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Detection of cnLOH as a sole abnormality in the diagnosis of myelodysplastic syndrome

CAP TODAY and the Association for Molecular Pathology have teamed up to bring molecular case reports to CAP TODAY readers. AMP members write the reports using clinical cases from their own practices that show molecular testing's important role in diagnosis, prognosis, and treatment. The following report comes from Seattle Cancer Care Alliance. If you would like to submit a case report, please send an email to the AMP at amp@amp.org. For more information about the AMP and all previously published case reports, visit www.amp.org.

AMP CAP TODAY case report

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Copy neutral loss of heterozygosity (cnLOH) is an acquired abnormality found in patients with cancer and hematologic disorders and can be detected by molecular techniques such as PCR-based analyses and hybridization-based chromosome genomic array testing (CGAT). We report a case in which cnLOH was the sole abnormality detected by CGAT in a patient with myelodysplastic syndrome. This case illuminates the importance of utilizing CGAT results, namely cnLOH findings, as one of the primary diagnostic indicators in order to expedite initial therapies.

Introduction. The 2008 World Health Organization criteria recognize the initial diagnosis of MDS as cytopenia of undetermined origin in the blood and greater than five percent blasts in the bone marrow, or less than 10 percent blasts in the bone marrow if unequivocal dysplasia is present along with a cytogenetic abnormality of: -5 or del(5q), -7 or del(7q), del(9q), del(11q), del(12p) or t(12p), -13 or del(13q), i(17q) or t(17p), idic(X)(q13), t(1;3)(p36.3;q21.1), t(2;11)(p21;q23), inv(3)(q21q26.2), t(3;21)(q26.2;q22.1), t(6;9)(p23;q34), or t(11;16)(q23;p13.3).[1] These abnormalities are detectable by conventional cytogenetics and fluorescence in situ hybridization techniques. CGAT and other molecular techniques are essential for detecting other subtle abnormalities and cnLOH, which have proved to be an indicator of acquired disease. However, cnLOH is not included in the WHO's list of recurring genetic abnormalities as evidence of MDS diagnosis.

In reporting CGAT results, our laboratory uses a filter size/resolution of 100 Kb for copy number gain and loss and 10 Mb for cnLOH abnormalities. We have reported cnLOH as a patient's sole clonal abnormality suggesting disease; the frequency of this occurrence in the general patient population is unknown. We hope this case report will help broaden the awareness that the detection of cnLOH is important for early classification, treatment, and monitoring of MDS.

Patient case. We present a case of a 61-year-old male with a history of glioblastoma multiforme diagnosed in 2009 **(Table 1,** page 2). The patient was treated for glioblastoma with local radiation, tolerated an autologous transplant, received nine cycles of temozolomide, and achieved remission. The patient was followed with routine MRI but continued to experience fatigue. In November 2010 and March 2011, a full examination of the patient's peripheral blood and bone marrow reported no abnormalities in myeloid blast, monocyte, or myeloid populations, or B or T cell populations. His results for conventional cytogenetics were consistently 46,XY[20] (no abnormalities) and normal FISH for chromosomes 5, 7, 8, 20, and the *MLL* locus at 11q23. CGAT was not performed at this time. During follow-up therapy, he continued to demonstrate extreme fatigue and his counts failed to rebound at a normal rate, which raised concern for aplastic anemia and treatment-related MDS. A brain MRI showed no evidence of tumor recurrence.

