

the serum levels of lactate dehydrogenase (LDH) and  $\beta_2$ -microglobulin ( $\beta_2m$ ), as shown in Table 2. Of all the serum factors studied, i.e. the sCD and the usual serum markers (LDH,  $\beta_2m$ , albumin, uric acid and C-reactive protein), sCD25 also showed the strongest correlation with tumor burden (data about albumin, uric acid and C-reactive protein are not shown in Table 2).

In conclusion, serum levels of sCD25, sCD8, sCD54 and sCD44 are roughly proportional to the burden of neoplasia, but sCD25 is clearly more sensitive as a marker of tumor burden than others sCD. sCD25 is also clearly a more sensitive marker of tumor burden than usual serum factors. Measurements of sCD25 can be indicated for stage assessment in all patients with NHL.

### Funding

This work was supported by grants XUGA90204A92 and XUGA90202A94 from the "Conselleria de Educación y Ordenación Universitaria de Galicia".

### Key words

Serum markers, tumor burden, sCD25, sCD8, sCD23, sCD54, sCD44, non-Hodgkin's lymphomas.

### Correspondence

M. Pérez Encinas, Servicio de Hematología, Hospital General de Galicia, C/Galeras s/n, 15705 Santiago de Compostela, Spain. Fax: international +34-981-540132.

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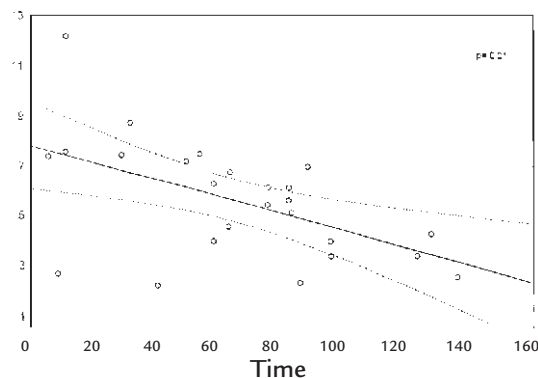
## Thyroid volume is progressively reduced as a sequela of neck irradiation for childhood Hodgkin's disease

GRAZIA BOSSI, DANIELA LARIZZA, GRAZIA SOMMARUGA,\* FRANCO CORBELLA,<sup>o</sup> CATHERINE KLERSY,<sup>#</sup> MAURIZIO ARICO

Departments of Pediatrics, \*Radiology, <sup>o</sup>Radiotherapy; <sup>#</sup>Biometric Unit, Scientific Direction, University; IRCCS Policlinico San Matteo, Pavia, Italy

**Thyroid volume reduction was observed, among 25 subjects off-therapy after Hodgkin's disease. The volume reduction was related to dose ( $p=0.014$ ) and time from radiotherapy ( $p=0.01$ ). The correlation was very specific since all patients with reduced volume had hypothyroidism, but not very sensitive since 25% of subjects with thyroid dysfunction had normal gland volume.**

As the thyroid gland is frequently within the field of neck irradiation for Hodgkin's disease (HD), patients treated in this way may have an increased risk of secondary thyroid carcinoma.<sup>1-3</sup> It is, therefore, recommended that the follow-up of these patients includes thyroid ultrasound examination<sup>4,5</sup> and monitoring of thyroid hormones.<sup>6</sup> We followed-up 25 children who had been treated for Hodgkin's disease; 22 of them received neck irradiation, while performing thyroid ultrasound screening, we also measured the gland volume, and this information was compared with that of the thyroid function.



