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Circulating immune complexes, complement and complement component levels in childhood Hodgkin's disease

W. E. BRANDEIS, CHARLOTTE TAN, Y. WANG, R. A. GOOD & NOORBIBI K. DAY *Sloan-Kettering Institute for Cancer Research, New York, USA*

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SUMMARY

Serum levels of circulating immune complexes (CIC) assayed by the Raji cell radioimmunoassay, total haemolytic complement (TCH50), C1q and C3 were correlated with clinical stage, histological type, age, sex and treatment of eighty-six children with Hodgkin's disease over a period of 4 years. Most significant findings were the changes of levels of CIC, TCH50, C1q and C3 during disease activity and following treatment. Significant perturbations were also seen in association with relapse. Levels of C and CIC were significantly elevated ($P<0{\cdot}001$) at the time of diagnosis prior to splenectomy and/or any treatment. In the group before treatment, 81% of CIC levels were above 16 μg/ml with a maximum value of 1120 μg/ml. During treatment 33% were still above normal with a maximum of 320 μg/ml. Within 1 year after cessation of treatment, 37% also remained above normal levels with a maximum of 240 μg/ml. At relapse prior to treatment, 63% were again elevated with a maximum of 1280 μg/ml. The most significant difference on TCH50 levels relates to treatment periods. Sera of patients with active disease who are previously untreated show elevation of TCH50 levels ($P<0{\cdot}001$) (average 127 CH50 u/ml). During and after treatment the TCH50 levels drop to 96 and 102 CH50 u/ml, as compared to normal control of 100 CH50 u/ml. In sera of patients at the first, second or third relapse, the combined TCH50 levels are significantly different from controls and across treatment periods ($P<0{\cdot}005$).

INTRODUCTION

Hodgkin's disease (HD) is characterized by a widespread involvement of the lymphoreticular system which leads regularly to marked perturbation of immunologic function, particularly to an impairment of cell-mediated immunity. The complement system is also affected in Hodgkin's disease. Although in earlier reports no statistical differences in complement levels between sera of Hodgkin's disease and those of healthy controls were observed (Southam & Goldsmith, 1951), in most recent investigations elevated levels of total haemolytic complement (TCH50) (Schier *et al.*, 1956; Rottino & Levy, 1959; Wagener & Haanen, 1977), C2 (Southam & Siegel, 1966), C4 (Balzola, Berruti & Segre, 1964) and C3 (Balzola *et al.*, 1964; Irunberry & Colonna, 1970) have been reported. Significantly elevated levels of TCH50, C4 and C3 were observed in sera of patients undergoing combined chemotherapy, total nodal irradiation and splenectomy (Weitzmann *et al.*, 1977).

Elevated levels of circulating immune complexes (CIC) were reported by Amlot *et al.* in sera of patients with HD who had generalized symptoms like night sweat, loss of weight and fever (status B) (Amlot, Slaney & Williams, 1976; Amlot *et al.*, 1978). Kavai *et al.* (1976) and Brown *et al.* (1978), on the other hand, were unable to confirm this correlation. Heier *et al.* (1977) studied sera of fifty patients with HD and showed that CIC levels are higher among the malignant lymphoma patients who had generalized

Correspondence: Dr Noorbibi K. Day, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA.

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symptoms than in the symptom-free patients. More recently, CIC have been shown to be present in sera of 50% of adult untreated patients with HD by Brown *et al.* (1978) After treatment, the level of CIC returned to normal (< 15 μg/ml) in thirty-nine of forty-one patients.

All the studies described above were performed in sera of adults with HD. To date there is no well-documented study on the complement perturbations and levels of CIC in childhood HD. Prior reports from this institution have stressed rather striking differences in immunologic cellular responses and distributions in children with HD as compared to adults (deSousa *et al.*, 1978; Tan *et al.*, 1978).

In the present study we have correlated complement and CIC levels with the clinical stage, histological type, age and sex of the patients and with the treatment of children with HD.