In March 2014 the patient requested evaluation at Seattle Cancer Care Alliance. A full examination of blood and bone marrow revealed concern for MDS with mildly increased my-

eloid blasts with mild immunophenotypic abnormalities and no evidence of glioblastoma multiforme **(Fig. 1A** and **B**, page 3). The CD34+ myeloid blasts represented 6.4 percent of the white cells by flow cytometry, and the abnormal cells by morphology were too low for the definitive diagnosis of MDS. The erythroid cells did show occasional irregular nuclear contours with megaloblastoid changes. The megakaryocytes were decreased in number with small hypolobulated forms. By morphology, the bone marrow blast count was three percent. The bone marrow biopsy showed a 30 percent cellularity. There were no ringed sideroblasts and no reticulin fibers. Flow cytometry showed a mildly increased myeloid blast population with mild immunophenotypic abnormalities. The cytogenetics and FISH continued to show normal results. However, results from the CGAT testing showed an abnormal result with clonal cnLOH of chromosome 11p of 38 Mb size in about 30 percent of cells **(Fig. 2**, page 4). No copy number aberrations were detected. Based on the lack of significant evidence of dysplasia by morphology, the patient's disease did not meet the WHO-defined criteria for MDS diagnosis. However, the traits that were highly suggestive of MDS were the clinical setting of low blood counts following therapy with oral temozolomide, the mild dysplasia present, the immunophenotypic abnormalities observed by flow cytometry, and the clonal 11p cnLOH observed by CGAT. The providers decided to not initiate therapy but watch the patient's progress closely.

The patient was reassessed in August 2014. The flow cytometry revealed a significant increase of CD34-positive myeloid blast cells to 15.7 percent, consistent with a myeloid stem cell neoplasm. The morphology showed 19.4 percent blasts, marked megakaryocytic hypoplasia, with megakaryocytic dysplasia, consistent with MDS **(Fig. 1C** and **D**, page 3). The cytogenetic, FISH, and CGAT all showed results consistent with those reported in March; the cnLOH of 11p continued to be this patient's sole detectable genomic abnormality while PCR results showed no mutations of *CEBPA, FLT3, KIT,* and *NPM1* genes (Table 1). At this point the patient was classified as secondary MDS RAEB-2 and started G-CLAM chemotherapy. When the patient was evaluated in September 2014, his counts appeared to be recovering. By November 2014 he had no evidence of MDS and achieved complete remission. The patient's platelet levels remained low and it was un-

Table 1. Summary of hematologic, cytogenetic, and molecular findings and therapies

Date	Flow results	Pathology/Morphology	Cytogenetics and FISH	Molecular/CGAT	Final BM diagnosis	Therapy
2009	Not available	UWMC Neuropathology: glioblastoma multiforme (WHO Grade IV)	Not tested	Not tested	Not applicable	Local radiation, autologous transplant, temozolomide chemotherapy
Nov. 2010 and March 2011	Normal myeloid blast, monocyte, myeloid, B and T cell populations	Erythroid hyperplasia; no overt features of MDS; no reticulin deposition; iron stores are increased	46,XY[20] Normal 5, 7, 8, 20; Normal *MLL*	Not tested	Normocellular with mild lymphopenia and thrombocytopenia of unknown etiology	No therapy
March 2014	Mildly increased myeloid blasts; CD34+ myeloid blasts at 6.4% of white cells	Normocellular marrow with adequate erythroid and myelopoiesis, megakaryocyte hypoplasia, and mild morphologic abnormalities in erythroid and megakaryocyte lineages	46,XY[20] Normal 5, 7, 8, 20	Abn. CGAT results w/ cnLOH of 11p in 30% of cells; no copy number aberrations (CNA)	Suggesting but not definitive for MDS	Watch and wait
June 2014	Not tested	Normocellular marrow with trilineage hematopoiesis; minimal morphologic dysplasia (<10% in all lineages), 4.5% CD34+ blasts by IHC	46,XY[20] Normal 3q, 5, 7, 8, 13, 20	(CGAT at an outside institution: normal)	Concern for secondary MDS but not diagnostic	Watch and wait
Aug. 2014	CD34+ myeloid blasts at 15.7% of the white cells, consistent with a myeloid stem cell neoplasm	Hypercellular marrow with 19.4% blasts; marked megakaryocytic hypoplasia, with megakaryocytic dysplasia; peripheral blood with 6.5% blasts	46,XY[20] FISH Not tested	Abn. CGAT results w/ cnLOH of 11p in 20% of cells; no CNAs; PCR: *CEBPA-, FLT3-, KIT-, NPM1-*	Secondary myelodysplastic syndrome refractory anemia with excess blasts (MDS-RAEB2)	Two cycles of G-CLAM
Sept. 2014	CD34+ myeloid blasts represent 6.6% of the white cells, consistent with a myeloid stem cell disorder	Markedly hypocellular (15% cellular), relative myeloid hypoplasia with ~4% blasts, suggesting persistent MDS	46,XY[20] FISH Not tested	Normal CGAT	MDS	Three cycles of Vidaza; transfusion
March 2015	No abnormal myeloid blast, monocyte, or myeloid population identified	No evidence of residual MDS	46,XY[20] FISH Not tested	Not tested	Complete remission with no evidence of MDS	No therapy

