

Pulmonary thrombi are not detected by 3D magnetic resonance angiography in adults with sickle cell anemia and an elevated tricuspid regurgitant jet velocity

Joshua J. Field,^{1*} Anusha R. Madadi,² Marilyn J. Siegel,³ and Vamsidhar Narra³

An elevated tricuspid regurgitant jet (TRJ) velocity is present in more than 30% of adults with sickle cell anemia (SCA) and is associated with a risk of death [1–4]. The contribution of pulmonary thrombi to an elevated TRJ velocity is not well defined. To evaluate the relationship between an elevated TRJ velocity and pulmonary thrombi, we performed 3D, contrast-enhanced magnetic resonance angiography (3D MRA) in nine adults with SCA. Of the six participants with an elevated TRJ velocity, 5 (83%) did not have thrombi in their pulmonary arteries. No individuals with a normal TRJ velocity had pulmonary thrombi. Based on this pilot study using 3D MRA images, thrombi are not present in large vessels in most individuals with a TRJ ≥ 2.5 m/sec, providing evidence thrombi may not significantly contribute to the pathogenesis of an elevated TRJ velocity in individuals with SCA.

Pulmonary thrombi are a common etiology of pulmonary hypertension in the general population, and several autopsy studies of individuals with SCA and pulmonary hypertension have reported pulmonary thrombi [5–7]. Of individuals with SCA and pulmonary thrombi, 66% involve large pulmonary arteries, indicating an embolic event; however, an autopsy study of individuals with SCA and histological evidence of pulmonary hypertension ($n = 20$) noted thrombi in the small pulmonary arteries of 33% of individuals [6]. These autopsy studies were published prior to reports describing the risk of mortality associated with an elevated TRJ velocity and thus they examined individuals with SCA and either symptomatic pulmonary hypertension or without a diagnosis of pulmonary hypertension, but with hypertensive changes on autopsy. There have been no autopsy studies examining the pulmonary vasculature of individuals based on the mild elevations in TRJ velocity that are now recognized to be clinically significant. Therefore, the contribution of pulmonary thrombi to the pathogenesis of a TRJ velocity ≥ 2.5 m/sec has not been thoroughly evaluated.

3D MRA is a technology that allows detailed visualization of the pulmonary arteries. The sensitivity and the specificity of 3D MRA imaging for detecting thromboemboli in the pulmonary vasculature is 81% and 100%, respectively [8]. The sensitivity of 3D MRA is high for the segmental arteries (87%), but decreases to 55% for the subsegmental arteries [8]. Prior imaging studies of the pulmonary vasculature in individuals with SCA using con-

ventional CT methods have demonstrated evidence of thrombi during episodes of acute chest syndrome (ACS), consistent with the process of vaso-occlusion [9]. Only one study, however, has evaluated the lung vasculature in individuals with SCA and an elevated TRJ velocity. Using CT angiography to examine the pulmonary vasculature of individuals with SCA and an elevated TRJ velocity, Linguraru et al. found no thrombi or large vessel disease [10].

In this study, we used 3D, contrast-enhanced MRA with a parallel imaging to evaluate the pulmonary vasculature of adults with SCA with and without an elevated TRJ velocity. We tested the hypothesis that thrombi are present more often in adults with SCA and a TRJ ≥ 2.5 m/sec when compared to those with a TRJ < 2.5 m/sec. Evidence to support our hypothesis would suggest that pulmonary thrombi contribute to an elevated TRJ velocity in some individuals with SCA.

We performed 3D MRA studies in nine individuals with SCA (HbSS or S β -thal⁰) (Table I). Two individuals were female (22%), and the mean age was 29.2 ± 9.6 years (range, 19–52). The mean TRJ velocity was 2.56 ± 0.25 m/sec (range, 2.2–2.9) and six individuals had a TRJ velocity ≥ 2.5 m/sec compared to three individuals who had a TRJ velocity < 2.5 m/sec. Out of the six participants with an elevated TRJ velocity, 5 (83%) had no evidence of pulmonary thrombi. None of the three individuals with a TRJ velocity < 2.5 m/sec had an evidence of pulmonary thrombi. The only study participant who demonstrated filling defects in the pulmonary vasculature was a 26-year-old man with HbSS who had 20 hospital admissions for pain and seven hospital admissions for ACS in the past 4 years. His TRJ velocity was 2.8 m/sec, and his 3D MRA demonstrated focal filling defects in the subsegmental artery of the apical segment of the right upper lobe and lateral segment of the right middle lobe. Of note, the segmental vessels were seen in the other study participants supporting the robustness of the MRA technique (see Fig. 1).

