THERAPY RELATED ACUTE NON-LYMPHOCYTIC LEUKEMIA: REPORT OF 4 CASES

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INTRODUCTION

Cancer chemotherapeutic agents have recently been increasing in use in both neoplastic and non-neoplastic conditions (DeVita *et al.*, 1981; Steinberg *et al.*, 1972). The desired drug effect on the latter, was the immunosuppressive property. Immediate and intermediate side effects are rather well known (Livingston, 1982). However, late treatment complications (Sieber and Adamson, 1975) were only recently recognised after having utilized aggressive form of treatment, which has led to an improvement in patient's survival. The most serious is the development of second neoplastic diseases, of which acute nonlymphocytic leukemia is the most commonly reported (Schmall *et al.*, 1982). This finding is not unexpected since experimental data have long indicated that some cytostatic drugs, particularly the alkylating agents, are carcinogenic in laboratory animals (Weisberger, 1977). However, evidence that these agents have similar effects in man has been anecdotal until recently, when a series of analytic studies have documented many incidences of acute nonlymphocytic leukemia (ANLL) in patients treated for Hodgkin's disease (Cadman *et al.*, 1977), multiple myeloma (Bergsagel *et al.*, 1979), non Hodgkin's lymphoma (Greene *et al.*, 1983), and ovarian cancer (Greene *et al.*, 1982). ANLL has also been reported following use of cytotoxic drugs for treatment of non malignant conditions (Casciato and Scott, 1979; Rosner and Grunwald, 1980). To differentiate it from de novo leukemia, this type of leukemia has been called therapy related or secondary leukemia. With the increasing number of long term survivors among cancer patients in Thailand, it was not surprising therefore to find therapy related acute nonlymphocytic leukemia cases. The case histories are reported herein.

CASE REPORTS

Case 1: Mrs. S.T. a 36-year-old Thai housewife, was admitted in May 1984 because of chest pain and pallor.

In January 1978, a classical radical mastectomy was performed for a stage II infiltrating ductal carcinoma of her left breast. Complete blood count (CBC) was normal. Postoperative radiation was given to the left chest and regional nodes. Total radiation dose was 5,000 rads in 5 weeks. Three weeks later, adjuvant chemotherapy with melphalan was begun. This was given for 5 consecutive days every 6 weeks for 27 months. Total dose of melphalan was 1,010 mg. In March 1982, a benign mass was excised from her right breast.

Anemia was first detected in September 1983. This slowly and progressively got worse, and in December her CBC showed hemoglobin (Hb) 8.8 gm/dl, hematocrit (Hct) 25 l/l; white cell count (wbc) 6.1×10^9/l, polymorphos 61%, eosinophils 1%, lymphocytes 26%, monocytes 9%, promyelocyte 1%, and myeloblast 1%, platelets 252×10^9/l.

In February 1984, she was admitted to another hospital because of fever, pallor chest pain and a 7 kg weight loss. CBC showed Hb 6.9 gm/dl; wbc 5.8 × 10^9/l with 8% myeloblasts; platelets 160 × 10^9/l. Bone marrow (BM) aspirate showed hypercellularity with adequate megakaryocytes. Erythropoiesis was megaloblastoid, and myeloid : erythroid (M:E) was 2.5 : 1. Granulopoiesis showed a left shift with 16% myeloblasts. There was evidence of dyshemopoiesis of the three cell lines as previously described (Bennett *et al.*, 1982). Blood transfusion, and chemotherapy which consisted of cytosine arabinoside and 6 thioguanine were given. Because of severe nausea from chemotherapy, she refused further treatment. There was no family history of cancer.

Examination on this admission was unremarkable except for a small left thyroid nodule and pallor. CBC showed Hb 8.5 gm/dl; wbc 3.5 × 10^9/l, polymorphos 16%, single lobed polymorphos 3%, band 8%, monocytes 1%, monocytoid 5%, lymphocytes 55%, myeloblasts 3%, monoblast 3%, and undifferentiated blast 6%; low platelets. BM aspirate did not show any significant change from the previous one. Iron stain was $4^+/4^+$ with ring sideroblast. She was considered to have refractory anemia with excess of blasts (RAEB) in transformation. Cytogenetic study of the bone marrow was not done. Direct Coombs' test was negative, and serum protein electrophoresis showed hyper - gammaglobulinemia. Prednisolone and vitamin B_6 were given but without improvement. Periodic blood transfusions had to be given. She expired a month later from sudden cardiac arrest. Autopsy was not permitted.