All tests performed on bone marrow unless otherwise specified.

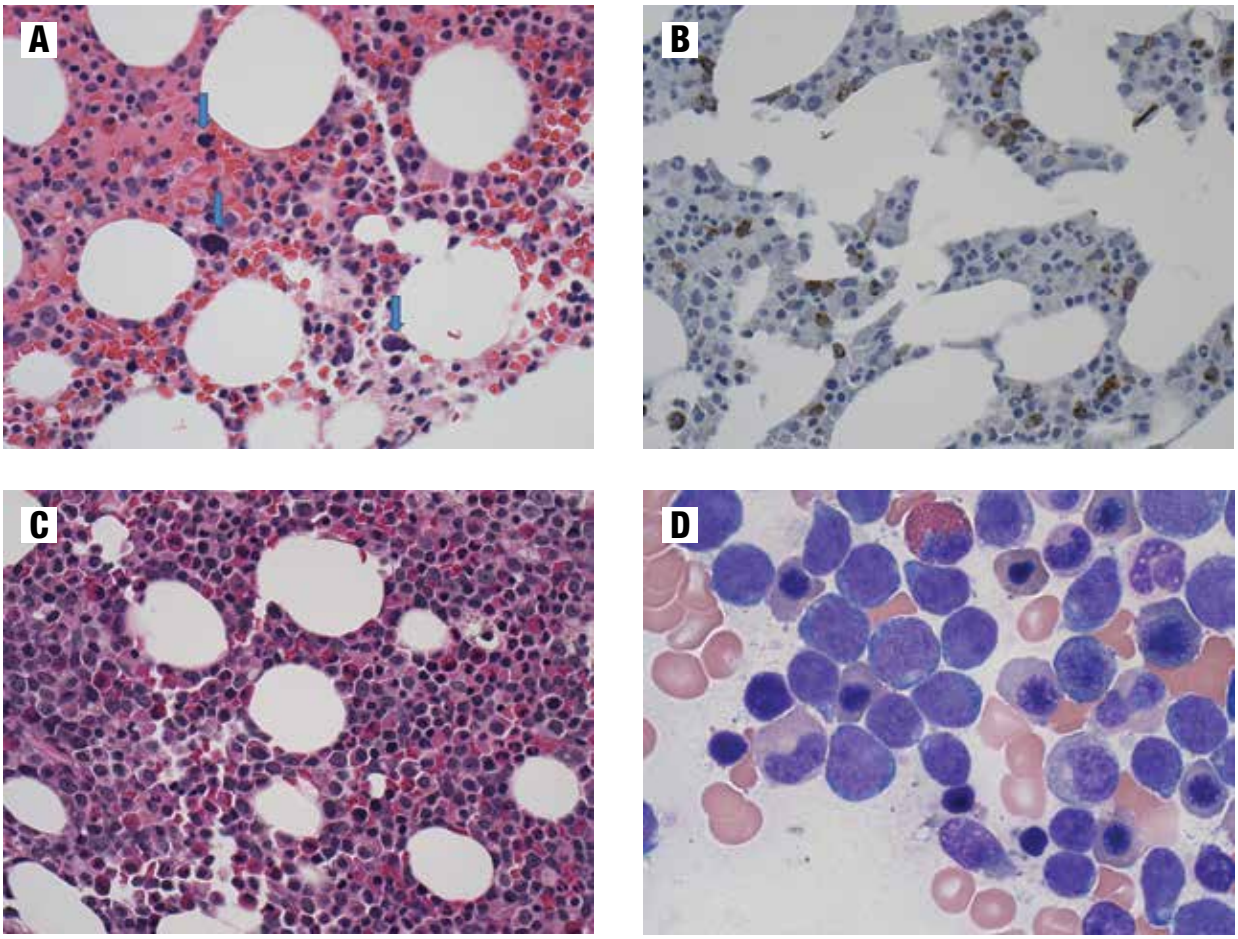

Fig. 1. Representative bone marrow morphological findings. **(A)** Bone marrow from the patient (same specimen as the CGAT) showing a hypocellular marrow with relative erythroid hyperplasia and dysplastic megakaryocytes (blue arrows). Stain: H&E, image taken with 40× objective. **(B)** Bone marrow from the patient (same specimen as the CGAT) showing a hypocellular marrow with increased scattered blasts (cells with brown pigment staining). Stain: CD34 immunohistochemistry, image taken with 40× objective. **(C)** Bone marrow core from the patient four months after CGAT study showing cnLOH. The marrow was hypercellular with increased immature mononuclear cells, which are the blasts. H&E-stained section, image taken with 40× objective. **(D)** Bone marrow aspirate from the patient four months after CGAT study showing cnLOH. This aspirate shows dysplastic features such as irregular nuclear contours and nuclear buds in the erythroid precursors with increased blasts (approximately 15 percent overall) in the marrow. Stained with Wright Giemsa, image taken with 100× objective.

clear if it was related to potential relapse of glioblastoma or minimal residual disease of MDS. Brain MRI in August 2015 confirmed brain tumor recurrence, and the patient died two months later without evidence of MDS.

