

References

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Chronic Myeloproliferative Disorders

**Anagrelide-associated cardiomyopathy in polycythemia vera and essential thrombocythemia**

A comprehensive database inquiry at our institutions identified 11 patients with echocardiogram-documented idiopathic cardiomyopathy that post-dated a diagnosis of either polycythemia vera or essential thrombocythemia. Anagrelide therapy was temporally associated with the particular complication in 6 patients, all of whom experienced symptomatic and/or objective improvement after drug discontinuation.

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Anagrelide, an oral imidazoquinazoline derivative, effectively lowers platelet count in a spectrum of chronic myeloproliferative disorders, including essential thrombocythemia (ET) and polycythemia vera (PV), with a proposed mechanism of action that involves interference with megakaryocyte differentiation.<sup>1,2</sup> Previously reported side effects of anagrelide have included headache, palpitations, diarrhea, fluid retention, and anemia.<sup>3</sup> Less frequent side effects include abdominal pain, nausea, dyspepsia, and rash. In addition, anecdotal reports of *idiopathic* congestive heart failure in patients receiving anagrelide therapy have surfaced but have not been formally studied.<sup>4</sup>

In a retrospective, IRB-approved study, the Mayo Clinic

Rochester database was queried and cross-referenced for diagnoses of *cardiomyopathy* or *heart failure* and *polycythemia*, *thrombocytosis*, or *thrombocythemia*. The initial inquiry yielded 434 cases, which were thoroughly reviewed to confirm the diagnosis of either PV or ET as well as to identify patients with an echocardiogram-documented *idiopathic* cardiomyopathy (ICM) that post-dated the hematologic diagnosis. A diagnosis of ICM required echocardiogram documentation of a reduced left ventricular systolic ejection fraction (EF < 50%) as well as the absence of both coronary artery disease and other causes of cardiomyopathy. Once a temporal association between anagrelide therapy and ICM was recognized during the initial phase of the study, the Mayo Clinic Jacksonville database was subsequently queried and cross-referenced for diagnoses of *congestive*, *heart*, and *anagrelide*.

The database search from the two Mayo Clinic centers resulted in the identification of 11 patients (9 females; age range 46-78 years; 7 PV and 4 ET) with echocardiogram-confirmed ICM that was recognized after the diagnosis of PV/ET at a median period of 9 years (range, 0.5-20). Anagrelide therapy, at standard doses with a median daily dose of 2 mg/day, was temporally associated with ICM in 6 of the 11 patients (4 from Mayo Clinic Rochester and 2 from Mayo Clinic, Jacksonville), all of whom were women and ranged in age from 33 to 77 years (Table 1).

Case #1 had a baseline EF of 69% one year before disease diagnosis. One year after anagrelide had been initiated, an EF of 50% was documented. Four years later, still on anagrelide therapy, the patient developed progressive dyspnea and palpitations and another trans-thoracic echocardiogram (TTE) revealed an EF of 30%. Anagrelide treatment was continued while the patient's symptoms progressed over the next two months and the EF further declined to 18%. Anagrelide was stopped at that point and four months later the patient's symptoms as well as the EF (25%) improved. Sixteen months after the diagnosis of ICM, the patient's EF remains abnormal at 28%.

Case #2 was treated with anagrelide for 6 months before experiencing acute onset congestive heart failure. TTE showed an EF of 25%. Upon cessation of anagrelide, the patient's symptoms improved.

Case #3 was on anagrelide therapy for just over three years when she developed fatigue and dyspnea on exertion; TTE showed an EF of 20%. Anagrelide was immediately discontinued, her symptoms improved and follow-up TTE one year later showed dramatic improvement of her EF to 66%.

**Table 1. Clinical and echocardiogram information in six patients, all females, with anagrelide-associated cardiomyopathy.**

Pt.	Diagnosis	Interval (months)		Before treatment	Ejection fraction (%)	
		Diagnosis to ICM	Anagrelide treatment to ICM		At ICM diagnosis	Off-anagrelide therapy for 0.2-16 months
1	PV	66	60	69	30 and then 18 on continued anagrelide treatment	28
2	ET	8	6	NA	25	NA
3	PV	120	36	NA	20	66
4	PV	156	13	73	35	55
5	ET	108	10	NA	35	44
6	ET	108	11	58	10	34

ICM, idiopathic cardiomyopathy; Pt, patient; NA, not available.

Case #4 had a TTE done one month prior to anagrelide therapy that showed an EF of 73%. She took anagrelide for just over one year before developing peripheral edema and dyspnea on exertion. TTE showed an EF of 30%. Anagrelide was discontinued leading to improvement in symptoms and a repeat TTE 3 months later showed an EF of 55%.

