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LONG TERM CONSEQUENCES ON T CELL LYMPHOPOIESIS OF HODGKIN'S LYMPHOMA THERAPY

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Summary: This is the first study to provide evidence of the durable impact of Hodgkin lymphoma treatment on naive central and thymic CD4⁺ T cells associated with immune-senescence, possibly affecting patients' vulnerability as they age.

To the Editor:

Hodgkin lymphoma (HL) is a hematologic malignancy of the lymphoid tissue that mainly affects young adults¹. Standard treatment for sub diaphragmatic stages I and II combines chemotherapy regimen followed by dose-reduced involved-field irradiation (including mediastinum). Adverse effects of treatment include early death, second neoplasm and organ dysfunction.¹ Nevertheless, the consequences of HL treatments on thymus involution remained poorly investigated. In this study, we investigated the long-term effects of thymic irradiation on the establishment and maintenance of the naive T-cell compartment and on the distribution of peripheral blood lymphocyte populations in 30 HL patients, 7 to 19 years after mediastinal radiotherapy (all in complete remission of Hodgkin's disease), compared to 60 age- and sex-matched healthy controls (HC), (summarized in Table E1 in this article's Online Repository at www.jacionline.org). Lymphocyte distribution was analyzed by FACS and thymic output was determined by measuring (i) T cell receptor excision circles (TRECs) and (ii) the frequency of naive CD4⁺ CD31⁺ thymic CD4⁺ T cells, which have higher TREC levels than naive CD4⁺ CD31⁻ central T cells.^{2, 3} (Methods section in this article's Online Repository at www.jacionline.org).

Results reported in Figure 1A and Table I showed that the CD3⁺ and CD3⁺ TCRαβ⁺ T cell counts were significantly reduced in HL patients compared to matched HC (p=0.02 and p=0.01, respectively) while the total lymphocyte counts were similar in both populations. The CD4⁺ T cell counts were dramatically lower in HL patients than in HC (p<0.001; mean decrease of 32%); in contrast, the CD8⁺ T cell counts were equivalent in both groups. HL patients exhibited (i) an increase in B cell counts (p=0.01), with an unaffected frequency of memory B cells, indicating an increase in naive B cells counts and, (ii) a marginal increase of NK cell frequency (p=0.04).

We next evaluated the long-term impact of treatments on CD4⁺ T cell homeostasis and thymic activity in HL patients. Compared to HC, HL patients exhibited a profound decrease in naive CD4⁺ CD45RA⁺ T cell numbers ($p<0.001$; mean decrease of 57%) while the numbers of memory CD4⁺ CD45RO⁺ T cells were equivalent in both groups ($p=0.21$) (Fig 1A and Table I). We also observed a significant increase in the frequency of CD4⁺ CD28⁻ T cells ($p=0.02$) in HL patients compared to HC (Table I), indicative of a chronic T cell activation.^{4, 5} Seven to nineteen years post-treatment, HL patients have lower absolute numbers of naive CD4⁺ CD31⁺ T cells ($p<0.001$; mean decrease of 59%) (Fig 1A and Table I; see Table E2, in this article's Online Repository at www.jacionline.org). Accordingly, TREC levels in peripheral leukocytes were lower in HL patients than in HC ($p<0.001$) (Fig 1B and Table 1; see Table E2); 18/30 patients had undetectable levels of TRECs (<100 TRECs/ 10^6 WBC), compared to 5/60 in matched HC (Fig 1B). During aging, the decrease of naive CD4⁺ CD31⁺ T cells correlates with the decline of TRECs.^{2, 6} Naive CD31⁺ T cells (Fig 1C) and TRECs levels (Fig 1D) became lower in HL patients earlier in life than in HC; TRECs were undetectable in 48 years-old HL patients but still detectable in HC at 70 years old and beyond (Fig 1D). Association between naive CD31⁺ T cells (Fig 1C) or TRECs levels (Fig 1D), as dependent variables, and age at analysis (22-72 years), revealed that the age-dependent decline in naive CD31⁺ T cells and TRECs was equivalent in both populations. Although the absolute number of naive CD31⁺ thymic T cells decreases with aging, the absolute number of naive T cells remains stable in the young and the elderly⁷, a result of a compensatory proliferation of peripheral naive CD31⁻ central T cells⁸. Naive CD4⁺ CD31⁻ T cells counts were dramatically lower in HL patients than in HC ($p<0.001$), whereas the CD31⁺/CD31⁻ T cell ratio was not affected in HL patients ($p=0.27$) (Table I). According to the age, the naive CD4⁺ CD31⁻ T cell counts remained relatively stable in HL patients and, as expected,⁷ in HC (see Fig E1, in this article's Online Repository at www.jacionline.org).