MATERIALS AND METHODS

Patient population. We studied eighty-six patients with Hodgkin's disease at the Memorial Sloan-Kettering Cancer Center during the period 1974–1977. Twenty-seven children were less than 10 years of age, fifty-nine were older than 10 years, with a median age of 12 years. The youngest patients were 4 years old (Fig. 1). Fifty-five were male and thirty-one female. The clinical and pathological staging was based on the Ann Arbor classification (Carbone *et al.*, 1971). For the histological staging, the nomenclature of the Rye Conference (Lukes *et al.*, 1966) was used. The groups of patients studied in relation to histologic type and the clinical stage are listed in Table 1. There were twenty-four patients in each of stages I and II, twenty-two patients in stage III and sixteen in stage IV. More patients in the more advanced stages of the disease had clinical symptoms, e.g. night sweat, loss of weight, or fever (status B). Fifty patients had nodular sclerosis (NS), eight lymphocytic predominance (LP), twenty-seven mixed cellularity (MC) and one child had lymphocytic depletion (LD). More male than female patients had LP, MC and LD (thirty-two males and four females), but the sexes were split evenly in the NS group (twenty-three male, twenty-seven female).

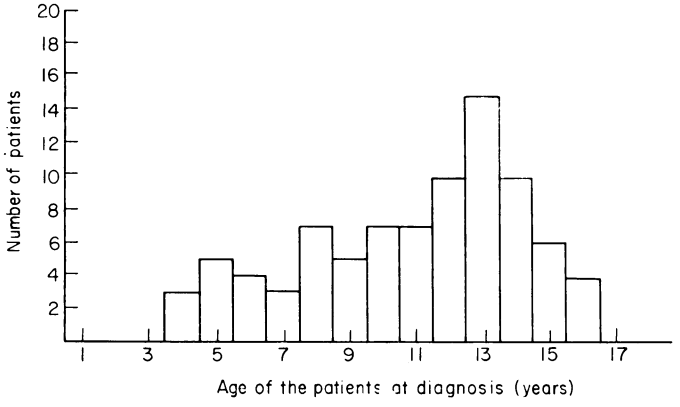

FIG. 1. Patient population of children with Hodgkin's disease.

The normal controls included thirteen healthy children aged 1–14 years without infections or malignancies. The serum values of TCH50, C components and CIC were the same for these children as our normal adult levels.

Splenectomy had been performed on most of the patients during the staging procedure. The treatment of the children was according to protocols published previously (Tan *et al.*, 1975).

Blood was drawn at the time of diagnosis before therapy was started, 3 months after initiation of treatment, and at intervals of 3–6 months thereafter. For our study, we used serum samples before 3 months, after commencement of treatment, and 1, 3, 5 and 10 years after cessation of therapy. When a patient had relapsed, a serum sample and the samples following renewed treatment were also analysed.

Patients' sera. Blood was drawn under sterile conditions, allowed to clot at room temperature for 1 hr, clarified by centrifugation in the cold, aliquoted and stored at −80°C until used. Complement studies were performed on 374 sera within 2 weeks after bleeding. The determinations of CIC were performed on 171 fresh frozen aliquots which had not been thawed previously.

Measurement of TCH50 and C components. The total haemolytic complement assay was carried out according to a method described previously (Day *et al.*, 1976). The results are expressed as CH50 units/ml. Immunochemical measurement of C1q and C3 was carried out by the Mancini technique using our own monospecific antisera prepared against both of these components (Day *et al.*, 1976).

TABLE 1. Histological types and clinical stages of childhood Hodgkin's disease

Stage	Nodular sclerosis	Lymphocytic predominance	Mixed cellularity	Lymphocytic depletion	Total
I A	8 (2)	7 (2)	3	1	19 (4)
B	4 (2)	—	1	—	5 (2)
II A	7	—	5 (2)	—	12 (2)
B	9 (1)	—	3 (3)	—	12 (4)
III A	4 (2)	1	4 (1)	—	9 (3)
B	8 (3)	—	5	—	13 (3)
IV A	1	—	—	—	1
B	9 (3)	—	6	—	15 (3)
All stages	50 (13)	8 (2)	27 (6)	1	86 (21)

Figures in parentheses indicate number of patients with relapse; A = patients without general symptoms; B = patients with general symptoms.

