Topoisomerase IIα mRNA and protein expression vs. *in vitro* drug resistance and clinical outcome in acute leukaemia

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Abstract. The objective of this study was to correlate the expression of topoisomerase (topo) IIα to *in vitro* drug sensitivity and to the clinical outcome in patients with acute leukaemia. Leukaemic cells were isolated from bone marrow or blood from 94 patients. Topo IIα mRNA (n=58) and protein (n=60) expression was determined by real-time RT-PCR and flow cytometry, respectively. In both groups, chemosensitivity testing by a bioluminescence ATP assay was performed to a variable extent for both topo IIα poisons and non-topo IIα targeting drugs. Topo IIα mRNA expression varied with relative values ranging from 0.03 to 14.20 (median 1.10). The median value for topo IIα protein-positive cells was 23% (range 0-99%). Cell samples from patients with a high (>median value) percentage of topo IIα-positive cells were significantly more sensitive to the topo IIα active drugs etoposide and daunorubicin, and showed a borderline value for idarubicin (p=0.08), while there was no difference for non-topo IIα targeting drugs. However, we did not find any significant differences in mRNA expression or the percentage of topo IIα-positive cells in patients who achieved complete remission after at most two induction courses compared with those who did not, nor did we find any difference in survival when patients with high mRNA expression/percentage of topo IIα-positive cells were compared with patients with low values. We conclude that expression of topo IIα, determined as percentage of topo IIα-positive cells, in leukaemic cells correlates to chemosensitivity *in vitro* against topoisomerase poisons but that it does not predict clinical outcome in acute leukaemia.

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Key words: topoisomerase IIα, acute leukaemia, drug resistance, prognosis, reverse transcriptase-polymerase chain reaction, flow cytometry

Introduction

A major problem in the treatment of acute leukaemia is intrinsic or acquired resistance to cytostatic drugs. Several mechanisms of such drug resistance have been identified in experimental tumour systems (1). The one most extensively studied is drug transport involving efflux pumps, among which p-glycoprotein has been shown to be of prognostic value (2). Another level of resistance, drug targets, may involve topoisomerases.

Topoisomerase IIα (topo IIα) is a nuclear enzyme, which is important for transcription, replication and mitosis. The enzyme introduces transient double-strand breaks, thereby catalyzing changes in DNA topology by passing a double-stranded DNA helix through this transient break site which is then religated (3). In normal cells, topo IIα is expressed in a cell cycle-dependent pattern with low levels in the G0/G1 phase and high levels in S/G2/M (4). There are data indicating that topo IIα expression in malignant cells is less cell cycle dependent, and that topo IIα could be significantly expressed in G0/G1 as well (5-8).

Topo IIα is the primary target for cytostatic drugs, such as anthracyclines, epipodophyllotoxins and amsacrine (9). These drugs convert the reversible double-strand break into an irreversible one, a 'cleavable complex' (10); and this DNA cleavage is considered the main mechanism to induce apoptosis, although other mechanisms could also be of importance (11). In different tumour cell lines, including leukaemic cells, low topo IIα content and/or activity has been correlated to drug resistance (12-16), although there have been contradictory results (17). Previous reports on topo IIα expression and clinical outcome have been negative regarding both mRNA (18,19) and protein (20) level, with one exception (21). In a previous study on acute leukaemia, we demonstrated that topo IIα protein is also expressed in the G0/G1 cell cycle phase, and suggested a correlation to clinical outcome (7).

The aim of the present study was to correlate topo IIα mRNA and protein expression to chemosensitivity *in vitro* and to further investigate the impact of topo IIα expression on response to induction therapy and prognosis in acute leukaemia.

Table I. Patient characteristics and topo IIα expression.