Discussion. cnLOH was the only detectable abnormality in this patient's molecular studies. CGAT is critical not only for the detection of cnLOH but also for submicroscopic genomic imbalances (copy number aberrations). These abnormalities are undetectable by conventional cytogenetics and FISH because they are below the threshold of detection size and resolution. In addition, because of a high degree of concordance with conventional cytogenetics and FISH, CGAT is effective at replacing imbalance FISH panels in the diagnostic setting.[2] In this case study, considering the lack of CNAs, chromosomal rearrangements, or common molecular aberrations detectable by PCR, the presence of cnLOH was the only genetic marker in which to follow this patient's disease progression.

The World Health Organization currently classifies tumors of hematopoietic and lymphoid tissue without reference to cnLOH results.[1,3] Although the mechanism leading to cnLOH has been postulated,[4] conclusions have yet to be drawn concerning patients with MDS and the association with cnLOH. In patients with AML, the presence of cnLOH is associated with a higher risk of disease recurrence and poorer patient outcomes.[2] This is a significant finding for patients with AML, yet we found an insufficient amount of literature outlining a comparable conclusion in patients with MDS. Because cnLOH can be detected only by CGAT (or SNP array) and not conventional cytogenetics or FISH, the current standard workup may underdiagnose some MDS patients. This case highlights the importance of CGAT findings of cnLOH and the need for future studies.