Measurement of CIC. For the detection of CIC, we used the Raji cell radioimmunoassay of Theofilopoulos, Wilson & Dixon (1976). 2×10^6 Raji cells in 50 μl minimal essential medium (MEM) were reacted with 25 μl of a 1:4 diluted test serum. After incubation and washing, the cells were reacted with ^{125}I-rabbit anti-human IgG for 30 min at 4°C. The radioactivity of the cell pellet was then determined in a gamma counter. The uptake of radioactive antibody was found by reference to a standard curve of various amounts of heat aggregated human globulin (AHG) (from 4 mg to 10 mg/ml). The amount of CIC in each sera tested is expressed as microgram AHG equivalent per millilitre of serum. For simplicity we will refer to these values as μg/ml. Levels from each of the thirteen normal controls were less than 16 μg/ml.

Aggregated human globulin (AHG). For the preparation of AHG, Cohn fraction II humans IgG (6 mg/ml) in phosphate buffered saline (0·1 M, pH 7·3) was used. After heating in a waterbath at 63°C for 30 min, the sample was centrifuged in a Damon PR-6000 IEC centrifuge (Fisher, Springfield. New Jersey) at 2000 r.p.m. for 30 min at 4°C and the upper third of the supernatant with the soluble aggregates was used in the assay. The protein content of the supernatant was determined by the Lowry method (Lowry *et al.*, 1951), and a final concentration of 4 mg/ml was used.

Radioiodination of rabbit anti-human IgG. Radioiodination of rabbit anti-human IgG with ^{125}I (New England Nuclear, Boston, Massachusetts) was performed using the chloramine T method of McConahey & Dixon (1966). The labelled anti-human IgG was dialysed extensively against phosphate buffered saline pH 7·2 for 36 hr. The specific activity of the ^{125}I-rabbit anti-human IgG (0·3 mg/ml) was 0·05–0·3 mCi/μg protein.

Biostatistical methods. Analyses of variance (Dunnett, 1970) were used on each of the determinations of TCH50, C1q, C3 and CIC to permit analyses of the effects of factors such as stage and type of disease, histology, age, sex, treatment and relapse status and their interrelationships with each other. A log transformation was first used on the CIC values to adjust for a skewed distribution. Due to the limited sample size three factors at a time at most were used in the factor analysis with combinations such as stage with sex and age or stage with status and treatment. Since this is a retrospective study, the factors are unbalanced and the multiple classification method of analysis of variance was used to analyse the effect of any factor after controlling for other factors. Spearman rank correlation was used to study relationship between pairs of TCH50, C1q, C3 and CIC determinations.

RESULTS

The data are grouped into periods before relapse and during the first, second, or third relapse. In each of these periods they are subdivided by treatment status into before treatment, during treatment and after cessation of therapy (within 1 year, 1 to 3 years, 3 to 5 years, 5 to 10 years, henceforth referred to as 1, 3, 5, 10 years after therapy). Analyses of variance were carried out using a combination of factors: age (under or over 10 years), sex, stages (I–IV), status (A, B), histology type, e.g. MC, NS, treatment (before, during, after), and relapse status (before any relapse, at relapse).

Since this is a retrospective study, fewer CIC values than complement component determinations were available for analysis. The sera of each patient with the most complete set of measurements nearest the midpoint of each period were used in the summary values given in Table 2.

Table 2 presents the mean, median, standard error (s.e.) and sample size of each of the treatment periods for TCH50, C1q and C3. We will consider in detail TCH50 values (Table 2), followed by a brief summary of changes in C1q and C3.

