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CASE REPORT

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Ultrastructural, cytogenetic, and molecular findings in mast cell leukemia: Case report

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Abstract

We report a *de novo* aleukemic form of MCL with a complex monosomic karyotype with LOH for multiple chromosomes and TP53 mutation. Additionally, whereas D816V KIT was not found, the c-Kit transmembrane domain p.M541L variant was detected which is the most common SNP of KIT gene in humans with controversial pathogenic role. In these cases, it is crucial to perform a rapid broad molecular study for an accurate diagnosis which could help to initiate targeted therapy.

KEYWORDS

acute leukemia, genomics, molecular biology, systemic mastocytosis

Mast cell leukemia (MCL) is an extremely rare and aggressive form of systemic mastocytosis (SM),[1] accounting for <1% of all adult SM, with ≥20% atypical and immature mast cells (MC) in bone marrow. There are two known forms of presentation for MCL, the classic case in which MCs account for ≥10% of the peripheral white blood cells and the more frequent aleukemic variant with less than 10% MCs in peripheral blood. *De novo* MCL should be distinguished from secondary MCL, evolving from other advanced SM. Diagnostic criteria and classification of SM have been updated in the 2017 World Health Organization classification.[2] Most cases of MCL have markedly high levels of serum tryptase, peripheral cytopenia, and/or leukocytosis with eosinophilia. Patients can develop any of the typical clinical features of SM due to organ impairment or MC activation, although skin lesions are not usually detectable. Atypical MCs show an abnormal antigen expression, characteristically CD2 and/or CD25 with the expression of concurrent immaturity markers.[1-3] Furthermore, most cases of aggressive SM harbor c-Kit tyrosine kinase domain mutations, most frequently D816V,[4] without recurrent chromosomal aberrations detected to date.[5] Therapeutic approaches are limited and, in general, prognosis of MCL is poor with less than one-year survival in most patients.[3]

We report the case of an 86-year-old Caucasian male referred to the emergency care with upper gastrointestinal bleeding due to esophageal ulcers (LA grade D esophagitis), which were successfully treated by endoscopy and with a proton-pump inhibitor, and concomitant severe acute thrombocytopenia, that prevented from taking a biopsy of the lesion. Initially, the thrombocytopenia was medically treated as immune-mediated without response. In addition, grade 2 normocytic anemia (Hb 80-99 g/L) persisted in the following blood tests, with no renal or hepatic failure and normal lactate dehydrogenase, a normal leukocyte total count (5.1×10^9/L [3.8-11.0]) with 71% neutrophil, 5% myelocyte, and 1% of atypical MCs and high levels of serum tryptase (501 μ/L [<15]).

Bosch-Vilaseca and Monter-Rovira contributed equally to this work.

Bone marrow was diffusely infiltrated by 60% of large atypical cells with loose chromatin and centric and round nuclei, atypical mast cell type I (MC I), and occasionally even larger ones with polylobed nuclei (promastocytes or atypical MC II) distributed in multiple aggregates.[6] Most of the atypical MCs exhibited a characteristic wide cytoplasm with azurophilic granules and multiple heavy inclusions or bizarre-looking vacuoles, not stained with May-Grünwald-Giemsa (MGG) (Figure 1A,B). Dysplastic features were absent in the peripheral blood and the bone marrow. These cells were positive for c-Kit (CD117) and CD2 by immunohistochemical staining. Myeloperoxidase was negative, chloroacetate esterase weakly positive, and toluidine blue stain revealed metachromatic granulated blast-like cells (Figure 1C,D). Electronic microscopy disclosed three distinct types of MCs (immature, mature, and activated MCs) characterized by its cytoplasmic granules which appear during their maturation (Figure 1E-G).

The immunophenotype of the neoplastic cells assessed by multiparametric flow cytometry was as follows: CD117,

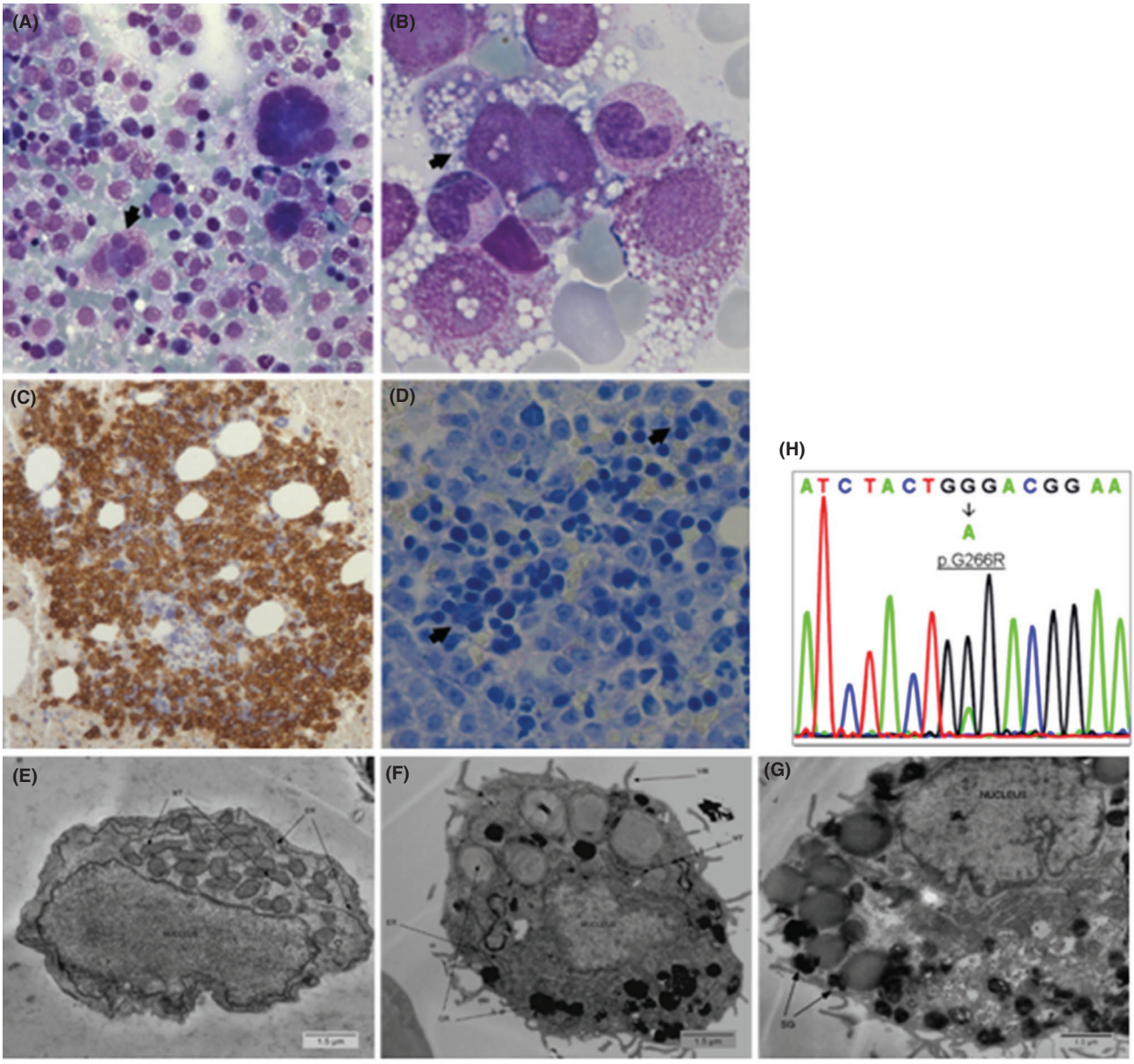

FIGURE 1 (A-F) Morphology and immunohistochemistry. A, BM infiltration by atypical MCs type I and type II (arrow). B, Amplified bilobed MC type II or promastocyte (arrow). C, c-Kit (CD117) immunohistochemical staining. D, Toluidine blue staining showing metachromatic MCs (arrows). (E-G) MCs ultrastructure at different maturation stages. E, Immature MC with irregular agranulated cytoplasm, mitochondria, and smooth ER. F, Mature MC with a lot of microvilli on cell surface and granules that vary in size, shape, and electron density. G, Activated MC granules clustered near to the exocytosis sites. H, Sanger sequencing of *TP53* exon 8 revealed the presence of a G→A base change resulting in a missense mutation G266R. BM, bone marrow; ER, endoplasmic reticulum; GR, granule; MC, mast cell; MT, mitochondria; SG, secretory granule

TABLE 1 (A) List of LOH as determined using CytoScan 750K Array; (B) Allelic variants identified by NGS