No.	Gender	Age	*De novo*/ secondary	FAB subtype	Leukocyte count x10^9	Karyotype	Cytogenetic risk group	Response[a]	BM/PB[b]	Topo II protein[c]			Topo II mRNA[d]
										Overall %	G0/G1 %	S/G2/M %	
1	F	33	*De novo*	M5a	10	Complex	Adverse	N	BM	71	69	95	
2	F	70	*De novo*	M2	193	Trisomy 8,13	Intermediate	R	BM	41	34	92	
3	F	67	*De novo*	M1	1	Normal	Intermediate	N	BM	8	3	43	
4	F	18	*De novo*	T-ALL	46	Complex		N	PB	0	0	3	
5	F	78	Secondary	M2	11	Complex	Adverse	NE	BM	13	6	55	
6	M	74	*De novo*	AML not spec	33	Normal	Intermediate	NE	BM	12	18	69	
7	F	61	*De novo*	M4	4	Normal	Intermediate	R	BM	89	88	91	
8	M	48	*De novo*	T-ALL	14	Normal		R	BM	30	24	81	
9	F	20	*De novo*	preB-ALL	5	Complex		R	PB	49	45	83	
10	M	46	Secondary	M2	2	Normal	Intermediate	R	BM	63	73	91	
11	F	39	*De novo*	M2	21	Normal	Intermediate	NE	PB	13	7	59	0.15
12	M	67	*De novo*	M4	18	Monosomy 22	Intermediate	NE	PB	62	61	89	
13	F	70	*De novo*	M1	2	n.d.		NE	BM	24	32	74	
14	F	83	*De novo*	M1	100	Normal	Intermediate	NE	PB	3	62	93	0.40
15	F	67	*De novo*	M2	24	Normal	Intermediate	NE	BM	14	6	79	2.40
16	F	36	*De novo*	M1	23	Normal	Intermediate	NE	BM	89	90	92	
17	F	78	Secondary	M1	94	Normal	Intermediate	NE	BM	6	13	69	
18	F	37	*De novo*	M2	51	Complex	Adverse	N	BM	22	15	84	
19	F	82	Secondary	M0	210	Normal	Intermediate	NE	PB	95	96	91	
20	M	54	*De novo*	M1	132	Normal	Intermediate	N	BM	28	22	82	1.12
21	F	78	*De novo*	M0	147	Failed		NE	PB	10	6	70	0.26
22	F	84	*De novo*	M1	63	Normal	Intermediate	NE	BM	21	13	44	
23	F	63	*De novo*	M4	165	Normal	Intermediate	R	BM	14	9	64	0.39
24	M	43	*De novo*	M1	12	Normal	Intermediate	R	PB	64	62	92	0.70
25	M	55	*De novo*	M1	28	Normal	Intermediate	R	BM	67	66	87	
26	F	60	*De novo*	M1	14	Trisomy 21	Intermediate	R	PB	17	13	66	4.53
27	F	56	*De novo*	M3	2	t (15:17)	Favourable	R	BM	1	1	1	
28	M	74	Secondary	M2	3	Monosomy 7	Adverse	NE	BM	5	1	44	1.66
29	F	81	Secondary	M0	110	t (9:22) Trisomy 8	Intermediate	NE	BM	15	6	60	
30	M	81	*De novo*	M1	5	n.d.		R	BM	6	3	24	
31	M	70	Secondary	AML not spec	10	Normal	Intermediate	N	BM	0	0	0	1.05
32	M	65	*De novo*	M1	9	Normal	Intermediate	R	BM	10	6	35	1.09
33	F	64	*De novo*	AML not spec	14	t (8:21), del (9)	Favourable	R	BM	71	69	89	
34	F	75	*De novo*	M4	22	Normal	Intermediate	R	PB	20	16	79	0.38
35	M	81	*De novo*	M2	4	Complex	Adverse	R	BM	2	1	31	0.86
36	F	83	Secondary	AML not spec	2	Trisomy 11, mono 21	Intermediate	NE	BM	0	0	0	0.77
37	M	44	Secondary	M2	147	t (9:22)	Intermediate	N	BM	2	0	5	
38	F	51	*De novo*	M3	1	t (15:17)	Favourable	R	BM	19	18	30	0.96
39	F	46	*De novo*	M2	50	Normal	Intermediate	R	BM	24	15	62	
40	F	65	*De novo*	M2	10	Complex	Adverse	R	BM	0	0	12	0.50
41	M	45	*De novo*	M5a	81	47XYY	Intermediate	R	PB	9	6	50	0.23
42	F	63	*De novo*	M1	5	Normal	Intermediate	R	BM	10	2	31	0.21
43	M	45	*De novo*	preB-ALL	6	Normal		R	BM	13	19	33	
44	M	35	*De novo*	M4	13	Complex	Adverse	R	BM	9	2	47	
45	F	33	*De novo*	M4	170	Inv (16)	Favourable	NE	BM	88	88	75	0.32
46	F	80	*De novo*	preB-ALL	16	Complex		R	BM	73	77	69	
47	F	44	*De novo*	M1/M2	13	t (8:21)	Favourable	N	BM	86	86	92	
48	F	48	*De novo*	M1	30	t (9:22) minor	Intermediate	R	BM	92	91	98	

Table I. Continued.