TABLE 2. Mean levels of TCH50, C1q and C3 before, during and after treatment in childhood Hodgkin's disease

Treatment	Time (years)	No.	TCH50 (u/ml) Mean	Median	s.e.	C1q (μg N/ml) Mean	Median	s.e.	C3 (mg % protein) Mean	Median	s.e.
No relapse											
Before		33	127	122	5·48	27	28	1·21	147	156	6·93
During		31	96	94	3·22	23	22	0·94	110	109	4·13
After	1	39	101	98	3·02	23	23	1·01	122	124	4·30
	3	31	106	100	5·10	26	25	1·28	120	119	6·70
	5	9	96	98	4·17	24	22	1·67	110	109	7·67
	10	7	96	98	6·66	24	24	4·09	118	114	11·62
Relapse 1											
Before		12	126	130	8·59	24	24	1·59	143	150	11·38
During		13	118	122	9·32	21	21	0·90	114	112	10·38
After	1	6	110	107	10·87	24	24	1·85	136	122	17·76
	3	6	98	90	7·73	29	30	2·97	133	101	19·26
Relapse 2											
Before		3	133	114	21·37	25	25	2·83	148	136	6·99
During		5	134	119	12·04	26	24	3·27	115	109	11·30
After	1	1	115	115	—	21	21	—	102	102	—
	3	1	84	84	—	37	37	—	84	84	—
Relapse 3											
Before		2	198	198	11·5	23	23	0·50	96	96	9·5
During		—	—	—	—	—	—	—	—	—	—
After		—	—	—	—	—	—	—	—	—	—
Normal Control		13	100	104	4·32	20	20	0·81	113	113	3·13

Data are grouped into periods (before any relapse, and during the first, second or third relapse) and subdivided according to treatment status (before, during and after treatment).

Total haemolytic complement levels

Analysis of variance showed no significant two and three-way interaction effects on the TCH50 levels between factors such as treatment periods, stage, disease status, histology type, age, or sex. For the individual factors, the most significant difference relates to the treatment periods. Sera of patients with active disease who are previously untreated show elevation of TCH50 levels above normal and above all the treatment periods ($P<0·001$) by the F-test. Mean serum levels for the before, during, and the pooled after-treatment groups are 127, 96 and 102 CH50 u/ml, respectively, as compared with a normal control of 100 CH50 u/ml (Table 2). Prior to initial treatment serum levels were elevated significantly from the normal control (Dunnett's test, $P<0·01$), and this is the only group that showed an elevation. In the sera of patients at the first, second or third relapse, the combined TCH50 levels were also significantly different from controls and across treatment periods (F-test, $P<0·005$). At the first relapse the mean level before treatment was 126, at the second relapse 133, and at the third relapse 198 CH50 u/ml (two patients). The levels during treatment after relapse were higher than the corresponding levels during treatment before the occurrence of any relapse. This is due partly to the fact that for some patients, especially those with multiple relapses, the first treatment given after relapse was not effective and alternate therapy had to be used. However, for patients in relapse who have received successful therapy, mean levels after treatment fell again to near normal values.

The mean levels across the stages are also significantly different (F-test, $P<0·001$). In the before relapse group the mean levels prior to treatment were 108, 138, 125, and 135 CH50 u/ml for stages I–IV respectively. Stages II–IB are all significantly higher than the normal control (Dunnett's test, $P<0·01$). However, the rank order of the levels for the different stages do not correspond with the stages; the

levels of stage II are always higher than those of stage III, especially in the group studied before treatment and the group analysed after 3 years of treatment. This rank order of stage II over stage III holds even when other factors such as sex and age, histology and disease status are controlled in the analysis of variance model in order to adjust for the unbalanced nature of the data. In the groups studied during and after treatment, the TCH50 levels fall within the normal range for stages I–III, but remained elevated in some patients in stage IV (Dunnett's test, $P<0{\cdot}05$).

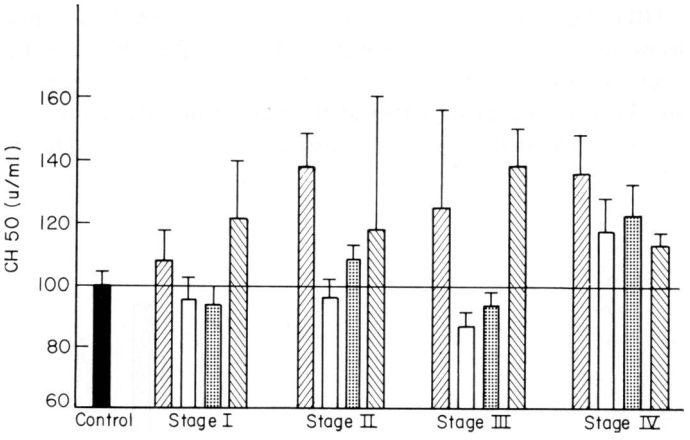

FIG. 2. Mean levels and standard error of TCH50 in patients' sera at stages I–IV of Hodgkin's disease, before (▨), during (□) and after 1 year of treatment (▦), and during the first relapse before treatment (▧).