Case 2: Mrs. P.C. (RH 072208) was a 54-year-old Thai housewife who was admitted on 7 December 1984 because of periodic fever for two months and hemoptysis for a day.

In November 1970, a diagnosis of IgG multiple myeloma was made. She was given daily cyclophosphamide and prednisolone, except during June 1975-December 1976 when her condition improved. Treatment was discontinued in May 1977. Total dose of cyclophosphamide given was 69,600 mg. In December 1976, she received 2,100 rads of radiation therapy to her sternum to relieve pain.

From July 1977 to September 1984, she was given a 6-weekly course of melphalan and prednisolone. Total dose of melphalan given was 1,588 mg. In October, she started to notice periodic low grade fever in the afternoons and pallor. Physical examination showed pallor with few ecchymoses. No organomegaly nor peripheral lymphadenopathy was observed. CBC showed Hb 7.1 gm/dl, Hct 20 l/l; wbc 1.9 × 10^9/l, polymorphos 19%, lymphocytes 64%, eosinophils 3%, myeloblasts 14%, platelets 34 × 10^9/l. Bone marrow smear showed no marrow particles, of the few cells seen, there were scattered myeloblasts, monoblasts, and immature plasma cells. Bone marrow clot section showed hypocellular marrow, decreased myeloid and megakaryocytic series with plasmacytosis. Cytochemical stains of immature cells in the buffy coat smear were positive for Sudan black and peroxidase, negative for PAS and nonspecific esterase. She was considered to have hypoplastic acute leukemia. Sepsis workup was negative. Serum protein electrophoresis failed to show monoclonal pattern. Roentgenogram of lateral skull still showed osteolytic bone lesions. Low dose prednisolone and lithium carbonate were given without success in bringing down the fever or causing leucocyte elevation.

She was admitted this time with a day history of hemoptysis. Chest roentgenogram revealed right upper lobe infiltration. There was tenderness of the right lower abdomen. Treatment was antimicrobial therapy and

appendectomy. Postoperative course was stormy with uncontrolled pulmonary and wound infection. *Pseudomonas aeruginosa* grew from the operative wound. She finally expired on 21 December. Autopsy showed multiple foci of pleomorphic plasma cell and plasmacytoid cell proliferation resulting in multiple osteolytic lesions and several greyish white nodules in the vertebra, ribs and sternum. The cellularity of remaining vertebral and sternal BM were about 85%. Blasts comprised approximately 40% of the marrow elements. Erythroblasts and mature myeloid cells were decreased. Iliac crest BM showed multiple foci of immature myeloid cells and areas of plasma cell proliferation in the hypoplastic marrow. Leukemic cell infiltrations were seen in the liver and spleen. Both lungs showed severe edema, hemorrhage and aspergillosis. Several mycotic thrombi were noted in the pulmonary blood vessels. The appendectomy wound showed necrotizing inflammation. Central hemorrhagic necrosis of the liver was noted. Both kidneys showed acute tubular necrosis.

Cytogenetic studies (13-14 weeks after last chemotherapy) of the BM showed hypoploidy (41-45 chromosomes), euploidy, hyperploidy (47 chromosomes) + XX and marker chromosomes in every clone (Fig. 1).