Based on the results of this small pilot study, few individuals with SCA and an elevated TRJ velocity demonstrate evidence of pulmonary thrombi. There are two potential explanations for the discrepant findings between our MRA examinations in individuals with an elevated TRJ velocity and autopsy studies that reported thrombi in the pulmonary vasculature of individuals

TABLE I. Demographic, Laboratory, and Echocardiographic Data in Nine Adults with Sickle Cell Anemia Who Underwent 3D, Contrast-Enhanced Magnetic Resonance Angiography

Participant	Age (years)	Gender	Phenotype	Hemoglobin (g/dL)	LDH (units/L)	TRJ velocity (m/sec)	Pulmonary thrombi present
Individuals with TRJ velocity ≥ 2.5 m/sec							
1	19	M	SS	8.5	645	2.9	No
2	23	M	SS	9.2	329	2.8	No
3	52	M	SS	8.4	549	2.6	No
4	28	M	SS	7.1	342	2.5	No
5	25	M	SBO	6.9	676	2.5	No
6	26	M	SS	10.7	229	2.8	Yes
Mean value \pm SD	28.0 ± 11.8	—	—	8.5 ± 1.4	462 ± 186	2.7 ± 0.2	—
Individuals with TRJ velocity < 2.5 m/sec							
7	31	F	SS	10.3	374	2.2	No
8	35	M	SS	7.3	582	2.3	No
9	25	F	SS	8.7	482	2.4	No
Mean value \pm SD	30.3 ± 5.0	—	—	8.8 ± 1.5	479 ± 104	2.3 ± 0.1	—

TRJ, tricuspid regurgitant jet; LDH, lactate dehydrogenase; SS, hemoglobin SS; SBO, hemoglobin S β -thalassemia⁰; SD, standard deviation.

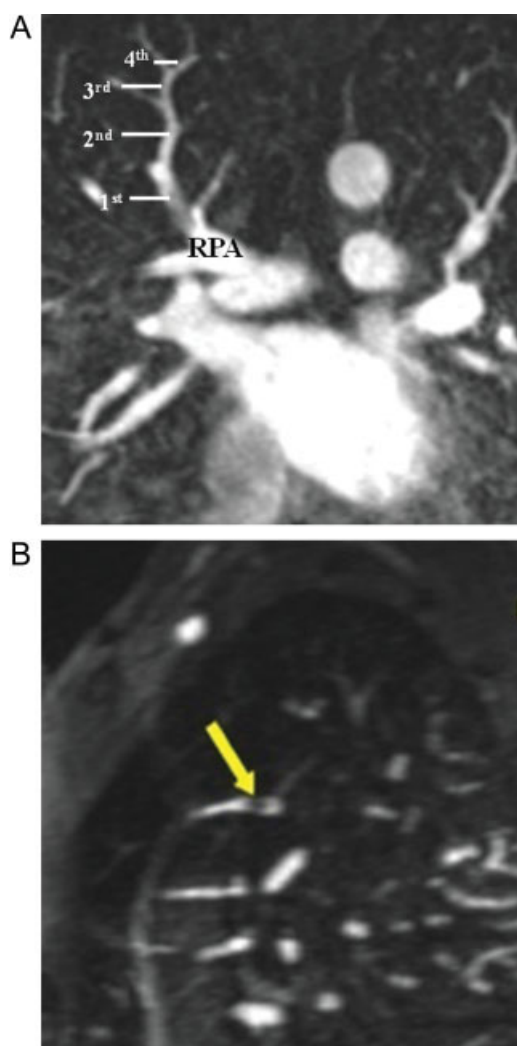


Figure 1. A: 3D, contrast-enhanced magnetic resonance image (3D MRA) of the right pulmonary artery (RPA) and 1st, 2nd, 3rd, and 4th segmental arteries. B: 3D, contrast-enhanced MRA image of Participant 6 (arrow) demonstrating a filling defect in a subsegmental artery of the apical segment of the right upper lobe. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

with SCA and pulmonary hypertension. First, a TRJ velocity of 2.5 m/sec is mild pulmonary hypertension and not diagnostic of pulmonary hypertension in the general population [11]. Thus, the absence of thrombi on 3D MRA imaging in this study may reflect the small increases in TRJ velocities that have been linked to mortality in this patient population. Prior autopsy reports of individuals with SCA and pulmonary hypertension likely represented the small group of individuals with SCA who had severe pulmonary hypertension and exhibited symptoms of right-sided heart failure. Second, the low prevalence of thrombi in our study could be due to the lower sensitivity of MRA for detecting thrombi in the subsegmental arteries, approximately 55% [8].

MRA has specific advantages compared to CT angiography, the usual imaging technique for diagnosing pulmonary thromboemboli. The MRA protocol avoids the significant radiation exposure associated with CT, a major disadvantage of CT. Another specific advantage of MRA compared to CT is that functional data can be obtained, if clinically indicated, reflecting the impact of an embolus on cardiac function and pulmonary blood flow velocity.

Limitations are present in this study. Our sample size of only nine participants increases the likelihood of a type II error. To definitively determine if pulmonary thrombi contribute to an elevated TRJ velocity, imaging studies in a larger cohort of individuals is necessary. A second limitation of our study is there was no correlative imaging or histologic studies to confirm the presence or absence of thromboembolic disease, but based on published

evidence of the high sensitivity of MRA for detection of pulmonary emboli, we believe that our results substantiate the absence of pulmonary emboli in main and segmental vessels in our study. However, it is possible that we failed to detect very small subsegmental pulmonary thrombi.