No.	Gender	Age	*De novo*/ secondary	FAB subtype	Leukocyte count x10^9	Karyotype	Cytogenetic risk group	Response[a]	BM/PB[b]	Topo II protein[c]			Topo II mRNA[d]
										Overall %	G0/G1 %	S/G2/M %	
49	F	80	*De novo*	M2	8	del16	Intermediate	NE	BM	16	10	64	1.75
50	M	71	*De novo*	M5a	260	Complex	Adverse	NE	BM	95	95	89	0.73
51	F	44	*De novo*	M1	52	Normal	Intermediate	N	PB	62	61	71	
52	F	76	*De novo*	M5a	2	Monosomy 17, del (5)	Adverse	NE	PB	99	99	98	
53	F	54	*De novo*	M2	215	n. d.		R	PB	58	54	84	
54	M	82	*De novo*	M1	1	Trisomy 8	Intermediate	NE	BM	85	92	87	
55	M	32	*De novo*	T-ALL	8	t(15;15), Inv (1), del (16)		R	BM	93	95	93	
56	F	40	*De novo*	M2	54	Normal	Intermediate	R	BM	80	80	94	2.57
57	F	71	*De novo*	M4	62	Normal	Intermediate	R	BM	83	83	76	0.27
58	F	27	*De novo*	M2	129	Normal	Intermediate	R	BM	71	72	44	0.11
59	M	71	*De novo*	M2	7	Failed		R	BM	47	47	47	
60	M	30	*De novo*	M1/M2	33	t (1:11)	Intermediate	N	BM	71	70	72	
61	F	66	*De novo*	M1	38	n. d.		R	PB				15.32
62	F	27	*De novo*	M4	85	Normal	Intermediate	R	PB				4.11
63	F	73	*De novo*	M2	39	Normal	Intermediate	R	PB				8.51
64	M	66	Secondary	AML not spec	3	Trisomy 8,11,15	Intermediate	R	BM				1.38
65	F	29	*De novo*	M4	60	Normal	Intermediate	R	PB				0.16
66	F	71	*De novo*	M2	20	Normal	Intermediate	R	PB				1.86
67	M	44	*De novo*	M1	1	Failed		N	BM				0.71
68	F	22	*De novo*	M6	14	Trisomy 8	Intermediate	R	BM				7.03
69	M	32	*De novo*	M3	30	t (15:17)	Favourable	R	PB				0.44
70	F	44	*De novo*	M1/M2	238	Monosomy 7	Adverse	N	BM				2.53
71	F	56	*De novo*	M2	25	Inv (16)	Favourable	R	PB				0.26
72	F	37	*De novo*	M6	2	Normal	Intermediate	R	BM				1.75
73	F	78	*De novo*	M1	32	Normal	Intermediate	R	PB				0.04
74	F	41	*De novo*	M1/M2	258	Normal	Intermediate	N	PB				0.82
75	F	56	*De novo*	M2	53	Normal	Intermediate	N	BM				3.04
76	M	47	*De novo*	M2	0	Normal	Intermediate	R	BM				2.31
77	M	42	*De novo*	M2	3	Normal	Intermediate	R	BM				0.96
78	M	34	*De novo*	M4	212	Trisomy 6	Intermediate	N	BM				1.72
79	M	43	*De novo*	M5a	1	Failed		R	BM				2.02
80	F	78	*De novo*	M1	108	Normal	Intermediate	R	BM				1.45
81	F	74	*De novo*	M2	1	Normal	Intermediate	R	BM				0.59
82	F	65	Secondary	AML not spec	17	t (3:21)	Intermediate	N	BM				2.36
83	F	70	*De novo*	M2	1	Trisomy 2	Intermediate	N	BM				0.33
84	M	74	*De novo*	M4	3	Failed		N	BM				0.24
85	M	71	*De novo*	M5a	12	Monosomy 13,17	Intermediate	R	BM				8.20
86	F	73	*De novo*	M1	3	Normal	Intermediate	R	BM				7.91
87	M	35	Secondary	M4	30	Complex	Adverse	N	BM				0.59
88	F	74	*De novo*	M2	60	Normal	Intermediate	R	BM				0.60
89	F	51	*De novo*	M6	36	inv (9), del (3)	Intermediate	R	BM				4.25
90	F	76	*De novo*	M1	16	Normal	Intermediate	R	BM				9.82
91	M	48	*De novo*	M1	25	Trisomy 13	Intermediate	N	BM				6.13
92	F	79	*De novo*	M4	36	Complex	Intermediate	R	PB				3.09
93	F	70	*De novo*	M0	14	Normal	Intermediate	R	BM				1.26
94	M	60	*De novo*	M1	10	Trisomy 8, mono 12	Intermediate	R	BM				3.14