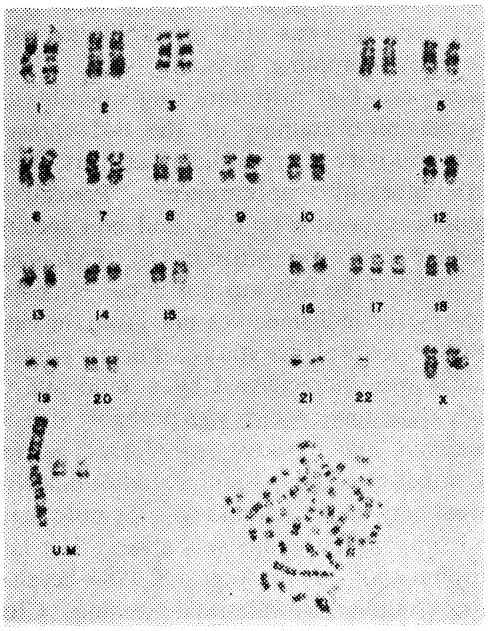

Fig. 1—G-banded karyotype from Case 2 showing abnormal chromosomes identified as 47, XX, −11, −11, +17, −22 and 3 unidentified marker (UM) chromosomes.

Case 3: Mrs. B.H. (RH 1222488) was a 62-year-old Thai housewife, who was admitted in June 1982 because of a pelvic mass. Exploratory laparotomy revealed a stage II B papillary serous cystadenocarcinoma of the left ovary. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was done without incidence. Preoperative CBC was normal. Postoperatively, adjuvant chemotherapy with melphalan was given for five days every month for a year. Total dose of melphalan given was 520 mg. No residual tumor was found in the second look operation performed in December 1983.

In April 1984, pallor was first noticed. Iron tablet was prescribed. The following month she was admitted because of fever and fatigue. Examination revealed marked pallor, liver and spleen nonpalpable. CBC showed Hb 5.4 gm/dl, Hct 13 1/1; wbc 5.8 × 10^9/l, polymorphos 63%, eosinophils 2%, basophils 2%, lymphocytes 25%, monocytes 10%, nucleated red cell (NRC) 2/100, decreased platelets. BM aspiration showed mild hypercellular marrow with marked decreased megakaryocytes. Erythropoiesis was increased with megaloblastic and dyserythropoietic changes, granulopoiesis showed 8% immature myeloid cells. A week later bleeding diathesis began. Corticosteroid was given without response. A course of vit. B_{12} injection was not successful in correcting her anemia. Direct Coombs' test was equivocal. Repeat BM aspiration a month

later showed 48% immature myeloid cells. Erythropoiesis was still megaloblastic with basophilic stippling in some NRC. Iron stain was 3-4+/4+ with rare ring sideroblasts. Cytochemical staining of the BM showed PAS positivity in erythroid precursors, some with block-like appearance. Peroxidase stain was positive < 5%, and non specific esterase stain was positive in 1%. Cytogenetic study of the BM was not done. Chemotherapy for leukemia was not given in view of poor performance status. Further clinical course was characterised by a moderate to high grade fever, which was probably due to leukemia, and repeated episodes of gastrointestinal bleeding. Barium studies failed to reveal any local pathology. Symptomatic and supportive therapy was given. Her last hospital visit was on August 8, 1984 because of fever and gastro-intestinal bleeding. CBC showed Hb 5.7 gm/dl, Hct 16 1/1; wbc 5.22 $\times 10^9$/l; polymorphos 56%, band 4% lymphocytes 40%; platelets 34 $\times$ 10^9/l; megaloblastic NRC 32/100 wbc. An incidental Hashimoto's thyroiditis was also found during the latter months of her illness. She probably expired not long after her last hospital visit.

Case 4 : Mrs. Y.N. (RH 1552305), a 45-year-old housewife, was admitted in March 1980 at a hospital because of an abdominal mass. Exploratory laparotomy revealed a stage I C mucinous cystadenocarcinoma of the right ovary. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. Adjuvant chemotherapy with monthly melphalan (3-6 days per cycle, 36-39 tab. per cycle) was given from March 1980-January 1982. Total drug given was 7,378 mg. There was no family history of cancer.