In summary, pulmonary thrombi are not present in many individuals with SCA and elevated TRJ velocity based on 3D, contrast-enhanced MRA imaging techniques. Thrombosis is not likely a significant contributor to the pathogenesis of an elevated TRJ velocity in adults with SCA. Based on the one participant in our study with pulmonary thrombi, evaluating individuals with an elevated TRJ velocity who require frequent hospitalizations for pain and ACS for pulmonary thrombi may be a reasonable strategy, but further studies are needed.

Methods

This study was approved by the Human Research Protection Office at Washington University School of Medicine and appropriate, informed consent was obtained from all participants before testing. Eligible participants were adults with SCA (HbSS or HbS β -thalassemia⁰) confirmed by hemoglobin analysis, age 18 years and older, who had undergone a 2D echocardiogram evaluation for TRJ evaluation within the past 6 months. We excluded individuals with a serum creatinine ≥ 1.5 mg/dL, who were pregnant or lactating and individuals with a contraindication to MRI (cardiac pacemaker, claustrophobia, aneurysm clip, or allergy to contrast).

Baseline 2D echocardiogram evaluations were performed when individuals were clinically stable, defined as no hospitalizations or emergency room visits for any reason within 2 weeks of the study, and no current increase in their baseline pain. Transthoracic echocardiography was performed using conventional clinical echocardiographic equipment with 2.5 or 3.5 MHz transducers. The results were interpreted by a single cardiologist who was blinded to the data. TRJ velocity was identified by color flow Doppler techniques and the TRJ velocity was recorded as the highest value obtained with a good quality Doppler envelope from either apical 4-chamber or parasternal views. An elevated TRJ velocity was defined as ≥ 2.5 m/sec.

Three-dimensional, contrast-enhanced MRA images were obtained by a 1.5 T whole body scanner equipped with 40 mT/m sonata grading system. Images were acquired after the injection of gadolinium chelate contrast agent and obtained with high resolution breath hold (14 sec) contrast-enhanced pulmonary MR angiography (repetition time 2.5 msec, echo time 1.0 msec, 20 degree flip angle, 384 by 288 matrix, a rectangular FOV according to patients size, bandwidth of 690 Hz/pixel, signal average = 1, plane resolution of 1.3×1.0 mm²). A test bolus was performed before each imaging study to ensure appropriate timing of contrast injection.

Acute thromboembolus was diagnosed if thrombotic material was directly visualized on more than one image in each of two planes. The level of a thromboembolus was categorized as central, lobar, segmental, or subsegmental. Standard nomenclature was used to identify segmental and subsegmental arteries [12,13]. MRA images were reviewed on a dedicated 3D imaging workstation (Vital Images, Minnetonka, MN). A consensus interpretation of two readers was performed.

Baseline laboratory values were obtained when individuals were well, had not reported an increase in their baseline pain, and had not been hospitalized for a pain or ACS episode for at least 2 weeks. Descriptive statistics were used to report the participant demographics, laboratory, echocardiographic, and MRA data.

¹Department of Internal Medicine, Washington University School of Medicine, St. Louis, Missouri

²Department of Internal Medicine, St. Luke's Hospital, Chesterfield, Missouri

³Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, Missouri

*Correspondence to: Joshua J. Field, Division of Hematology, Department of Internal Medicine, Box 8125, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO, 63110

E-mail: jfield@dom.wustl.edu

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Atypical presentations of Sweet's syndrome in patients with MDS/AML receiving combinations of hypomethylating agents with histone deacetylase inhibitors

Cristina Alencar,¹ Matthew Abramowitz,² Samir Parekh,¹ Ira Braunschweig,¹ Mark Jacobson,³ Lewis Silverman,⁴ and Amit Verma^{1*}

Epigenetic silencing of tumor suppressors is seen in Myelodysplastic Syndrome (MDS) and even though demethylating agents have improved survival in this disease, a majority of patients are still resistant to these therapies. In an effort to improve response rates, newer combinations of histone deacetylase (HDAC) inhibitors and hypomethylating agents are being used to reverse aberrant epigenetic silencing in MDS. The combinations of these agents may result in unanticipated toxicities. We report three cases of Sweet's syndrome (SS) associated with the use of 5-Azacitidine/Decitabine in combination with HDAC inhibition. They were characterized by atypical pathological appearance with relatively lesser number of invading neutrophils, presumably because of the underlying bone marrow failure. One case was characterized by fulminant and aggressive nature of SS, which became refractory to standard immunosuppressive treatments and result in mortality. SS should thus be considered a part of differential diagnosis of skin lesions in patients receiving combination of epigenetic therapies and should be treated aggressively.

The first case was a 44-year-old man with high-risk myelodysplastic syndrome (MDS), enrolled in a Phase I clinical trial evaluating a combination of 5-azacitidine and vorinostat. Initial presentation included rapidly appearing skin nodules (Fig. 1A) with fevers, vomiting, and diffuse abdominal pain after the 2nd cycle of treatment. The patient developed acute renal failure and stridor requiring intubation because of involvement of vocal cords with nodular lesions. Biopsy of the skin nodules (Fig. 1B) revealed dense, hemorrhagic, and neutrophilic dermatitis with massive subepidermal and intraepidermal edema, consistent with a diagnosis of Sweet's syndrome (SS). Interestingly, the amount of neutrophilic involvement of the skin was quantitatively less than previous reports, possibly because of the neutropenic state of the patient. This episode responded to high dose steroids with resolution of nodules and systemic symptoms. The patient achieved a complete remission of his MDS on therapy but developed a recurrence of the skin nodules with 5th cycle. This episode again responded to oral prednisone. He developed a third relapse when on decitabine treatment at the time of relapse of his MDS. During the last relapse, the patient became refractory to high dose steroids and eventually died from acinetobacter pneumonia and sepsis.

The second case was a 47-year-old woman with refractory acute myelogenous leukemia with complex karyotype, who failed two induction treatments.

While awaiting a matched unrelated donor transplant, the patient was started on combination of 5-azacitidine 75 mg/m² plus valproic acid (2.5 gm/day) [1]. After the second dose of azacitidine, patient developed a skin rash in the abdominal area (Fig. 1C) and right thigh with fever requiring hospitalization. Both skin lesions were papula-nodular that subsequently became ulcerated. Biopsy of skin (Fig. 1D) revealed acute infiltration of the dermis by neutrophils consistent with SS. Patient got treated with topical antibiotics and no steroids were necessary, and azacitidine was stopped. Lesions healed in about three weeks.

The third case was a 50-year-old man with Hepatitis C infection also enrolled in a Phase I clinical trial with 5-azacitidine and vorinostat. Patient had received seven cycles of the above combination with complete response. He was started on the same combination after relapse of his MDS and then developed a macula-papular, erythematous rash in the neck and upper back area (Fig. 1E). The biopsy again was consistent with atypical appearance of SS, similar to the first two cases. The patient's lesions responded to a short course of oral prednisone.

SS was first reported in eight cases of women with fever, neutrophilia, plaques on the limbs, and dermal infiltration by mature neutrophils in 1964 by Robert Douglas Sweet [2]. Most of the patients responded well to steroids. SS often occurs in association with a systemic disorder. Neoplasms are responsible for 10–20% of the cases and hematological malignancies are more common than solid tumors. Leukemia is responsible for 42% of the hematological malignancies associated with SS, and the most common solid tumors reported are breast, genito-urinary tract, and gastro-intestinal tract cancers [3]. Although drug-induced SS is an uncommon event, granulocyte colony-stimulating factor (G-CSF), all-*trans* retinoic acid, antibiotics (trimethoprim/sulfamethoxazole), and vaccines have been most commonly implicated [4,5]. Three cases of SS associated with bortezomib have also been recently described [6,7].

To date, there is no report of SS associated with hypomethylating agents (azacitidine, decitabine). As MDS has been associated with SS [8,9], the association with the use of azacitidine/decitabine or histone deacetylase (HDAC) may be casual. Of note, patients were not getting G-CSF, which is commonly implicated in drug-associated SS. SS is generally treated with glucocorticoids and nonsteroidal anti-inflammatory drugs. In refractory cases, dapsone, potassium iodide, colchicine, doxycycline, clofazimine, and indomethacin have been used. Our first case of SS relapsed while on tapering doses of prednisone and became refractory to high dose steroids. The refractory and fulminant nature of this syndrome

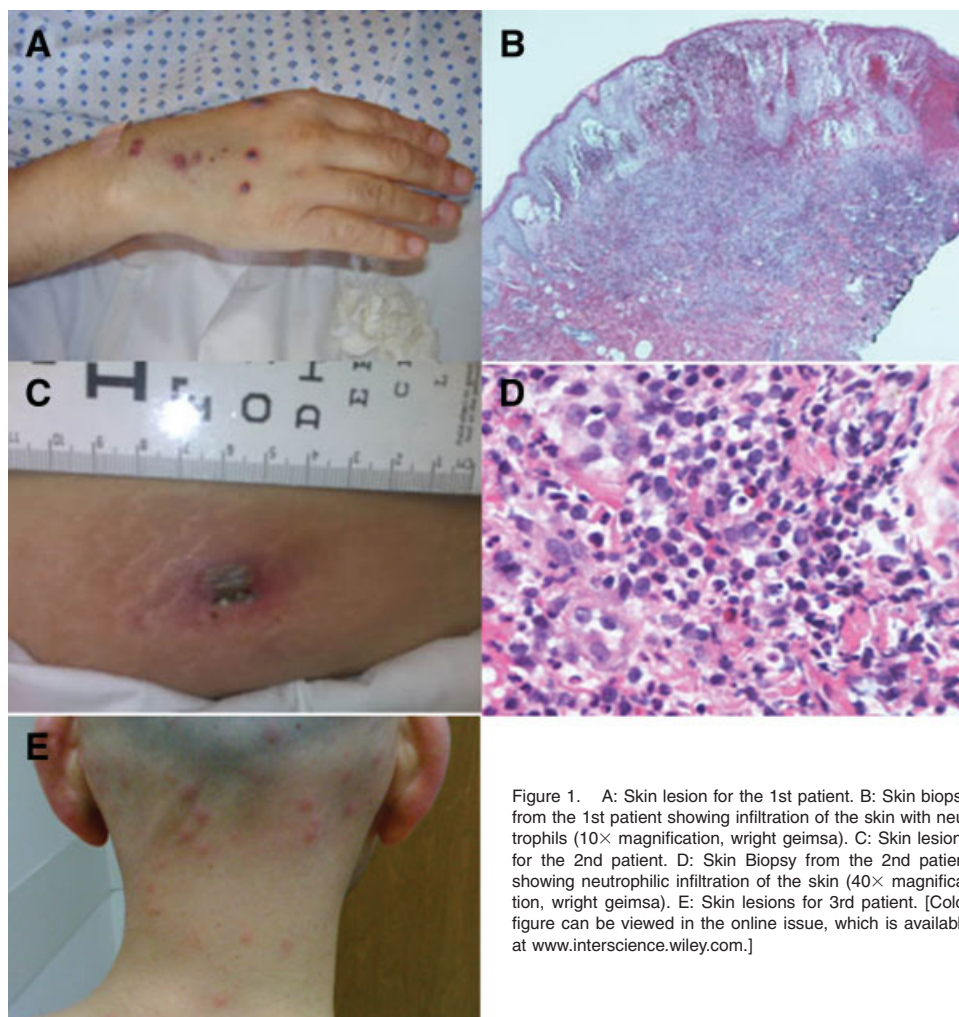


Figure 1. A: Skin lesion for the 1st patient. B: Skin biopsy from the 1st patient showing infiltration of the skin with neutrophils (10 \times magnification, wright geimsa). C: Skin lesions for the 2nd patient. D: Skin Biopsy from the 2nd patient showing neutrophilic infiltration of the skin (40 \times magnification, wright geimsa). E: Skin lesions for 3rd patient. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

could be related to therapy with epigenetic modifiers. The differential diagnosis for skin lesions in these patients can include fungal and other infective lesions, leukemic involvement of the skin, and drug reactions. Because of the atypical pathological appearance of Sweet's lesions in this setting, it is important that this should be noted in the differential and treated early and aggressively. Furthermore, as DNMT inhibitors and HDAC inhibitors are showing promise in MDS, these observations need to be tested in larger cohorts in the future.

¹Department of Oncology, Albert Einstein College of Medicine
Bronx, New York 10461

²Department of Nephrology, Mount Sinai School of Medicine, New York

³Department of Pathology, Albert Einstein College of Medicine
Bronx, New York 10461

⁴Department of Oncology, Mount Sinai School of Medicine, New York

*Correspondence to: Amit Verma
Albert Einstein College of Medicine
Bronx, NY 10461

E-mail: averma@aecom.yu.edu

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FLAIE (fludarabine, cytarabine, idarubicin, and etoposide), a four drug induction chemotherapy for adult acute myeloid leukemia: A single center experience

Anna Candoni,^{1*} Erica Simeone,¹ Mario Tiribelli,¹ Michele Malagola,² Domenico Russo,² and Renato Fanin¹

The target of this pilot study was to evaluate the complete remission (CR) rate and the safety profile of a four drug induction chemotherapy regimen (FLAIE) in previously untreated AML. Fifty consecutive patients were included between 2003 and 2005. All were younger than 65 years (median age of 51 years). FLAIE included: fludarabine (25 mg/m²), cytarabine (2 g/m²), and etoposide (100 mg/m²) on days 1–5, idarubicin (6 mg/m²) on days 1, 3, and 5. Patients were evaluated for response rate, treatment-related adverse events, overall survival (OS), and disease-free survival (DFS). After FLAIE, CR occurred in 64% of patients (32/50); three patients (6%) achieved a partial remission and 15 (30%) were nonresponders. There was only one induction death (2%). Infections occurred in 29/50 patients (58%). Grade II–III WHO oral mucositis was reported in 11 patients (22%). Median time to neutrophil ($>1 \times 10^9/l$) and platelet ($>50 \times 10^9/l$) recovery was 24 and 26 days, respectively. Supportive therapy consisted of a median of 13 packed red cell units and 9 platelets units. The probability of 1-year OS and DFS were 68% and 48%. The FLAIE regimen appeared to have acceptable toxicity, but these results suggest that the addition of etoposide to FLAI scheme does not improve CR rate of FLAI alone.