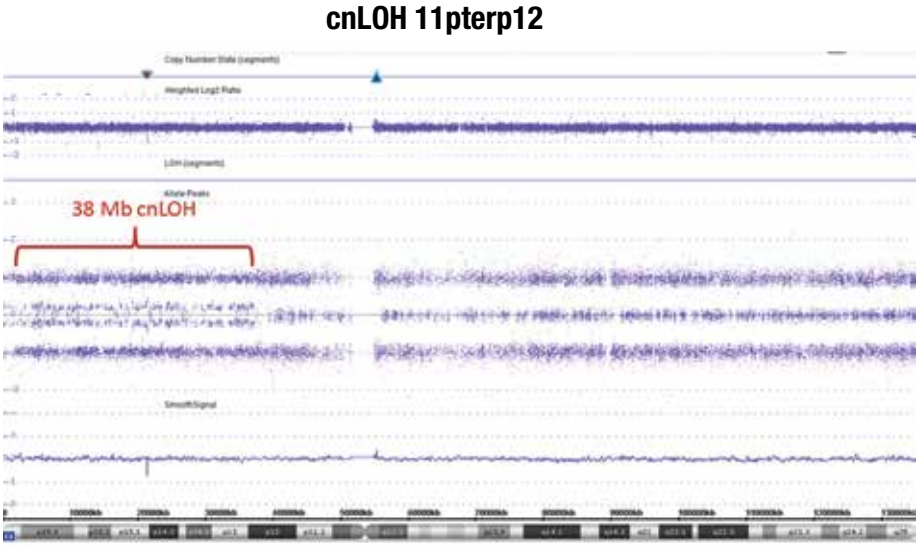

Fig. 2. Chromosome genomic array testing (CGAT) identified copy neutral loss of heterozygosity (cnLOH) of the short arm of chromosome 11 as the sole molecular abnormality. The X axis denotes genomic location (short arm 11p on the left and long arm 11q on the right separated by the centromere region with no probe coverage; see chromosome 11 ideogram on the bottom), while the Y axis denotes log2 ratio of the copy number (upper panel) and the allelic track (middle panel). Each blue/purple dot corresponds to a probe on the array. Chromosome 11q shows the normal allelic track pattern whereas 11p demonstrates cnLOH (splitting of the middle track) from the telomere (11pter) to band 11p12 (38 Mb in size) in approximately 30 percent of cells. There is no copy number aberration evident.

Conclusion. This case study raises the question whether the progression to high-grade MDS could have been avoided for this patient if therapy had been initiated in March 2014 considering the cnLOH as diagnostic evidence for his disease. The intriguing findings from this patient warrant the World Health Organization's consideration of cnLOH as part of the diagnostic criteria similar to other cytogenetic abnormalities. The literature currently available for associations of cnLOH as a sole abnormality with MDS is limited, but the detection of cnLOH has proved valuable in the diagnosis of subtle disease.[2,4] A comprehensive evaluation including the CGAT findings of cnLOH can help providers classify disease types and prompt diagnosis and initial therapy. □

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Test yourself

Here are three questions taken from the case report. Answers are online now at www.amp.org/casereviews *and will be published next month in* CAP TODAY.

1. The 2008 WHO guidelines recognize which abnormalities as criteria for diagnosis of MDS? Cytopenia of undetermined origin in the blood *and*

a) >five percent blasts in the bone marrow.
b) <10 percent blasts in the bone marrow if unequivocal dysplasia is present *and* cnLOH.
c) <10 percent blasts in the bone marrow if unequivocal dysplasia is present *and* monosomy 5.
d) <10 percent blasts in the bone marrow if unequivocal dysplasia is present *and* t(2;11)(p21;q23).
e) Answers A, C, and D.
f) All of the above.

2. At a point in this patient's disease progression, he demonstrated:

a) A FISH abnormality of monosomy 5.
b) Higher than 10 percent abnormal cells by flow cytometry.
c) A cytogenetic abnormality of del(7q).
d) An abnormality detected by CGAT (chromosome genomic array testing) at higher than 40 percent.
e) A copy number aberration by CGAT (chromosome genomic array testing).

3. Which is true regarding copy neutral loss of heterozygosity (cnLOH)?

a) It is detectable by conventional cytogenetics and FISH.
b) The mechanism leading to cnLOH has been reviewed.
c) It is associated with a higher risk of disease recurrence in patients with acute myeloid leukemia.
d) It is detectable by molecular techniques such as PCR-based analyses and hybridization-based CGAT (chromosome genomic array testing).
e) Answers B, C, and D.