[a]R, responder; N, non-responder; NE, not evaluable. [b]BM, bone marrow sample; PB, peripheral blood sample. [c]Percentage topo IIα-positive cells. [d]Arbitrary units.

Materials and methods

Patients. Bone marrow (BM, n=70) or peripheral blood (PB, n=24) samples, collected from 94 patients with newly diagnosed acute leukaemia, were investigated after ethics committee approval and informed consent. The patients had a mean age of 58 (range 18-84); 61 were women and 33 were men. Seventy-six patients had *de novo* AML and 12 had AML secondary to myelodysplastic syndrome (n=4), CML (n=3), Hodgkin's lymphoma (n=1), essential thrombocythemia (n=1), or cytostatic treatment for other reasons (n=3). Three patients had pre-B-ALL and 3 patients had T-ALL, all *de novo*. Cytogenetic analysis was routinely performed for 85 patients. Based on criteria derived from the MRC AML 10 trial (22), 7 patients with AML were defined as having favourable karyotype whereas 61 and 11 patients had intermediate or adverse karyotype, respectively. For patient data in detail, see Table I.

Samples from 58 patients were vitally frozen and used for real-time RT-PCR. Forty-nine of these patients were evaluable for clinical response to induction treatment. Fresh samples from 60 patients (including 24 of the patients who provided samples for RT-PCR) were used for flow cytometry. In this group, 40 patients were evaluable for clinical response. In both groups, patients who were not evaluable for clinical response received only palliative treatment due to poor performance status; or, in a few cases, died early during induction treatment.

Patients with AML who were evaluable for clinical response to induction treatment received at least one course of induction therapy containing an anthracycline or mitoxantrone (1 patient received amsacrine) in combination with cytarabine. Thirty patients received an additional drug which in 14 cases was etoposide. When a second induction course was given, it contained amecrine instead of anthracycline or mitoxantrone in 17 cases, and etoposide was added in 23 cases. Patients with ALL received treatment according to the Swedish Adult ALL Group protocol, which includes daunorubicin in the first induction course and amsacrine in the second induction course.

Complete remission (CR) was defined as ≤5% bone marrow blasts after recovery from induction therapy. In three cases, response was defined as CR despite the presence of 5.5-6% blasts. Clinicians had considered these patients to be in CR and the patients remained in CR during follow-up. A patient was defined as a responder if CR was achieved with at most two induction courses and as a non-responder otherwise.

Laboratory methods. For real-time RT-PCR, vitally frozen mononuclear cells, continually collected at the participating hospitals, were used. The leukemic cell line CEM, cultured with 89% RPMI, 10% fetal bovine serum and 1% L-glutamine, was used to construct standard curves. Thawed patient sample cells and fresh CEM cells were washed in Dulbecco's PBS (Gibco, Invitrogen, Paisley, UK) before RNA isolation. RNA was isolated using QIAamp RNA Blood Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, and was stored at -80˚C. The quality and quantity of RNA was determined using RNA chips with RNA 6000 Nano Reagents & Supplies (Agilent Technologies, Waldbronn, Germany) with a Bioanalyzer (Agilent Technologies).

cDNA was synthesized from 400 ng RNA from each patient sample or cell line to a volume of 40 μl, which was stored at -20˚C. For cDNA synthesis, Omniscript RT kit (Qiagen) or first-strand synthesis kit for RT-PCR (Roche, Mannheim, Germany) with OligodT primer, was used. To perform a real-time PCR, a mix containing Taqman Universal PCR Master mix (Applied Biosystems), primer (1 μM), probe (0.25 μM), 2 μl cDNA, and water to a total volume of 25 μl was prepared and the samples were amplified in duplicates using ABI PRISM 7700 (Applied Biosystems, Weiterstadt, Germany). Primers and probes for the house-keeping gene ß-actin and topo IIα are shown in Table II.