Finally, we evaluated the impact of doses, time since radiotherapy, age at the treatment, modalities of chemotherapy and disease stage, on the naive CD31⁺ thymic T cell numbers and TRECs levels. Results showed a more pronounced decrease in thymic output in HL patients receiving radiation doses of 40 Gy than in patients receiving a dose <40 Gy ($p=0.044$ for naive CD4⁺ CD31⁺ T cells; $p=0.004$ for TRECs levels) (Fig 1E-F). Patients treated at older ages have a more pronounced decrease in TRECs levels than patients treated younger (in univariate ($p=0.002$) and multivariate analysis – see Table E2) and most of the patients treated after 34 years of age had undetectable TRECs levels (see fig E2, in this article's Online Repository at www.jacionline.org). The cut-off value for age at treatment associated with a lower number of naive CD31⁺ T cells was explored using ROC analysis of sensitivity and specificity at different cut-off values. The number of CD31⁺ naive T cells was lower in patients treated beyond the age of 34 years than in patients treated younger (area under the ROC curve=0.73). Although TRECs levels were slightly lower in HL patients treated with ABVD than with other chemotherapies ($p=0.007$), naive CD31⁺ T cell number was not dependent on the type of chemotherapy ($p=0.33$) (see fig E2). Finally, multivariate analysis showed that male patients had a

more pronounced decrease in naive CD4⁺ CD31⁺ T cells than female patients (p=0.007) (See Table E2). No association was observed between the decrease in thymic output and time since radiotherapy and the stage of HL.

Here we thus show that, long after treatment, HL patients exhibit a persistent and dramatic alteration of CD4⁺ T cell homeostasis and a profound alteration of thymic function evidenced by a decrease in central, thymic naive T cells and TRECs, while memory CD4 T cell counts remained unaffected. We report that signs of immune senescence (decrease in naive T cells and TRECs and increase in CD28⁻ CD4⁺ T cells) occur earlier in HL patients than in healthy subjects. High doses of radiotherapy and treatment beyond the age of 34 years exacerbated the reduction in thymic output.

In conclusion, the age-dependent thymic involution is protracted in these patients, a setting that suggests a premature loss of thymic function. These results suggest that, with aging, patients may become more vulnerable to infections and aging-associated immune disorders.

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Key words: naive T cells, thymopoiesis, radiotherapy, Hodgkin lymphoma, immune senescence, TRECs, homeostasis.