These observations are illustrated in Fig. 2, which shows mean and standard errors of the levels of TCH50 of normal controls and patients' sera at stages I–IV, before, during and after 1 year of treatment, and during the first relapse before treatment. As shown, the TCH50 levels were elevated at the time of diagnosis. For stages II–IV these levels dropped during therapy to near normal value, and remained there until a relapse occurred. At stage IV the levels were still elevated during and 1 year after treatment. Only two relapse patients were studied in the stage IV group.

In general, female patients had higher TCH50 levels than males in each of the treatment periods. In the group studied prior to treatment the mean value of fifteen females was 139, while the mean value of eighteen males was 117 u/ml (F-test, $P<0{\cdot}05$). In those studied during treatment the means of female patients were still higher than those of the males, but both fell within the normal range.

No significant difference was observed between the patients of age groups greater than 10 years and those younger than 10 years and no significant difference could be assigned to the histology types NS and MC.

In some patients a more complete set of TCH50 data at 4-month intervals was available. Differences between one period and the next for each patient were calculated. The most dramatic difference again is between the TCH50 levels at time of initial diagnosis and 4 months later. The means and s.e. of the drop in TCH50 levels are 15 ± 9 ($n = 6$), 35 ± 13 ($n = 9$), 42 ± 12 ($n = 7$), 34 ± 4 ($n = 3$), for stages I–IV respectively. For stages I–III the levels at 4 months after treatment and beyond remained within the normal range until relapse occurs. For stage IV where the course of therapy may last more than 2 years, nine of the eighteen determinations within 4–24 months after the start of the therapy show elevations above 120 CH50 u/ml.

Levels of C1q and C3

The summary, statistics of mean, median s.e. and sample size of C1q and C3 determinations are given in Table 2. The subgroups are those defined earlier for TCH50. Stages II–V are significantly elevated from controls (C1q, $P<0{\cdot}01$ and C3, $P<0{\cdot}04$).

Levels of CIC

Fewer CIC determinations were available than corresponding complement components given in Table 2. Twenty-one determinations were made at the time of initial diagnosis, twenty-two during treatment, and twenty-eight after the cessation of treatment, eight at relapses prior to treatment and sixteen at relapses during or after treatment.

Like the complement components, the most significant correlation with the CIC levels is the treatment period (F-test, $P<0{\cdot}001$). The geometric means for the patients with disease prior to any relapse is 122 μg/ml; it then drops to 27 μg/ml during treatment and 22 μg/ml in all of the after treatment periods combined. The CIC level is again elevated to a mean of 66 μg/ml during all relapses combined prior to initiation of treatment. Prior to treatment, either at the time of first diagnosis or at relapses, the CIC levels are both significantly elevated from the normal control, 16 μg/ml (Dunnett's test, $P<0{\cdot}01$). (Data not presented.)

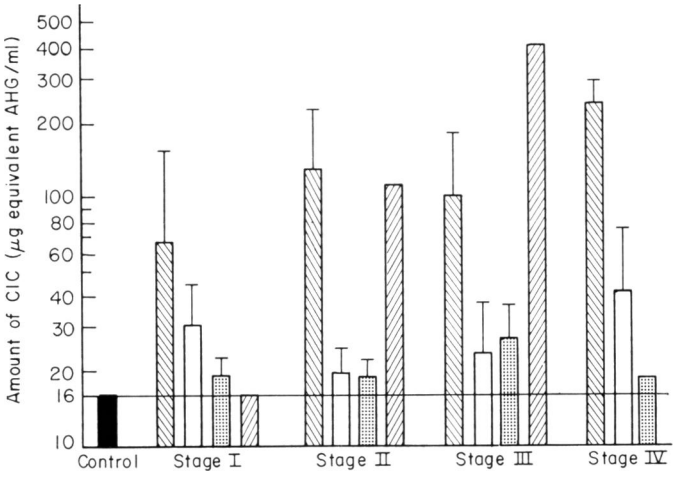

FIG. 3. Levels of CIC of patients during initial Hodgkin's disease, before (▨), during (□) and after 1 year of treatment (⊡), and of patients during all relapses before treatment (▨).