Standard curves were constructed for ß-actin and topo IIα by serial dilutions of the purified (QIAquick Gel Extraction kit, Qiagen) amplification products using cDNA from the leukemic cell line CEM. Patient samples were related to the standard curves, giving a relative quantification of the gene product. Topo IIα expression (mean value of the duplicates) was normalized by division with the ß-actin expression. This quotient was used for statistics. Flow cytometry was performed as previously described (7).

Chemosensitivity *in vitro* was tested by a bioluminescence ATP assay as previously described (23). Briefly, ATP levels in a specific cell type are relatively constant in living cells but degrade rapidly if the respiratory cycle is disturbed. The level of ATP is therefore proportional to the number of viable cells in a sample. ATP levels are determined in cell samples cultured with cytostatic drugs, and expressed as a percentage of living cells in comparison to samples cultured without cytostatic drugs, that is, a resistant cell sample will achieve a high percentage value in this test. Every group tested for a certain drug was divided into two groups, using as cut-off the median value for topo IIα mRNA expression or percentage of topo IIα-positive cells, respectively. Thus, for every tested drug, one group with 'low' topo IIα mRNA/percentage of topo IIα protein-positive cells and one group with 'high' topo IIα mRNA /percentage of topo IIα protein-positive cells were compared according to chemosensitivity *in vitro*.

Statistics. The Mann-Whitney test was used for comparison of mRNA and protein expression in the groups defined by clinical response, cytogenetic risk and *de novo*/secondary AML. Survival curves were calculated according to Kaplan-Meier, and the log-rank test was used for comparison of survival. Student's t-test for independent groups was used for comparison of chemosensitivity *in vitro* in different groups of patient samples. Statistics were calculated using SPSS 11.5 for Windows (SPSS Inc., Chicago, IL).

Results

Topo IIα mRNA. Topo IIα mRNA expression varied widely, with relative values ranging from 0.04 to 15.32 arbitrary units (median 1.10) and a skewed distribution (Fig. 1). No statistically significant differences were found between topo II mRNA expression in samples from patients in different cytogenetic risk groups or from patients with *de novo* and secondary AML.

Topo IIα protein. The overall expression of topo IIα varied, with median 23% positive cells (range 0-99%) and a bimodal distribution (Fig. 2). Cells in the S/G2/M cell cycle phase expressed topo IIα to a higher extent than cells in the G0/G1

Table II. Polymerase chain reaction (PCR) primers (forward and reverse) and probes.

ß-actin forward	CTGGCTGCTGACCGAGG
ß-actin reverse	GAAGGTCTCAAACATGATCTGGGT
ß-actin probe	CCTGAACCCCAAGGCCAACCG
Topo IIα forward	CGCTTATCCTGACTGAGGGAGAT
Topo IIα reverse	CTAAGAGGGAAAACCCCATATTTG
Topo IIα probe	TCAGGCCTTGGTGTGGTT

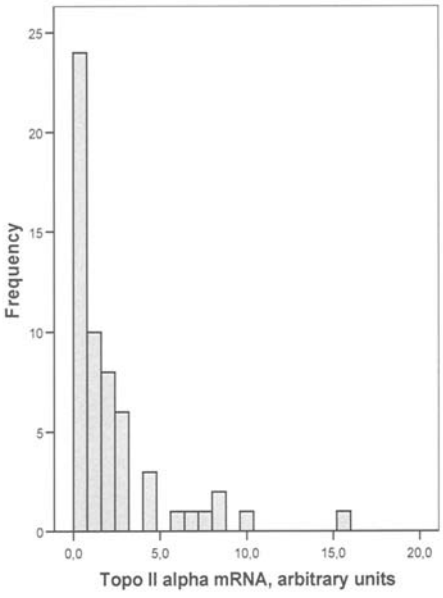

Figure 1. Frequency histogram of topo IIα mRNA (arbitrary units) in 58 patients with acute leukaemia.

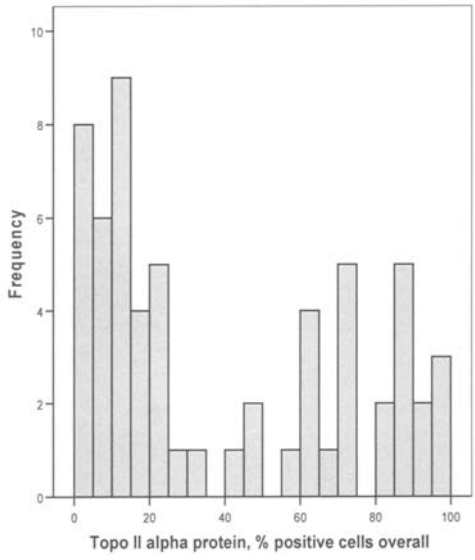

Figure 2. Frequency histogram of overall percentage of topo IIα protein-positive cells in 60 patients with acute leukaemia.