A			
Chrom.	**Cytoband/Location**	**Start (Mb)[a]**	**End (Mb)[a]**
3	3p21.31-p21.2	47 002 139	50 797 870
5	5q34-q35.2	167 244 759	176 110 659
7	7p21.2-p15.3	14 753 209	22 086 697
7	7q11.21	62 569 501	66 530 404
7	7q31.31	117 485 006	120 694 229
9	9q31.1-q31.2	107 580 656	111 256 573
10	10q22.1-q22.2	73 731 650	77 131 019
B			
Gene	**AA variant**	**cDNA variant**	**VAF**
KIT	p.M541L	c.1621A>C	0.57
TP53	p.G266R	c.796G>A	0.075
GATA2	p.A164T	c.490G>A	0.54
EZH2	p.D185H	c.553G>A	0.48
ANKRD26	p.Q20R	c.59A>G	0.56

Abbreviations: AA, aminoacid; Chrom, chromosome; VAF, variant allele frequency.

[a]Nucleotide position of the origin and the end of the aberration in the reference genome (hg19).

CD2, CD25, CD34, HLA-DR, and CD123 were positive, whereas CD45, MPO, and TdT were negative.

Conventional cytogenetic studies revealed a complex monosomic karyotype: 35,XY,-1,-3,-5,add(5)(q32),-7,-10,-11,-12,-13,-15,-16,-17,-18,+mar1[2]/35,XY,i(1)(q10),-3,-5,add(5)(q32),-7,-10,-11,-12,-13,-15,-16,-17,-18[2]/46,XY[16] (Figure S1). DNA arrays performed with an Affymetrix platform and Cytoscan 750K confirmed these findings along with the detection of loss of heterozygosity regions (LOH) (Table 1A).

Mutation screening with conventional PCR techniques, as previously described, ruled out mutations in *KIT* D816V, *FLT3*, *NPM1*, *CEBPA*, *MLL*, *IDH1*, *IDH2*, as well as the rearrengements *CBFB-MYH11* and *RUNX1-RUNX1T1*.

An amplicon-based targeted next-generation sequencing (NGS) myeloid panel of 42 genes (Haloplex TM, Agilent Technologies) routinely used in our institution based on published studies of Bullinger *et al* group detected a *KIT* M541L variant in exon 10 (transmembrane domain)[7] and a *TP53* missense pathogenic mutation that was confirmed by Sanger sequencing (Figure 1H). Other concurrent allelic variants of *GATA2*, *EZH2,* and *ANKRD26* genes were detected (Table 1B). All these variants have previously been described and reported as polymorphisms of uncertain pathogenic significance.

Therefore, the patient was diagnosed with *de novo* aleukemic MCL, according to the 2017 WHO criteria. Due to a rapid clinical deterioration, midostaurin was started, before obtaining the final molecular studies results. Despite this, the clinical status worsened and the patient died one month after diagnosis.

To sum up, we report an aleukemic form of MCL case with a complex monosomic karyotype. We also identified LOH regions of chromosomes 3, 5, 7, 9, and 10.[8] Of note, our patient harbored a *TP53* mutation which has already been defined as an early transforming event in hematologic malignancies.[9] As it has been reported, our patient lacked the D816V *KIT* mutation; however, a c-Kit transmembrane domain p.M541L variant was detected and it could modify the phenotype of a neoplastic myeloid progenitor transformed by *TP53* mutation. In fact, this *KIT* allelic variant in exon 10 is the most common SNP found in the *KIT* gene in humans and its pathogenic role is controversial.[7] The contributing effects of the other variants remain to be elucidated.[5,10,11]

Further MCL cases should be analyzed with the newly available techniques before drawing more precise conclusions about the genomic landscape of this infrequent disease. In these cases, it is crucial to perform a rapid broad molecular study for an accurate diagnosis which could help to initiate targeted therapy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

AB-V and AM-R: conceived and designed the analysis, collected the data, performed the analysis, and wrote the paper; SC-W: contributed data or analysis tools (electronic microscopy study); EB and MC: contributed data or analysis tools (molecular analysis); GO, ÁR, CM, MP, and MLB: aided in interpreting the results and worked on the manuscript. JFN: conceived the study and were in charge of overall direction and planning. All authors discussed the results and commented on the manuscript.

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SUPPORTING INFORMATION

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