From January-March 1985 she was admitted to another hospital because of anemia. CBC showed Hb 8.6 gm/dl, Hct 25 1/1; wbc 10.5 $\times$ 10^9/l, polymorphos 52%, band 4%, meta 5%, eosinophils 3%, lymphocytes 36%, NRC 133/100 wbc; slight decreased platelets. Peripheral blood (PB) smear showed many fragmented red cells, occasional tear drop poikilocyte and echinocyte, rare polychromasia, few bilobed NRC; few hyposegmented polys; and few giant platelets. Some platelets had hypogranular and vacuolated cytoplasm. BM aspirate showed hypercellular marrow with marked erythroid hyperplasia. Most normoblasts were in basophilic or polychromatophilic stages. Some had multilobed nuclei and basophilic stippling. Granulopoiesis was markedly decreased with only few mature cells seen. There were less than 5% blasts. Megakaryocyte was adequate. Some had basophilic cytoplasm and single lobed nucleus. Iron stain was not done. Coombs' test, ANF, LE prep, anti DNA were negative. B_1c was 1540 µgm/ml. No specific therapy was given.

From 24 April 1985, she began to complain of weakness and low grade fever. She went to the latter hospital where another bone marrow was done. There was no significant change in the marrow picture from the previous one. Iron stain showed 2-3+/4+ with ring sideroblast. PB smear showed a further decrease in the number of leucocytes and platelets and an increase in NRC. Diagnosis of sideroblastic anemia, diabetes mellitus. and abscesses at labia majora and forehead were made. She was given prednisolone and fluoxymesterone for a few days. She later received 3 units of packed red cell at another hospital, where all medicine were discontinued.

She was admitted to Ramathibodi hospital on 1 May 1985 because of continued fever, pallor, and abscesses at forehead, labia and right axilla. A few petechiae, small ecchymoses and 3 cm hepatomegaly were observed. CBC revealed Hb 9.2 gm/dl; wbc 12.6$\times 10^9$/l, polymorphos 37%, band 17%, eosinophils 3%, lymphocytes 25%, monocytes 2%, mye-

locytes 3% promyeloblasts 4%, myeloblasts 4%, NRC 12/100 wbc; decreased platelets. BM aspirate yielded only marrow blood. BM imprint showed scattered myeloblast, and immature cells with fine reticulated chromatin, some with plasmacytoid cytoplasm. Serum globulin was normal. BM biopsy showed hypercellularity (cellularity 80%), increased erythroid series, myeloid hyperplasia and immaturity (<20% immatured cells), adequate megakaryocyte and increased iron store. She was considered to have refractory anemia with excess blasts. Cytogenetic studies from marrow blood revealed 42, X, –5, –6, –8, –11, –13, –15, –18, –19, +5 markers in 15 cells; 43, XX, –5, –6, –8, –11, –15, –18, –19, +5 markers in 40 cells. Double minutes were found in some cells (Fig. 2).

Penicillin G sodium and amikacin were given for infection with good response locally but without subsidence of fever. Nonsteroidal anti-inflammatory drug was given, but was able to normalize her temperature only briefly. Further clinical course was marked by fever and recurrent skin infection. PB neutrophils showed normal leucocyte alkaline phsophatase and peroxidase activities. Chemotherapy was not given. As of August 1985, she is still alive, but doing very poor. Her CBC, however, still showed only a few blasts. There were only few NRC in the PB, which was in sharp contrast to the findings in January.

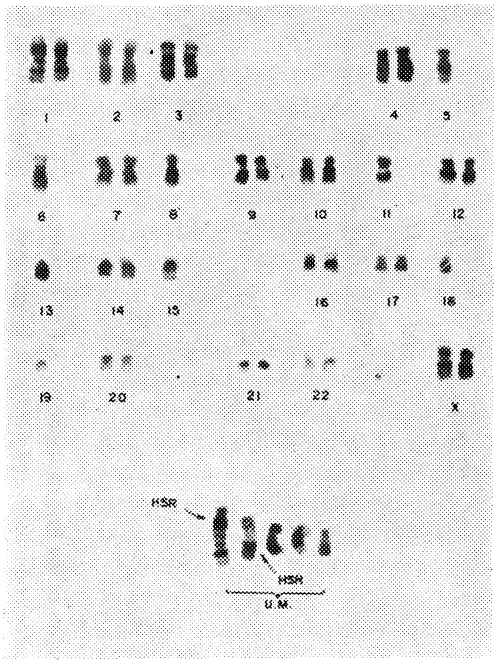

Fig. 2—G-banded karyotype from Case 4 showing abnormal chromosomes identified as 43, XX, –5, –6. –8, –11, –13, –15, –18, –19, and 5 unidentified marker (UM) chromosomes. Arrows indicated marker chromosomes which bear homogeneously staining regions (HSR).