Case #5 was treated with anagrelide for 10 months before developing chest pain. Coronary angiogram was normal, but TTE showed an EF of 35%. Anagrelide was stopped with improvement in symptoms. Follow-up TTE one month later showed an EF of 44%.

Case #6 had a TTE done 9 months into anagrelide treatment (EF = 58%). Two months later, still on anagrelide therapy, she developed symptoms of heart failure. TTE showed an EF of only 10%. Anagrelide was stopped leading to immediate clinical improvement and a repeat TTE, performed within a week, showed a substantial improvement in EF (34%).

Treatment in the 5 patients with ICM that was not associated with anagrelide therapy consisted of either phlebotomy alone (3 patients) or other cytoreductive agents (2 patients). The clinical course of ICM in these 5 cases was stable in terms of both symptoms and EF.

The observations from the current study strongly suggest a potentially reversible drug-induced cardiomyopathy in anagrelide-treated patients with either PV or ET. The mechanism of action is currently unknown but may involve the drug's known cardiovascular effects including positive inotropism, vasodilatation, and tachyarrhythmia.<sup>5</sup> In regards to this last effect, the reversible nature of anagrelide-associated ICM is reminiscent of tachycardia-induced cardiomyopathy.<sup>6</sup>

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## Acute Myeloid Leukemia

### ABC transporter ABCA3 is expressed in acute myeloid leukemia blast cells and participates in vesicular transport

Drug resistance is a major issue in the treatment of acute myeloid leukemia (AML), and drug efflux by ATP-binding-cassette (ABC) transporters is one of the main mechanism involved in this resistance. We determined the prevalence of the intracellular transporter ABCA3 in specimens from patients with AML, and addressed its biology with attention to intracellular compartmentalization.

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We recently described a subpopulation of leukemic progenitor cells with high intrinsic drug efflux capacity, and detected the expression of ABCA3 in the cells of the AML-SP1 leukemia model.<sup>1-3</sup> Whereas expression of ABCA3 was absent or low in normal hematopoietic tissues, reverse transcription polymerase chain reaction (RT-PCR) revealed expression of the transporter in 81% and 88% of mononuclear cells from adult and pediatric patients (n=33), respectively (Figure 1A). The median blast count of the samples was 80% (range 5% to 98%), and the blast percentages did not correlate with ABCA3 levels (correlation coefficient -0.05). The expression varied broadly with a trend towards higher levels in specimens from patients with relapsed or resistant disease (median 28.4%, n=6) than in specimens from those with primary disease (median 12.5%, n=27;  $p=0.16$  two-tailed t-test for two samples with unequal variances). With the mean ABCA3 expression levels of FAB classes being 18.5% in M1 (n=4), 13.0% in M2 (n=7), 13.5% in M4 (n=8) and 13.3% in M5 (n= 4), there was no segregation with FAB subtypes, nor with the prognostic implications of the karyotype. Immunocytology with a polyclonal antibody against ABCA3 was performed on seven cases and showed high staining intensities in two, intermediate/low in three, and no staining in 2 cases. The corresponding RT/PCR were values 150 and 32% in the two cases with high staining intensity, 27%, 2.1% and 11.9% in the cases with intermediate/low staining and 0% in the two with no staining. The immunostains showed a vesicular pattern of ABCA3 in the cytoplasm of the leukemic blasts (Figure 1B). Overexpression of ABCA3 in 293A cells augmented the number and size of acidic vesicles, as visualized by the fluorescent dye *lysotracker red*<sup>®</sup> specific for acidophilic organelles. Exploiting the fluorescent characteristic of daunorubicin, which is quenched when distributed into a non-nuclear compartment of cytoplasmic organelles,<sup>4</sup> we observed quenching of daunorubicin fluorescence in ABCA3 expressing cells which suggested that daunorubicin translocates from the nucleus into an extranuclear cellular compartment (Figure 2A). It is not yet clear whether the decrease in nuclear daunorubicin is due to an increased extranuclear compartment of the cells, transport the daunorubicin by ABCA3, or both mechanisms. To address the volume of the extranuclear space we established a flow cytometric assay with *lysotracker red*<sup>®</sup> fluorescence as an indicator of lysosomal mass per cell. In this assay, a staining ratio (LSR) was defined by the mean fluorescence intensity of a given blast cell population divided by the fluorescence intensity of normal lymphocytes stained and measured on the flow cytometer in strict parallel: