# Chemotherapy for acute myeloid leukemias with cytosine arabinoside, daunorubicin, etoposide, and mitoxantrone may cause permanent oligoasthenozoospermia or amenorrhea in middle-aged patients

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The aim was to follow-up gonadal functions in long-term survivors of acute myeloid leukemias (AML) after intensive chemotherapy based on high-doses of cytosine arabinoside (Ara-C) and anthracyclines in the study UHKT-911.

Adult patients were treated with at least 3 cycles of chemotherapy including 1–3 courses of Ara-C 10x2000 mg/m$^2$/12 h and daunorubicin (DNR) 2x45 mg/m$^2$/d. Spermiologic examinations were performed in 7 men by the classic microscopic method and results were evaluated according to the WHO criteria. Two patients (42- and 47-year-old) after DNR and Ara-C chemotherapy had nearly normal spermiologic findings. The semen of a 49-year-old patient contained normal numbers of spermatozoa with decreased velocity when examined 1 year after chemotherapy but 4 years later exhibited oligoasthenozoospermia. The patient received 4 cycles of Ara-C and DNR plus one cycle with etoposide 350 mg/m$^2$ and mitoxantrone 30 mg/m$^2$. Semen examination of two patients 55- and 59-year-old showed permanent oligoasthenozoospermia with only sporadic progressively motile spermatozoa which might not be compatible with fertilization by sexual intercourse. They received the same chemotherapy including cumulative doses of etoposide 500 mg/m$^2$ and mitoxantrone 36 mg/m$^2$. Semen of two patients after allogeneic bone marrow transplantation exhibited severe oligoasthenozoospermia with no motile spermatozoa. Permanent amenorrhea developed in two women (42- and 46-year-old) during chemotherapy with DNR, Ara-C, etoposide, and mitoxantrone which was not the case in three women (29–40 years old) treated without etoposide and mitoxantrone.

Intensive chemotherapy with high-doses of Ara-C and DNR plus one cycle of etoposide and mitoxantrone may cause permanent gonadal dysfunction in middle-aged patients with AML.

*Key words: acute myeloid leukemia, high-doses of cytosine arabinoside, daunorubicin, etoposide, oligoasthenozoospermia, premature menopause*

Chemotherapy with alkylating agents may cause permanent azoospermia in men and permanent amenorrhea in women in a dose-dependent manner [11]. Other chemotherapeutic agents including e.g. those used for treatment of patients with acute leukemias are believed to induce only temporary severe gonadal dysfunction which is usually completely reversible after the end of chemotherapy [7, 13, 16]. Intensive chemotherapy with high-doses of cytosine arabinoside (Ara-C) alone or in combination with anthracyclines has improved disease-free or overall survival in patients with acute myeloid leukemias (AML). This improvement was described in various types of cytogenetic [12] or biological [9] categories of AML. Although high-doses of Ara-C based regimens are widely used for patients with AML in most hematological centers their influence on male and female fertility was not described.

The aim of our study was to perform andrological and gynecological examinations of long-term AML survivors treated with a regimen containing high-doses of Ara-C and daunorubicin (DNR) in the study ÚHKT-911 [9, 10]. Furthermore, we compared results of these examinations with those of AML survivors after allogeneic bone marrow transplantation carried out in the same time and institute.

## Patients and methods

Patients with various types of acute myeloid leukemias (AML) were diagnosed according to the French-American-British classification criteria [2] with the use of the described methods [8]. Patients were treated with 4–5 chemotherapy cycles according to the ÚHKT-911 study at the Institute of Hematology and Blood Transfusion (UHKT) in Prague in years 1991–1995 [9, 10]. Induction chemotherapy consisted of these treatment types:

a) standard: standard doses of Ara-C (Alcysten, Spofa, Czech Republic) 150–200 mg/m$^2$ per 3-h infusion every 12 hours for 7 days and 3–4 doses of DNR (Rubomycin, Medexport, Russia) 45 mg/m$^2$ per day on days 1, 3, 5, (and 7).

b) HD: high-doses of Ara-C 2000 mg/m$^2$ per 3-h infusion every 12 hours for 5 days and DNR 45 mg/m$^2$ per day on days 4 and 5.

c) EMi: etoposide (Vepesid, Bristol-Myers, Germany) 100 mg/m$^2$ per day for 5 days and mitoxantrone (Refador, Spofa, Czech Republic) 10–12 mg/m$^2$ per day on days 1, 3, and 5.

Patients in complete remission (CR) received 2–4 consolidation cycles (1–3 HD and one standard or one EMi cycle) according to their tolerance of chemotherapy [9, 10].

Three patients in their 1st or 2nd CR were treated with HLA-matched allogeneic bone marrow transplantation (AlloBMT) from their brothers after myeloablative preparative regimens containing high-doses of alkylating agents.

Semen samples were produced in the clinic by masturbation after at least 4-days sexual abstinence. Semen analyses were performed on fresh specimens by the classic microscopic method [6] in patients at least 1 year after the end of chemotherapy and were evaluated according to the WHO criteria [17]. Informed consent for the treatment and the andrological or gynecological examination was obtained from all patients. The study and all examinations were approved by the Institutional Ethical Committee on Human Experimentation in accordance with the Helsinki Declaration of 1975.

## Results

*Male patients treated only with chemotherapy.* Five male patients were treated only with chemotherapy. Their characteristics, the given cumulative doses of cytostatics, and spermiologic findings are summarized in Table 1. Two of 3 patients with pathological findings (patient 3 and 5) were examined twice to confirm initial results. Pathological findings are underlined in the Table 1.

Two patients (patient 1 and 2, Tab. 1) who were 42- and 47-year-old at examination, respectively, exhibited normal or nearly normal (a decreased ejaculate volume) spermiologic findings.

The patient 3 was examined twice. For the first time it was 1 year after the end of chemotherapy at his age of 49 years when only the decreased propulsivity index 800 and the decreased average spermatozoal velocity 14 μm/s were found. Other sperm characteristics were normal (Tab. 1). The second andrological examination of the patient 3 was carried out 5.5 years after chemotherapy at his age of 54 years when his ejaculate volume, sperm concentration, motile sperm count, propulsivity index, and average spermatozoal velocity were decreased (Tab. 1).

The patients 4 and 5 were 55 and 59 years old at examination, respectively, and exhibited oligozoospermia and severe asthenozoospermia with only sporadic progressively motile

**Table 1. Male patients' characteristics, cumulative doses of cytostatics and spermiologic findings**

| | Normal range | Units | Patient 1 | Patient 2 | Patient 3 | | Patient 4 | Patient 5 | |
|---|---|---|---|---|---|---|---|---|---|
| AML FAB type | | | M3 | M2 | M4 | | M5 | M2 | |
| Age at examination | | years | 42 | 47 | 49 | 54 | 55 | 59 | |
| Time after therapy | | years | 1.2 | 1.0 | 1.0 | 5.5 | 5.0 | 4.1 | 4.5 |
| Cytosine arabinoside | | mg/m$^2$ | 45 600 | 62 800 | 45 600 | | 45 600 | 47 600 | |
| Daunorubicin | | mg/m$^2$ | 450 | 540 | 510 | | 530 | 510 | |
| Mitoxantrone | | mg/m$^2$ | 0 | 0 | 30 | | 36 | 36 | |
| Etoposide | | mg/m$^2$ | 0 | 0 | 350 | | 500 | 500 | |
| Ejaculate volume | ≥ 2.0 ml | | 2.0 | **1.5** | 2.0 | **1.5** | 2.0 | 5.0 | 3.0 |
| Sperm concentration | ≥ 20.0 x 10$^6$/ml | | 72 | 130 | 68 | **15.0** | **1.5** | **8.0** | **12.0** |
| Motility | ≥ 50% | | 60 | 70 | 65 | 50 | **sporadic** | **sporadic** | **sporadic** |
| Motile sperm count | ≥ 20.0 x 10$^6$ | | 86 | 137 | 88 | **12** | | | |
| Time of passing | ≤ 60 s | | 52 | 20 | **140** | **2 280** | | | |
| Propulsivity index | ≥ 1 000 | | 1 750 | 5 000 | **800** | **45** | | | |
| Average velocity | ≥ 20.0 μm/s | | 32 | 50 | **14** | **5** | | | |
| Normal morphology | ≥ 30% | | 58 | 55 | NE | 44 | NE | NE | 44 |

Abnormal findings are underlined. NE – not examined.

spermatozoa (Tab. 1). Their propulsivity index and average spermatozoal velocity were not measurable and motile sperm count could not be calculated because of only sporadic progressive motility of spermatozoa. Spermiologic findings in patients 4 and 5 would probably lead to their infertility by sexual intercourse.

*Male patients after allogeneic bone marrow transplantation*. Two patients were treated with chemotherapy and sibling HLA-matched allogeneic bone marrow transplantation (AlloBMT) after myeloablative conditioning regimen.

Semen examination of the 27-year-old patient with AML M6 was performed 6 years after his AlloBMT. His preparative regimen for AlloBMT consisted of fractionated total body irradiation 10 Gy plus cyclophosphamide 2x60 mg/kg i.v. at his 2nd CR. His ejaculate volume was 0.6 ml with less than 1 million/ml of spermatozoa without any progressive motility. This patient did not succeed in obtaining his semen for cryopreservation before chemotherapy, when he was 19-year-old, probably because of his bad clinical status and psychic distress caused by adverse diagnosis.

The 40-year-old patient with RAEB-T/AML M4 was examined 4.5 years after his AlloBMT with the Bu4Cy2 (busulphan 16 mg/kg + cyclophosphamide 120 mg/kg) myeloablative preparative conditioning regimen. His ejaculate volume was 1.8 ml with no spermatozoa found in his semen after centrifugation (azoospermia).

*Gonadal functions in females after chemotherapy.* We followed-up 5 women, 29–46 years old, treated with chemotherapy in the study (Tab. 2). Three younger women (patients 1–3; 29, 31, 40 years old) who had been treated with DNR and Ara-C did not experience permanent amenorrhea during or immediately after this treatment. However, permanent amenorrhea developed during chemotherapy in two older middle-aged females (42 and 46 years old) who were treated with higher cumulative doses of Ara-C and DNR including one cycle of etoposide and mitoxantrone (Tab. 2).

Furthermore, permanent amenorrhea developed in the patient 2 after myeloablative preparative regimen (total body irradiation 10 Gy plus cyclophosphamide 2x 60 mg/kg i.v.) given before bone marrow transplantation from her HLA-identical brother in her 2nd CR at the age of 32.5 years (Tab. 2).

**Table 2. Female patients' characteristics, cumulative doses of cytostatics and menopause onset**

| | Units | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 |
|---|---|---|---|---|---|---|
| AML FAB type | | M4 | M4 | M4 | M2 | M2 |
| Age at chemotherapy | years | 29 | 31 | 40 | 42 | 46 |
| Cytosine arabinoside | mg/m$^2$ | 37 600 | 46 000 | 25 800 | 62 400 | 62 800 |
| Daunorubicin | mg/m$^2$ | 460 | 470 | 445 | 420 | 440 |
| Mitoxantrone | mg/m$^2$ | 0 | 0 | 0 | 30 | 30 |
| Etoposide | mg/m$^2$ | 0 | 0 | 0 | 500 | 500 |
| Age of menopause | years | none at 36 | none at 32 | 46 | 42 | 46 |
| Menopause onset | | still none | at 32.5 after BMT | 6 years after CHT | during CHT | during CHT |

BMT – bone marrow transplantation, CHT – chemotherapy.

## Discussion

This study has demonstrated that permanent severely impaired spermatogenesis leading to probable *in vivo* infertility was found in two of five examined middle-aged men, and premature menopause in two of five women after combination chemotherapy with non-alkylating agents. Unfortunately, the reported 5 men had not been examined before chemotherapy and thus pretreatment semen analyses from them were not available. However, they all had fathered at least one progeny and reported no fertility problems. Higher age of men up to 60 years does not usually lead to changes in sperm quality similar to those found in our two patients [14]. Thus the administered chemotherapy itself appears to be the main cause of impaired semen findings.

The median age of menopause onset in Czech women is about 51 years with a wide range of 40–53 years that is considered normal [5]. Permanent amenorrhea developed in two women 42 and 46 years old during chemotherapy that seems to be the major causal factor. However, their age may be a contributing factor as demonstrated in women with breast cancer treated with combined CMF chemotherapy containing alkylating agent cyclophosphamide plus methotrexate and fluorouracil [4].

Chemotherapy for AML based on repeated cycles of high-doses of Ara-C and DNR plus an additive toxic effect of one cycle with etoposide and mitoxantrone may lead to premature menopause or severe asthenozoospermia that might not be compatible with fertilization via sexual intercourse. It seems that the sole combination of daunorubicin and high-doses of Ara-C did not cause changes of spermiologic findings (patients 1 and 2) or premature menopause (patients 1–3). The addition of one cycle with etoposide and mitoxantrone in the described doses with the previously given higher doses of Ara-C and DNR were the most important factors for gonadal toxicity in men over 50 years and in women over 40 years. The influence of this combined chemotherapy on gonadal functions of younger patients is not known.

Although the number of male patients with AML in our study is small – 7, it is comparable to the

numbers of reported patients with AML (4–8 patients) in published studies describing no permanent gonadal dysfunctions after chemotherapy but with lower doses of non-alkylating agens [7, 16]. On the basis of our original findings we recommend semen cryopreservation before the start of chemotherapy in patients with AML. However, the semen collection by masturbation was unsuccessful in two our young patients aged 19 and 30 years at diagnosis before the start of chemotherapy.

Radiation therapy and high doses of alkylating agents are usually included into bone marrow or peripheral blood stem cell transplantation preparative regimens and cause infertility in more than 70% of men and 80% of women [15]. Semen analyses in our two transplanted males and premature menopause at the age of 32.5 years in one female after myeloablative preparative regimens corresponded well with the previously described severely impaired fertility of transplanted patients [1, 11, 15].

The advent of assisted reproductive technologies (ART) as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) increased the success rate for achieving a pregnancy [3, 11]. These techniques could not be probably used in one our transplanted patient with no spermatozoa in his ejaculate (azoospermia) but could be used in patients with severe asthenozoospermia after chemotherapy in whom few residual viable spermatozoa may be sufficient for successful use of ICSI. Repeated andrological examinations should be performed in the transplanted case with the finding of azoospermia, because 3x repeated semen examination is necessary for the establishment of the diagnosis of ejaculatory azoospermia. Furthermore, testis sperm extraction from patients with postchemotherapy ejaculatory azoospermia may provide spermatozoa that can be successfully used with ICSI to get healthy offspring [3].

We conclude that in long-term AML survivors we have unexpectedly observed permanent severe asthenozoospermia in men over 50 years and permanent amenorrhea in women over 40 years as undesirable complications of intensive chemotherapy without alkylating agents. Alkylating agents had been previously thought to be the only chemotherapeutic agents causing permanent gonadal dysfunction. However, the severity of gonadal dysfunction after the given intensive chemotherapy in the study seems to be lower than that in patients treated with stem cell transplantation after myeloablative conditioning regimens containing alkylating agents.

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