DISCUSSION

From the above case histories, it can be seen that these four patients had a common feature. All were treated with chemo and radiotherapy (RT) for their primary malignant diseases. Common chemotherapeutic agent used was melphalan. Chemotherapy (CT) was given for a period in all except Case 2, who has been receiving medication until the last few months. After a variable lapsed period, hematologic disorders ranging from myelodysplastic syndrome (MDS) to ANLL developed. All except Case 2 were in clinical remission of their primary diseases. None except Case 1 received specific treatment for their hematologic disorders because of the predictably poor treatment result. As of August 1985, all except Case 4 were dead. The clinical features of four patients were summarized in Tables 1-2. It could be seen that they were consistent with the previously described secondary ANLL, which has recently been shown to be a pleuripotent stem cell disease (Tabilio *et al.*, 1984).

Besides the rather distinct clinical features, cytogenetic studies of the bone marrow cells were also done on two of our four patients because previous studies had indicated that the karyotypic findings would be useful for both diagnosis and prognosis of the disease

Table 1

Summary of 4 cases of therapy induced myelodysplastic (MDS) acute leukemic syndromes.

Case No.	Age,* Sex	Primary disease	Previous treatment			Interval from initial P_x to MDS (mo.)	Activity of 1° dis. at MDS	Specific P_x	Survival (from Dx of MDS) (mo.)	Cause of death
			RT (r)	CT (mg)	Duration (mo.)					
1.	30, F	CA breast	5,000		5/4	68	in remission	AraC + 6TG	9	cardiac arrest
				mel 1,010	27					
2.	40, F	multiple myeloma		ctx 69,600	60	167	not remission	nil	3	uncontrolled infection and bleeding
			2,000		2/4					
				mel 1,588	86					
3.	62, F	CA ovary	–	mel 520	12	22	in remission	nil	? 4**	? bleeding**
4.	45, F	CA ovary	–	mel 7,378	22	58	in remission	nil	7+	still alive (August' 85)

*Age at diagnosis of the first malignancy, RT = radiation, CT = chemotherapy, mo = months, mel = melphalan, ctx = cyclophosphamide, Arac = cytosine arabinoside and 6TG = 6 thioguanine, **on assumption, lost to follow up.

Table 2

Initial hematologic and marrow findings correlated with certain clinical features of 4 cases with myelodysplastic (MDS) acute leukemic syndrome.

Case No.	Hb/Hct	wbc ($\times 10^9$/l)	% blast	platelets ($\times 10^9$/l)	NRC (/100 wbc)	BM findings	BM karyotype	Major clin. manifest.	Time to leukemic conversion	Survival (months)
1.	8.8/25	6.1	1	252	–	RAEB in transformation	ND	pallor fever chest pain	never convert	9
2.	7.1/20	1.9	14	34	–	ANLL and myeloma	hyper-, eu-, hypoploidy with marker chromosomes	pallor fever	leukemia from the outset	3
3.	5.4/13	5.8	–	decreased	2	RAEB	ND	pallor fever bleeding	2	?4
4.	8.6/25	10.5	–	slight decreased	133	RA	hypoploidy with marker chromosomes	pallor fever skin infection	still in MDS*	still alive (7+)

RAEB = refractory anemia with excess of blasts, RA = refractory anemia, ND = not done, *as of August '85.

(Sandberg *et al.*, 1982). Although we can not say with certainty, that the karyotype of Case 2 were those of the leukemic cells in view of the remaining myeloma cells in the bone marrow; the complexity of the changes were more than those previously reported in untreated myeloma (Ferti *et al.*, 1984). Complexity of the karyotypic changes seen in our patient suggested karyotypic instability, which appeared to be the feature of secondary ANLL. Besides, it also indicated poor prognosis (shortest survival). After this period of considerable chromosomal variation, one stable clonal karyotype may ultimately emerge (Sandberg *et al.*, 1982). Incidentally, various karyotypic changes seen in our patient were similar to those previously reported in a similar type of patient (Rosner and Grunwald, 1980). Cytogenetic findings in Case 4 were more uniform, showing absence of several chromosome numbers including chromosome number 5; which has previously been shown to be one of the four chromosomes non randomly involved in secondary ANLL (Sandberg *et al.*, 1982). With all the available evidences, we, therefore, believe that all of our four cases suffered from therapy related acute myelodysplastic-leukemic syndrome.