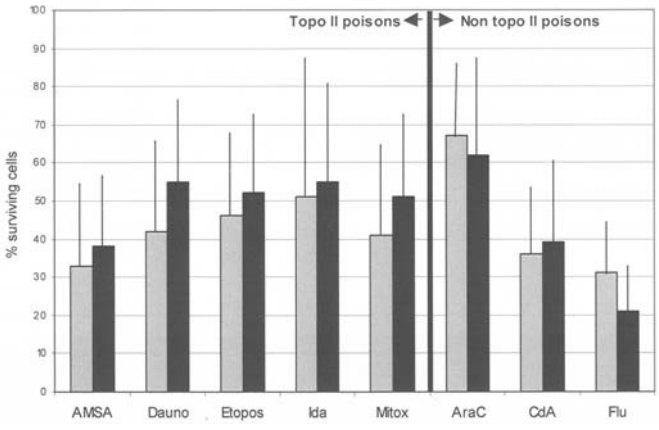

Figure 3. *In vitro* sensitivity to different cytostatic drugs in patient samples with high (grey bars) or low (black bars) expression of topo IIα mRNA. The groups of high and low expression were defined by dividing each group of patients, tested for a certain drug, into two groups with median expression as cut-off. Sensitivity is expressed as a percentage of living cells after incubation with the drug, i.e. a relatively low percentage indicates relatively more sensitive cells. Vertical lines indicate standard deviation for each sample. AMSA, amsacrine (n=44); Dauno, daunorubicin (n=46); Etopos, etoposide (n=31); Ida, idarubicin (n=44); Mitox, mitoxantrone (n=43); AraC, cytarabine (n=37); CdA, cladribine (n=31); Flu, fludarabine (n=25).

cell cycle phase; median 70.5% (range 0-98%) positive cells vs. 20.5% (0-99%).

In samples from patients with favourable karyotype (n=5), the proportion of topo IIα-positive cells had a median of 71% (range 1-88%) overall, 69% (1-88%) for cells in the G0/G1 phase, and 75% (1-92%) for cells in S/G2/M, while samples from patients with intermediate karyotype (n=35) showed positivity with a median of 21% (0-95%) overall, 18% (0-96%) in G0/G1, and 71% (0-98%) in S/G2/M. Samples from patients with adverse karyotype (n=9) showed positivity with a median of 13% (0-99%) overall, 6% (0-99%) in G0/G1 and 55% (12-98%) in S/G2/M. These differences were not statistically significant.

Overall, samples from patients with secondary AML (n=9) showed topo IIα positivity with a median of 6% (0-95%), and samples from patients with *de novo* AML (n=45) with a median of 24% (0-99%), p=0.04. For cells in G0/G1, the median was 6% (0-96%) for secondary AML and 32% (0-99%) for *de novo* AML, p=0.04, while for cells in S/G2/M, the median was 55% (0-91%) for secondary AML and 74% (0-98%) for *de novo* AML, not significant (n.s).

Topo IIα mRNA and protein. Topo IIα mRNA expression and percentage of topo IIα-positive cells were determined in 24 patients. There was no correlation between mRNA expression and percentage of topo IIα-positive cells.

Chemosensitivity in vitro. Patient samples with low topo IIα mRNA tended to be less sensitive to topoisomerase poisons, but not to other drugs (Fig. 3). This tendency was stronger for samples with a low percentage of topo IIα protein-positive cells, and reached statistical significance for daunorubicin and etoposide (Fig. 4). Since RT-PCR and flow cytometry were performed in different patient groups, it was not possible to make a direct comparison between the groups tested for topo IIα mRNA and percentage of topo IIα protein-positive cells, respectively.