Fig. 3 presents the CIC values of the patients during their initial disease episode before treatment, during treatment and within 1 year after cessation of treatment, and for patients at all relapses before treatment. In the before treatment group, seventeen (81%) of the twenty-one values are above 16 μg/ml, with a maximum value of 1120 μg/ml. During treatment seven (33%) of the twenty-two determinations are still above normal, with a maximum of 320 μg/ml. Within 1 year after cessation of treatment, seven (37%) of nineteen values also remain above normal levels with a maximum of 240 μg/ml. At relapse prior to treatment, five (63%) of eight values were again elevated, with a maximum of 1280 μg/ml.

Table 3 shows the mean, median, range and sample size of the CIC values for different stages of HD for the different treatment periods. Apparent differences were observed across the stages, with higher levels being found in the stage IV groups, but these differences are not significant. These relationships are illustrated in Fig. 4. No significant effects of age, sex, or disease status were found. The overall geometric mean of fifty-one nodular sclerosis patients is 43 μg/ml, as compared with a value of 25 μg/ml in twenty mixed cellularity patients (F-test, $P<0{\cdot}02$). Within these groups of patients before relapse and before treatment, eleven NS patients have a geometric mean of 205 μg/ml, while six MC patients have a geometric mean value of 53 μg/ml. The cumulative frequency distributions of these two histological types are given in Fig. 5.

TABLE 3. Value of mean, median, range and sample size of circulating immune complexes at different stages and different treatment periods of Hodgkin's disease

	Stage I				Stage II				Stage III				Stage IV			
Treatment	Mean	Median	No.	Range	Mean	Median	No.	Range	Mean	Median	No.	Range	Mean	Median	No.	Range
No relapse																
Before	148	128	4	16–320	196	160	5	23–480	261	100	7	16–1120	279	216	5	160–560
During	35	36	3	16–52	27	16	8	16–90	42	16	5	16–144	112	16	6	16–320
After:																
1 year	33	16	6	16–50	20	16	4	16–32	49	16	8	16–240	19	19	1	19
3 years	24	16	5	16–30	16	16	5	16	16	16	3	16	68	68	2	16–120
Relapse 1																
Before	16	16	1	16	112	112	1	112	400	400	1	400	—	—	—	—
Normal Control	16	16	13	16	—	—	—	—	—	—	—	—	—	—	—	—

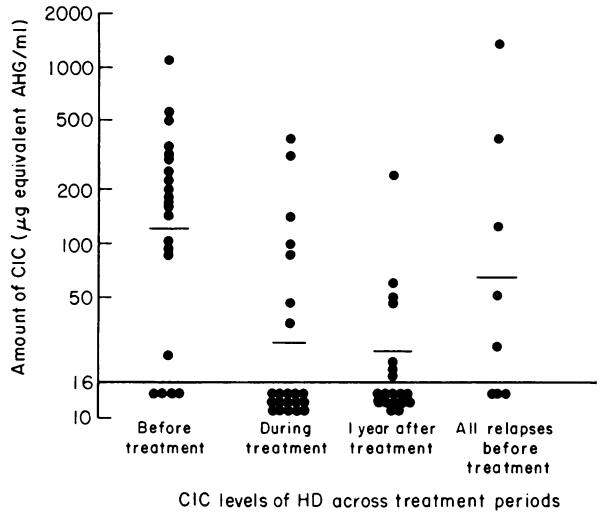

FIG. 4. Mean, median, and range of CIC values and sample size for different stages of Hodgkin's disease at different treatment periods. (——) Geometric mean.

