiple techniques to confirm complex and poorly differentiated acute leukaemias. Additionally, this case adds to the existing body of literature for triphenotypic acute leukaemia as defined by the most recent diagnostic criteria, for which there are only scarce data available.

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Vancomycin variable *Enterococcus* (VVE), *E. faecium*, harbouring the vanA gene complex

Sir,

In this article we describe the isolation from a blood culture specimen of a vancomycin variable *Enterococcus* (VVE), *Enterococcus faecium*, harbouring the vanA gene complex and a methicillin resistant *Staphylococcus aureus* (MRSA). We highlight the importance of using molecular methods for detecting VVE from sterile sites.

VVE are *E. faecium* harbouring the vanA gene or vanB gene complex but being phenotypically vancomycin susceptible. VVE vanA was first described in 2011.¹ VVE can only be detected by molecular methods and cannot be cultured on selective vancomycin containing media. Different clones of VVE have caused nosocomial outbreaks.² This makes the detection of VVE important in clinical samples in order to ensure relevant antibiotic treatment and in screening samples to mitigate nosocomial spread. VVE detection is challenging; the clone is likely to be unrecognised, which facilitates further spread. VVE outbreaks through transmission in health care settings have been described in previous studies. Hammerum et al. describe a clonal shift in vanA *E. faecium* isolates from clinical samples obtained from patients in Denmark from 2015 to the first quarter (Q1) of 2019. They report in 2019 that VVE ST1421-CT1134 vanA *E. faecium* became the most dominant vanA *E. faecium* clone and has spread throughout Denmark.² In Australia in 2018 the Australian Group of Antimicrobial Resistance (AGAR) reported 10 VVE that harboured the