**Figure 1. Regression line and 95% CI of the thyroid volume measured by us in patients evaluated at different times after completion of treatment for childhood Hodgkin's disease.**

**Table 1. Presenting features, treatment modalities and thyroid evaluation in 25 subjects treated for childhood Hodgkin's disease**

Pts.	Sex/age	Chemotherapy	RT dose on neck	Laryngeal protection	Age at evaluation	FT4	FT3	Basal TSH	Stim.	Thyroid volume (mL)	
						(nv 7-19 pg/mL)	(nv 2.3- 4 pg/mL)	(nv 0.3- 3.8)	TSH (peak)	observed	age-related mean±SD
1	F/8	MOPP x 8; ABVD x 3	22.5	no	21	3.1	0.6	3	17.5	2.9	6.3±1.5
2	M/7	MOPP x 3; ABVD x 3	25.5	yes	20	6.6*	2.5	6.1	26.9	4.6	6.3±1.5
3	M/11	MOPP x 3; ABVD x 3	26	no	22	6	2.9	3.7	18.7	3.7	6.3±1.5
4	M/13	MOPP x 3; ABVD x 3	42	yes	23	5.4	2.6	1.5	18.7	3.6	6.3±1.5
5	F/12	MOPP x 5; ABVD x 5	26	yes	20	8.1	1.8	4.7	22.3	5.6	6.3±1.5
6	M/6	MOPP x 3; ABVD x 3	25.5	yes	13	12.4	3.2	1.3	4.8	5.3	6.1±1.6
7	M/2	ABVD x 3	-	-	9	11.5	3.6	3.4	23.2	7.2	3.6±1.3
8	F/12	MOPP x 3; ABVD x 3	25	no	20	8.4	1.9	1.2	11.8	4.2	6.3±1.5
9	M/7	ABVD x 3	30	no	15	1*	2.6	15.2	100	2.5	6.3±1.5
10	M/11	MOPP x 3; ABVD x 3	-	-	18	9.9	4	2.1	10.2	6.3	6.3±1.5
11	M/7	MOPP x 3; ABVD x 3	26	yes	15	8.2	3.4	2.3	20	5.8	6.3±1.5
12	M/6	ABVD x 3	25.5	no	13	7.8	3.3	3.7	19.8	6.3	6.1±1.6
13	M/8	ABVD x 3; CEP x 11	25/37.6°	yes	15	6.2*	2.6	12.1	77.8	2.7	6.3±1.5
14	M/13	MOPP x 3; ABVD x 3	26	yes	19	9.9*	2.3	6.8	38.6	4.7	6.3±1.5
15	M/9	ABVD x 3	20	yes	14	5.6	2.4	3.6	12.8	6.9	6.3±1.5
16	M/6	MOPP x 1; ABVD x 2	20.8	yes	11	7.4	2.4	1.2	9.9	4.1	4.9±1.5
17	F/15	MOPP x 3; ABVD x 3	20	no	21	7.3	2.5	1.5	16.9	6.4	6.3±1.5
18	F/9	MOPP x 1; OPPA x 1; COPP x 2	-	-	14	6.6	3.3	2.6	41	7.6	6.3±1.5
19	M/12	MOPP x 3; ABVD x 3	20	yes	17	9.9*	2.3	4.2	35.5	7.3	6.3±1.5
20	F/12	MOPP x 3; ABVD x 3	20	yes	16	5.3*	3	11	103	2.3	6.3±1.5
21	F/14	ABVD x 3	20	no	17	7.5	2.2	1.8	13.8	8.8	6.3±1.5
22	M/11	MOPP x 3; ABVD x 3	20	no	15	5.8	2.9	9.5	29.1	7.5	6.3±1.5
23	F/13	ABVD x 3	20	yes	14	9.1	3	2.6	27.5	12.2	6.3±1.5
24	F/13	MOPP x 3; ABVD x 3	36	yes	15	9.1	3.6	2.4	14.8	7.6	6.3±1.5
25	F/8	MOPP x 3; ABVD x 3	23.6	yes	9	8.4	3.3	2.3	22	7.4	3.6±1.3

ABVD = adriamycin + bleomycin + vinblastine + imidazole carboxamide; OPP = nitrogen mustard + vincristine + procarbazine + prednisone; OPPA = vincristine + procarbazine + prednisone + adriamycin; COPP = vincristine + procarbazine + prednisone + cyclophosphamide; CEP = CCNU + etoposide + prednisone; \*on replacement therapy with thyroxine; °neck was irradiated twice, once during front-line mantle irradiation, and again after disease relapse.