FIGURE 1

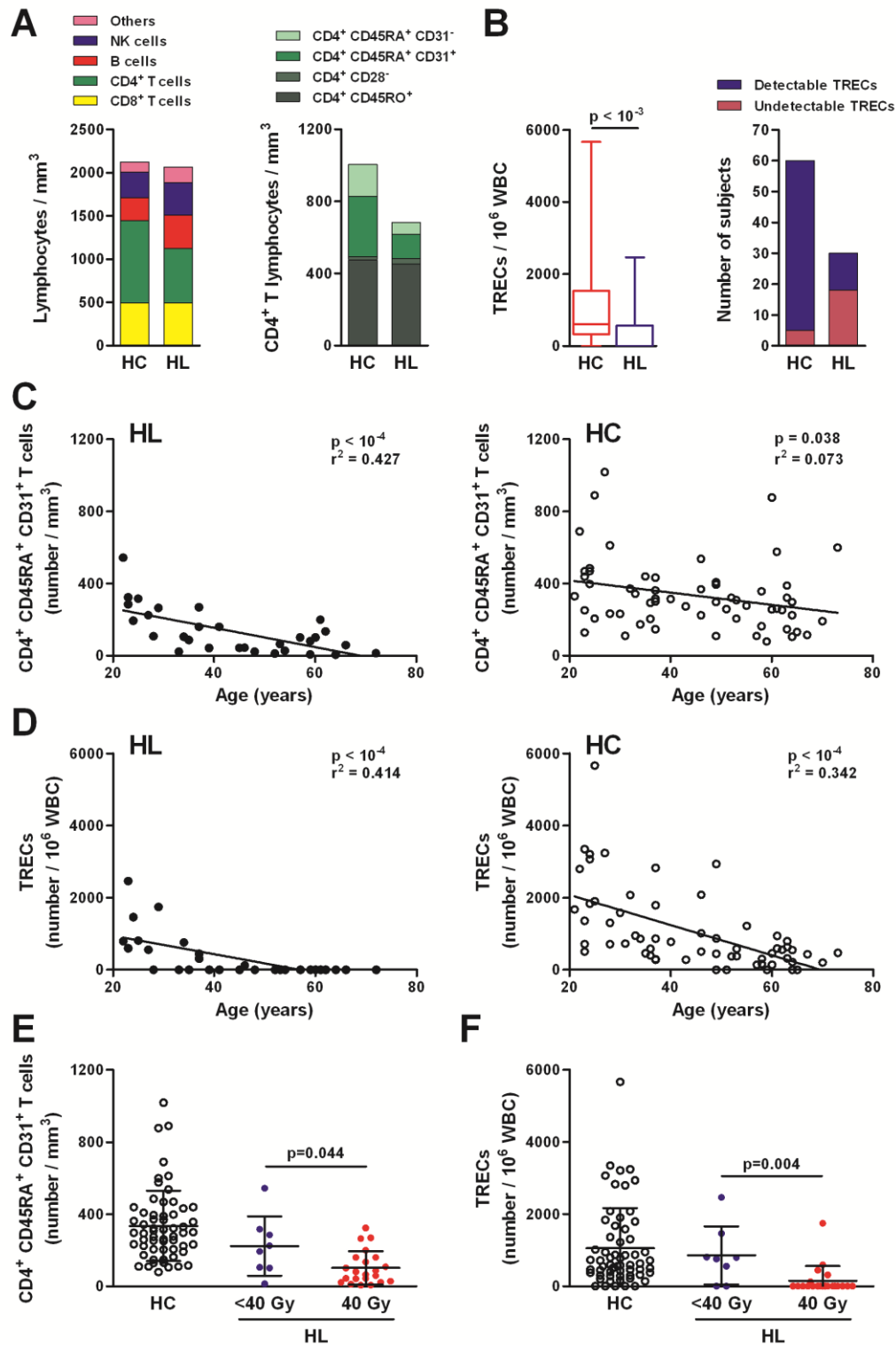
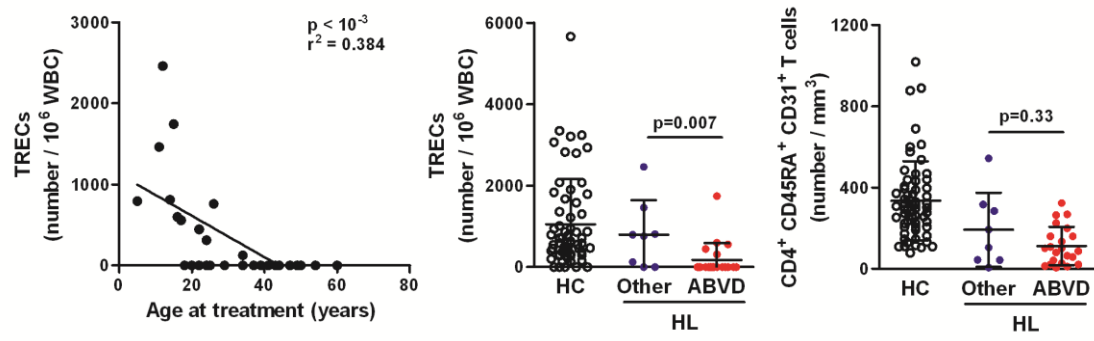


Figure 1. Immune status and thymic output in Hodgkin's lymphoma (HL) patients and healthy controls (HC). (A) Lymphocyte subsets. (B) Analysis of TRECs expression. Number of naive CD4⁺CD31⁺ T cells (C and E) and TRECs number (D and F) depending on age and treatment.