Mechanism of drug carcinogenesis is not yet completely understood at present. The most widely held concept is drug induced chromosomal damage (Sieber and Adamson, 1975), and the chromosomal changes found in this disease seems to support it. Melphalan is a bifunctional alkylating agent that binds to DNA and produces chromosomal aberration as well as sister chromatid exchange in peripheral lymphocytes of cancer patients (Einhorn *et al.*, 1982). The drug is now considered to be a potent mutagen and a human carcinogen (IARC, 1980). Antimetabolites, with a different mode of action, are relatively safe. Chromosomal damage may lead to mutation. This, probably in conjunction with (an) other carcinogenic insult (s) (from treatment or environment) result (s) in malignant transformation (Yunis, 1983). However, genetic susceptibility and proficiency of DNA repair are probably very important in determining the final outcome of the involved tissue (Margison and Saffhill, 1979). Since malignant transformation process takes time, it is not unexpected to find a mean interval from the initial drug exposure to development of AL to be about 3-5 years in all reported series (Casciato and Scott, 1979; Rosner and Grunwald, 1980). Interestingly, mean latent periods for radiation leukemogenesis and for chemotherapy induced solid tumors are longer at 5-7 (Casciato and Scott, 1979) and 10 years (Coleman, 1982) respectively. Median total drug dose (mg) and median latent period (month) for the development of second malignancy were 1,550 and 48 for melphalan, and 47,000 and 54 for cyclophosphamide respectively (Schmall *et al.*, 1982).

Risk of developing secondary ANLL depends on the primary disease. Thus, in multiple myeloma, the risk was as high as 17.4% at 50 months (Bergsagel *et al.*, 1979). In ovarian cancer; the risk was $9.6 \pm 3.3\%$ at 7 years (Greene *et al.*, 1982), and in breast cancer it was only 0.3% (Rosner and Grunwald, 1980). The risk could possibly be reduced since we now know that there are certain strong predisposing factors to secondary ANLL, e.g. use of alkylating agents, aggressiveness of treatment and prolonged cytotoxic therapy. Thus, until we have a better understanding of the disease, we should avoid these factors as much as possible, especially in postoperative adjuvant (when tumor burden is low) and in non-neoplastic conditions. Since this complication almost always occurs long after the offending drugs have been discontinued; ways have to be found to monitor adverse drug effect during therapy, so that they can be discontinued immediately at the slight evidence of abnormality. Unfortunately, nothing concrete has

so far been found. However, one could possibly use the human tumor clonogenic assay to select the appropriate drugs prior to treatment, thereby avoiding therapeutically useless but potentially toxic drugs (Coleman, 1982).

To the best of our knowledge, this complication has not been reported previously in Thailand. This could simply be due to unawareness or absence of long term survivors. However, with increasing number of long term survivors from effective treatment of primary diseases, it is expected that more cases will be found. We believe that careful study of this condition may one day lead to a better understanding of leukemogenesis.

SUMMARY

Four cases of acute myelodysplastic - nonlymphocytic leukemia secondary to cytotoxic agents were reported. Primary diseases were breast cancer (1 patient), ovarian cancer (2 patients) and multiple myeloma (1 patient). All except one (with multiple myeloma) were in clinical remission of their primary diseases. Common cytotoxic agent used was melphalan. Median total drug dose and median latent period from diagnosis of primary diseases were 1299 mg and 63 months respectively. None with the exception of one received specific treatment. All died except one who is in a very poor condition. Survival from the diagnosis of hematologic diseases ranged from 3-9 months. Clinical features, cytogenetic findings, pathogenetic mechanism and risk of the disease were briefly discussed.

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