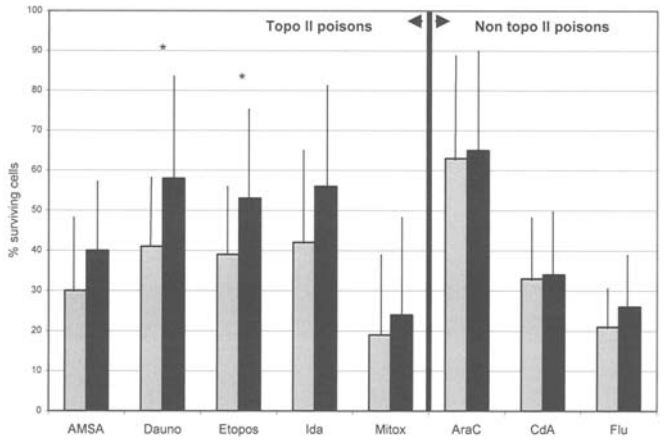

Figure 4. *In vitro* sensitivity to different cytostatic drugs in patient samples with high (grey bars) or low (black bars) percentage of topo IIα protein-positive cells. The groups of high and low expression were defined by dividing each group of patients, tested for a certain drug, into two groups with median percentage as cut-off. Sensitivity is expressed as a percentage (mean value of the group) of living cells after incubation with the drug, i.e. a relatively low percentage indicates relatively more sensitive cells. Vertical lines indicate standard deviation for each sample. AMSA, amsacrine (n=39); Dauno, daunorubicin (n=37); Etopos, etoposide (n=38); Ida, idarubicin (n=38); Mitox, mitoxantrone (n=32); AraC, cytarabine (n=34); CdA, cladribine (n=29); Flu, fludarabine (n=32). The difference in sensitivity was statistically significant for daunorubicin and etoposide (p=0.02 and p=0.04).

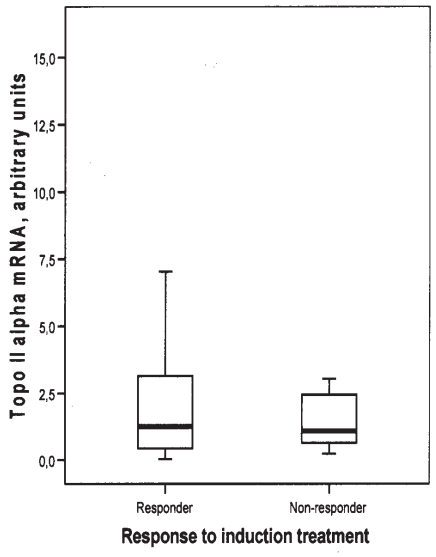

Figure 5. Topo IIα mRNA (arbitrary units) in samples from responders (n=37) and non-responders (n=12). For definition of responders see text.

Clinical outcome. Of the 49 clinically evaluable patients tested for topo IIα mRNA, 37 were defined as responders and 12 as non-responders. Median topo IIα mRNA expression was almost the same in the responder group (1.26; range 0.04-15.32) and in the non-responder group (1.08; 0.24-6.13) (Fig. 5).

Survival was investigated by dividing the patients into 2 groups with the median topo IIα mRNA expression as cut-off. Median survival was 21 months (95% CI: 3-39) in the group with low topo IIα mRNA expression and 15 months (95% CI: 6-25) in the group with high topo IIα mRNA expression (n.s.). Similar results were obtained when patients who were not evaluable for response to induction therapy were excluded (data not shown).

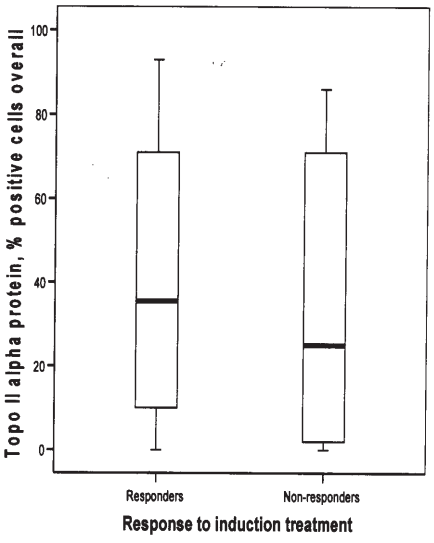

Figure 6. Overall percentage of topo IIα protein-positive cells in samples from responders (n=30) and non-responders (n=10). For definition of responders see text.