Table I: Characteristics of Hodgkin's lymphoma patients and healthy controls

	Healthy subjects mean [sd]	Hodgkin's lymphoma patients mean [sd]	
Lymphocytes, (cells/mm ³)	2123 [576]	2065 [1106]	<i>P</i> = .24
CD3 ⁺	1531 [425]	1231 [649]	<i>P</i> = .02
CD3 ⁺ CD4 ⁺ CD8 ⁻	954 [302]	633 [293]	<i>P</i> < .001
CD3 ⁺ CD8 ⁺ CD4 ⁻	492 [197]	493 [334]	<i>P</i> = .45
CD3 ⁺ CD4 ⁺ CD28 ⁻	18 [23]	30 [53]	<i>P</i> = .35
CD4 ⁺ CD28 ⁻ /CD4 ⁺ (%)	2% [2.8]	4%	<i>P</i> = .02
CD3 ⁺ CD4 ⁻ CD8 ⁻ TCRαβ ⁺	16 [10]	11 [6]	<i>P</i> = .01
CD4 ⁺ /CD8 ⁺ ratio in TCRαβ ⁺	2.4 [1.2]	1.6 [0.6]	<i>P</i> = .003
CD3 ⁺ TCRαβ ⁺ CD4 ⁻ CD8 ⁺	448 [177]	444 [316]	<i>P</i> < .001
CD3 ⁺ TCRαβ ⁺ CD8 ⁻ CD4 ⁺	949 [302]	630 [293]	<i>P</i> < .001
CD3 ⁺ TCRγδ ⁺ CD4 ⁻ CD8 ⁻	48 [46]	64 [45]	<i>P</i> < .001
CD3 ⁺ CD4 ⁺ CD45RA ⁺	512 [304]	202 [154]	<i>P</i> < .001
CD3 ⁺ CD4 ⁺ CD45RA ⁺ CD31 ⁺	335 [195]	134 [125]	<i>P</i> < .001
CD3 ⁺ CD4 ⁺ CD45RA ⁺ CD31 ⁻	177 [146]	67 [46]	<i>P</i> < .001
CD4 ⁺ CD45RA ⁺ CD31 ⁺ /CD4 ⁺ CD45RA ⁺ CD31 ⁻ T cell ratio	2.3 [1.16]	2 [1.29]	<i>P</i> = .27
CD3 ⁺ CD4 ⁺ CD45RO ⁺	475 [153]	454 [249]	<i>P</i> = .21
CD3 ⁻ (CD56 ⁺ and/or CD16 ⁺)	294 [159]	372 [307]	<i>P</i> = .16
CD3 ⁻ (CD56 ⁺ and /or CD16 ⁺)/lymphocytes (%)	13.8% [6]	17.5 % [10]	<i>P</i> = .04
CD19 ⁺	265 [112]	382 [301]	<i>P</i> = .01
CD19 ⁺ /lymphocytes (%)	12% [3,5]	17.7 % [7]	<i>P</i> < .001
CD19 ⁺ CD27 ⁺ /CD19 ⁺ (%)	30.9 (%) [12]	19 (%) [9]	<i>P</i> < .001
TRECs number/10 ⁶ WBC			
At least 100	n=55	n=12	
Less than 100	n=5	n=18	
Mean [sd]	1057 [1108]	647 [1750]	<i>P</i> < .001
Median [interquartile range]	610 [345-1470]	0 [0-598]	

WBC, white blood cells.



Legend Fig E1.

Analysis of TRECs numbers in Hodgkin's lymphoma patients according to the age at diagnosis (left panel). Analysis of TRECs (middle panel) and naive CD4⁺ CD31⁺ T cell (right panel) according to the type of chemotherapy. Horizontal bars indicate mean values \pm sd.