Standard front-line induction chemotherapy for acute myeloid leukemia (AML) is based on the combination of cytarabine plus an anthracycline (daunorubicin or idarubicin), with or without the addition of a third drug [1–4]. CR can be obtained in about 70–75% of younger patients [1–4]. The factors associated with poor response to induction therapy are advanced age, unfavorable karyotype, secondary leukemia, high leukemic burden and over expression of multidrug resistance (MDR)-related proteins, such as P-glycoprotein (PGP), that reduce the sensitivity of tumor cells to various anticancer drugs [1–8]. The use of an additional agent to the standard two-drug induction therapy has led to conflicting results. However, several studies have shown the efficacy and low toxicity of the addition of fludarabine to conventional AML induction regimen [9–17]. The inclusion of the cytotoxic agent etoposide to the cytarabine plus anthracycline schema seems to improve CR rates and DFS, but no conclusive data are available [2,18].

To date, no studies are available including etoposide in a fludarabine-based induction therapy. We report about our experience with a four-drug (fludarabine, cytarabine, idarubicin, and etoposide = FLAIE) induction regimen in a group of 50 consecutive AML patients, younger than 65 years (median age 51 years; range: 21–63) and previously untreated. All patients were treated between January 2003 and December 2005. According to the French–American–British (FAB) classification, 21 patients had M4–M5 AML, 19 M1–M0 AML, 7 M2 AML, and three patients were not classifiable (post-MDS AML). Fourteen cases (28%) had a secondary leukemia. Overall, 38 patients (76%) were at high risk for the presence of one or more of the following features: therapy-related or secondary AML, unfavorable karyotype (complex cytogenetic abnormalities or any abnormality involving chromosomes 3, 5, 7, or 11), peripheral blood blast count $>30 \times 10^9/l$, over expression of P-glycoprotein by flow cytometric analysis (mean fluorescent index > 6) [1,3,5,7,8,15].

All the 50 patients were evaluable for response after FLAIE induction therapy. Only one case of induction death was reported (2%). The CR rate was 64% (32/50); three patients achieved a partial remission (PR) (6%) and 15 were nonresponders (30%). Response to induction was not

affected by risk at diagnosis (58% of CR rate in the standard risk group and 66% of CR rate in the high risk group) or by PGP over expression (58% of CR rate in PGP-positive cases and 65% in PGP-negative cases, $P = 0.7$).

Documented infections and/or fever of unknown origin (FUO) were observed, as expected, in most cases. In particular, infections occurred in 29 of 50 (58%) patients, including 18 episodes of bacteremia (nine due to Gram-positive bacteria, six Gram-negative bacteria, and 3 polymicrobial) and 14 cases of pneumonia (four *Aspergillus* sp. pneumonia); 26 episodes of FUO, Grade II–III WHO, were recorded. Infectious death after FLAIE occurred in only one patient who developed septic shock and multiorgan failure due to *Enterobacter cloacae* bacteremia. Oral mucositis Grade II–III (WHO) was reported in 11 patients (22%), and oral herpes simplex virus reactivation was documented in 16 cases (32%). No patient experienced Grade IV WHO hepatic toxicity, but 10 (20%) had a Grade II–III WHO transient elevation in liver function tests, specifically bilirubin and/or transaminases. Enteritis with diarrhea and abdominal pain, Grade II–III WHO, was reported in 15 cases (30%). No treatment-related cardio toxicity was observed. As expected, all patients experienced Grade IV WHO hematological toxicity; median time to neutrophil ($>1 \times 10^9/l$) and platelet ($>50 \times 10^9/l$) recovery was 24 (range: 18–42) and 26 (range: 20–45) days, respectively. Supportive treatment consisted of a median of 13 packed red cell units (range: 10–28) and nine platelet units (range: 5–15). G-CSF was used in 22 patients (44%), because of prolonged myelosuppression and/or severe infection, for a median of 10 days (range: 2–16). No life-threatening bleeding occurred. After FLAIE treatment, median time to hospital discharge was 36 days (range: 26–62). After a median follow-up time of 24 months (range: 1–70), 19 (38%) patients are alive (all in CR), whereas 31 (62%) are dead. Nineteen patients died of AML (refractory or relapsed), one died during induction therapy (septic shock from *Enterobacter cloacae*) and three after a consolidation course, whereas in CR (two invasive *Aspergillosis* and pneumonia, and one *Pseudomonas* sp. pneumonia). Finally, eight patients died for early or late transplant-related mortality (TRM). Probability of 1-year OS and DFS was 68% (95% CI: 42–88) and 48% (95% CI: 28–62), respectively (Fig. 1A,B).

Thirty-nine of 50 patients (78%) received a hematopoietic stem cell transplantation (HSCT). Median time between FLAIE and HSCT was 6.3 months (range 3.5–31). Four patients (8%) underwent an autologous HSCT, whereas 35 (70%) received an allogeneic transplant (13 from a sibling donor and 23 from a matched unrelated donor). The median follow-up after HSCT was 15 months (range 1–66), with 16/39 (41%) patients alive and in continuous CR.