Of the 40 clinically evaluable patients tested for topo IIα protein expression, 30 were defined as responders and 10 as non-responders. There was no significant overall difference in percentage of topo IIα-positive cells between samples from responders and non-responders (median 35% and 25% positive cells respectively; Fig. 6), and the same was true for cells in G0/G1 (29% and 18.5%) and S/G2/M (67.5% and 71.5%). Survival was compared between patients with low and high topo IIα expression, using the median (23% positive cells) as cut-off. Median survival was 7 months (95% CI: 0-16) in the group with <23% positive cells and 10 months (95% CI: 6-14) in the group with >23% positive cells (n.s.). Results regarding survival were similar when patients who were not evaluable for response to induction treatment were excluded (data not shown).

Discussion

This study included bone marrow and blood samples from 88 patients with AML and six patients with ALL. The presented results on clinical outcome did not change when patients with ALL were excluded from the statistical calculations (data not shown). PB and BM samples were both used in this study. There are data indicating that normal PB mononuclear cells express very low amounts of topo IIα mRNA (24). In this study, there was no statistically significant difference between topo IIα mRNA expression in PB and BM samples (data not shown), indeed some of the highest relative values were found in PB samples (Table I).

The reported difference in topo IIα protein positivity in *de novo* and secondary AML does not allow any firm conclusion since the number of patients with secondary AML was small. Still, one could speculate about down-regulation of topo IIα in secondary AML as a contribution to poor prognosis.

Chemosensitivity testing *in vitro* showed, as could be theoretically expected, that samples with a lower percentage of topo IIα-positive cells were more resistant against topo IIα poisons, particularly daunorubicin and etoposide, but not against non-topo IIα targeting drugs. The same pattern, although less evident, was seen for topo IIα mRNA expression. This supports the hypothesis that downregulation of topo IIα can be a resistance mechanism against these drugs, also in the clinic.

However, samples from responders did not express significantly more topo IIα mRNA or protein than samples from non-responders. This is in accordance with the results of Kaufmann *et al* (20), who investigated topo IIα at the protein level using Western blotting in 41 newly diagnosed AML patients, Galmarini *et al* (19) who determined topo IIα mRNA by RT-PCR in 77 AML patients, and by McKenna *et al* (18) in a study of mRNA expression in 16 AML patients.

In this study, there was no difference in survival for patients with 'high' or 'low' expression of topo IIα mRNA or protein. Again, this is in agreement with Galmarini *et al* (19). In contrast, however, Lohri *et al* (21), who also investigated topo IIα mRNA expression, using quantitative RT-PCR in samples from 57 patients with AML, found that the group with high topo IIα mRNA expression had a significantly better prognosis, defined both as progression-free survival and as overall survival.

The failure to demonstrate a correlation between topo IIα expression and clinical outcome could have several explanations. As AML patients are generally treated with both anthracyclines and cytarabine, the anthracycline's, and thereby topo IIα's, effect on clinical outcome could be obscured.

Another explanation could be heterogeneously distributed topo IIα (20). The mRNA expression is determined on average and there could be subpopulations of cells, with very low expression of topo IIα, which are responsible for poor response to induction treatment or relapse. Our flow cytometry method determines the percentage of topo IIα-positive cells but not the average or individual cell content of topo IIα protein, and this could possibly explain the lack of concordance between topo IIα mRNA expression and percentage of topo IIα-positive cells. The *in vitro* data on chemosensitivity support the theory that subpopulations with low topo IIα protein content could be of importance for resistance to topoisomerase poisons, and that the percentage of topo IIα-positive cells determined by flow cytometry reflects this resistance better than the average mRNA expression determined by RT-PCR.

Another factor of importance could be post-translational modification of topo IIα activity. It has been demonstrated that cell-cycle specific phosphorylation is important in regulation of human topo IIα enzymatic activity (25,26), and that formation of 'cleavable complexes' is reduced in a mutated HL-60 leukaemic cell line with impaired phosphorylation of topo IIα in the presence of topoisomerase-targeting drugs (VP-16 and amsacrine) (27) compared to unmutated HL-60. Another mechanism that could enhance enzymatic activity is the formation, together with other proteins, of a 'toposome' during mitosis (28). Obviously, neither of these possible activity regulators are taken into account in the present study.

In conclusion, despite a correlation to chemosensitivity *in vitro*, this study could not verify any predictive value of topo IIα mRNA or protein expression on clinical outcome in acute leukaemia. Since topo IIα is the main target for important cytostatic drugs in treatment of acute leukaemia, further attempts to link topo IIα to clinical response are reasonable, preferably with methods taking heterogeneous distribution and enzyme activity into account.

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