None of the patients developed symptoms related to thyroid dysfunction or overt thyroid enlargement; one patient had a single thyroid nodule and regional lymph node enlargement. Thyroid function (FT4, T4, FT3, T3, basal and stimulated TSH) was normal in 9 of the 25 patients (36%), including 6 of the 22 (27%) who had received neck irradiation and 3/3 patients not irradiated (Table 1); 8 patients (32%) had low FT3 and FT4 levels, with increased basal TSH. Increased TSH response to TRH was present in 7/8 subjects. In 6 cases, thyroid replacement therapy was given, another became euthyroid and the other is still under evaluation. The remaining 8 patients (32%) had low FT3 and FT4 levels, with normal basal TSH. After TRH stimulation, TSH response was raised in only two of these eight.

**Ultrasound study.** Sixteen patients (64%) had a normal thyroid, 9 had parenchymal cysts (n=4) or inhomogeneity (n=5); none of the patients showed parenchymal nodules. Thyroid volume was inferior to age-standardized volumes in 9/25 patients (36%) (Table 1). Thyroid volume was similar in male and female patients, and in patients older or younger than 15 years at the time of assessment. Although patients with low FT4 and high TSH values tended to have a

lower thyroid volume, the difference was not statistically significant. Conversely, thyroid volume was significantly lower in patients with a radiotherapy dose >20 Gy and in patients off-therapy for >5 years. In the regression analysis none of the following were significantly associated with a lower thyroid volume: sex, radiotherapy site, radiotherapy dose (as a continuous variable), age at completion of treatment and age at current evaluation, levels of free T4 or of TSH, while time off-therapy, radiotherapy dose (cut-off 20 Gy), and, marginally, chemotherapy were significant. In the multivariate analysis only time off-therapy remained significantly associated with thyroid volume (p=0.01). This model showed that thyroid volume tended to decrease the longer the time since completion of treatment (Figure 1) and the more aggressive the chemotherapy and radiotherapy used.

In conclusion, thyroid ultrasound follow-up study for screening of thyroid nodules may provide additional information on thyroid volume, and this may be related to thyroid function. The thyroid volume was significantly inferior to age-standardized in 36% of the patients. Time elapsed from treatment completion was the only independent risk factor for this event.

### Correspondence

Dr. Maurizio Aricò, Department of Pediatrics, IRCCS Policlinico S. Matteo, 27100 Pavia, Italy. Fax international +39-382-502601 • E-mail: aricom@ipv36.unipv.it

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### Chlorambucil synergizes with purine analogs in inducing *in vitro* cytotoxicity in B-cell chronic lymphocytic leukemia