Despite the remarkable advances in cellular and molecular characterization of AML blasts and the growing insights in disease pathogenesis, to date, the “classical” combination of cytarabine plus an anthracycline is still widely accepted as common induction therapy for AML patients [1,19]. In the recent years, various strategies have been tested to improve the results in the treatment of AML patients. In particular, efforts have been done to improve the CR rate of induction chemotherapy by adding different drugs to the standard regimen and to optimize postremission therapy [1–3]. The purine analog fludarabine, initially used in lymphoid malignancies, has shown promising results in AML. In the last 15 years, fludarabine-based induction regimens have been tested in patients with relapsed AML and, more recently, at disease onset

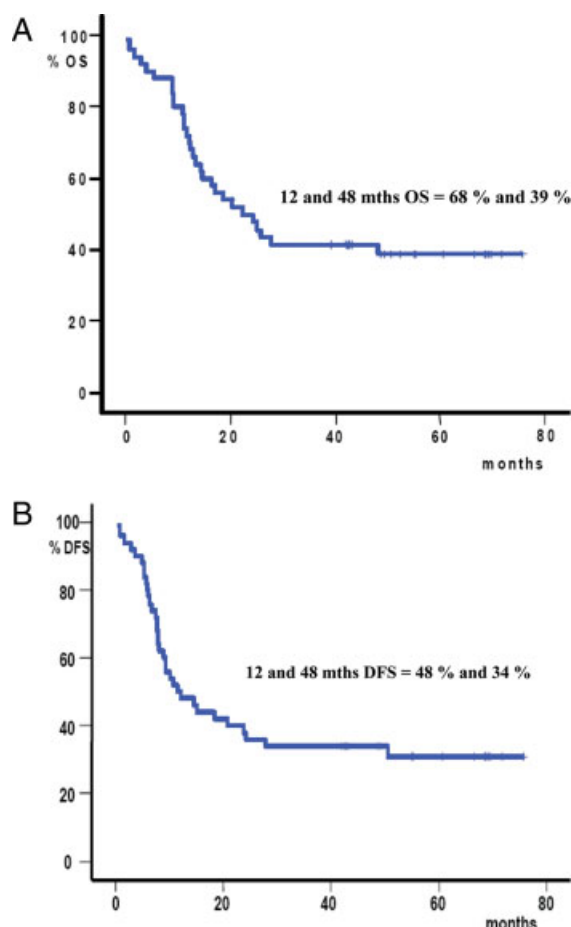


Figure 1. (A) Overall survival curve and (B) disease free survival curve in 50 cases. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I. Characteristics of Patients Treated with FLAIE (AML02 Protocol) or FLAI (AML99 Protocol)

	FLAIE (AML02 protocol)	FLAI (AML99 protocol)	P value
Total number (sex)	50 (19M/31F)	57 (34M/23F)	0.7
Median age (range), years	51 (21–63)	49 (19–60)	
Over 60	8/50 (16%)	0/57	0.01
FAB subtype			
M0–M1	19/50 (38%)	11/57 (19%)	
M2	7/50 (14%)	12/57 (21%)	
M4–M5	21/50 (42%)	28/57 (49%)	
M6	0/50 (0%)	1/57 (2%)	
Sec	3/50 (6%)	5/57 (9%)	
High risk at diagnosis	38/50 (76%)	40/57 (70%)	0.5
Unfavorable karyotype	13/50 (26%)	22/57 (39%)	0.2

TABLE II. Toxicity Data and Outcome of Patients Treated with FLAIE (AML02 Protocol) or FLAI (AML99 Protocol)

	FLAIE (AML02 protocol)	FLAI (AML99 protocol)	P value
Complete remission rate	32/50 (64%)	42/57 (74%)	0.3
Early death	1/50 (2%)	1/57 (1.75%)	0.9
PMN > $1 \times 10^9/l$			
Median (range), days	24 (18–42)	24 (19–44)	0.6
PLT > $50 \times 10^9/l$			
Median (range), days	26 (20–45)	22 (18–30)	0.1
Mucositis (Grade II–III WHO)	11/50 (22%)	8/57 (14%)	0.3
Enteritis (Grade III–IV WHO)	2/50 (4%)	3/57 (5%)	0.9
Documented infectious and febrile episodes (number of episodes)	58	60	
Hospitalization			
Median (range), days	36 (26–62)	34 (24–56)	0.6
Overall survival			
1 year	68% (95% CI: 42–88)	62% (95% CI: 40–87)	
4 year	39% (95% CI: 16–44)	32% (95% CI: 19–45)	

[9,13,15,16,20–26]. The rationale of fludarabine inclusion in cytarabine—containing courses is based on the synergic activity of the two drugs that has been proved both in vivo and in vitro [25–27]. Between 1997 and 2002, a three-drug induction course including fludarabine, cytarabine, and idarubicin (FLAI) has been tested at our Center, first in a pilot study (AML 97 protocol) and subsequently in a multicenter phase III study (AML 99 protocol) [15,16]. In the latter protocol, efficacy and safety of FLAI was compared with ICE (idarubicin, cytarabine, and etoposide) induction regimen. In AML 97 and AML 99 studies, induction chemotherapy with FLAI course attained CR rates of 72 and 74%, respectively [15,16]. Moreover, the AML 99 randomized study (FLAI arm vs. ICE arm) clearly showed that FLAI was more effective (CR rate) and less toxic than ICE for induction of remission but the OS and DFS were similar in the two arms [16]. To improve the results attained with FLAI (CR rate, OS and DFS), we tested the addition of etoposide as a fourth drug of induction regimen (FLAIE schema). Etoposide was chosen on the basis of its proved efficacy in younger and elderly patients with AML [2,28,29]. In the previous studies in AML setting, etoposide has been associated with different antineoplastic agents but, to the best of our knowledge, never with fludarabine.