Table E1: Characteristics of Hodgkin's lymphoma patients and healthy controls

	Healthy controls	Hodgkin's lymphoma patients	<i>P</i>
Sex			
Male	22	11	<i>P</i> = 1
Female	38	19	
Age at analysis			
Mean age, years [sd]	44.1 [15.5]	43.8 [15.2]	<i>P</i> = 1
20-30	15	7	
31-45	14	8	
>45	31	15	
* Stage of Hodgkin's lymphoma			
II	NA	7	NA
III	NA	23	
† Chemotherapy regimen			
ABVD	NA	22	NA
VB- VP	NA	4	
Other	NA	8	
‡ Autoimmunity			
Yes	1	2	<i>P</i> = .25
No	59	28	
§ Thyroid abnormality			
Yes	7	19	<i>P</i>= .0001
No	52	9	
NA	1	2	
Numbers of infections per year			
Less 2 infections	58	23	<i>P</i>= .03
2 or more infections	1	5	
NA	1	2	

* Patients were staged according to the Ann Arbor classification (Stage II, involvement of two or more lymph node regions on the same side of the diaphragm; Stage III, involvement of lymph node regions on both sides of the diaphragm, which may include the spleen).

†ABVD: doxorubicin, bleomycin, vinblastine, dacarbazine; VB-VP: vinblastine, bleomycin, etoposide (VP16), and Prednisone; Other: EBVM (Epirubicin, bleomycin Vinblastin, Methotrexate), VABEM (vindesine doxorubicin, carmustine, etoposide, methylprednisolone), BEACOPP baseline (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone), OPPA (vincristine, doxorubicin, prednisone, procarbazine), BEAM (carmustine, etoposide, cytosine arabinoside, melphalan).

‡ Autoimmunity is defined with an autoimmune disease as systemic lupus erythematosus or rheumatoid arthritis etc.

§ Hypothyroidism or hyperthyroidism measured by thyroid function tests.

|| Numbers of infections are defined as respiratory infections (eg, sinusitis, otitis, bronchitis) in one year and the need for antibiotics.

Table E2: Factors associated with the number of CD31^{+thymic} naïve cells and the TRECs status in multivariate analysis. This multivariate analysis describes different factors associated with the number of CD31^{+thymic} naïve cells (a and b) and with a small amount of TRECs (c and d) in the population including either Hodgkin's Lymphoma patients (HL) and healthy controls (HC) (a and c) or among HL patients (b and d). A positive regression coefficient means that the factor is associated with higher levels of CD31^{+thymic} naïve cells or a risk of having a small amount of TRECs. A negative coefficient means a decrease of these parameters.

a) Factors associated with the number of CD31^{+thymic} naïve cells in HL and HC.

	Coefficient	p	95%CI
Male	96.80	0.007	[26.2, 167.4]
Age at analysis	-3.60	0.002	[-5.8, -1.3]
Hodgkin's Lymphoma	-202.70	0.000	[-268.9, -136.5]

b) Factors associated with the number of CD31^{+thymic} naïve cells in HL.

	Coefficient	p	95%CI
Age at treatment	-5.10	0.000	[-7.2, -3]
Thyroid abnormality	-72.30	0.030	[-137, -7.6]

c) Factors associated with a small amount of TRECs (less than 100 copies/10⁶ WBC) in HL and HC

	Coefficient*	p	95%CI
Male	-1.45	0.081	[-3.09, 0.17]
Age at analysis	0.10	0.002	[0.03, 0.17]
Hodgkin's Lymphoma	4.06	0.000	[2.08, 6.05]

d) Factors associated with a small amount of TRECs (less than 100 copies/10⁶ WBC) in HL

	Coefficient*	p	95%CI
Age at treatment	0.15	0.007	[0.04, 0.27]
Thyroid abnormality	1.47	0.231	[-0.94, 3.89]
Number of previous chemotherapies	0.80	0.519	[-1.63, 3.23]

* With TRECs status analysis, the coefficients have a special meaning: the antilogarithm of the coefficient equals the odds ratio.