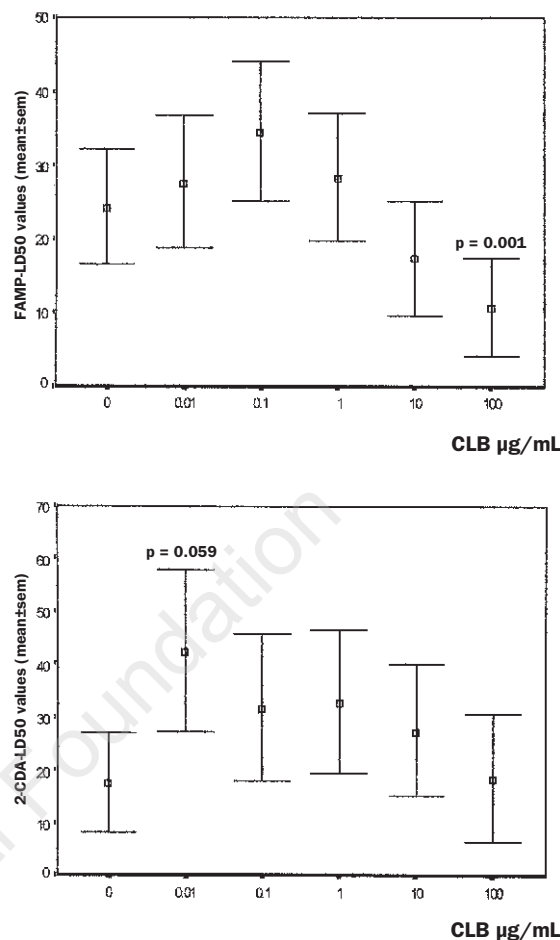
IDA CALLEA,\* GIUSEPPE CONSOLE,\* GIUSEPPINA SCULLI,<sup>o</sup> MONICA FILANGERI,\* GIUSEPPE MESSINA,\* FORTUNATO MORABITO\*

\*Centro Trapianti di Midollo Osseo e Terapia Sovramassimale Emato-Oncologica, Divisione di Ematologia; <sup>o</sup>Centro per le Microcitemie e Biologia Molecolare, Dipartimento di Emato-Oncologia, Azienda Ospedaliera Bianchi-Melacrino-Morelli, Reggio Calabria, Italy

**Combinations of different drug concentrations of CLB+FAMP and CLB+2-CDA were synergistic in, respectively, 42.9% and 34.8%. At leukemic cell survival  $\leq 50\%$ , 16.4% and 23.4% of all combinations were synergistic in the 2-CDA and FAMP groups, respectively. A significantly higher mean value of antagonistic interactions was observed in the 2-CDA group ( $p=0.037$ ).**

Fludarabine (FAMP), 2-chlorodeoxyadenosine (2-CDA) and chlorambucil (CLB) induce apoptosis in chronic lymphocytic leukemia (CLL) B-cells.<sup>1,2</sup> In this study we examined whether CLB improved *in vitro* CLL cell chemosensitivity to either FAMP or 2-CDA. The results indicate that CLB synergizes *in vitro* with both purine analogs.

Samples from 23 CLL patients were tested. Lymphocytes were separated as previously described.<sup>3-5</sup> CLB (Sigma, St Louis, Mo, USA), FAMP (Fludara, Schering AR, Germany) and 2-CDA (Leustatin, Ortho



**Figure 1.** *In vitro* effect of several CLB concentrations on FAMP- (A) and 2-CDA- (B) LD<sub>50</sub> values at MTT assay. Statistical analysis was performed by Wilcoxon matched-pairs signed-ranks test. The difference of the mean values was significant between CLB= 0 µg/mL and CLB = 100 µg/mL ( $p=0.001$ ) in the FAMP group. A border line significance was observed in the 2-CDA group between CLB = 0 µg/mL and CLB=0.01 µg/mL ( $p=0.0592$ ).

Biotech, USA) were employed at described concentrations.<sup>3-5</sup> MTT assay was performed as previously described.<sup>3-5</sup> The lethal dose (LD)<sub>50</sub> values, leukemic cell survival (LCS) and drug interactions were calculated by home made software.<sup>3-5</sup>

FAMP-LD<sub>50</sub> values were significantly lower with 100 µg/mL concentrations of CLB; conversely higher 2-CDA-LD<sub>50</sub> values were observed with 0.01 µg/mL of CLB (Figure 1). The interactions between CLB and either 2-CDA or FAMP, tested in respectively 420 and 525 combinations, were synergistic in 146 (34.8% of the total) and 225 (42.9% of the total) (Table 1). Similar percentages of additive interactions (15.7% and 18.8%) were detected with both purine analogs, while a higher percentage of antagonistic interactions was observed among the 2-CDA group. At LCS  $\leq 50\%$ , 23%