The clinical and biological features of the patients treated with FLAIE (Protocol AML02) were comparable with those of the patients in our previous FLAI based protocol (Protocol AML99) (Table I). The CR rate (63%) and 4 years OS (39%, 95% CI: 16–44) and DFS (34%, 95% CI: 17–48) of FLAIE induction course did not prove to be superior than those attained with FLAI regimen (CR rate 74%, 4 years OS and DFS 32% and 31.5%, respectively) as previously reported by Russo et al. [16]. However, hematologic recovery and extrahematologic toxicities of FLAIE were similar to what we observed in AML99 protocol, with a low induction death rate (2%) (Table II). Similarly, the incidence of other nonhematologic severe toxicities was quite low. Gastrointestinal severe adverse events were rare (only 4% of Grade III WHO), and the rate of infectious complication was in line with our and others previous experiences [15,16].

In conclusion, taking into account the small sample size and the limitations of this study, our data suggest that the addition of etoposide to the FLAI induction regimen (FLAIE) did not increase efficacy in terms of CR rate in AML patients younger than 65 years. Nonetheless, these data show that FLAIE is feasible with overall safety and time to hematological recovery similar to the historical control (FLAI).

Methods

FLAIE regimen consisted of fludarabine (25 mg/m²/day, days 1–5), cytarabine (2 g/m²/day, days 1–5), etoposide (100 mg/m²/day, days 1–5) and idarubicin (6 mg/m²/day, days 1, 3, and 5). After induction therapy, all patients were planned to receive two consolidation courses with cytarabine (2 g/m²/day, days 1–5) and idarubicin (12 mg/m², days 1,3, and 5). Baseline biologic studies included bone marrow examination with immunophenotype, karyotype, and molecular analyses. Karyotype was considered normal if no clonal abnormalities were seen in at least 20 metaphases, or unfavorable if complex cytogenetic abnormalities or any abnormality involving chromosomes 3, 5, 7, or 11 were detected. In positive cases, cytogenetic and molecular analyses were performed also before consolidation, before and after transplant, and in all relapsing cases. MDR-related proteins (PGP, MRP, LRP) expression were assessed, as previously described, in all patients at diagnosis and in case of relapse [6].

Blood counts and biochemistry were determined three times a week during the follow-up period. A bone marrow aspiration was performed at days 7 and 15 to document leukemic blasts clearance. Final determination of remission status was assessed by blood and bone marrow examination at the time of hematologic recovery, or at a maximum of 45 days from the start of FLAIE. Response criteria were those of National Cancer Institute revised by the International Working Group [30]. CR was defined as absence of any tumor and <5% bone marrow blasts with an absolute neutrophil count (ANC) $>1 \times 10^9/l$, platelets $>100 \times 10^9/l$, and transfusion-independence. PR was defined as 5–15% blasts in bone marrow of adequate cellularity with evidence of trilineage regeneration. Patients who did not meet the criteria for CR or PR were categorized as nonresponders. Therapy-related toxicity was evaluated according to the World Health Organization (WHO) guidelines. Early death was defined as death occurring during induction therapy or before hematological recovery. Patients were followed up until progression or death. The primary end points of the study were CR rate and treatment-related toxicity after FLAIE. Secondary end points were OS and DFS.

Data were analyzed as of March 31, 2009, with a median follow-up time of 24 months (range 1–70 months). Survival curves were constructed using the Kaplan-Meier method. OS was measured from the time of diagnosis until death from any cause. DFS defines the time from CR until disease relapse or death. Patients who did not relapse were censored at date of death or date of last follow-up, as appropriate. Continuous variables were checked by descriptive statistics (arithmetic mean, standard deviation, median, minimum and maximum). Comparisons were performed by the Fisher's exact test and by Student's *t*-test. All *P* values were two sided and were considered significant when <0.05 . Data were analyzed using NCSS60 software (NCSS Company, Kaysville, UT).

¹Chair and Division of Hematology, Bone Marrow Transplantation Unit, Department of Clinical and Morphological Research, University of Udine, 33100 Udine, Italy

²Chair of Hematology, University of Brescia, Spedali Civili di Brescia, Brescia, Italy

Correspondence to: Anna Candoni, Division of Hematology and Bone Marrow Transplantation Unit, University of Udine, Italy.

E-mail: candoni.anna@aoud.sanita.fvg.it

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