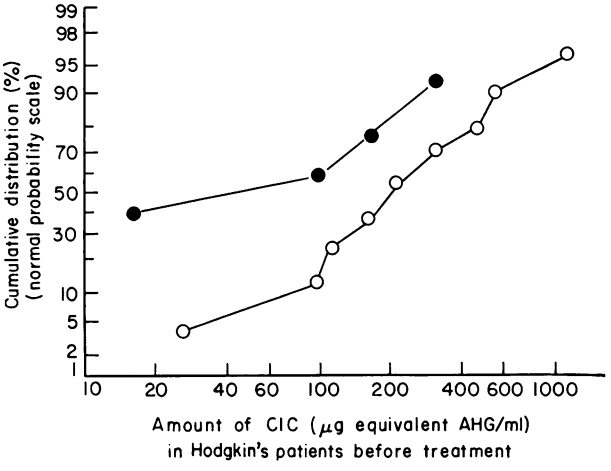

FIG. 5. Cumulative CIC frequency distributions of patients with nodular sclerosis (○—○) and mixed cellularity (●—●).

DISCUSSION

This is the first study of perturbations of complement and levels of circulating immune complexes in childhood Hodgkin's disease. In the present analysis, sera of eighty-six children with HD were investigated with respect to changes of total haemolytic complement TCH50, C1q, C3 and CIC over a period of 4 years. These data were correlated with the clinical stage, histological type, age and sex. The most significant findings were alterations of the levels of TCH50, C1q, C3 and CIC in the presence of disease activity and following treatment. Significant changes were also seen with relapses. Complement levels were significantly elevated ($P<0{\cdot}001$) at the time of diagnosis prior to splenectomy and/or treatment. If the patients are subdivided by stage of disease, the serum TCH50 levels in patients with stage II, III and IV are significantly higher ($P<0{\cdot}01$, 0·005, 0·01, respectively) than levels of TCH50 in healthy controls. Although the TCH50 levels of the different stages also differ significantly from one another, they did reveal a linear trend, because the mean values of patients in stages I–IV are 108, 138, 125 and

135 CH50 u/ml, respectively. TCH50 levels of stage II and IV are more alike and more elevated than those of stages I and III. During therapy most of the elevated levels in our study returned to normal values except those of patients with stage IV. In this group, the complement levels remained elevated after treatment even though 3 years passed following successful treatment that induced complete clinical remission. In contrast, C levels in sera from children of stages I, II and III fell to normal limits and remained there as long as 10 years following successful treatment and cessation of therapy.

In sera of patients in stages I, II and III who relapsed, the complement values were found to increase significantly ($P<0{\cdot}01$), although they were not found to be different from the values noted at diagnosis or before treatment. We noted that treatment of relapses does not necessarily lower the elevated TCH50 levels to normal. However, 1 and 3 years following treatment that brought complete clinical remission to one of the relapsed patients, all values had been normalized. This interesting finding suggests that disease activity may be present in patients with advanced Hodgkin's disease in spite of clinical inactivity. Certainly, each patient should be studied further and efforts made to elucidate the basis of the persistent increase in TCH50 levels. The differences of TCH50 values between male and female patients are significant ($P<0{\cdot}05$), sera of female children with HD having higher levels than sera of males. No association of complement component levels, histological type, age of patients or clinical status with (B) or with (A) general symptoms was evident.

Several investigators have studied complement levels in sera of adult HD patients. The first report (Southam & Goldsmith, 1951; Libansky & Jezkova, 1963) revealed no significant differences in sera of adult HD when compared with healthy controls nor were changes seen that could be attributed to treatment such as radiation or nitrogen mustard (Southam & Goldsmith, 1951). Further, in those studies the levels of C did not seem to be related to the condition of relapse or remission in patients (Libansky & Jezkova, 1963). On the other hand, several investigators have reported C levels in adults with HD (Schier *et al.*, 1956; McKenzie, Coesky & Hetrick, 1967; Lichtenfeld *et al.*, 1976; Wood *et al.*, 1958; Irunberry & Colonna, 1970; Wagener & Haanen, 1977) consonant with our findings. In a detailed study Rottino & Levy (1959) demonstrated that TCH50 levels were elevated in twenty-one of thirty-seven patients with active disease whereas only six of twenty-three patients in complete remission showed elevations. They found no correlation of serum C levels with age, sex or stage, although they and others (Wood *et al.*, 1958) showed a good association of the levels of complement with other acute phase reactants (CRP, ESR, gammaglobulin and fibrinogen). The finding of elevated C values is not explained readily by present knowledge regarding the stimulation of C and C components. Whether increased C levels can contribute to immune defense against HD is unknown. Their increase, however, might represent a non-specific response to the developing neoplasm (Maness & Orengo, 1977) since in our study these elevated levels drop significantly when remission is induced by effective initial treatment. Our findings of persistently elevated C levels after treatment of both stage IV and relapsed patients from stages I–III suggest an association with continuing disease, as mentioned earlier. Once again, this observation indicates that in the relapsed patients subclinical disease activity may be present. Both of these new findings require detailed investigation.

Circulating immune complexes were determined by the Raji cell radioimmunoassay (Theofilopoulos, Wilson & Dixon, 1976). We have shown repeatedly that increased CIC levels are associated with sediments between 19S and greater, evidence entirely compatible with the concept that the Raji test in these patients is measuring circulating immune complexes (Day, unpublished observations). Others, however, have sometimes found material ranging from 10–30S suggesting that sometimes different sizes of complexes may be detected by the Raji as well as other tests for CIC. The CIC levels in the present study were elevated significantly at the time of diagnosis before treatment was started and they increased progressively from stages I–IV from 148, 196, 261 and 279 μg/ml, respectively. During treatment these values dropped and remained near the normal values and sometimes to very low levels—even less than normal when the patients were in complete remission often over a 10 year follow-up. At the time of exacerbation, however, the CIC levels again were increased significantly ($P<0{\cdot}001$). The highest CIC levels observed in this study were seen in sera of children with a histological diagnosis of the nodular sclerosing form of HD. These levels were significantly different when compared with serum CIC

levels of patients with HD of mixed cellularity. No differences in CIC levels could be found between children with HD who were older than 10 years and younger children, between males and females or between different expressions of generalized symptoms (status A and B).

Prior studies reveal conflicting data concerning CIC levels in adult HD. Whereas Amlot and co-workers (Amlot *et al.*, 1976, 1978) in a study of sixty-two patients showed elevated levels in HD and reported a close correlation of CIC levels with symptoms of night sweats and fever, i.e. patients with poor prognosis, Kavai *et al.* (1976) and Brown *et al.* (1978) and our present studies cannot confirm this. The reason for this may be a difference in the techniques employed for CIC determinations. Long *et al.* (1977), using the Raji cell radioimmunoassay, found that thirty-four of ninety patients with HD have serum CIC levels greater than 10 μg/ml. In an interesting study these investigators showed that the CIC in the sera bound to a monolayer culture of cells from a patient with HD.

Our findings of CIC in childhood HD are comparable to our recent observations of CIC levels in sera of patients with lung cancer, neuroblastoma (Brandeis *et al.*, 1978), of adult colon–rectal disease and breast cancer, where CIC levels are greater in advanced disease than in the earlier stages.

It now seems most important to analyse the circulating immune complexes and to determine them in detail by physical and chemical analyses. In a detailed study of our patient with CIC, cryoglobulinaemia and non-hereditary angio-oedema, we showed the cryoglobulin to contain lymphocytotoxic antibody against lymphocyte surface membranes of both leukaemic and normal lymphocytes. Membrane antigen was also present (Day *et al.*, 1976). Similar studies of the composition of CIC in children and adults with HD seem very much in order. Long term follow-up of the findings from the present investigation that advanced disease (stage IV) and relapsed patients showing distinct perturbations of complement must also be pursued. However, based on our observations to date, CIC and TCH50 levels may be of value in prognosis of childhood HD and may serve to complement methods for staging and determining the efficacy of treatment in this disease.

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